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Otis Laboratory Accomplishments 2017

Buzzards Bay MA • Salinas CA • Bethel OH

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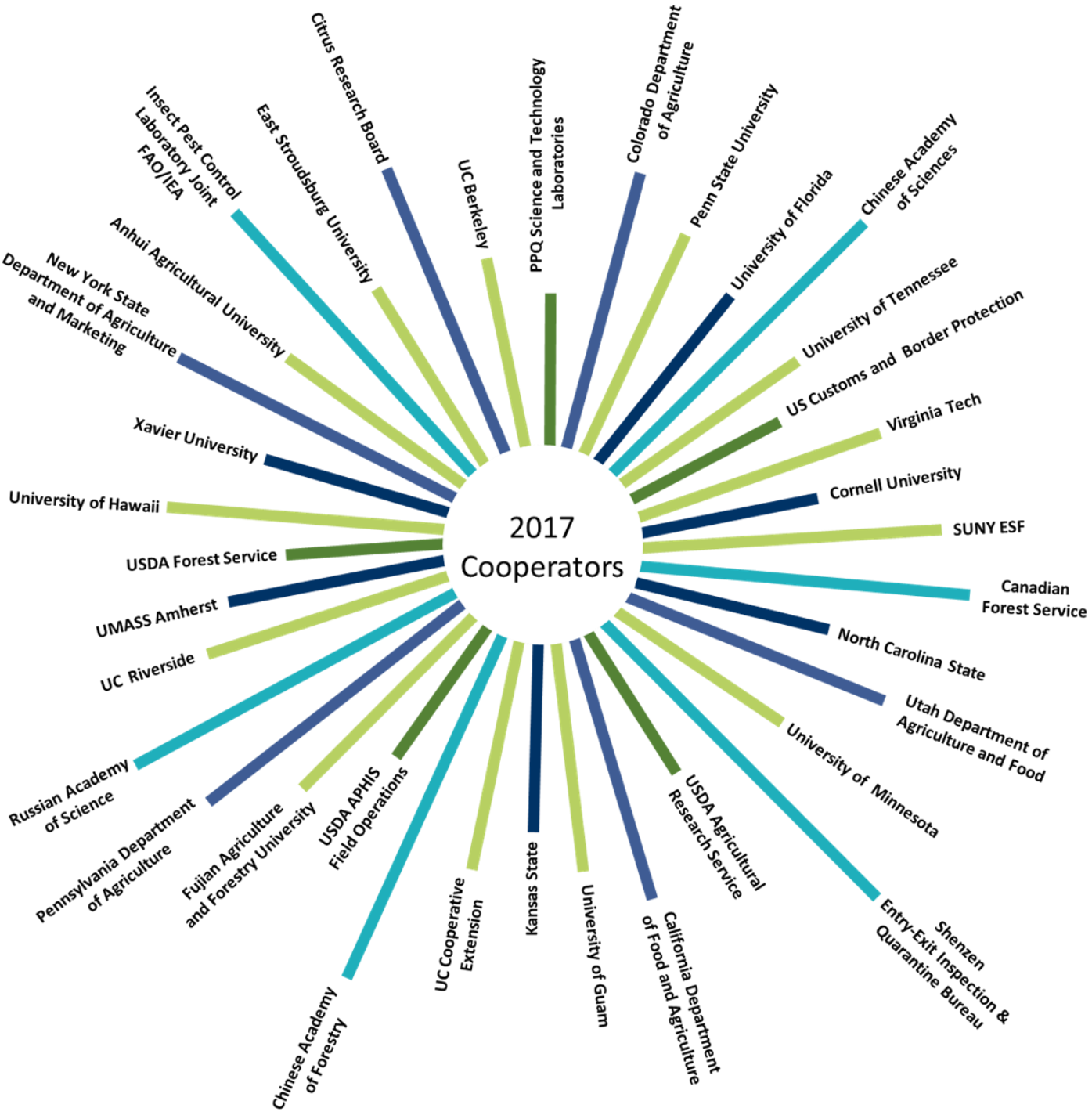
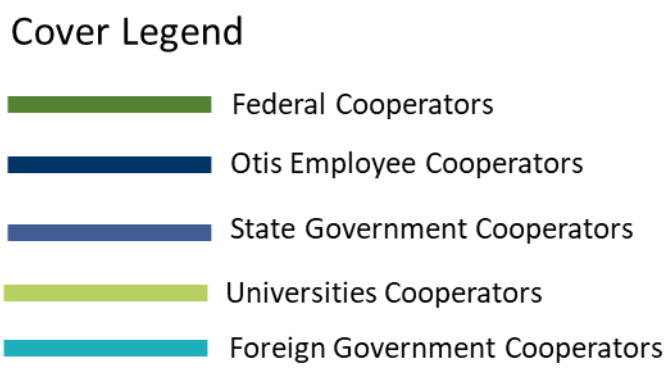
OTIS LABORATORY ACCOMPLISHMENTS 2017

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Commodity Treatment

Biological Control

Insecticide Technology

Pest Management

Rearing

Survey, Detection, and Analysis

Behavior and Chemical Ecology

Behavioral Ecology and Survey Technology

Chemical Ecology and CAPS Lure Support

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Quantitative Risk Analytics

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Collaborative Relationships: A Formula for Success

Director: Scott Pfister

Assistant Director: Scott Myers

The Merriam-Webster dictionary defines collaboration as working jointly with others, or together, especially in an intellectual endeavor, or to cooperate with an agency or instrumentality with which one is not immediately connected. Collaborative relationships are essential and pivotal to the pursuit of new knowledge and to the methods development work conducted by Otis Laboratory. Towards this end, this year's annual report is dedicated to our collaborators and their important contributions to APHIS' mission.

The Otis Laboratory, Salinas, CA Field Station, and Bethel, OH satellite laboratory have many collaborative relationships internally within PPQ and externally with other state and federal agencies, universities, and international institutions. We exist to provide tools and knowledge to PPQ programs, which makes the Office of the Deputy Administrator, Policy Management and Field Operations our most important customers.

Internally, laboratory scientists and technicians are members of numerous Cross Functional Working Groups where they receive input and direction on what the methods development needs are for PPQ programs. It is critical that the work done at the Otis Laboratory serves to support and resolve existing programmatic needs. Collaborating with other Science & Technology laboratories allows scientists to exchange ideas and techniques in many areas such as treatments, molecular diagnostics, analytics, biological control, trapping and lure development. We strive to compliment, not compete, with other laboratories in bringing solutions to PPQ.

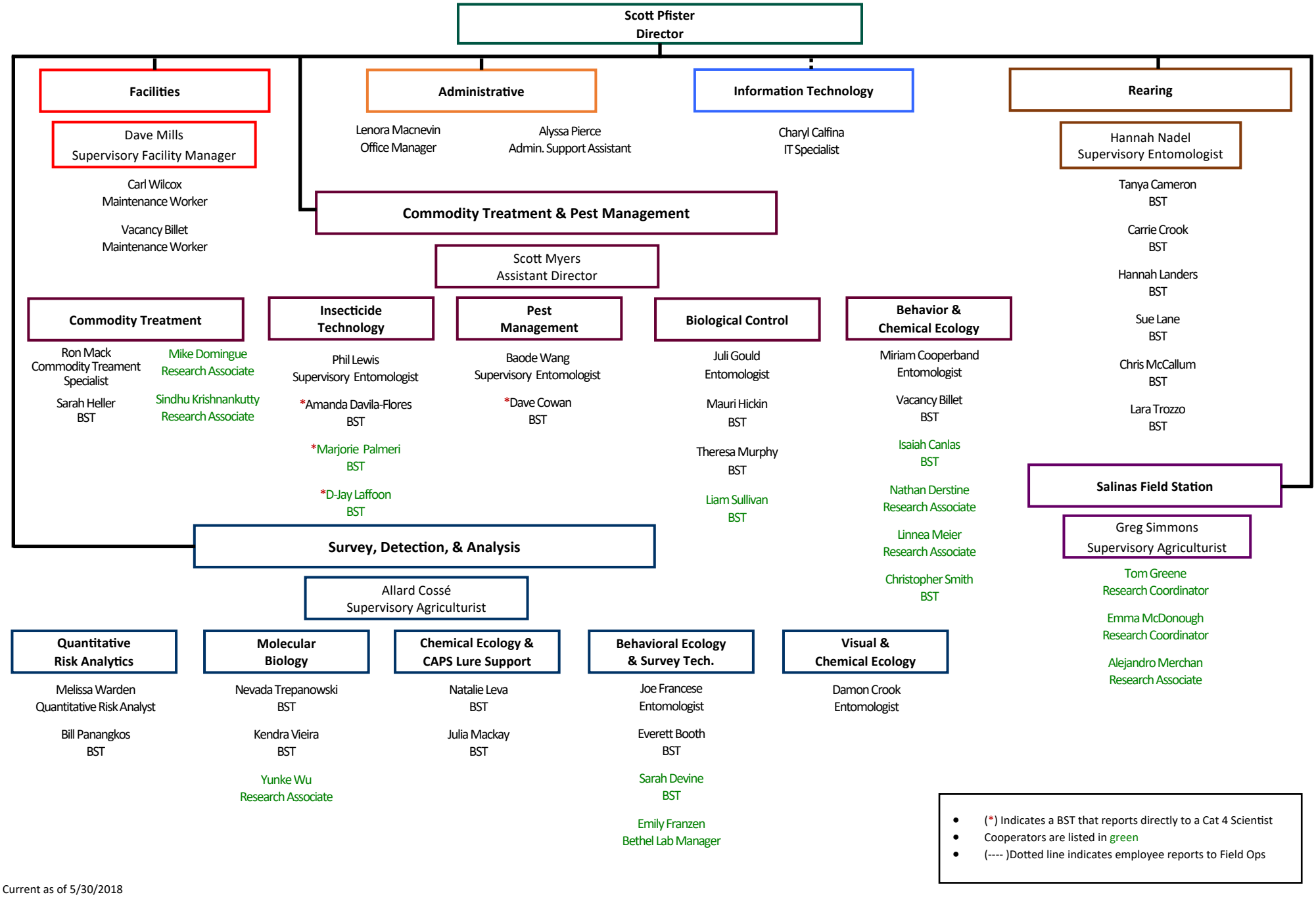
It is important to maintain strong working relationships with other federal agencies such as the Forest Service, Agriculture Research Service (ARS), National Institute of Food & Agriculture, and Customs and Border Protection (CBP). Some of the problems we face cut across agency mission areas, and it is important to coordinate intra agency efforts to ensure that we are all pulling together for a robust national response. For example, some of our current projects strive to bring novel solutions to successfully utilizing biological control agents for forest pests and protecting U.S. agriculture from khapra beetle, respectively with the ARS Centers for Beneficial Insect Research in Newark, Delaware and Grain Health Research in Manhattan, Kansas. Additionally, the Salinas Field Station supports a biological control laboratory in Michigan, which maintains a year-round EAB colony for research, by providing ash leaves when they are unavailable during the Michigan winter.

Partnerships with universities across the U.S. provide a means to remain abreast and in sync with the latest research. Currently, Otis Laboratory maintains cooperative agreements with over a dozen institutions of higher learning across the U.S. such as the University of California, Cornell, University of Massachusetts, Xavier, Kansas State, University of Florida, University of North Carolina, and Virginia Tech. Research Associates employed by many of these universities work in the Otis Laboratory, or at one of its satellite locations, on many different projects serving to protect our nation's agricultural and forestry resources. A few of these cooperative projects include 1) investigating the biology and development of lures for *Euwallacea* spp., khapra beetle, and spotted lantern fly, 2) the rearing and identification of intercepted Coleoptera and siricids by CBP at ports of entry, 3) population genomics of Asian gypsy moth and Asian longhorned beetle (ALB), 4) regulatory treatments for light brown apple moth, and 5) the development of monitoring and control methods for exotic invasive forest pests.

Considering that one of PPQ's main goals is to detect and respond to exotic pests introduced into the U.S., having international collaborators is essential. Some examples include a collaborative project with the International Atomic Energy Agency Insect Pest Control Laboratory in Austria, which has provided mitigations and treatment options for many fruit fly species of concern. Another is the important collaborations with numerous universities in China which allow us to study and work on mitigations for several important pests such as ALB and emerald ash borer within their native range.

At Otis Laboratory, building collaborative relationships, both internally and externally, for PPQ is a key element to successfully managing many of the invasive pests threatening our agricultural and forestry resources. As you read through this year's annual report, please take a moment to reflect on how the vast majority of the research discussed was only made possible through such ongoing relationships.

Photo taken in Otis' new workshop on 6/1/18 by Charyl Calfina



- (*) Indicates a BST that reports directly to a Cat 4 Scientist
- Cooperators are listed in green
- (----)Dotted line indicates employee reports to Field Ops

Current as of 5/30/2018

Evaluation of cold tolerance between wild population and laboratory reared Mediterranean fruit flies

Vanessa S. Dias[1], Florence Maxwell[1], Camilo Rivera[1], Amanda A. S. Cardoso[1], Carlos Caceres[1], Marc Vreysen[1] and Scott W. Myers[2]

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Phytosanitary cold treatments for exotic fruit flies are commonly used for the safe trade of agricultural commodities throughout the world. In particular, citrus is well adapted to the use of cold treatment as a phytosanitary measure since many varieties are tolerant of the treatment temperatures used. International shipping times also allow for treatments to be conducted in-transit, minimizing delays in bringing product to market.

The high level of efficacy typically required by importing countries for establishing phytosanitary measures such as cold treatments requires the testing of large numbers of fruit flies, typically tens of thousands. For this reason, researchers developing treatment schedules for these commodities typically rely on the use of laboratory reared cultures to determine the most tolerant life stage and to establish the optimal treatment temperature and duration to meet phytosanitary requirements. Since many economically important fruit flies have well established artificial diets and rearing protocols, this can be done economically with relatively minimal time investment. However, one concern is that this approach relies on the assumption that laboratory and wild populations respond similarly to cold treatment. It has been suggested that flies reared at constant temperatures of 25°C or greater may be more susceptible to cold treatments than their wild counterparts, and that a treatment developed using less tolerant organisms may not adequately control wild flies. For these reasons, researchers will occasionally replenish or replace laboratory reared cultures with additions of wild collected flies. However, there have been no studies that have made comparisons of cold tolerance between laboratory reared and wild flies, and little is known about the efficacy of colony replenishment to correct for any potential difference.

The current study was done to determine if wild and laboratory reared colonies of the Mediterranean fruit fly, *Ceratitis capitata*, vary significantly in their tolerance to cold at typical treatment temperatures (Figure 1).

Figure 1. *Ceratitis capitata* on tangerine.

The experiments were performed at the Insect Pest Control Laboratory of the FAO/IAEA Agriculture and Biotechnology Laboratories in Seibersdorf, Austria. The Mediterranean fruit flies used in this experiment all originated from Valencia, Spain. The original laboratory colony was at generation 23 at the time of this study. The collection used for wild comparison was made in 2017. Parental flies of the wild population were reared in fruit for one generation prior to their use in this study, increasing the population size to provide enough flies to conduct the experiment.

Results showed no significant differences in survival of third instar larvae across each of the three treatment durations. Very few larvae survived following the eight day cold treatment, and just a single larva was still moving in the wild population after the 15 day treatment (Table 1). While this larva was moving and therefore counted as a survivor, it failed to pupariate and did not survive to the adult stage.

These results suggest that there may be more potential for larvae from the recently collected "wild" population to survive the 15 day treatment. However, we cannot draw a definitive conclusion based on a single larva and the relatively low number of flies used in this experiment. Work planned for 2018 will continue to evaluate these two populations to improve our ability to measure any potential differences in cold tolerance between these two groups. In addition, we plan to collect wild *C. capitata* from other parts of the world (e.g., Argentina, Morocco), and then compare the wild sources with long standing laboratory colonies.

Table 1. Mean mortality (± SE) of wild collected and laboratory reared culture of Mediterranean fruit fly larvae following cold treatment at 1.1 ± 0.1°C in oranges and tangerines.

Population	Total no. treated	Replicates	Time at 1.1 ± 0.1°C		
			0 days	8 days	15 days
Lab (F23, F24)	1,811	6	21.62 ± 8.81	99.86 ± 0.14	100.00 ± 0.00
Wild (F1, F2)	1,846	5	36.75 ± 15.58	99.13 ± 0.75	99.67 ± 0.33

Update: Development and harmonization of phytosanitary treatments for exotic tephritids fruit flies

Scott W. Myers[1], Guy Hallman[2], Lincong Wang[3], Florence Maxwell[2], Carlos E. Cáceres Barrios[2] and Marc J.B. Vreysen[2]

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A number of tephritid fruit flies pose a great threat for entry into the U.S., and approved phytosanitary treatments are lacking for many important species. New treatment schedules approved for this group may be used by many developing countries to export their produce to the U.S. safely, reducing the risk of introduction and protecting U.S. agriculture. In addition, frequent fruit fly outbreaks in the U.S. and the resulting domestic quarantines require that phytosanitary treatments be available for growers to move fruits and vegetables to domestic and international markets.

The FAO/IAEA Agriculture and Biotechnology Laboratories in Seibersdorf, Austria have more than a dozen cultures of exotic fruit fly species of economic importance, multiple population sources of key species, and the ability to obtain virtually any tropical tephritid. The laboratory provides the ideal environment to develop new phytosanitary treatments, collect additional data on existing treatments, and make treatment efficacy comparisons across species, populations, and host plants to standardize treatment schedules for a number of important commodities.

Since 2009 USDA APHIS has successfully collaborated with scientists from USDA ARS and FAO/IAEA at the Seibersdorf facility to develop a number of cold treatments for high risk tephritids. These proactive treatments can be used to ensure safe trade despite future quarantines resulting from discoveries of exotic fruit flies in the U.S. and in the countries of our trade partners, as has happened on various occasions in the past.

Developing harmonized phytosanitary treatment schedules across countries, pest species, and commodities would provide a number of benefits for USDA APHIS and domestic specialty crop producers in the U.S. In particular, they would help reduce the probability that exotic fruit flies are introduced through commercially imported fruits and other host commodities.

In addition, they would provide important tools that could be used to move host commodities domestically when new exotic fruit fly incursions do occur. In 2017, two studies were completed that examined 1) comparison of tolerance to vapor heat treatment in three populations of *Bactrocera dorsalis* and 2) confirmatory testing of cold treatment for *Zeugodacus tau*.

Comparison of tolerance to vapor heat treatment in three populations of *Bactrocera dorsalis*

A number of current vapor heat treatment schedules used for *B. dorsalis* vary in their severity depending on origin of the host commodity. A study was performed to compare populations of *Bactrocera dorsalis* from China, Kenya, and Thailand for tolerance to vapor heat treatment (47°C and 95% RH) in mangoes. Treatments were conducted in an environmental chamber using mangoes that were infested by placing fruit in cages with adult flies (Figure 1). The egg stage was targeted as the most tolerant as determined from previous studies. Fruit was treated in replicates of six infested mangoes per population for 150 to 180 minute durations. Following treatment, mangoes were held at 25°C and then dissected to detect the presence of survivors developing to third instar larvae. Infestation rates were estimated based on larval counts from an equal number of untreated control fruit.

Figure 1. Mango placed in rearing cage with adult *Bactrocera dorsalis* for ovipostion.

Results showed that the population from Kenya was slightly less tolerant when estimates of LD 90 (90% mortality) seed surface temperature were compared. However, at LD 95 (95% mortality) and Probit 9 (~99.9% mortality) levels there were no significant differences among the three populations (Figure 2). Since the higher levels of control are what is most relevant for phytosanitary treatments, these results suggest that vapor heat treatments for *B. dorsalis* in mango are broadly applicable across geographic regions.

Cold treatment of *Zeugodacus tau*

Zeugodacus tau is a polyphagous fruit pest of economic importance in Asia. A colony of *Z. tau* was obtained from China and reared in the laboratory on zucchini fruit, as cucurbits are favored host plants that infest easily by placing the fruit in cages with flies. Studies to determine the most cold-tolerant stage found the third instar to be the most tolerant stage. Confirmatory testing to establish a cold treatment schedule was initiated in mandarins. Infestation by placing mandarins in cages with ovipositing flies resulted in low infestation rates, with very few fruit producing larvae. Therefore, infestation was done by injecting eggs collected from zucchini fruit under the peel of mandarins. Following treatment, fruits were carefully dissected and all larvae were removed and counted. Survival was determined by larval movement one day after fruit were removed from the treatment chamber. Results found four survivors in 5,003 treated larvae after 20 days at 1.1 ± 0.1°C. An additional experiment to evaluate a 22 day treatment found no survivors from a total of 2,207 treated. Currently, the most severe USDA cold treatment schedule used for tephritids only is the schedule for *Anastrepha ludens* (T107-b) which requires 18, 20, and 22 days at 0.56, 1.1, and 1.67°C, respectively. While this study did not test treatments at temperatures other than 1.1°C, these results suggest that T107-b would not be adequate for *Z. tau*, and that two additional days should be added to provide quarantine level control.

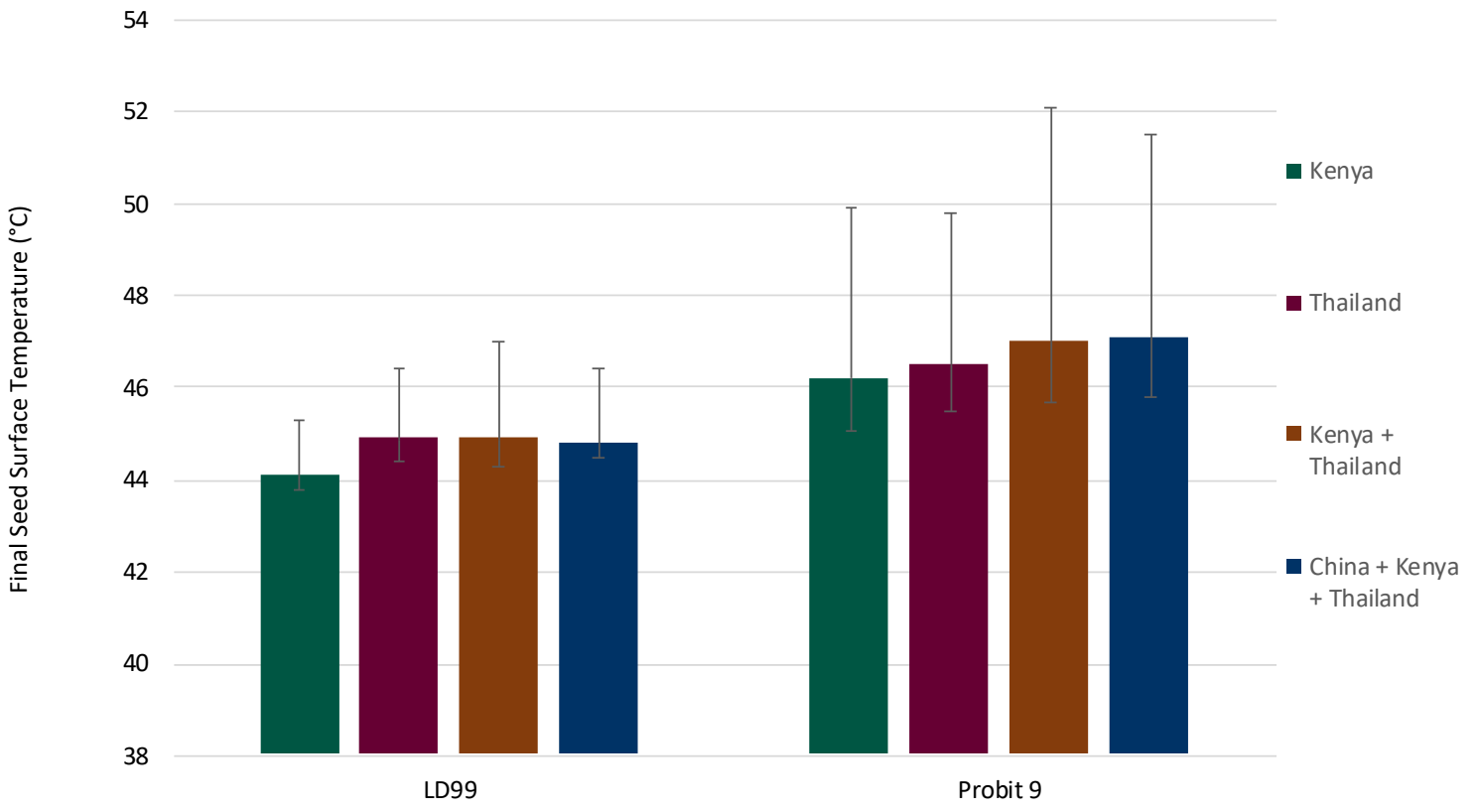

Figure 1. Probit model mortality estimates (95% Fiducial limits) of final seed surface temperatures in vapor heat treatment of mangoes infested with 24 h-old eggs of three populations of *Bactrocera dorsalis*.

Evaluation of methyl bromide alternative fumigants for efficacy against pinewood nematode

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Introduction

The fumigation of logs for export from the U.S. represents one of the largest quarantine and pre-shipment use exemptions for methyl bromide (MB). Log fumigations require high doses of MB to kill pinewood nematodes, PWN, *Bursaphelenchus xylophilus*, and the oak wilt fungus—the two most important pests for log exports. The phase-out of MB has made the fumigant more expensive and makes its availability in the future uncertain. In addition, federal and state air quality regulations restrict MB use and thus have reduced the volume of log exports from the U.S.

The use of MB continues for log exports from the U.S. and, to a lesser degree, domestic movement of logs, firewood, and other wood products under the quarantine and pre-shipment use exemption because no alternative treatments are commercially available for these commodities. The development and adoption of efficacious and economically viable alternative fumigants would facilitate compliance with the Montreal Protocol and provide a number of additional benefits to industry, and to domestic and international regulatory agencies to facilitate international trade.

The alternative fumigant products, sulfuryl fluoride, phosphine, and ethanedinitrile, currently offer the best available options to provide effective quarantine level control of wood pests with minimal disruption to current industry practices, infrastructure, product quality, and economics. We used small scale fumigations in a laboratory environment to evaluate these three products for efficacy against pinewood nematode.

Methods

The efficacy of each fumigant was evaluated in a two part process. First, pine chips inoculated with PWN were treated to establish baseline dose-toxicity relationships. Secondly, white pine blocks (9 × 9 × 15 cm) with bark on one surface were used to simulate logs in small scale experiments. Blocks were sealed on all sides without bark to assess the fumigants ability to penetrate through the bark and control nematodes (Figure 1A). Fumigations were performed at 20°C using 10 L glass jars (Figure 1B) held inside environmental chambers. Headspace fumigant measurements were made at regular intervals during the fumigation via gas chromatography and resulting data was used to calculate concentration × time (C×T) products. Post treatment evaluations of chips and wood blocks were made at six and 21 day intervals using standard sterile techniques and Baermann funnels to extract live nematodes.

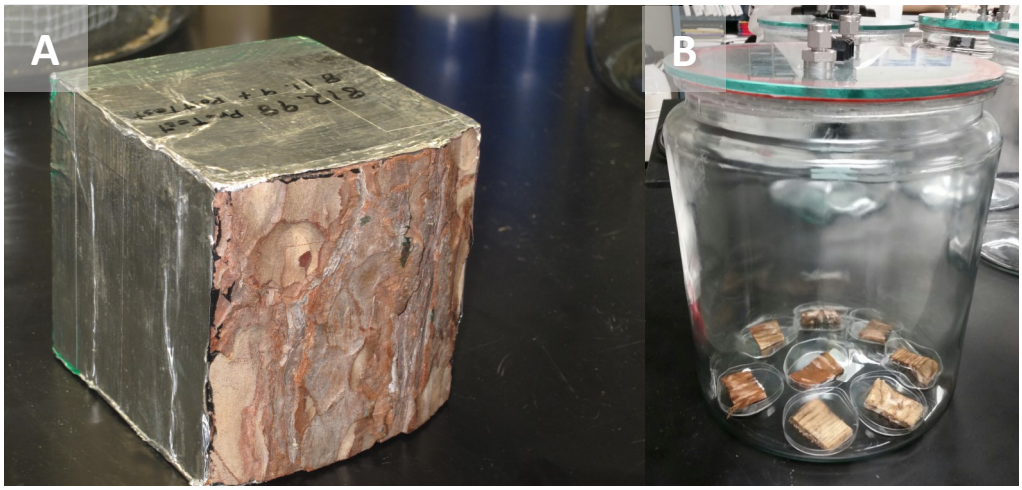

Figure 1. A) Wood blocks sealed on five sides to simulate log fumigations. B) Fumigation of wood chips inoculated with pinewood nematode.

Results and discussion

Each of the methyl bromide alternative fumigants was able to provide complete control of nematodes in pine chips at one or more of the treatment schedules tested (Table 1). Control of nematodes in the pine blocks simulating logs was more challenging. A summary of results from the block studies is provided in Table 2. Methyl bromide fumigation did not prevent recovery of nematodes in the 16 hour duration treatment, but it was successful when following the 48 hour schedule. Neither sulfuryl fluoride nor phosphine completely controlled nematodes at the schedules evaluated in this study, suggesting they are not promising treatments for pine log exports.

The ethanedinitrile treatment, however, was successful in preventing recovery of nematodes at all rates tested in two separate experiments. This indicates it may be a promising treatment, however, ethanedinitrile is not currently registered for use on logs in the U.S.

Future studies will focus on ethanedinitrile to gather additional data on efficacy on PWN in freshly cut pine. In 2018 we plan to conduct further evaluations of efficacy using the block method described here at several additional doses and at lower temperatures. Additionally, we plan to inoculate and treat whole log sections of white pine in larger chambers to determine efficacy under experimental conditions that more closely approximate commercial log fumigations.

Table 1. Minimum treatment conditions to provide complete control of pinewood nematode in white pine chips fumigated at 20°C.

Fumigant	Treatment duration	Initial dose (mgL^{-1})	Mean CxT ($h\text{-}mgL^{-1}$)
Sulfuryl fluoride	48 h	70	3419.8
Phosphine	14 d	2.2 (1500 ppm)	673.1
Ethanedinitrile	24 h	30	753.5

Table 2. Efficacy of methyl bromide and alternative fumigants in controlling pinewood nematode in fumigations of white pine blocks at 20°C.

Fumigant	Treatment duration	Initial dose (mgL^{-1})	Mean CxT ($h\text{-}mgL^{-1}$)	Pre-treatment PWN/g dry wood ± SE	21 day PWN/g dry wood
Methyl bromide	16 h	120	1,845	718.0 ± 422.5	112.3 ± 40.8
	48 h	120	5,258	231.7 ± 173.0	0.0 ± 0.0
Sulfuryl fluoride	48 h	140	6,994	258.2 ± 17.1	135.0 ± 120.0
	48 h	160	7,878	318.1 ± 305.0	283.5 ± 141.5
	48 h	180	8,943	44.5 ± 27.5	545.5 ± 148.5
Phosphine	20 d	2.2* (1500 ppm)	732	579.0 ± 272.0	266.4 ± 153.2
Ethanedinitrile	24 h	40	930	208.6 ± 101.1	0.0 ± 0.0
	24 h	60	1,445	170.3 ± 42.6	0.0 ± 0.0
	24 h	80	1,972	259.2 ± 81.4	0.0 ± 0.0

*Additional phosphine gas was added after 10 days to return the treatment to 1,500 ppm.

Optimization of lures for khapra beetle

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Introduction

Khapra beetle, KB, *Trogoderma granarium*, is a stored products pest that is currently quarantined in the U.S. because of the potential danger it presents to the U.S. grain industry and world food security. Their larvae feed on a wide range of dry food products and often damage large proportions of material they infest. This project evaluates the efficiency of commercially available lures and traps for KB with the objectives of optimizing detection in the monitoring programs already in place, and assisting in management of any potential introductions. In addition to evaluating lures and traps for KB, warehouse beetle, WB, *Trogoderma variabile*, was evaluated in parallel studies to assess whether this closely related species could act as a behavioral surrogate for KB. We focused on the effects of lures on larvae because little is currently known about the responses of both species to semiochemicals. Because the larval period can be quite prolonged and the window to catch adults is limited, improved traps for larvae would be beneficial to ongoing detection and monitoring efforts.

Behavioral effects of the lures on larvae were evaluated in a series of laboratory bioassays that are capable of distinguishing between various behavioral phenomena, including: attraction, preference, arrestment, and trapping efficacy. The lures tested included the Trece™ KB pheromone lure and three kairomone baits: the Trece™ 5 Storgard oil kairomone lure (oil), the Insects Limited™ PantryPatrol Gel (gel), and the Insects Limited™ Dermestid Tab bait (tab) (Figure 1). In some experiments, simple wheat germ was used as a positive control. Each odor was evaluated individually in each assay, despite the fact that odors are often used in combination in trapping efforts. Later studies will evaluate these interaction effects. Assays were conducted using larvae that were grouped as early (~2 mm) and late (~5 mm) instar.

Attraction assay

The first assay was performed in a small scale wind tunnel (Figure 2A), which pushed air over a 10.5 x 14 cm white paper arena, where a single insect was placed to evaluate its mobility in response to different treatments. The propensity of the larvae to move upwind toward the odor source would indicate oriented attraction. In the wind tunnel assay, large KB and WB larvae tended to walk upwind more often than smaller larvae for all of the treatments with the exception of the oil.

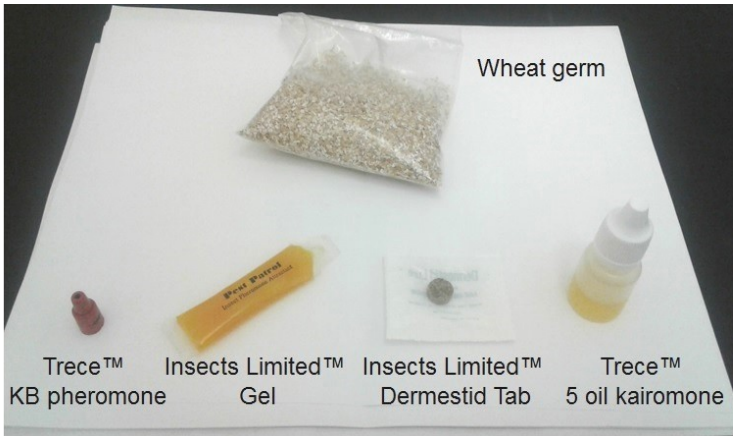

Figure 1. Kairomone and pheromone lures used for attraction of *Trogoderma* stored product pests for monitoring traps.

This tendency was especially pronounced for the pheromone lure, which was not very attractive to smaller KB larvae, but among the most attractive lures to larger larvae.

For both large and small larvae of KB, the gel and tab baits were the only treatments consistently more effective at eliciting upwind movement than wheat germ alone or blank controls. Overall, the responses by WB to odors in this assay were not as pronounced. The small WB larvae responded most strongly to the KB lure, but this only increased the response rate by 10% relative to the negative control. The large WB larvae had the strongest response to the Dermestid Tab bait & KB pheromone, but again, this only increased the response rate by 10%.

Preference assay

Another assay involved a two-way choice between baits or blank controls involving two 35 ml vials connected with a 4 cm long x 1 cm diameter PVC tube (Figure 2B). The goal of this assay was to assess preference of WB and KB larvae for each odor source. A single insect was placed in a hole in the tube and a choice was noted if it moved into either vial within 5 minutes. Most of the kairomone odors were attractive to smaller and larger KB and WB larvae when compared to controls, but there were several differences between the species. The tab bait was more consistently attractive to WB, while the gel was more consistently attractive to KB. The pheromone lure was generally not attractive to either species, with the exception of larger KB larvae.

Arrestment assay

Arrestment was measured by a Petri dish assay where baits were placed under holes on opposite ends of the arena (Figure 2C&D). The amount of time spent on either half of the Petri dish within five minutes was recorded. For KB, the results were much different for larger larvae compared to smaller larvae in this assay. All three of the commercially available kairomone baits were highly effective at eliciting a greater time on the side of the Petri dish where the odor was introduced, but wheat germ and the pheromone lure were not similarly effective. However, for smaller larvae the pheromone and gel elicited a positive response, but the other lures all had a negative effect. Overall, this experiment might suggest that the gel would be most effective in encouraging KB larvae to remain near baited traps.

Trapping experiment

A close-range trapping experiment was also performed. The experiment was set up in 18 L plastic dishpans (Figure 2E), with each including a box trap (IL-2300-10, Insects Limited, Westfield, IN) and different bait treatments. The trap, including the treatment, was placed in the arena in one corner, while 10 larvae were released in the opposite corner. The total numbers of larvae in each trap after 24 hours were recorded. For this assay the differences between WB and KB responses were particularly pronounced. The proportion of larvae found in the traps was much greater for KB than WB.

Amongst the different treatments, approximately 20 to 30% of small and 40 to 55% of large KB larvae were found in the traps, while only 1 to 4% of small and 5 to 20% of large WB larvae were found in the traps. Furthermore, for large KB, there were no significant differences in the numbers found in traps with different odor treatments, including the control, but there were differences among the treatments for smaller larvae. The treatment with the most captures for small KB was the gel, and these captures were significantly greater compared to the tab bait, which was the treatment with the fewest captures. Conversely, for small WB larvae, there were no differences among treatments, but there were for larger larvae. For the larger WB larvae, all three of the commercially available kairomone traps were significantly more attractive than control traps or those with the pheromone lure.

Conclusion

At this point, because of the numerous differences in results between the different assays, it does not seem likely that the WB can be used in place of KB to assess potential behavioral responses to semiochemicals in the laboratory. Fully considering the results from each assay for large and small larvae, the commercially available gel lure is likely to be most effective. Field trapping of KB is needed to confirm that it is the most effective lure since WB cannot be used as a surrogate for such investigations.

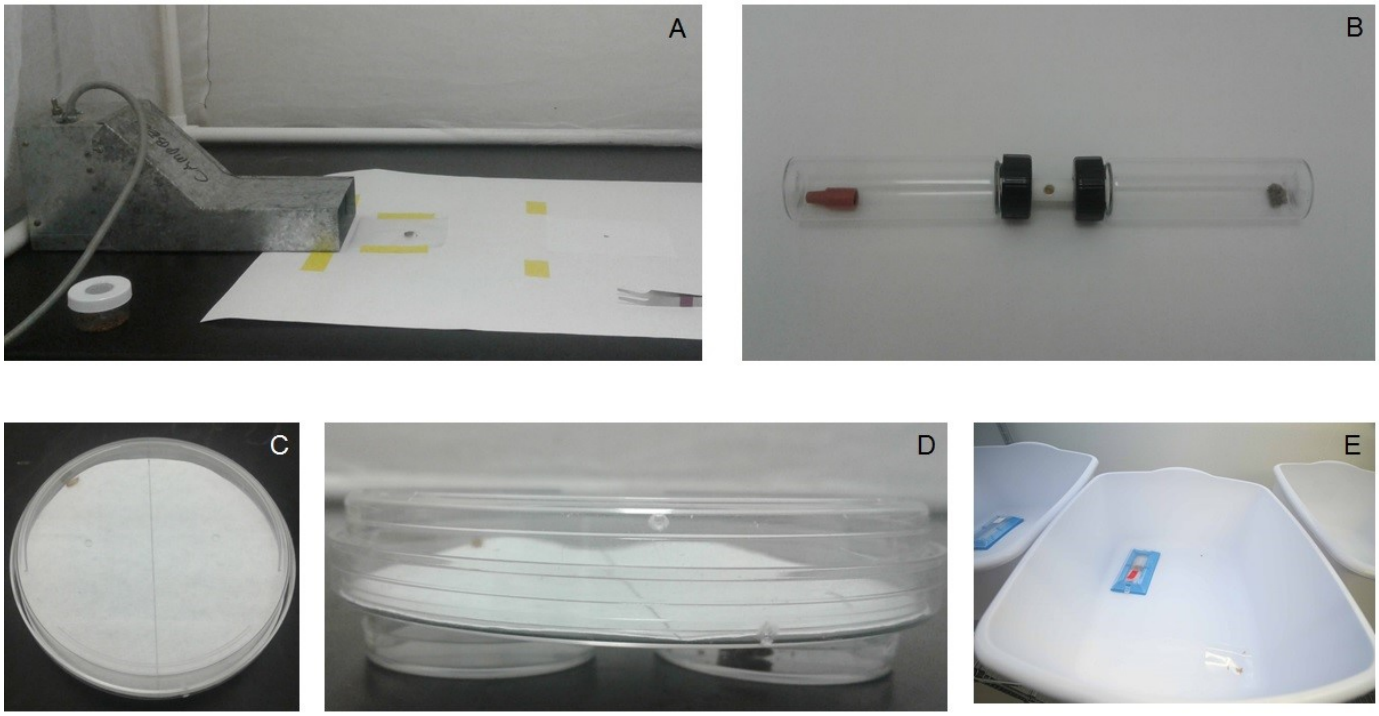

Figure 2. Laboratory bioassays used to assess the effects of semiochemical attractants, which include: A) a mini-wind-tunnel, B) a two-way olfactometer, C&D) an arrestment assay where odor sources are placed in wells below holes on each side of a Petri dish, and E) trapping in 18 L plastic containers.

Khapra beetle larval behavioral responses to whole body extracts

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The khapra beetle, KB, *Trogoderma granarium*, is a pest of a wide array of stored grain products. It currently has an extensive distribution throughout warm and arid regions of Eurasia and Africa. It is a quarantine pest in the U.S. and has a history of being intercepted at ports of entry. Eradications of this pest at certain locations have been successful. Preventing establishment of the khapra beetle in the U.S. is crucial for maintaining access to products in export markets. Various kairomone attractants have been developed for monitoring the species. Additionally, a pheromone produced by adult females is used in current APHIS-PPQ monitoring efforts [1].

Little research has been done on whether there are pheromones that affect the movement of larvae, which could potentially be deployed in management strategies. It is known that carbon dioxide and certain food odors can be attractive, while many short chain (3-7 carbon) alcohols and organic acids can be repellant [2]. Furthermore, it is readily observed that KB larvae, as well as those of other *Trogoderma* species, aggregate even on non-food sources such as papers placed in laboratory colony jars (Figure 1).

The question of whether there may be semiochemicals that mediate this process has not been previously investigated. These authors researched this possibility in KB, as well as two related species, the warehouse beetle, WB, *Trogoderma variabile*, and larger cabinet beetle, LCB, *Trogoderma inclusum*. Hexane extracts were obtained from late instar larvae that were killed by being frozen for 48 hours at -20°C (Figure 2). Extracts were made at a ratio of 4 ml of hexane per 1 g of larvae of each species. These extracts have been analyzed using gas chromatography coupled with mass spectrometry (GC-MS) (Figure 3), which indicated the presence of a number of compounds with fragmentation patterns that are consistent with those cuticular hydrocarbons. Previously published research confirms that similarly prepared extracts using dichloromethane contained a number of cuticular hydrocarbons [3]. There were no noticeable differences among the traces for any of the different species, with each containing a region of several compounds indicative of hydrocarbons, all at similar retention times. There were also a number of other compounds in the extracts, including fatty acids and sterols.

Figure 1. KB larvae assembling in clusters on paper placed in a laboratory colony jar.

Figure 2. Hexane extracted from KB larvae. The extract is contained in hexane in the vial on the left and was removed from the vial on the right, containing the dead larvae.

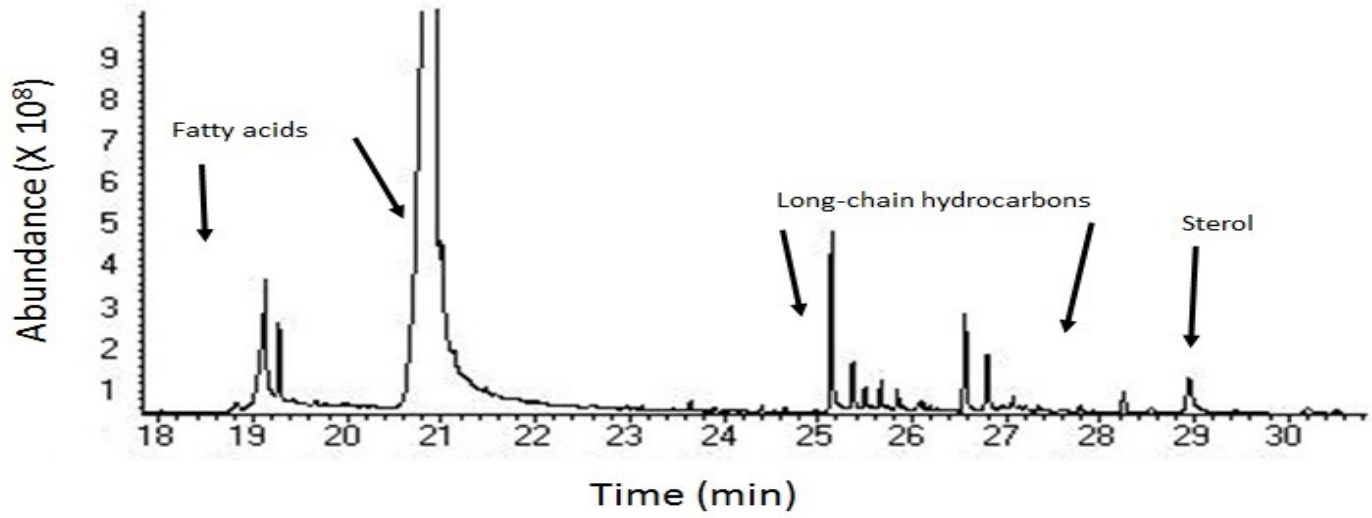

Figure 3. Gas chromatography trace from an LCB larval extract showing ion abundance for compounds separated.

For assessing behavioral effects, two choice experiments were performed inside 15 cm glass Petri dish arenas, with a filter paper fully covering the bottom surface (Figure 4). Within each arena, two smaller 3 cm filter papers were placed. Each of the papers were folded three times in parallel to present a corrugated surface that the insects could go under if they chose, as well as crevices that may be physically attractive for aggregating. In each replicate, one paper received a 100 µl aliquot of one of the extracts, while the other received 100 µl of hexane as a control. This is a dose of roughly five larval equivalents. Ten late instar larvae were placed in the arena and allowed to acclimate overnight in a dark room.

For all three species, it was found that larvae were more likely to be found on the side of the Petri dish with the hexane control rather than the conspecific larval extract. They were also more likely to be on or near the filter paper treated with the control versus the larval extract. Thus repellency of the conspecific extract was demonstrated, rather than the attraction that was expected given the observations of behavior in laboratory colonies.

It should be noted that the dose was higher than a single larva produces and thus the concentration of these chemicals may indicate a biologically unrealistic situation. Furthermore, it is also possible that production of other particular compounds was elicited by the stress of the larvae being frozen to kill them before the extraction. Identification of the causal factor for such repellency may provide a product useful for the management of KB.

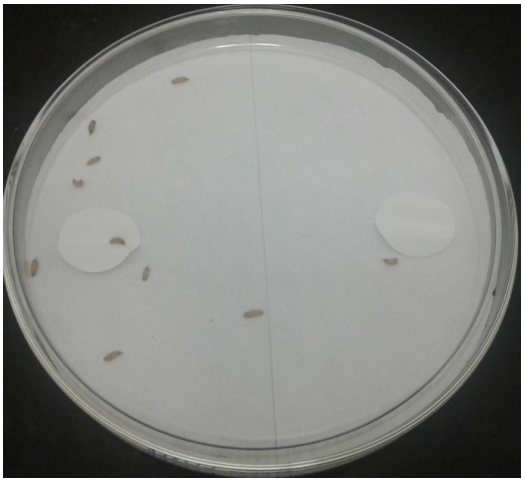

Figure 4. Bioassay showing that, in this instance, KB larvae preferred to remain on the left side of the arena, but without substantial clustering on the smaller treatment paper.

References

1. Barak AV. Development of a new trap to detect and monitor khapra beetle (Coleoptera: Dermestidae). Journal of Economic Entomology. 1989;82(5):1470-1477.
2. Spangler HG. Reactions of the larvae of the khapra beetle and *Trogoderma parabile* to certain food substances and organic compounds. Journal of Economic Entomology. 1965;58(2):212-218.
3. Dubis E, Maliński E, Hebasupwska E, Święeka M, Nawrot J, Pihlaja K, et al. The composition of cuticular hydrocarbons of the khapra beetles, *Trogoderma granarium*. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry. 1987;88:911-915.

Deltamethrin treated packaging for the control of khapra beetles

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The khapra beetle, KB, *Trogoderma granarium*, is a serious pest of stored products and is the only stored products pest that is currently quarantined in the U.S. KB larvae feed on a wide range of dry food products of plant and animal origin including cereal grains, dried fish, and museum specimens [1]. Although, KB was not historically classified as a penetrating or invading insect species, it is in the same family and genus as two strongly penetrating species, *Trogoderma variabile* and *Trogoderma glabrum* [2]. Therefore we suspect KB to also be a penetrator and a major concern for packaged grain. Improved packaging technologies, such as long-lasting insecticide-treated packaging material, may help farmers in developing countries protect their cereal grains from insect infestations for extended periods of time. By using insecticide incorporated packaging material, it may be possible to keep the integrity of the stored grain inside of the bag and protect against insects penetrating/invading from the outside.

We evaluated the use of Zerofly® Storage Bags, which are deltamethrin-treated polypropylene storage bags. They are commercially available from Vestergaard Frandsen Inc. and are currently used throughout African regions, India, and Vietnam. The insecticide is embedded in a woven polypropylene material at a rate of 3 g per kg (3000 ppm). The bags are advertised to offer two-year protection and are considered to be "Food Safe" under U.S. and E.U. regulations. They are used for the storage of grains, pulses, and seeds and are designed to kill insects upon contact and movement on the treated material. The first objective was to determine the effect of the deltamethrin-treated packaging material on adult KB after progressively longer exposure (1, 2, 5, and 7 days). The second experimental objective was to determine the effects of short term exposure (8 hours, 1, 2, 3, and 4 days) on late instar KB larvae from the deltamethrin-treated packaging material. The third objective was to determine the effect of continual exposure on early and late instar larvae from the deltamethrin-treated packaging material in comparison to an untreated laminated woven bag.

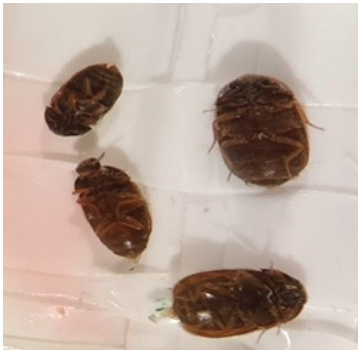

Figure 1. Affected khapra beetle larvae shown on deltamethrin-treated polypropylene storage bags.

Adult khapra beetles were categorized as "*affected*" if incapacitated, usually with their ventral side upward, but still moving their legs and mouthparts (Figure 1). When adults were exposed to deltamethrin-treated material, all were either *affected* or killed by the contact. The percentage of mortality increased with longer exposure periods, as the adult khapra beetles transitioned from being *affected* to dead.

The short term exposure of larvae had similar results. After longer exposure to the treated packaging material, fewer adults emerged and the percentage of dead larvae increased. Mortality was significantly greater for all larvae exposed to the treated bags versus control bags, regardless of exposure duration. Furthermore, under conditions of continued exposure, we observed no difference between susceptibility to the deltamethrin-treated packaging materials between early or late instar khapra beetle larvae. Dead larvae were observed to be blackened and desiccated (Figure 2). The combined adult emergence was approximately 3%, and larval death was approximately 97%. However, roughly 25% of early instar larvae and 55% of late instar larvae matured to adulthood when exposed to the untreated packaging material, while the remaining larvae failed to develop into adults (<5% mortality).

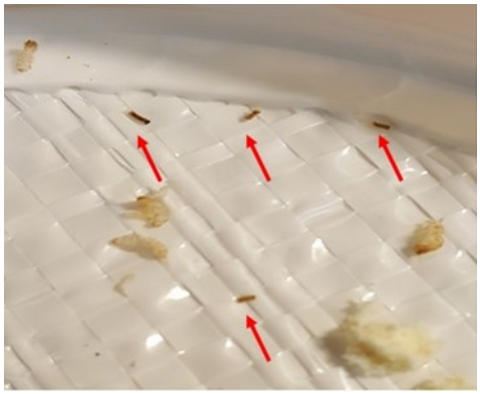

Figure 2. Dead khapra beetle larvae, indicated by red arrows, after continual exposure on deltamethrin-treated packaging material.

References

1. Hagstrum DW, Klejdysz T, Subramanyam Bh, Nawrot J. Atlas of stored-product insects and mites. American Association of Cereal Chemists International, St. Paul, MN; 2013. pp. 123.
2. Highland, HA. Protecting Packages Against Insects, In: Gorham, J.R. (Ed.), Ecology and Management of Food-Industry Pests. Assoc. Official Anal. Chemists, Arlington; 1991. pp. 345-356.

Feasibility of using steam and vacuum to control infestations of coconut rhinoceros beetle, *Oryctes rhinoceros*, in mulch

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The coconut rhinocerous beetle, CRB, *Oryctes rhinoceros*, currently poses an expanding threat to palm trees on the Hawaiian Island of Oahu. Adult beetles lay eggs in decaying matter such as downed logs, mulch and organic debris. Movement of this material risks spreading the infestation to other parts of Oahu, other Hawaiian Islands, and the U.S. mainland. Commercial mulch operations are directly impacted by the presence of CRB larval stages in their yard material. Therefore, these businesses would welcome any mitigation measure that would sterilize mulch material to the degree that it could be safely moved without the risk of insect spread.

Figure 1. The original treatment design was a flexible polymer bag hung from scaffolding to simulate a top loading arrangement. This arrangement did not work because it allowed too much compression on the insects.

Cooperators Zhangjing Chen and Mark White of Virginia Tech received Farm Bill funding to expand the utility of vacuum steam application for mulch materials. The objective was to develop a silo arrangement that could be placed at commercial mulch yards so that material could be top loaded, sterilized with vacuum steam, and subsequently emptied into a waiting truck for movement off site.

The first step in this process was to develop a small scale system and prove the concept at Virginia Tech. A flexible polymer bag was custom made and hung from scaffolding to simulate a top loading arrangement (Figure 1). Due to the difficulty of obtaining field collected CRB grubs from Hawaii, Japanese beetle, *Popillia japonica*, were field collected in Tennessee and used as a testing surrogate. During the experiments, it was determined that the vacuum inside the flexible chamber resulted in additional compacting forces that crushed the test larvae. In order to obtain a result that would better approximate a preferred rigid commercial silo, a stainless drum was chosen and placed inside a larger vacuum chamber (Figure 2).

Figure 2. The final treatment design simulated a rigid commercial silo. A stainless drum was placed inside a larger vacuum chamber.

A central steam distributing manifold constructed of perforated PVC pipe was inserted in the middle of the test barrel. Wooden stakes were also inserted into the barrel to support the thermocouple probes at selected locations. Two *P. japonica* larvae were placed in small, cut sections of bamboo that were wrapped in cheese cloth. This arrangement provided protection from crushing, but also allowed vacuum and steam penetration. A total of 10 bamboo pieces (20 larvae total) were placed at selected vertical locations in the barrel as the mulch was added. Once the barrel was completely filled with mulch and seeded insects, the vacuum steam chamber was closed and testing was initiated.

A test matrix was developed that considered six replicate tests at each of three separate vacuum levels (100, 250, 400 mmHg) with controls. The objective was to heat all probes to a minimum 56°C for 30 minutes hold time. The result indicated that the treatment cycle ranged from 42 to 50 minutes, with an average treating time of 45.7 minutes. A typical temperature profile is expressed in Figure 3. All 18 tests resulted in complete mortality of *P. japonica* larval stages. Control specimens were examined and all remained alive.

Based on the success of these results, another Farm Bill suggestion will be submitted in FY18 to work with grub stage CRB in mulch, using a scaled up commercial grade silo on location in Hawaii.

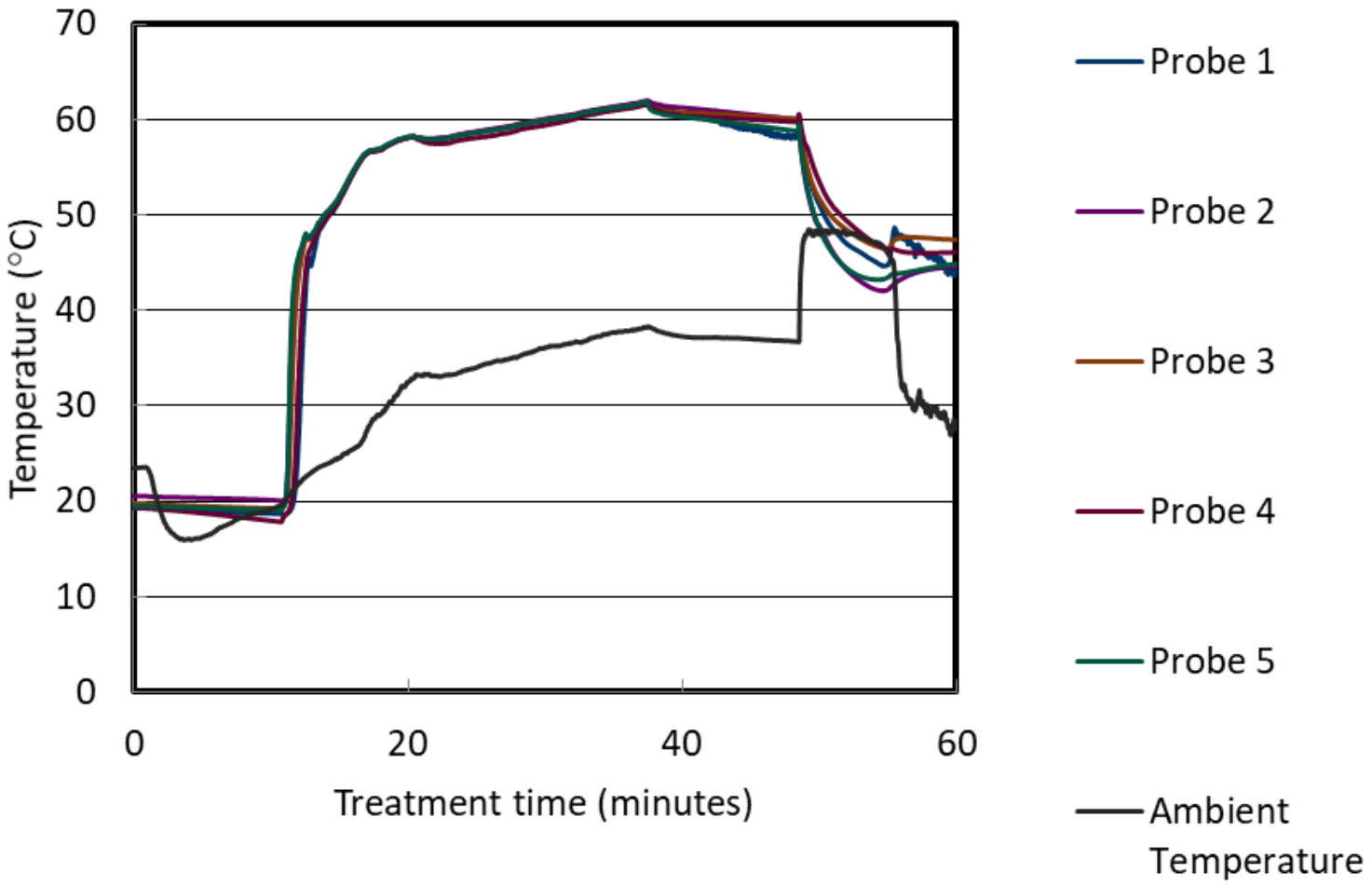

Figure 3. A typical temperature profile throughout the barrel of mulch.

Optimization of commercial radiofrequency for phytosanitary heating of solid wood packaging material

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[4]RF Kiln Tech Limited, Ontario, Canada

ISPM-15, the international standard for treatment of solid wood packing material (SWPM), was amended in 2013 to include a dielectric heating option using either radiofrequency (RF) or microwave (MW). In the *Otis Laboratory 2016 CPHST Laboratory Report*, we reported that researchers at Penn State, APHIS PPQ S&T CPHST, and RF Kiln Tech teamed up to design, fabricate and place a commercial scale RF testing chamber at Penn State's Forest Products Laboratory. The oven tube with RF generator cabinet and automatic wood loading track is seen in Figure 1.

Figure 1. The commercial RF testing chamber designed by researchers at Penn State, APHIS PPQ S&T CPHST and RF Kiln Tech Limited.

Once loaded in the oven, the main electrode is hydraulically pressed down onto the stack for improved electrical contact, and accessory electrodes and temperature probes are distributed throughout the stack (Figure 2). This unit was placed at Penn State for the purpose of optimizing commercial RF treatment for bulk processing of pallet components. The commercial chamber will be used to research topics such as heating uniformity and the impact of chamber design on heating efficiency in order to provide reliable RF treatment schedules to industry.

Initial trials to investigate the effectiveness of an added pressure component (15 psi) to the RF treatment on a bulk stack of 55 yellow poplar, *Liriodendron tulipifera*, cants (3.5" x 7" x 8' long) illustrated the advantage of adding pressure to the treatment.

Figure 2. A view of the inside of the RF chamber before a run begins. The orange temperature probes and metallic accessory electrodes are located throughout the experimental stack of wood.

An initial trial without a pressure component resulted in an overall treatment time in excess of 290 minutes to reach 60°C for a one minute hold at most locations within the stack (Figure 3). In the next trial, 15 psi of pressure was added later in the testing period, reducing the treatment time to 170 minutes (Figure 4). The addition of pressure later in the treatment facilitates better moisture migration and steam retention allowing for a reduced treatment time due to greater heating uniformity. Future experiments will continue to explore the manipulation of pressure for commercial advantage, as will vacuum and electrode configuration. Various insulations and reflective paints applied directly to the oven shell will also be evaluated in an effort to decrease heat loss and improve overall energy efficiency.

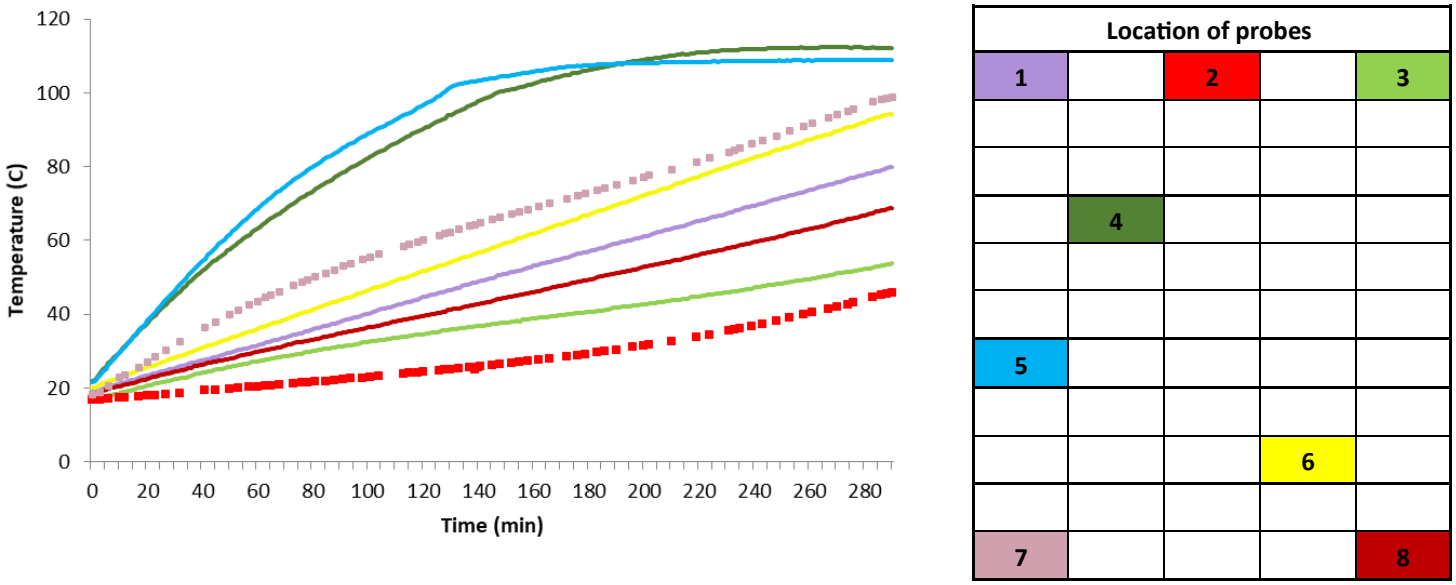

Figure 3. The results of trial 6, an RF treatment test run on yellow poplar cants without any pressure. The key on the right shows the locations of the temperature probes located throughout the stack.

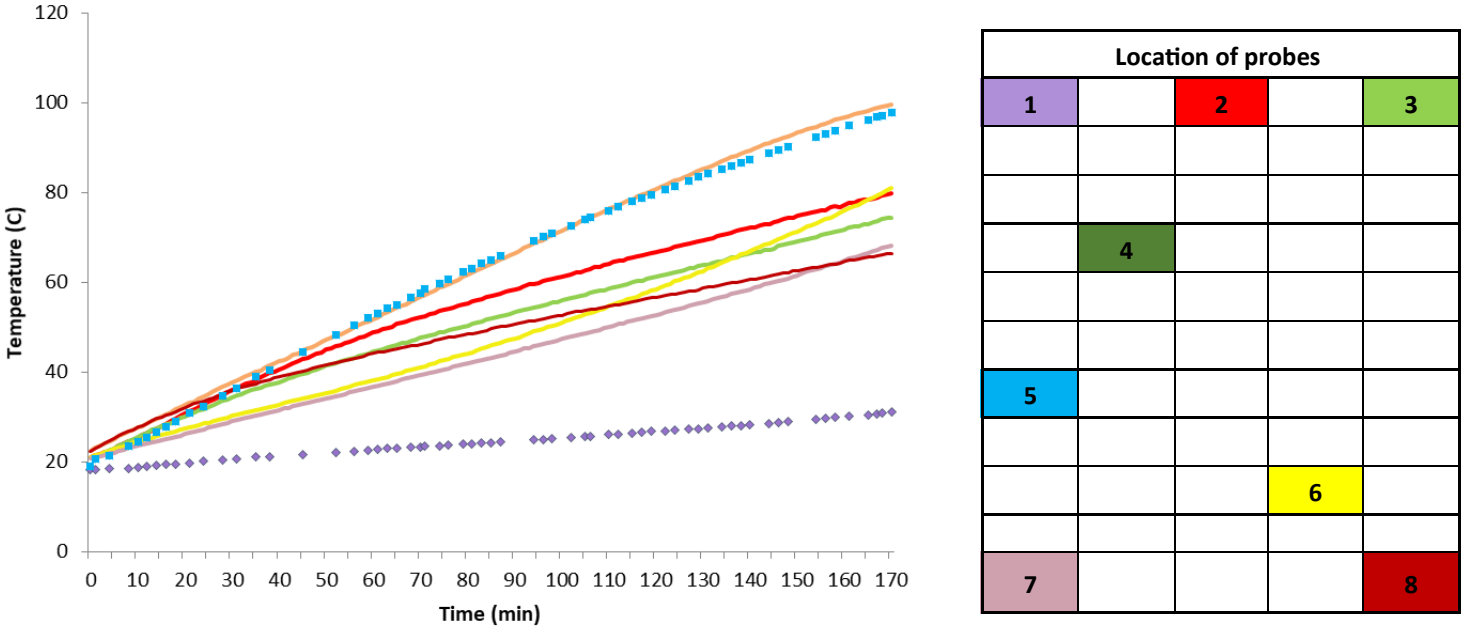

Figure 4. The results of trial 7, an RF treatment test run on yellow poplar cants with the addition of 15 psi. The key on the right shows the locations of the temperature probes located throughout the stack.

Integrating insecticide treatments and biological control for controlling emerald ash borer in urban environments

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Introduction

Emerald ash borer, EAB, *Agrilus planipennis*, parasitoids are establishing in many states; parasitism rates are increasing and the density of EAB is declining. However, by the time EAB infestations are discovered the population numbers can be in the millions against which only thousands of parasitoids are released. Thus many of the large ash trees in forest environments have died due to EAB damage despite parasitoid releases. Treatment of large ash trees is not economically feasible in most forest settings. However, in urban environments, city managers often choose to protect a portion of the trees from EAB damage by injecting systemic insecticides. This research centers on evaluating the effectiveness of combining insecticide and biocontrol treatments. Our hypothesis is that chemical treatment will protect the ash long enough for the parasitoid populations to increase sufficiently in the environment to continue protecting the ash (particularly larger ash trees). This report describes preliminary results after the first three years of a multi-year study.

Release of parasitoids

Three species of EAB parasitoids were released in the U.S. in each of three cities: Syracuse, NY; Naperville, IL; and Boulder, CO. We released two larval parasitoids, *Spathius galinae* (~2,000 females released) and *Tetrastichus planipennisi* (~5,000 females released), and one parasitoid that attacks EAB eggs, *Oobius agrili* (~2,200 females released) in 2015 and 2016. *Spathius galinae* was released again in 2017. Parasitoids were released in semi-natural areas within a 1 to 1.5 km release plot, and a non-release control plot of the same size was also established for sampling. In Naperville and Boulder the releases were done in the center of the release plot. Due to logistical constraints, releases were made at the northern edge of the release plot in Syracuse.

Data collection

Fifty trees in three categories (treated indefinitely, treated once, and not treated) were established in each of the release and control locations. Every year we recorded the health of each tree as it pertained to infestation by EAB (crown class, bark splits, epicormic shoots, d-shaped exit holes) and in the first two years we collected two branches from a sub-set of the trees and recorded EAB density and levels of parasitism. To document the dispersal of parasitoids, 30 yellow pan traps were placed on infested but living ash trees on a grid throughout both the release and control plots.

The insects caught in the pan traps were collected weekly and sorted to identify EAB parasitoids. We report here on the data from the yellow pan trap sampling. The branch sampling and ash health assessment data are still being analyzed.

Establishment and dispersal of parasitoids

Syracuse, NY

In 2015, the first year of sampling, five *T. planipennisi* adults were recovered in four traps (Figures 1, 2). Surprisingly, the parasitoids were not recovered in the traps closest to the release points but up to 2 km away, although still within the release plot. The number of traps with *T. planipennisi* increased to 13 in 2016 and 21 in 2017, with the first recoveries of parasitoids in the control plot in 2017.

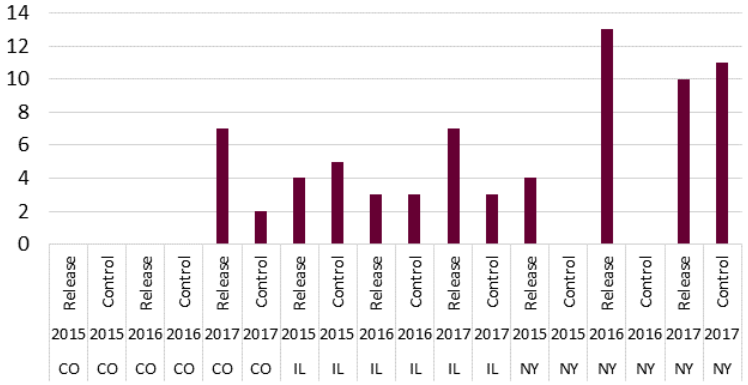

Figure 1. The number of yellow pan traps recovering at least one *T. planipennisi* in release and control plots over three years, in three states.

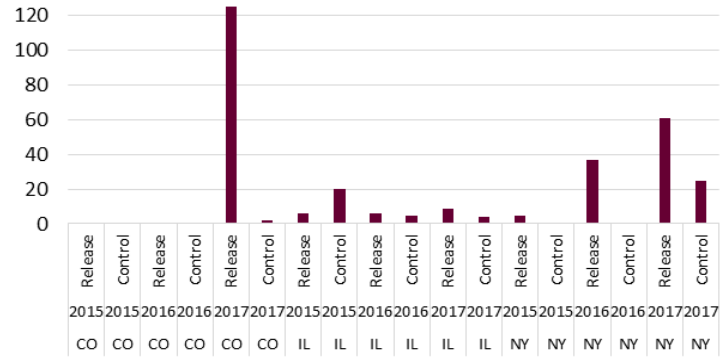

Figure 2. The number of individual *T. planipennisi* recovered in yellow pan traps in release and control plots over three years, in three states.

The numbers of *T. planipennisi* recovered increased from five in 2015 to 37 in 2016 and to 86 in 2017, with 11 traps in the control plot capturing 25 parasitoids. Figure 3 shows the distribution of positive (and negative) recoveries. While recoveries were limited to the release plot in 2016, by 2017 the population was well distributed throughout both the release and control plots and *T. planipennisi* had been recovered 6.5 km from the release locations. A single *S. galinae* was recovered in 2016, but *O. agrili* was never found.

Naperville, IL

As in Syracuse, only *T. planipennisi* was recovered in yellow pan traps in Naperville, IL and the insects had moved a good distance (2.5 km) within the first summer. Unlike Syracuse, however, neither the number of traps recovering parasitoids nor the number of parasitoids recovered increased appreciably from 2015 to 2017. This could be due to the generally poorer condition of the trees in the area and the possible collapse of EAB populations in non-treated trees. *Tetrastichus planipennisi* was well spread out throughout both release and control plots in 2015 and remained so through 2017 (Figure 4).

Boulder, CO

No parasitoids were recovered in 2015 in Boulder, CO and only a single *O. agrili* was collected in 2016. Studies of overwintering EAB have shown that most EAB overwinter as J-larvae in Boulder and are not available to *T. planipennisi* when they emerge in the spring, which could explain the lack of establishment. However, in 2017, after two years without any collections, 127 *T. planipennisi* were recovered from nine traps. The number of individuals was greater than any other city and the number of traps with positive identifications was comparable to Naperville. In addition, the parasitoid had spread to the control plot, 2 km from the release point (Figure 5).

Conclusions

Tetrastichus planipennisi has shown a remarkable ability to establish and spread throughout urban environments. We have had less success capturing *S. galinae* and *O. agrili* in the yellow pan traps, but branch sampling should pick up the larval parasitoids. In 2018, we will scrape ash bark to recover EAB eggs to determine levels of parasitism. In the next year or two, we will determine if *T. planipennisi* is sufficiently established and will start withholding insecticide treatment from half of the treated trees. We will then continue to monitor ash health and EAB density and parasitism to determine the validity of our hypothesis.

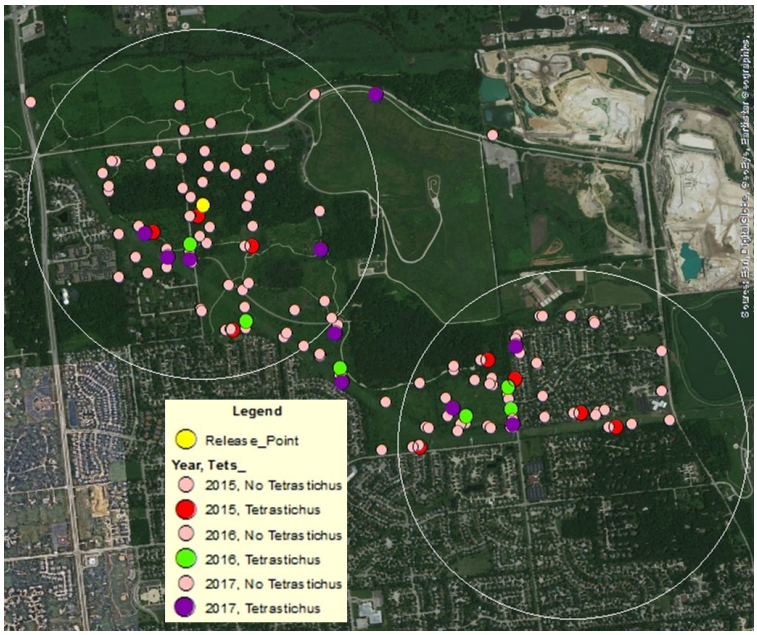

Figure 4. Distribution of *Tetrastichus planipennisi* in yellow pan traps in Naperville, IL.

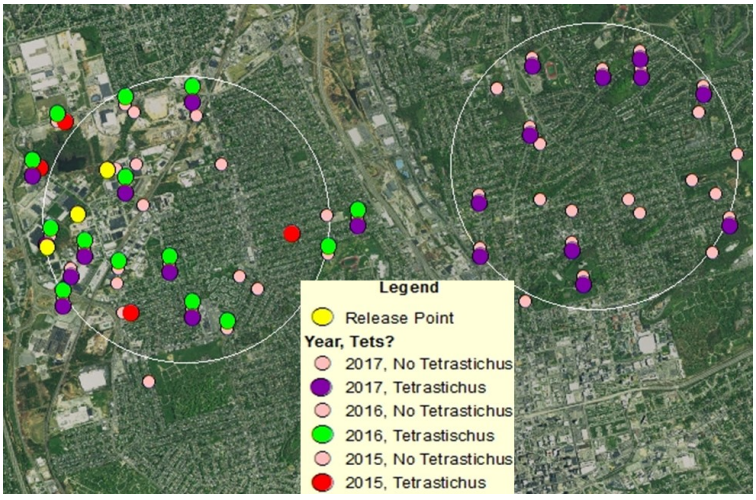

Figure 3. Distribution of *Tetrastichus planipennisi* in yellow pan traps in Syracuse, NY from 2015 to 2017.

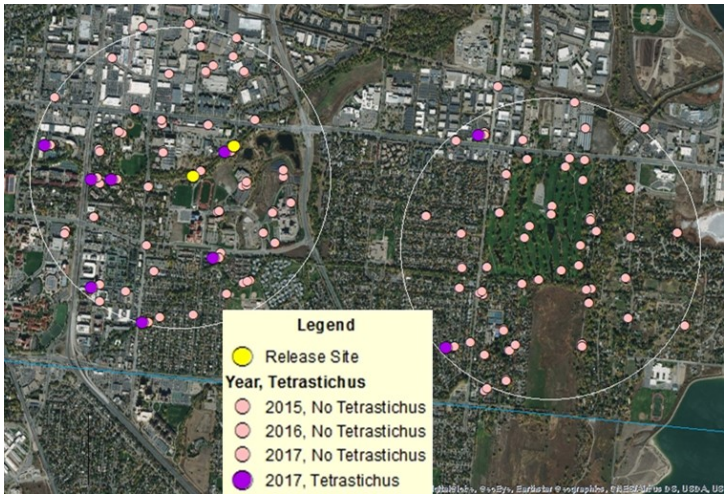

Figure 5. Distribution of *Tetrastichus planipennisi* in yellow pan traps in Boulder, CO.

Foreign exploration for Asian longhorned beetle parasitoids

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Introduction

As Asian longhorned beetle, ALB, *Anoplophora glabripennis,* populations decline they will become increasingly more difficult to detect and eradicate. Specific natural enemies may be more efficient at locating ALB in low density satellite populations; thereby complementing eradication efforts. Additionally, if new populations become established in the U.S. natural enemies could be useful for classical biological control.

From 2015 to 2017, researchers in China set up sentinel willow, *Salix* sp., or box elder, *Acer negundo*, logs containing ALB eggs and young larvae for attracting parasitoids. After exposure, sentinel logs were recovered from the field and dissected in the laboratory for signs of parasitoid emergence and identification. In the summer of 2017, a subset of parasitoids were brought to the Otis Laboratory Insect Containment Facility in an attempt to start colonies of these species.

Creation and set-up of sentinel logs

Fresh logs were placed in wet floral foam bricks and stored in plastic chambers with approximately 20 male and female ALB adults. Fresh branches of box elder were provided for ALB feeding. After allowing 1 to 1.5 weeks for ovipostion, the sentinel logs were removed from the chamber and waxed on both ends. Sentinel logs were then caged (to exclude avian predation) and hung in willow stands in both Beijing and Shanghai and in poplar plantations in Hunchun. All experimental sites were in managed -natural forests.

Recovery and dissection of sentinel logs

After exposure in the field for a month, the sentinel logs were recovered and dissected in our cooperator's Beijing laboratory. The fate (alive, dead, or parasitized) of each ALB egg or larva was recorded. The parasitized hosts, or any stage of parasitoids found in host oviposition sites or host larval galleries, were collected and reared individually in glass vials in the laboratory under 25°C, 55 to 65% RH and photoperiod 16:8 light-day conditions. The parasitoids were identified to species after they developed into adults. Parasitism rates were also calculated based on the dissection data.

Parasitoids collected from sentinel logs

Table 1 provides a summary of the parasitoids recovered from 2015 to 2017. In the first year, four species of parasitoids were recovered and identified from the sentinel logs. Five new parasitoid species were recovered in the subsequent year. In 2017, one new parasitoid species was recovered although its identity is currently unknown. Additionally, a subset of parasitoids were hand carried under the Otis permit for unidentified parasites of invertebrates from Asia to the Otis Laboratory Insect Containment Facility for rearing. Ten *Oxysychus* sp. individuals (Figure 1) and six *Anastatus* sp. individuals (Figure 2) emerged—enough to test rearing methods. Unfortunately, these colonies were not successfully started. The goal for 2018 is to continue to attempt starting new colonies of ALB parasitoids in Otis and China for further biocontrol studies.

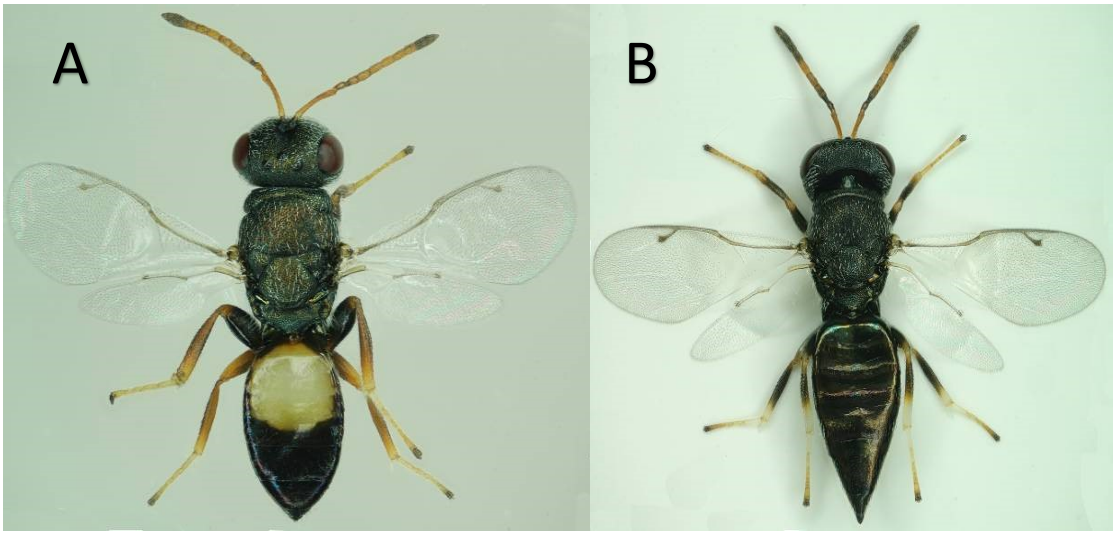

Figure 1. Parasitoid *Oxysychus* sp. (Hymenoptera: Pteromalidae) recovered from Beijing. A) male and B) female.

Figure 2. Parasitoid *Anastatus* sp. (Hymenoptera: Eupelmidae) recovered from Beijing.

Table 1. Summary of parasitoids of early stage ALB recovered from sentinel logs and their parasitism rates for 2015 through 2017.

Parasitoid	Host stage	Recovered site	Host no.	Parasitism rate (%)	Recovered site	Host no.	Parasitism rate (%)	Recovered site	Host no.	Parasitism rate (%)
			2015			2016			2017	
***Eurytoma* sp.**	Egg or 1st instar larva	Beijing	380	12.37	Beijing	1,156	6.45	Beijing	906	2.43
***Oxysychus* sp.**	Egg or 1st instar larva	Beijing	380	12.37	Beijing	1,156	11.83	Beijing	906	5.19
					Shanghai	784	15.13	Shanghai	452	7.52
***Anastatus* sp.**	Young larva	Shanghai	422	4.03	—	—	—	Shanghai	452	1.33
Ichneumonid	Young larva	Hunchun	1,716	0.47	—	—	—	—	—	—
					—	—	—	—	—	—
***Zolotarewskya* sp.**	Young larva	—	—	—	Beijing	1,156	0.53	Shanghai	452	2.21
					Shanghai	784	4.96			
***Callocleonymus* sp.**	Young larva	—	—	—	Beijing	1,156	2.35	Shanghai	452	0.22
		—	—	—	Shanghai	784	1.42			
***Bracon* sp.**	Young larva	—	—	—	Beijing	1,156	3.35	Beijing	906	5.96
		—	—	—						
***Spathius* sp.**	Young larva	—	—	—	Beijing	1,156	6.6	—	b	—
		—	—	—	Shanghai	784	5.67	—	—	—
***Sclerodermus* sp.**	Young larva	—	—	—	Beijing	1,156	1.08	Beijing	906	0.22
		—	—	—	Shanghai	784	0.71			
Unknown	—	—	—	—	—	—	—	Beijing	906	0.22

Update: The life cycle of *Anastatus orientalis*, an egg parasitoid of spotted lanternfly

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Introduction

Spotted lanternfly, SLF, *Lycorma delicatula*, is an invasive sap-sucking insect native to China, India, and Vietnam. It was first reported in Berks County, Pennsylvania in 2014 and despite extensive eradication efforts, it has continued to spread in the U.S. Exploration and research for an effective biological control agent has been ongoing for the last two years. An egg parasitoid from China, *Anastatus orientalis*, is currently being examined. In 2016, we showed that *A. orientalis* is able to continually parasitize egg masses when reared under long-day conditions (25°C, 65% RH, 16:8 light-day cycle). We also found that the majority of parasitoids did not enter summer diapause. It is important for a biocontrol agent to have synchrony with its host and for this particular parasitoid, a summer diapause period is necessary because SLF egg masses are not present in the summer months. The lack of summer diapause caused some concern because it may indicate that an alternate host is necessary, and parasitoids that could attack native species are not good candidates for biological control. In 2017, life cycle studies of *A. orientalis* were conducted under Pennsylvania-climate conditions to determine if the parasitoid would enter a summer diapause under typical field conditions. Under these conditions, there was 100% parasitism of egg masses and 100% of *A. orientalis* entered summer diapause.

Diapause in the laboratory

In 2017, 50 SLF egg masses infested with *A. orientalis* were shipped from China and reared in a growth chamber under Berks County, Pennsylvania temperature and day length conditions. Only four of the 50 egg masses contained *A. orientalis*, and 40 *A. orientalis* adults emerged. Those 40 adults were split into exposure groups at a 5:1 (female to male) ratio and given an SLF egg mass collected in Pennsylvania (Figure 1A).

Exposures were conducted under Pennsylvania climate conditions and the growth chamber parameters were altered daily to reflect those conditions. All parasitoids entered a summer diapause (Figure 1B). Emergence occurred 18 weeks later, which would coincide with fall in Pennsylvania, when SLF adults begin laying eggs again. This demonstrated that synchrony with the presence of SLF egg masses is possible as parasitoids were only active during two short periods: 1) spring, just before SLF nymphs emerge and 2) fall, just after SLF egg masses are laid (Figure 2).

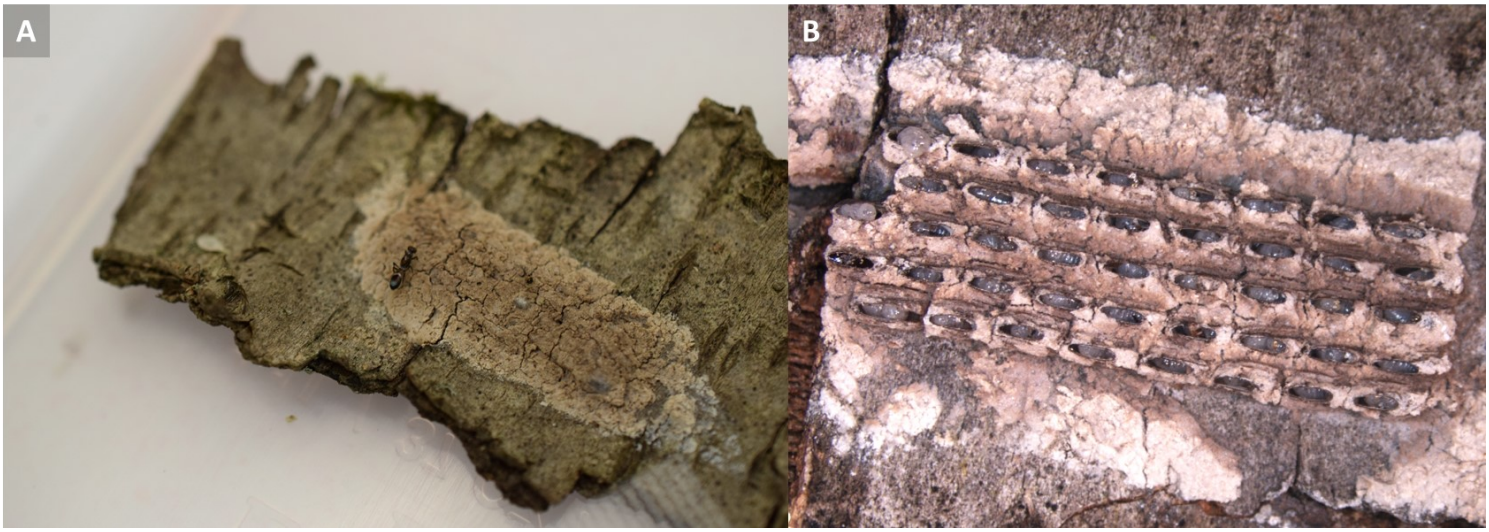

Figure 1. A) *Anastatus orientalis* female probing an SLF egg mass at the Otis Laboratory quarantine facility, Buzzards Bay, MA. B) An SLF egg mass showing 100% parasitism of *A. orientalis* and 100% of the *A. orientalis* larvae in summer diapause.

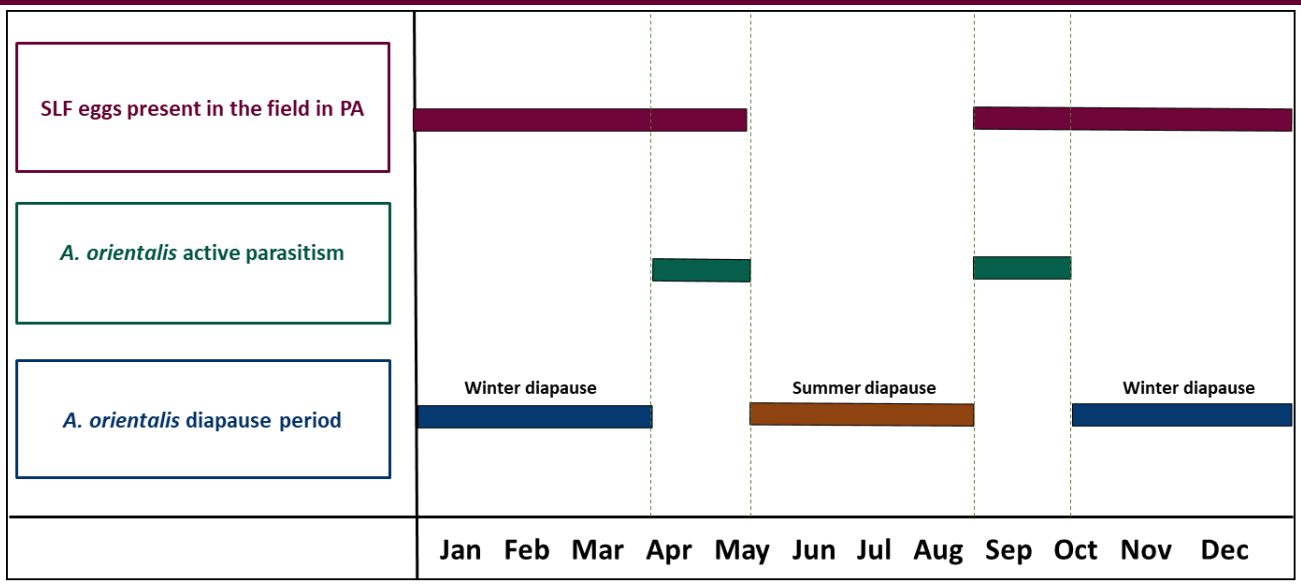

Figure 2. Synchrony of A. *orientalis* emergence and the presence of spotted lanternfly egg masses (red). The diapause period in 2017 shows diapause in the cold months (blue) and a summer diapause (orange) when SLF nymphs and adults are feeding in the field but egg masses are not present. *Anastatus orientalis* have two active periods of parasitism (green) that coincide with the presence of SLF egg masses in the spring and fall.

Observations from the native range

Summer diapause was also observed in the native range of *A. orientalis*. In July 2017, SLF egg masses were collected in Beijing, Shanghai, and Xinxiang, China to assess summer diapause. *Anastatus orientalis* in diapause were found in all three locations. High mortality in SLF egg masses with *A. orientalis* was also observed, which may have been the result of host feeding. Studies of this additional parasitoid induced mortality are underway.

Conclusion

Rearing *A. orientalis* under PA-climate conditions creates an environment that best mimics what the parasitoids will experience in the field. Summer diapause was induced by decreasing day length and temperature, instead of using a constant 25°C temperature and a long-day light cycle (16:8 light-day). This is extremely important because it shows that the parasitoid will not be active in the summer months when SLF egg masses are not present and will actively parasitize masses in the spring and fall. In 2018 extensive specificity testing will be conducted on *A. orientalis*.

Furthermore, another potential SLF biological control agent, *Dryinus* sp., was discovered in 2017. This nymphal parasitoid was recovered on sticky bands in Beijing, China (Figure 3). More research will be conducted in 2018 to examine this parasitoid's life cycle and potential as an additional biocontrol agent of SLF.

Figure 3. A sticky band from Beijing, China showing an SLF nymph with a *Dryinus* sp. larva emerging from underneath the wing pad.

Facilitating spread of the microsporidian pathogen *Ovavesicula popilliae* for long-term suppression of Japanese beetle

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Introduction

The introduction of Japanese beetle, JB, *Popillia japonica* (Figure 1), into western states via air cargo transport and other commerce activities continues to be a threat that would significantly impact agriculture in California, Oregon, and other non-infested states [1]. The air cargo industry in the Midwest expends significant resources to reduce the risk of unintentional shipment of live Japanese beetles. Costs include construction, maintenance and storage of exclusion devices (Figure 2), pesticide treatments, and staffing cost of safeguarding personnel during loading of west-coast bound planes. Airports go to great lengths and expense to treat/reduce JB host material and may even restrict the planting of certain crops that attract beetles in the vicinity of an airport. Establishing an effective biological control agent would reduce the likelihood of inadvertent movement of JB to west coast states and would provide a long-term strategy for JB control at airports. *Ovavesicula popilliae*, a host-specific microsporidian (fungal-like) pathogen (Figure 3), has been shown to significantly increase JB winter mortality and to reduce female fecundity by 50 to 60%. Within five years of the pathogen being introduced, JB populations have been observed to decline by 75% [2].

Figure 1. Japanese beetle life stages.

Figure 2. An exclusion device used to prevent Japanese beetle from flying into airplanes during cargo loading operations.

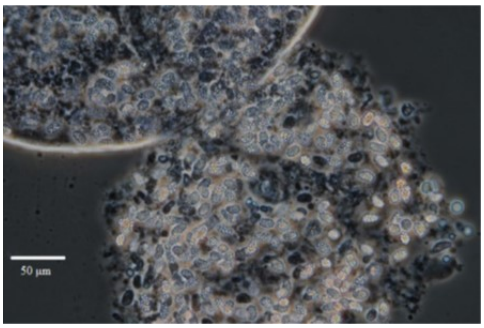

Figure 3. The pathogen *Ovavesicula popilliae* infecting the malpighian tubules of Japanese beetle.

Methods

Japanese beetle adults were trapped and collected in Michigan where the rates of *Ovavesicula* infection in JB are high. Adult beetles (11-16,000 per release site) were frozen for several days and then shipped under an APHIS 526 permit to APHIS PPQ Field Operations personnel who seeded the JB cadavers into the ground as a means of releasing the pathogen into our study areas. Release sites were located near where JB were being trapped at an airport. Prior to the releases, live JB adults were collected at each of the sites, frozen and sent to Michigan in order to determine a baseline infection rate of the pathogen. Additionally, cooperators from 14 other states voluntarily collected JB at 17 locations for pathogen analysis.

Results

JB adults from the Michigan collection sites were determined to have infection rates of 50% or greater. The *Ovavesicula* pathogen was successfully released at seven sites in five states (Iowa, Illinois, Missouri, Nebraska, Ohio) and the infection rate of this pathogen was determined in JB populations from collections made at 24 sites in 19 states (Table 1).

Conclusions

The *Ovavesicula* pathogen was found to be present at very low levels (0-1%) in JB population samples collected at the airport release sites, except for the Ohio site where an unexpected 26% of the JB adults were infected.

Ovavesicula was initially found in Connecticut in 1988 and infection rates between 9 and 16% are noted from collections originating from New England states. High infection rates (>50%) have been achieved in Michigan through many years of augmentative releases and have resulted in significant impacts on the growth and viability of JB populations [2].

Further augmentative releases of infected JB adults at the study sites as well as continued monitoring for the presence of the pathogen will continue in 2018. There are also plans to develop a molecular detection tool for the pathogen so that infection determination work will not have to be done by the laborious physical dissection of individual JB malpighian tubules.

References

1. Oregon Department of Agriculture. Economic risk analysis: Oregon and the Japanese beetle. http://www.oregon.gov/ODA/shared/Documents/Publications/IPPM/JapaneseBeetleEconomicRiskAssessment.pdf accessed 2/23/2017. ODA 2017; Oregon.gov/ODA
2. Smitley DR, Jo Y-K, Hudson I. Association of *Ovavesicula popilliae* (Microsporida:Ovavesiculidae) with winter mortality of larvae and reduced fecundity of female Japanese beetles (Coleoptera: Scarabaeidae). J Econ Entomol. 2011;40(3):589-596.

Table 1. Pathogenicity of Japanese beetle larvae assessed by Michigan State University. Cargo airports are shown in green print and release sites are underlined. The total number of larvae dissected and the number found to be infected with *Ovavesicula popilliae* are shown for each location.

State	Location (introduction)	Dissected (n)	Infected (n)
Arkansas	Various – XNA, UAF, WP, WF, EWA	466	1
Colorado	Pueblo Zoo & Golf – PZ, PGC, FGC	263	7
Connecticut	Bradley Intl Airport – CONN	96	10
Delaware	Dover Air Force Base – DOV	46	1
Illinois	Rockford airport – ROC	95	1
Indiana	Indianapolis Intl Airport – INDY	20	0
Iowa	Des Moines Intl Airport – DES	102	0
Kentucky	Louisville Intl Airport – SDF	28	1
Massachusetts	Otis Lab – OTIS	96	10
Maine	Various – MEA, MEB, MEC	242	29
Michigan	Golf Courses – EH, BP, OL, W	204	73
Michigan	Various – HTC, AW	140	2
Minnesota	Golf Courses – HL, OG, CO, OG	409	5
Missouri	Airports – LAM, KCA	180	1
Nebraska	Eppley Airfield – EPP	96	1
Ohio	Wilmington Air Park – WAP	82	21
Oklahoma	Golf Course & Nursery – GLN, TM	184	0
Oregon	Cedar Mill area – CEDAR	105	3
Tennessee	Nursery & Airport – CWN, BNA	177	15

Efficacy of bark sprays and tree injection in controlling spotted lanternfly

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Introduction

Spotted lanternfly, SLF, *Lycorma delicatula*, a sap feeding insect native to Taiwan and China, was first detected in the U.S. in 2014. Commodities that could be impacted in Pennsylvania include the state's grape, apple, and stone fruit ($180 million annually) as well as pine and hardwood lumber sales ($12 billion annually). Field populations are currently known to be present in 13 counties in southeastern Pennsylvania, with seven counties added in November, 2017. Ongoing eradication efforts include outreach and education activities, host tree removal, and using the preferred host, tree of heaven, *Ailanthus altissima*, as a trap tree via the application of a water soluble insecticide to the lower portion of the tree trunk. Insecticides of this type are readily absorbed by plants and transported in its tissues.

Methods

Two of these insecticides (dinotefuran and imidacloprid) were applied alone and in combination via a bark spray application using a one-gallon sprayer at the maximum labeled rates. Trunk injectable formulations of dinotefuran, imidacloprid, and emamectin benzoate (also water soluble) were applied to individual trees by pressurized basal tree injection using 2 ml of product per diameter inch of tree as previously described [1].

Treatments were applied in late August to landscape *Ailanthus* trees that were located at a single property in Douglassville, PA that had a heavy SLF infestation.

Each treatment was comprised of two trees between 5 and 8 inches in diameter (6.4 inches average, 1.0 inch = SD), and two untreated trees were included as controls. Tarps were placed under the trees and dead lanternfly were counted regularly for six weeks (Figures 1, 2).

Figure 1. Tarp lay out used to capture spotted lanternfly.

There were 19 mortality observations made during the study and these were used to generate mortality averages for each treatment (Table 1). Data were log transformed and means were separated using the Tukey HSD test at $p = 0.05$.

Figure 2. Symptoms of insecticide intoxication and paralysis are evident four hours following a tree injection.

Results

Adult SLF are susceptible to dinotefuran and imidacloprid applied by either method and over 34,000 dead SLF adults were counted during the six week period of this study, with one tree accounting for about 30% of the total (Figure 3).

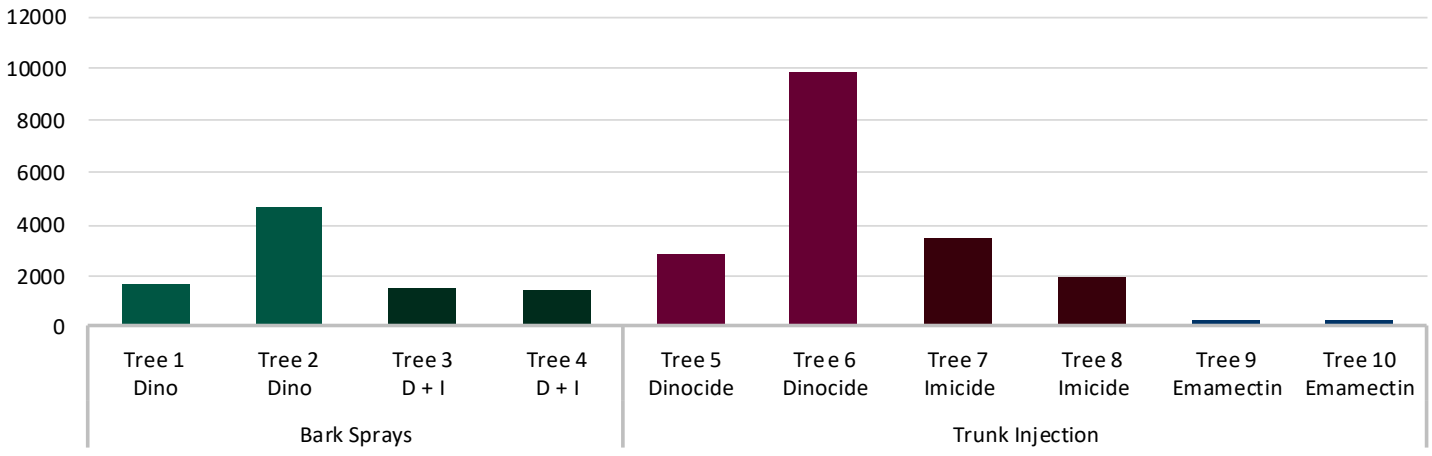

Figure 3. Spotted lanternfly adult mortality totals over a six week period by insecticide and treatment method.

In contrast, emamectin benzoate was not effective against spotted lanternfly, although average mortality for this insecticide (11.4 ± 1.9) was significantly higher than mortality in the control group (2.7 ± 0.4) where $T_{74} = 4.42$, $p < .001$. The bark spray treatment with both insecticides had a lower average mortality as compared to dinotefuran alone ($F_{1,75} = 10.88$, $p < .002$) while the average number of SLF adults killed by trunk injection treatments (237.8 ± 34.9) was significantly higher as compared to trees receiving bark spray treatments (102.2 ± 13.4) where $F_{1,151} = 11.08$, $p < .002$.

Mortality data were highly variable over time (one overnight observation counted greater than 1,800 dead SLF from Tree 6) and varied greatly by individual tree with some trees attracting significantly more SLF adults over the course of the study than others ($F_{9,184} = 32.11$, $p < .001$; Table 1). This disparity is also highlighted by significant differences in mortality between trees belonging to the same treatment pair (e.g., Trees 1 and 2, Trees 5 and 6) (Table 1).

Conclusions

During the six weeks of observation, SLF adults were readily killed by both bark spray and trunk injection treatments of dinotefuran and imidacloprid, but were not susceptible to the other water soluble insecticide tested, emamectin benzoate. Mortality of the insects was apparent soon after treatments were applied. In the case of several of the trunk injection treatments, dead and dying SLF were abundant on the tarps in as little as four hours post-treatment (Figure 2). New SLF adults continually migrated to the treated trees during the course of the study as prior arrivals were killed by the insecticides and dropped to the tarps.

While this study noted significant differences between and within the treatments, some of this was influenced by the uneven distribution and attraction of SLF to certain trees and by there being only two trees in each treatment group. Previous work observed a significant decline in dinotefuran residues after several months when trees are trunk injected [1]. This is a concern for the treatment program as an early season trunk injection application may not remain effective throughout the SLF field season, which can extend beyond six months. Further work is planned for 2018 to improve on this work and to identify superior treatment methods for SLF control.

Table 1. Summary data and statistical comparison of spotted lanternfly adult mortality when exposed to treated trees over a six-week period. ANOVA comparisons were made using the Tukey HSD test; averages followed by the same letter are not statistically different ($p = .05$).

		Area tarped (sq ft)	Average by Date n=19 (±SE)
Bark Spray Applications	Tree 1 Dinotefuran	432	84.2 ± 13.7 *CD*
	Tree 2 Dinotefuran	520	243.6 ± 36.1 *AB*
	Tree 3 Dino+Imi	441	78.4 ± 11.7 *CD*
	Tree 4 Dino+Imi	325	74.5 ± 15.5 *D*
Trunk Injection Applications	Tree 5 Dinotefuran	270	148.7 ± 31.9 *BCD*
	Tree 6 Dinotefuran	504	517.7 ± 110.1 *A*
	Tree 7 Imidacloprid	140	183.1 ± 28.3 *ABC*
	Tree 8 Imidacloprid	324	101.6 ± 19.6 *CD*
	Tree 9 Emamectin	441	10.8 ± 3.1 *E*
	Tree 10 Emamectin	324	11.9 ± 2.4 *E*

Reference

1. McCullough DG, Poland TM, Anulewicz AC, Lewis P, Cappaert D. Evaluation of *Agrilus planipennis* control provided by emamectin benzoate and two neonicotinoid insecticides, one and two seasons after treatment. J Econ Entomol. 2011;104(5):1599-1612.

Testing red maple floral structures for imidacloprid using ELISA

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Study purpose

A research study in Ohio is investigating an alternative control strategy for the Asian longhorned beetle, ALB, *Anoplophora glabripennis,* eradication program. The study aims to determine the feasibility of significantly reducing the number of at-risk host trees that are treated at the periphery of the ALB infestation where lightly infested trees are located. The ALB program expressed interest in any potential impact the chemical treatments might have on pollinators that could forage on the pollen of treated trees. Red maple, *Acer rubrum,* was selected to investigate this issue as it is a common tree within the ALB quarantine zone in Ohio and is a known early-season source of pollen.

Materials and methods

Imidacloprid levels were quantified from the floral structures of 21 individual red maple trees that were sampled between February 23rd and March 2nd, 2017 in Ohio. At that time, over 100 degree days had accumulated in that region of Ohio and red maples were in full bloom [1]. All of the trees were producing male flowers except for one tree (A1-29) that produced female flowers (Figure 1). Seven of the samples were collected from adjacent untreated control trees.

The treated trees had been trunk injected with imidacloprid either four months prior to sampling (Sites A3 and G) or nine months prior to sampling. Samples were processed using protocols previously published by the Insecticide Technology group at Otis [2,3]. Assays were performed at 25x dilution in addition to 20x dilution in order to remove several false positive results that appeared in samples taken from two control trees (G-15 and I-C2) (Table 1). Non-quantifiable (NQ) indicates low levels of imidacloprid are present, but an exact value cannot be determined with this assay method [4].

Figure 1. Red maple floral structures.

Table 1. Imidacloprid levels (ppb) determined from red maple floral structures. Samples in green were collections taken from untreated (control) trees. Non-quantifiable (NQ) indicates presence but an exact value cannot be determined by the assay.

Treatment Site	Tree #	Result at 20x (ppb)	Result at 25x (ppb)
A1	11	NQ	0.0
	29*	507	310
	C1	0.0	NQ
A2	9	224	157
	30	NQ	NQ
A3 (Fall)	11	NQ	0.0
	31	NQ	NQ
	35	0.0	0.0
	53	302	244
E1	63	NQ	0.0
	69	176	NQ
	76	411	302
	C1	NQ	0.0
G (Fall)	3	NQ	0.0
	12	182	NQ
	15	181	0.0
	21	NQ	0.0
I	19	259	153
	24	345	186
	C1	NQ	0.0
	C2	148	0.0

*Indicates female tree

Discussion

Of the 14 treated trees from which floral structures were collected, five male trees and one female tree tested positive for imidacloprid at the 25x dilution rate. Average residue values in flowers from treated trees did not differ statistically for those treated four months prior in the fall (73.8 ± 48.9 SD) or the previous spring (150.9 ± 36.6 SD) where $T_{12} = -1.26$, $p = .23$ and NQ samples were assigned an average value of 125 ppb. The average residue levels of imidacloprid seen in this study are well below lethal levels that have been estimated for honey bees [5].

An additional safety factor for pollinators is that imidacloprid is primarily transported to the leaves and secondarily to flowers. One study found that imidacloprid levels in the anthers was an order of magnitude lower as compared to intact male flowers, thus reducing pollinator exposure to imidacloprid [6]. That study also found that imidacloprid was absent in the nectar and in the forager bees that were collected from treated red maple trees during flowering.

The findings in this study and toxicity studies of previous research suggest that imidacloprid levels in male red maple flowers do not present a high risk to result in acute mortality of early season honey bees [5,6].

References

1. Ohio State University [Internet]. Plant Phenology Calendar; c2015 [cited 2018 Febr 15]. Available from: http://www.oardc.ohio-state.edu/gdd/
2. Lewis P, Turcotte R. Use of Chemical Protection and Host Tree Reduction to Control an Emerald Ash Borer Infestation in West Virginia. J Biodivers Manage Forestry 2015;4(1):1-5. doi: 2327–4417.1000136
3. McCullough D, Poland T, Anulewicz A, Lewis P, Cappaert D. Evaluation of *Agrilus planipennis* (Coleoptera: Buprestidae) Control Provided by Emamectin Bezoate and Two Neonicotinoid Insectcides, One and Two Seasons after Treatment. Journal of Economic Entomology 2011;104(5):1599-1612.
4. Envirologix Inc [Internet]. Pamphlet for EP-006- Imidacloprid Quantiplate Kit; c.2015 [cited 2017 Oct 11]. Available from: https://www.envirologix.com/wp-content/uploads/2015/05/EP006-Imidacloprid-101215.pdf
5. Mullin CA, Frazier M, Frazier JL, Ashcroft S, Simonds R, vanEngelsdorp D, Pettis JS. High levels of Miticides and Agrochemicals in North American Apiaries: Implications for Honey Bee Health. PLoS One 2010; 0009754.
6. Johnson J. The Role of Pesticides on Honey Bee Health and Hive Maintenance with an Emphasis on the Neonicotinoid, Imidacloprid. University of Maryland, Baltimore 2012; PhD dissertation.

Determining pesticide content of ash foliage used for emerald ash borer rearing

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Study purpose

The Emerald Ash Borer Biological Control Rearing Facility in Brighton, Michigan uses host emerald ash borer, EAB, *Agrilus planipennis*, to rear parasitoids with the goal of establishing these biological control organisms in states that are being impacted by the borer. Ash is fully deciduous in the Northeast and thus the winter rearing operation relies on Shamel ash, *Fraxinus uhdei*, from California. In 2016, following several instances of increased EAB mortality, the rearing facility began to suspect that the supplemental Shamel ash foliage could have been contaminated with pesticides. The Insecticide Technology group at Otis is capable of testing for the presence of two commonly used insecticides, imidacloprid and dinotefuran. Between 2016 and 2017, three sets of foliage from Shamel ash sourced from California, and one set of ash foliage directly from the Brighton rearing facility were analyzed to determine purported pesticide content.

Methods

Imidacloprid levels were quantified from leaf samples received in 2016 and 2017. The presence of dinotefuran was also assessed for the second set of foliage received in 2017.

Untreated foliage from ash trees grown in the greenhouse and the Otis Laboratory were used as controls for all analyses. Samples were processed using protocols previously published by this group [1,2].

Results

Five of the eleven samples that were analyzed by Otis in 2016 tested positive for low levels of imidacloprid, ranging from 0.14 ppm to 0.20 ppm (Table 1). The remaining six samples had negligible levels of insecticide, just at the detection limit of the assay method [3]. Samples received in the fall of 2017 did not have any imidacloprid (Table 2). The mortality observed in the EAB colony at that time may have been caused by other insecticides that had been applied to or around the trees.

Table 1. Imidacloprid concentration in ash leaf samples in 2016 (20x dilutions unless noted). Non-quantifiable (NQ) values are at the limit of what this assay method can determine.

Sample Source	Sub Sample ID	ELISA Run #1	ELISA Run #2	Final Value
Otis Control Foliage	Ash 1	NQ	—	NQ
	Ash 2	0.0 ppm	—	0.0 ppm
Brighton Lab	Bag 1	0.20 ppm	—	0.20 ppm
	Bag 2	NQ	—	NQ
	Bag 3	NQ	—	NQ
California	Bag 1-1	0.17 ppm	—	0.17 ppm
	Bag 1-2	n/a	NQ	NQ
	Bag 2-1	0.15 ppm	—	0.15 ppm
	Bag 2-2	0.30 ppm (40x)	0.15 ppm	0.15 ppm
	Bag 3-1	—	NQ	NQ
	Bag 3-2	—	NQ	NQ
	Bag 4-1	NQ	—	NQ
	Bag 4-2	0.141 ppm	—	0.14 ppm

Table 2. Imidacloprid concentration in ash leaf samples collected in September, 2017. Non-quantifiable (NQ) values are at the limit of what this assay method can determine.

Sample Source	Sub Sample ID	Result (ppb) and Dilution			
Sacramento Bag A	1	0.0	30x	—	
	2	0.0	30x	—	
Sacramento Bag B	1	0.0	30x	—	
	2	0.0	30x	—	
Otis Control Foliage	Ash 1	0.0	30x	0.0	20x
	Ash 2	0.0	30x	NQ	20x

There are a number of granular turf products that are used to augment and enhance the growth of trees and lawns, and many of these contain insecticides (e.g., Bayer Advanced Complete Insect Killer [beta-cyfluthrin], Ortho Grub B Gone [bifenthrin], Bayer DeltaGard [deltamethrin] and Syngenta's Demand G [lambda-cyhalothrin]. The second set of samples received in late 2017 did not have detectable levels of dinotefuran, however both samples from San Diego, California had high levels of imidacloprid (Table 3). The value for the December San Diego sample is an approximation but was not processed further since the value was so high. Residue levels of this magnitude indicate there had been a recent pesticide application on or very close to the trees the samples came from.

Recommendation

In order to alleviate future instances of EAB mortality, it might be worthwhile to identify an insect in California that readily feeds on ash foliage. Prior to shipments, these insects would be bagged onto the trees being used for foliage collections and so could provide an indication of any nearby pesticide applications. For the most part, insects in the order Lepidoptera (moths and butterflies) are not very sensitive to imidacloprid, so a suitable and readily available insect surrogate would first need to be identified.

Table 3. Imidacloprid and dinotefuran content in ash leaf samples collected in November/December, 2017.

Sample Source, date	Imidacloprid	Dilution	Dinotefuran	Dilution
Sacramento, 11/30	0.0 ppm	25x	0.0 ppm	10x
San Diego, 11/29	3.6 ppm	200x	0.0 ppm	10x
San Diego, 12/5	~24 ppm	200x	0.0 ppm	10x

References

1. Lewis P, Turcotte R. Use of Chemical protection and host Tree Reduction to Control an Emerald Ash Borer Infestation in West Virginia. J Biodivers Manage Forestry. 2015;4(1):1-5. doi:2327–4417.1000136
2. McCullough D, Poland T, Anulewicz A, Lewis P, Cappaert D. Evaluation of *Agrilus planipennis* (Coleoptera: Buprestidae) Control Provided by Emamectin Benzoate and Two Neonicotinoid Insecticides, One and Two Seasons after Treatment. Journal of Economic Entomology. 2011;104(5):1599-1612.
3. Envirologix Inc. Pamphlet for EP-006- Imidacloprid Quantiplate Kit. https://www.envirologix.com/wp-content/uploads/2015/05/EP006-Imidacloprid-101215.pdf accessed 10/11/2017. Envirologix 2015; Portland, ME.

Asian gypsy moth female flight distance and the ability of late instar larvae to tolerate food deprivation

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Introduction

Lymantria dispar asiatica, is one subspecies of the Asian gypsy moth (AGM) complex, whose females possess flight ability. AGM as well as *L. mathura*, *L. monacha*, and *L. xylina* are targets of survey and detection efforts as well as offshore vessel certification programs, as many have been found on vessels entering U.S. and Canadian ports. The goal of this project is to further assess the risk of *L. dispar asiatica* through collecting more data on the biology and behaviors of *L. dispar asiatica* in Russia. In 2017, studies focused on two aspects that could increase ability of colonizing new areas: 1) the flight distance of female moths and 2) the ability of late instar larvae to tolerate food deprivation.

Flight distance of female moths

To determine the flight distance of female moths, field experiments were conducted in western Sayan Mountains in Yermakovsky district, Krasnoyarsk Krai of Russia. Live female moths of *L. dispar asiatica* (also referred to as Siberia gypsy moth) were collected from the field site by using a white screen with light sources (Figure 1), powered by a generator. These females were marked using different colors of ink (Figure 2) for release at different distances and placed into cardboard boxes.

Figure 1. White cloth screen with lights for recapture of female adults.

Figure 2. An adult female was color-marked with red ink.

Then, those females were released at distances of 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 km from the illuminated screen (Figure 3). Releases were conducted from July 27 to August 3, 2017. The times of moth release and moth return to the illuminated screen were recorded for all moths. The screen remained illuminated from 10:00 pm to 2:30 am.

The results from the female flight distance study indicate that moths returned to the illuminated screen from release distances as far as 3.0 km. However, only a small percentage of females returned to the lighted screen during the 4.5 hour illumination period:

- 4 out of 600 at 1.0 km
- 0 out of 300 at 1.5 km
- 0 out of 600 at 2.0 km
- 0 out of 140 at 2.5 km
- 3 out of 860 at 3.0 km
- 0 out of 300 at 3.5 km

Figure 3. Female adults were placed on top of a post at different distances from the illuminated screen.

We do not know why only a small portion of female moths returned to the illuminated screen during this period, since all of the released moths were captured not long before the study, using the same method. Nonetheless, the results of this study demonstrated that female moths can fly up to 3.0 km in a short period of time, with or without stopping.

Late instar larvae tolerance to food deprivation

To assess the ability of late instar larvae of *L. dispar asiatica* to tolerate food deprivation, AGM larvae from Siberia and *L. dispar dispar* from the U.S., North American gypsy moth, NAGM, were reared under laboratory conditions for their first three larval instars on *Betula pendula* leaves. Thirty-five 4th instar larvae that all molted during the same day were used for each treatment group. Larvae were randomly assigned to four experimental groups that varied by the amount of food provided (100%, 75%, 50%, and 30% of daily relative consumption amount for a fully fed larva). Individual larvae were reared separately in a Petri dish. Larvae were checked to determine: 1) instar number and duration, dynamics of larvae biomass, and mortality, 2) time of pre-pupation, pupa and adult emergence, and 3) relative daily growth rate (RGR). Additionally, female moths were dissected within one day post emergence and the amount of eggs for each female was determined. The results from the food deprivation study of both the AGM and NAGM late instar larva are summarized below.

- As food became less available, mortality of both sexes of NAGM as well as female AGM increased (Figure 4), but mortality of AGM males did not change substantially.
- Mortalities of both female and male AGM were lower than that of NAGM as less food became available.

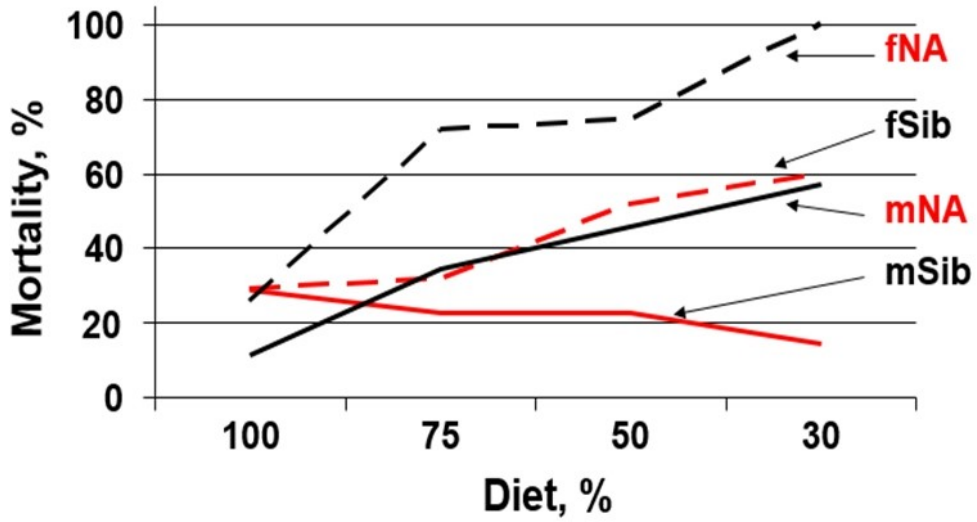

Figure 4. Mortality from the 4th instar larva to adult emergence for gypsy moth from Siberia (AGM), and from the U.S. (NAGM) when provided with different amounts of host plant (*Betula pendula*) materials, i.e., 30%, 50%, 70%, and 100% (fSib and mSib denote female and male AGM from Siberia, fNA and mNA denote females and males of NAGM collected from New Jersey, U.S.).

- No NAGM larvae survived to become female pupae in this test when only 30% amount of food was provided. Therefore, AGM appears to be more tolerant to food deprivation than NAGM.
- For both AGM and NAGM, in this case, the weight increase from the beginning of 4th instar to pupa generally became smaller when less food was available (Figure 5), thus resulting in smaller pupae when insufficient food was provided. The effect of food deprivation is more obvious in females of both subspecies, especially for NAGM from the U.S.
- Food deprivation has more effect on fecundity of NAGM than on AGM, although food deprivation resulted in smaller sizes of female adults for both AGM and NAGM. When provided with 100% of their food requirements, normally both AGM and NAGM female pupae are around 1.5 g. At this weight, an NAGM female produced approximately 175 more eggs than an AGM female does, but NAGM eggs are presumably smaller. However, as less and less food became available, lighter adults emerged from smaller pupae. When females emerged from pupae weighing only approximately 0.4 g, fecundity of both AGM and NAGM was almost identical (Figure 6).

Summary

The above results indicate that female AGM from Siberia, Russia are capable of flying at least 3.0 km in less than one -hour. This implies that for a vessel called at a port even for a short time during the risk period in the evening, there is potential that female AGM could fly to the vessel to lay egg masses on the superstructure, and thus, poses great risk.

The results from the food deprivation study indicate that although AGM females are relatively less fecund in terms of eggs per unit of female body weight than NAGM females, they likely devote more resources to individual eggs and female flight muscles, and are therefore less susceptible to food shortages. The mortality of AGM larvae is also lower than that of NAGM when less food is available. Therefore, AGM may be able to colonize a new area with less preferable food sources more successfully than NAGM.

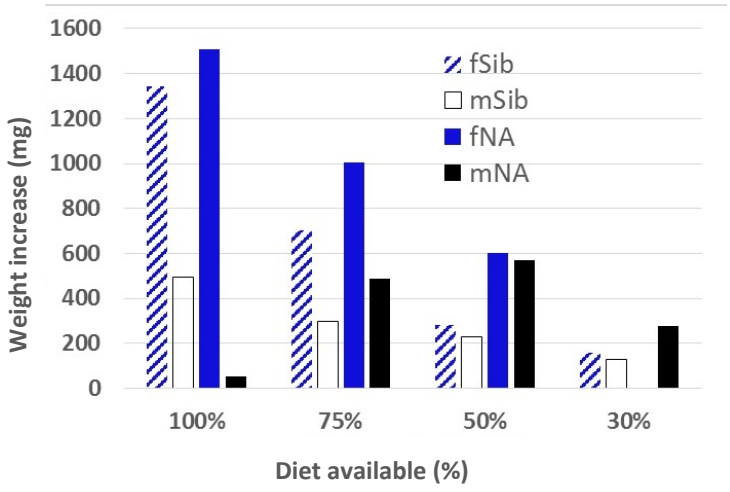

Figure 5. Weight increases (mg) from the beginning of 4th instar to pupa when provided with different food amounts for AGM and NAGM (fSib and mSib denote female and male AGM from Siberia, fNA and mNA denote female and male NAGM from the U.S.).

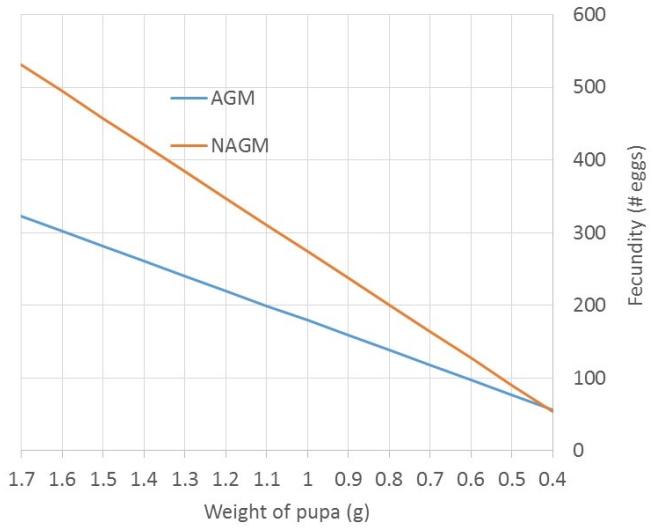

Figure 6. Plot of fecundity of AGM and NAGM females from pupae of different weights.

Trapping of L*ymantria* species and biology of *Lymantria xylina*

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Introduction

Egg masses of several species in the genus *Lymantria* such as *L. dispar asiatica*, *L. xylina*, and *L. mathura*, have been found on vessels entering U.S. ports, and are targets for survey and detection efforts as well as offshore vessel certification programs. This is a multiyear project with the overall goal to further assess the risk of these species or subspecies and to improve risk mitigation strategies. The objectives for this project, conducted in China in 2017, are to evaluate: 1) trapping efficacy and population levels using disparlure and xylinalure as baits for several *Lymantria* species, 2) egg-laying behavior of *L. xylina*, and 3) the effect of extreme environmental conditions on egg-hatching.

Trapping efficacy and population levels

To evaluate trapping efficacy with disparlure and xylinalure for several *Lymantria* species, milk carton traps baited with disparlure or xylinalures were placed at a total of 25 sites in Beijing, Tianjin, Chongqing, and in the provinces of Liaoning, Hebei, Shanxi, Shandong, Henan, Anhui, Jiangsu, Zhejiang, Sichuan, Hubei, Fujian, and Yunnan. For each site, 5 to 10 traps were placed, and the distance between the traps at each site was at least 100 m. Depending on the latitude, and therefore the estimated adult flight period, traps were placed from late May to mid-June and were checked once every two to seven days. Trap capture counts were stopped after three consecutive counts of no targeted *Lymantria* moths. Molecular methods were used to confirm the identity of captured moths when it was not possible to identify the insect solely based on morphological characteristics.

For evaluating risk period of *Lymantria* species, our results confirmed that disparlure baited traps at these sites may also capture males of a few *Lymantria* species such as *L. xylina*, *L. dissoluta*, and *L. marginata* in addition to *L. dispar asiatica*, while xylinalure appears to attract only *L. xylina*. A total of 195 traps were placed at the above mentioned sites, and a total of 4,259 AGM males were captured in these traps. Based on the number of AGM male moths captured at each site, AGM population levels were determined to be similar at most sites in China in 2017 when compared with the number captured in 2016. However, there were two exceptions, Dalian of Liaoning province and Liuan of Anhui province, where the number of captures almost doubled in 2017.

Egg-laying behavior of *L. xylina*

To assess the pathway risk of *L. xylina* through marine vessels, it is necessary to have a better understanding of the biology of this insect. In fiscal year 2017, we focused our studies on the egg-laying behavior of *L. xylina*, and continued evaluating the effect of extreme environmental conditions on egg hatch. Field surveys were conducted in Pingtan Island of Fujian province to determine the egg laying preference on different plant species. Additionally, we evaluated the location of egg masses on one of *L. xylina*'s primary hosts, *Casuarina equisetifolia*, in China. In the laboratory in Fujian, China, newly emerged adults were placed in cages with twigs of different species as well as on paper cards, bricks, and metal sheets to determine whether the moth deposits egg masses onto substrate similar to that on a marine vessel.

The results from field surveys indicate that on the primary host, *C. equisetifolia*, the majority (95.3%) of *L. xylina* egg masses were found on branches, while 4.4% of egg masses were found on the main trunk and less than 0.3% of egg masses were found on leaves. Most of the egg masses (71.7%) were laid on parts of the plant located 2 to 4 m above the ground.

Approximately 0.1% of egg masses were also found on the following plants: *Ligustrum sinense*, *Digitaria somguinalis*, *Murraya exotica*, *Bidens pilosa*, *Mussaenda pubescens*, and *Acacia confusa*. During the field survey, a few egg masses were found on metal fences and bricks (Figure 1). In the laboratory cage study, females laid egg masses on colored paper cards as well as the metal and nylon screen mesh of the cage.

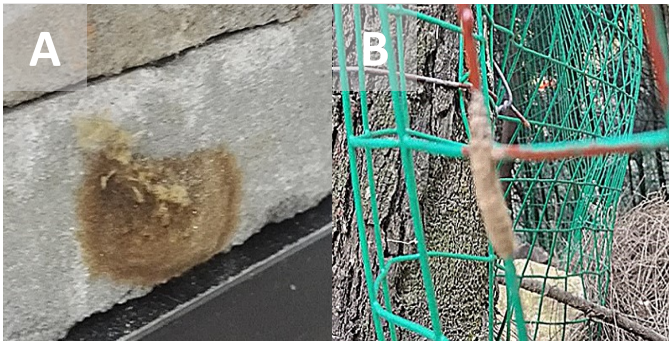

Figure 1. Egg masses of *Lymantria xylina* were found on A) brick and B) metal screen fence in Pingtan, Fujian province of China.

Effect of extreme environmental conditions on egg hatching

The effect of adverse environments on egg hatching, with a focus on extreme temperature and exposure to extreme salinity, were evaluated for *L. xylina*. Effects of low temperature on egg survival and egg hatching was evaluated by placing egg masses in low temperature chambers at -20°C, -15°C, -10°C, -5°C, 0°C, and 5°C for 3 hours and 1, 5, 10, 15, 20 and 25 days. There were five egg masses, each with an average of approximately 600 eggs in each temperature treatment. The effects of high temperature on egg mass survival was evaluated by placing egg masses in climate chambers at 30°C, 35°C, 40°C, 45°C, and 50°C for 3 hours and 1, 5, 10, 15, 20 and 25 days. In both extreme low and high temperature tests, eggs were placed in room temperature conditions after exposure. Tolerance to extreme salinity was examined by immersing egg masses into 11 different solutions of varying NaCl concentrations (0‰ to 54.2‰) for 3 hours to 17 days, at room temperature. In all cases, egg hatching was checked daily from late March to late June.

As for the effect of extreme temperatures on egg hatching, the results indicate that, generally, the longer the eggs are exposed to high temperature, the lower the hatching rate. This is most notable when eggs are exposed to temperatures ≥ 40°C for more than 120 hours. Extreme low temperatures have a similar effect on egg hatching; when temperature is lower than -15°C, egg hatching rate was as low as ~10%, even when only exposed for one day. When placed at -10°C for more than five days, less than 1.5% of eggs hatched. Exposure to less extreme temperatures has much less adverse effects on egg hatching. For example, the average hatch rate was 73.4% when placed at -5°C for 10 days and 63.7% when placed at 35°C for 10 days.

In regards to the effect of immersion in saline on egg hatching (Figure 2), the general trend observed was that the higher the saline concentration and the longer eggs were immersed, the lower the hatching rate. However, no significant correlation can be inferred, statistically. Egg hatching rate was higher than 10% in all, but one (54.2% for 408 hours) combination of saline concentration from 0 to 54.2% and length of immersion from 3 to 408 hours. When only considering the open ocean salinity (3.5%), egg hatching rate (y) can be estimated based on the length (x, hours) of immersion using the equation:
$y = -0.147\ln(x) + 1.1167$ ($R^2 = 0.7922$).

Conclusion

Several species of *Lymantria* including *L. dispar asiatica*, and *L. xylina* in southern China can be captured using the disparlure baited traps. However, xylinalure is more attractive to *L. xylina* than disparlure.

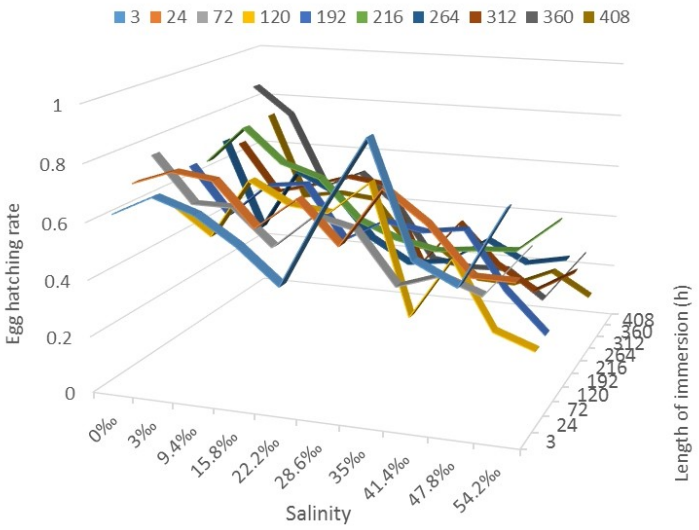

Figure 2. Effect of immersion length and salinity on egg hatching rate.

Trap capture data in recent years does not seem to indicate natural population cycles of these two species, this is likely the result of the management practices in place in different areas of China. Female *L. xylina* moths were found to lay eggs on other tree parts such as tree trunks as well as substrates such as bricks and fences that are similar to surfaces of vessel superstructure and containers; therefore, they pose a greater risks than moths that only lay egg masses on small branches or twigs. *L. xylina* egg hatching was found to be adversely affected by prolonged exposure to extreme temperatures and salinity. However the egg hatching rate was generally not affected significantly when exposed to less extreme temperature (≥ -5°C and ≤ 35°C) and salinity (≤ 3.5%) for ≤ 10 days, indicating that eggs could remain viable during some vessel voyages across the Pacific Ocean from eastern Asia to North America.

In the coming years, we plan to continue trapping studies to determine population levels of *Lymantria* species in different areas of China and to compare population cycles of AGM in China to that of Russia and Japan, where AGM populations reportedly show clear cyclic trends. For *Lymantria* species, especially, *L. xylina*, the focus will be developing light traps for trapping females in the evening as it is the female that may potentially lay egg masses on vessels. More studies including field surveys will be conducted to collect more data for analyzing egg-laying sites of *L. xylina*.

Host evaluation of velvet longhorned beetle

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Introduction

The velvet longhorned beetle, VLB, *Trichoferus campestris* (Figure 1), is an exotic wood borer native to China, Japan, Korea, Mongolia and Russia. It has also been found in quite a few European countries. In North America, the insect was detected in Quebec, Canada in 2002 and has been found in Illinois, Colorado, Utah, New Jersey, New York, Ohio, and Pennsylvania in the U.S. There have been quite a few reports of the host plant species of the beetle that is based primarily on the study by Iwata and Yamada [2], who indicated that about 40 genera of conifers and angiosperms can be host plants of VLB and concluded that this species can potentially attack most woody plants. However, whether the beetle attacks live healthy trees of the above stated species remains largely unconfirmed. The reference was published 27 years ago, when the synonym, *Hesperophanes campestris*, for VLB was still used. Possible problems include misidentification of host tree species or the beetle, and different definitions of "full host". For wood-boring insects "full host" should be defined as a tree species that the beetle is able to complete its life cycle. In this study we conducted a survey and field cage studies in several locations in Utah as part of our on-going VLB host range evaluation to determine whether the beetle attacks live host trees of different species.

Figure 1. A VLB adult emerged from caged section of a peach tree in Pleasant Grove, Utah.

Materials and methods

The caging studies began in May, 2014. We deployed wire sleeve cages on branches and trunks of suspected host trees in Adam's Orchard. The sleeve cages were designed to catch adult VLB as they emerged from wood. A total of 80 sleeve cages were installed (Figure 2A&B), 40 on old trees of *Prunus avium* (cherry), 20 on *Malus pumila* (apple trees), and 20 on *Prunus persica* (peach trees). In addition, a few cages were installed on birch trees showing suspect damage at Nunn's Park.

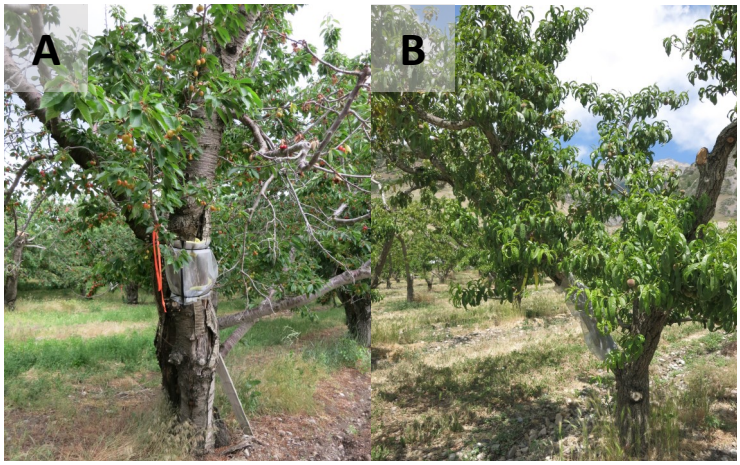

Figure 2. A) A cage was installed on the trunk of an old cherry tree from which two VLB adults emerged late. B) A cage was installed on a branch of a peach tree from which six VLB adults emerged late.

Sleeve cages were checked for signs of VLB emergence every few days during peak flight period in early June, and were later checked on a weekly bases until mid-August. In July 2014, a few additional sleeve cages were installed on suspected infested old cherry, peach, birch, pine, and poplar trees in Adam's Orchard, McRiley's Golf Course, and Nunn's Park.

In 2017, 96 wire-screen sleeve cages were installed on sections of the following tree species: *Acer negundo*, *A. saccharinum*, *Betula occidentalis, Elaeagnus angustifolia*, *Gleditsia triacanthos*, *Juglans* sp., *Populus* sp., *Prunus avium*, *P. cerasifera*, *P. persica*, *Robinia pseudoacaciam*, *Styphnolobium japonicum*, *Tilia* sp., and *Ulmus* sp. A total of 64 cages were installed at Adam's Orchard; 29 on peach, 20 on apple, and 16 on cherry. An additional 20 cages were installed on dead or dying trees, which had multiple exit holes, similar to VLB exits holes.

All trees were categorized as live, partially dead, or dead.

Live trees were assessed as H1, H2, or H3:

- H1 was used if there were no signs of injury
- H2 was used if there were some signs of injury (i.e., <1/3 dead branches)
- H3 was used if between 1/3-1/2 of dead branches had signs of injury

Partial dead trees were trees where more than half of the total branches were dead.

Dead trees were assessed as D1, D2, or D3:

- D1 was used if there were freshly dead branches, with some dry green leaves
- D2 was used if there were dead branches but live roots
- D3 was used if there were no signs of dry green leaves or live roots

Trees such as the Japanese pagoda tree, apple, oak, honey locust, willow, pear, and birch were also inspected for signs of VLB infestation in the following public areas within 1.5 miles of previous trap catches: Mick Riley Golf Course, Adam's Orchard, Anderson Park, Battle Creek Park and Hill Creek Park in Pleasant Grove, Lindon City Center Park, Murray City Park and Arboretum, Murray Cemetery, and Creekside Park in Holladay (Figure 3).

Figure 3. Sites in Utah where surveys were conducted and cages were installed for VLB host evaluation.

Results and discussion

For sections of trees caged in 2014, a total of 22 beetles emerged from the caged section of trees in 2014, while 27 emerged in 2015. Among them, 21 were from old cherry trees and 18 were from peach trees. A total of 17 exit holes were found on old cherry trees and two on peach trees. All these trees were live, although some were stressed, thus confirming that live cherry and peach trees are acting as hosts for VLB.

For sections of trees caged in 2017, a total of two VLB adults emerged from two peach trees, one from a healthy tree, and one from a stressed tree. Nine beetles emerged from two dying Russian olive trees.

No VLB were caught in cages installed on other trees, although *Dicerca caudata* adults were caught in cages on birch and linden trees. The sleeve cages at Mick Riley's Golf Course and Nunn's Park did not catch any beetles.

Numerous healthy apple trees, which is reportedly a preferred host, were also examined for exit holes but no VLB were found**.**

We have yet to find any beetles emerge from twigs, branches or major stems of trees less than 2 inches in diameter, which is different from our previous study in China, where beetles were found emerging from twigs of apple trees around 1 inch in diameter.

Cages that were installed in May, 2014 also had beetle emergence in the summer of 2015, which means that for at least some VLB, the completion of development in host trees in Utah spans two years.

Although adults emerged from two logs taken from a dying Norway maple, *Acer platanoides*, in Mississauga, Ontario, Canada [2], it does not clearly show that VLB is able to infest live trees and complete development on living trees of that species. In our caging study, we demonstrated that VLB may attack live trees such as cherry, peach, and Russian olive in the U.S. as well as complete development on them. In future studies, we will include more species in another field survey. We plan to cage paired live beetles with live trees in field, dwarf or bonsai trees (including species and different sizes of candidate trees) in addition to field sleeve cage studies. Genetic studies will also need to be carried out to determine whether there are differences in host suitability in different populations (or subspecies) of VLB.

References

1. Iwata R, Yamada F. Notes on the biology of *Hesperophanes campestris* (Faldermann) (Col., Cerambycidae), a drywood borer in Japan. Material und Organismen. 1990;25(4):305-313.
2. Erin B-A, Troy K, Jean T. Discovery of *Trichoferus campestris* (Coleoptera: Cerambycidae) in Ontario, Canada and first host record in North America. The Canadian Entomologist. 2014;146:111-116.

Host range evaluation of Asian longhorned beetle

Baode Wang[1] and Ruitong Gao[2]

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[2]Chinese Academy of Forestry, Beijing, China

Introduction

Asian longhorned beetle, ALB, *Anoplophora glabripennis*, was first detected in the U.S. in 1996. Since this detection in New York State, great eradication efforts have been implemented at sites in several states where the beetle has subsequently been found. Although we have gained a better understanding of ALB host range by conducting studies and analyzing survey data from the U.S. there are still some tree species that have the potential to serve as an ALB host that still need to be evaluated. The ALB host list still needs to be refined to the species level so that resources can be better allocated for the management of ALB in quarantine areas in the U.S. We directed our focus in 2017 to tree species that are listed in the U.S. as "occasional to rare hosts" (*Albizia julibrissin, Cercidiphyllum japonicum* and *Platanus* spp.) or as "questionable U.S. records" (such as *Hibiscus syriacus* and *Morus* sp.) [1]. Additionally, *Catalpa bungei* was evaluated in China, where it was anecdotally reported to be an ALB host, since we could not obtain a closely related North American species, C. *bignonioides* and *C. speciose*, to plant in our "common garden" in China.

Approaches and results

In 2017 we continued our host evaluation in a "common garden" setting as well as by caging sections of trees with paired beetles (i.e., 1 male and 1 female per cage) in field sites in China. We also surveyed trees for signs of ALB infestations in several provinces and major cities in China.

Cage studies

We started caging paired beetles on tree sections in June and July of 2016. Beetle longevity, egg pits made, active egg sites, and development of individual beetles were recorded. The species in this study include *C. bungei*, *C. japonicum*, and *H. syriacus*.

2016 on C. bungei

- Beetles were caged without twigs of *Acer* sp. for feeding the preliminary conclusion is none of the ALB larvae survived. Details below:
    - July, 2016: 217 egg pits were made by the beetles
    - July and September, 2016: only 34 active sites were found
    - October, 2016: 16 active sites were found
    - April, 2017: one active egg site
    - October, 2017: no active egg sites or exit holes

2017 on C. bungei

- Beetles were caged with a section of the main trunk (approximately 1.8 m, 8-9 cm in diameter) (Figure 1) and were provided newly collected twigs of *Acer truncatum* in the cage for ALB adult feeding. The test is ongoing, but it appears that no ALB larvae have survived.

Figure 1. Caging beetles with a section of a *Catalpa bungei* tree.

2017 C. bungei

- ALB larvae were inserted under tree bark (Figure 2). The evaluation is still on-going. However, it appears that the inserted larvae will be able to complete development. Details below:
    - September 4, 2017: 13 of the 1st instar, 42 of the 2nd instar, and four of the 4th instar ALB larvae were inserted
    - October 22, 2017: six of 13 1st instar, 24 of 42 2nd instar, and four of four 4th instar still produced fresh frass (Figure 3), respectively
    - These trees will be checked again in 2018 starting in May

2016 on C. japonicum

- Beetles were caged. The evaluation is on-going. However, it appears only a very small percentage (approximately 2%) of larvae might be able to complete development. Details below:
    - July, 2016: 193 active sites out of the 511 ALB egg pits
    - October, 2016: 147 active egg sites
    - April, 2017: 42 active egg sites
    - Late October, 2017: four active egg sites were obvious, no exit holes
    - The trees will be monitored until beetle emergence or until October, 2018

2017 on C. japonicum

- Beetles were caged with different sizes of trees. The evaluation is still ongoing. Details below:
    - June 26, 2017: eight smaller trees of 3 cm DBH, four larger trees of 27 cm in DBH
    - August 15, 2017: eight active egg sites were found on smaller trees, and 67 active egg sites were found on larger trees
    - October 22, 2017: no active sites were found on smaller trees, 58 active sites on larger trees
    - All these trees will be checked again in 2018 starting in May

For *H. syriacus* caged with beetles in 2016, quite a few egg pits were made by the beetles caged in June, 2016, but only nine active egg sites were found on five of the 25 trees. Only one active egg sites was found when checked in October, 2016, and then no active sites were found and there was no ALB emergence in 2017. We conclude that this species is unlikely as a suitable host of ALB.

Similar caging studies were also conducted for trees of several species starting in 2017 in the "common garden" in Beijing. Cages were installed on trees of the following species on June 26, 2017 with a pair of male and female ALB adults: *Juglans nigra*, *Platanus occidentalis*, *Tilia cordata*, *Liridendron tulipifera*, and *Morus macroura*. Freshly collected twigs of *Acer mono* were placed in all cages for adult feeding.

Figure 2. Artificial larva insertion site on the trunk of a *Catalpa bungei* tree.

Figure 3. Frass produced by the inserted 4th instar larva on a *Catalpa bungei* tree seven weeks after it was inserted under the bark of the tree.

The caged section of trees were last checked on October 18, 2017, when 30 of the 32 initial active egg sites on *P. occidentalis* still had fresh frass, indicating these larvae were still alive. Only two of the eight active egg sites on *T. cordata*, two of the 24 on *M. macroura,* had fresh frass, while no fresh frass could be seen on caged sections of *J. nigra* and *L. tulipifera* by then.

Studies in a "common garden" setting

We continued monitoring trees including *Koelreuteria paniculata*, *Liridendron tulipifera*, *Alnus incana*, *Acer platanoides*, *A. mono*, *Aesculus chinensis*, *Fraxinus Americana*, and *Populus* spp for ALB infestation in the "common garden" in Beijing from mid-March to late August. However, no new ALB egg sites, nor exit holes were found, although one dead ALB adult were found under a tree of *A. mono*. We did notice that quite a few new exit holes of the emerald ash borer (EAB), *Agrilus planipennis* on ash trees. This "common garden" was set up in 2003 and with 20 species of trees from the U.S. and no pesticides or herbicides have been used. Despite seeing quite a few exit holes before 2010 on tree species such as *A. mono*, *Aesculus chinensis*, and *Koelreuteria paniculata,* it appears as though the ALB population has declined.

Site surveys

Trees in the following genera were surveyed in 2017 for ALB infestations in different locations (Beijing, Tianjin, Chongqing and the province of Hebei, Henan, Shandong, Shanxi) of China: *Acer*, *Armeniaca*, *Carpinus, Catalpa*, *Cerasus*, *Crataegus*, *Fraxinus*, *Koelreuteria*, *Lagerstroemia*, *Malus*, *Platanus*, *Populus, Prunus*, *Pyrus*, *Quercus*, *Salix*, and *Ulmus*. The following species were found to be infested by ALB in several locations: *Acer negundo*, *A. saccharinum*, *A. truncatum*, *Fraxinus velutina*, *Koelreuteria paniculata*, *Platanus occidentalis*, *P. x acerifolia*, *Salix babylonica*, *S. capitata*, *S. matsudana*, and *Ulmus pumila*.

Discussion

The field caging studies conducted in China allowed us to collect information on the suitability of living trees acting as ALB hosts for some of the rare, occasional, and questionable host species in the U.S. Results from previous years suggest that *Albizia julibrissin* may not be a suitable host for ALB. In this study, the results suggests that *H. syriacus, C. bungei*, *J. nigra*, and *L. tulipifera* may also not be suitable as ALB hosts. The results also confirm that although it is possible for *C. japonicum* to be a host for ALB, it may not be a good host. Host evaluations for ALB through caging studies will be continued for *C. japonicum*, *Platanus* spp., *Morus* sp., and *Tilia cordata* in the coming years. We may also add a few rare and questionable ALB host species to the caging study. Surveying trees in different areas for ALB infestation can be helpful in identifying and determining tree species that may act as ALB hosts in a given environmental condition.

In our common garden in Beijing of mixed tree species, ALB populations levels have been declining despite the restriction of pesticide applications and the limit of natural enemies. In fact, recent surveys have found almost no naturally occurring ALB within this garden.

Reference

1. Wang, B. *Asian longhorned beetle: Annotated Host.* [Online]. Available from: https://www.aphis.usda.gov/plant_health/plant_pest_info/asian_lhb/downloads/hostlist.pdf [February 8, 2018]

Asian longhorned beetle switch host feeding studies to evaluate host suitability

Dave Cowan[1] and Baode Wang[1]

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Introduction

An ongoing concern of the Asian Longhorned Beetle Eradication Program is that the removal of preferred hosts, such as striped maple, *Acer pensylvanicum,* and red maple, *A. rubrum*, from a quarantine area could cause beetles to move to less preferred host trees. If these less preferred hosts of Asian longhorned beetle, ALB, *Anoplophora glabripennis*, are capable of supporting beetle reproduction, these trees could serve as natural refuge following preferred host removal, further complicating eradication efforts. In an effort to address these concerns, three studies were designed to investigate how a shift to a suboptimal food source from a preferred host affected adult longevity, feeding, fecundity and egg hatch. The results of these studies provide information that enables APHIS to refine the Asian longhorned beetle: Annotated Host List [1] and allow the ALB Eradication Program to focus resources on tree species that support beetle reproduction.

Methods

These studies were conducted over a 16 month period. Beetles were separated by age group, seven to 14 days and 15 to 30 days post emergence, and placed as male-female pairs on potential host tree species; striped maple was used as a control.

Experimental host tree species included:

- Common hackberry, *Celtis occidentalis*
- Katsura, *Cercidiphyllum japonicum*
- American sycamore, *Platanus occidentalis*
- Autumn olive, *Elaeagnus umbellata*
- Bigtooth aspen, P*opulus grandidentata*
- Pear, *Pyrus* sp.
- Cherry, *Prunus* sp.
- American beech, *Fagus grandifolia*

Prior to the study, beetles were individually reared on striped maple. During the study, paired beetles were held in mating containers with four twigs (15 cm long, 0.2-0.7 cm in diameter) as a food source and provided with an artificial ovipositional substrate (cardboard 2.5 x 8.0 x 17.5 cm wrapped in multiple layers of cheese cloth) (Figure 1). Beetles were held under a 15:9 light-day photoperiod, at 23°C and 55% RH. Mortality, feeding, and oviposition were monitored for the full lifespan of the beetles. The study concluded when no surviving beetles remained. Eggs laid during the course of the study were removed from the mating containers and held in an incubator at 26°C for up to five weeks and monitored for hatching.

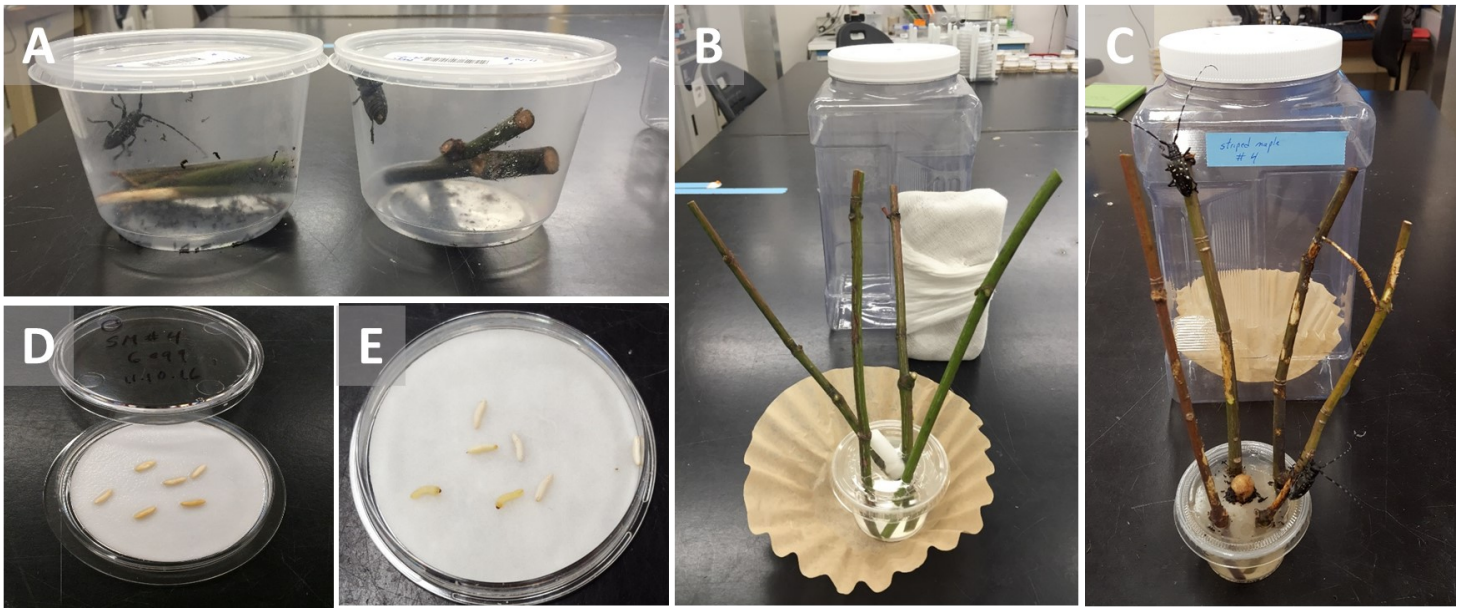

Figure 1. Study setup: A) Beetles held separately prior to enrollment. B) Beetle mating pair condo with artificial ovipositional substrate and twig food source. C) Feeding damage. D) Eggs removed for hatching in incubator. E) Hatched larvae.

Results

Beetles survived on all study tree species, with at least half of the beetles on each species lasting for more than 35 days (Figure 2). Beetles fed on bigtooth, aspen, and katsura all survived as well as the control beetles fed on striped maple. Female beetle lifespans were not affected by switch host feeding, however male longevity significantly decreased on all species except katsura and American beech.

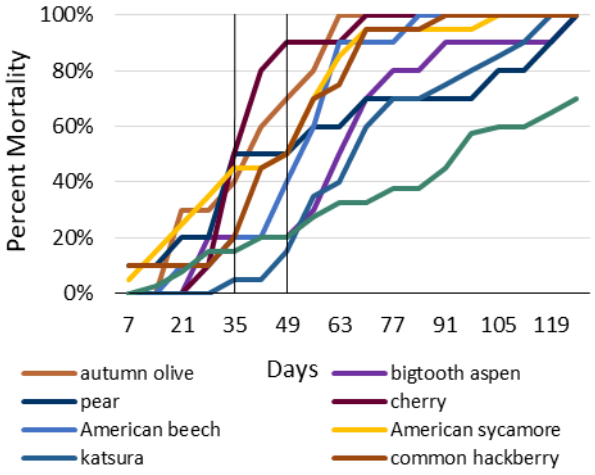

Figure 2. ALB percent mortality by tree species out to 126 days.

Mated beetle pairs fed on all species of trees in the study. However, beetles fed significantly less on the study tree species than on striped maple, except in the case of katsura and American beech (data available upon request). The average number of eggs oviposited by beetle pairs was greatest in those fed on striped maple, but beetles that fed on all other species were able to produce eggs (data available upon request). Amount of feeding was correlated with egg production in all studies (Figure 3).

All of the host tree types tested resulted in production of viable eggs, as evidenced by hatch rate (Figure 4). In Study 3, the oviposition by beetles fed on striped maple was less than what would normally be expected. The premature death of one of the females contributed to decreased oviposition total but this may have also been attributed to seasonal fluctuations in food quality or colony vigor. Oviposition by the beetles fed on American beech and cherry may have been similarly affected.

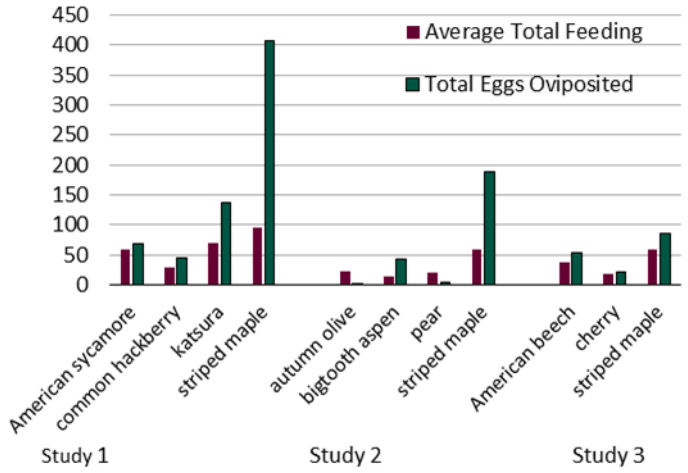

Figure 3. Average total feeding and total eggs oviposited by mat-

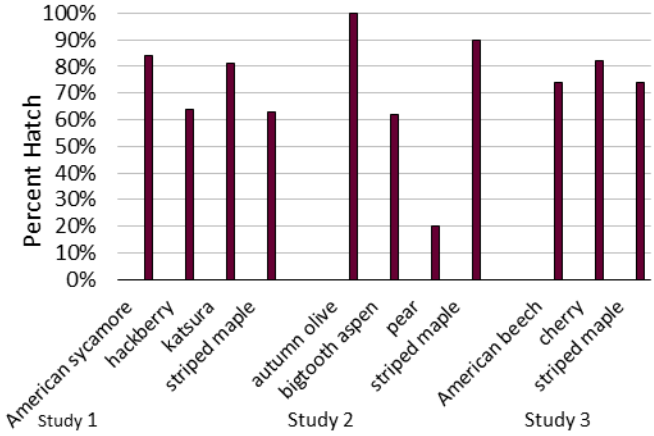

Figure 4. ALB percent hatch by study and tree species.

Conclusion

The ability of ALB to survive and reproduce on alternative tree species following removal of a preferred host is a critical factor in determining the efficacy of an eradication program. Taken together, these studies demonstrate that: male longevity is negatively impacted by a switch in host feeding, both male and female ALB adults survived and fed on all host species provided in this study. The number of oviposited eggs varied. Viable eggs were produced by mated beetle pairs fed on all host species in this study. These data show that even non-preferred hosts can support beetle reproduction and that they should be taken into account during eradication efforts. Further studies are warranted in order to determine if similar oviposition would occur in live trees as compared to the artificial oviposition substrate used in these studies.

Reference

1. Wang B. *Asian longhorned beetle: Annotated Host.* [Online]. Available from: https://www.aphis.usda.gov/plant_health/plant_pest_info/asian_lhb/downloads/hostlist.pdf [February 8, 2018]

Asian longhorned beetle colony production

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Introduction

Asian longhorned beetle, ALB, *Anoplophora glabripennis*, persists in status as one of the most destructive wood boring pests of maple and other hardwoods in North America. The Otis Insect Containment Facility continues to rear four colonies of ALB from the following locations: China, Ohio, Worcester (Massachusetts), and New York. A mixed colony comprised of individuals from each of the four locations also remains at the facility. These colonies support research and the development of control and eradication methods for the beetle at isolated locations. The average weekly production levels, artificial diet rearing methods, and individual insect tracking procedures were reported in *Otis Laboratory 2016 CPHST Laboratory Report* and have not been modified during the last year.

Outreach and research conducted with Otis ALB colonies

The colonies maintained at Otis help support public outreach and training efforts as well as research in a number of fields (Table 1).

Research projects facilitated by these ALB colonies in 2017 are described below:

- Chemical ecology studies: adults were exposed to full spectrum lighting (Figure 1) to enhance pheromone production and develop an efficient ALB monitoring trap
- Chemical ecology studies: testing adult volatiles from ALB reared on various host woods rather than artificial diet (pg. 99)
- Biocontrol non-target-host testing: ALB larvae were used to rear a candidate biocontrol agent, the parasitic beetle *Dastarcus helophoroides*
- Biocontrol research: “sentinel logs” (host wood samples infested with ALB larvae) were prepared
- ALB host specificity testing (pg. 46)

Table 1. Numbers of live and preserved ALB life stages reared and provided in 2017 to institutions in the U.S.

Purpose	Eggs	Neonates	Older Larvae	Pupae	Male Adults	Female Adults
Training/Outreach	180	150	220	45	380	332
Chemical Ecology	—	—	—	—	141	183
Biocontrol	—	—	98	10	20	20
Host-specificity	—	—	—	—	84	88
Colony	4,577	3,770	2,948	2,789	802	562
Total	4,757	3,920	3,266	2,844	1,407	1,165

Rearing trials

Trials investigating new ALB rearing techniques involving the use of willow, *Salix* spp., bolts began in 2017. Willow was felled in western Massachusetts and cut into 41 cm-long bolts. Willow is easy to propagate and maintain in a laboratory with full-spectrum lighting (Figure 2), making it an ideal host for rearing in the containment facility when research requires a natural host.

Rearing in willow is carried out by placing an early instar ALB larva in a drilled hole at the top of the bolt for two to three months to feed (Figure 3). Bolts are then chilled for 11 weeks at 10°C, as required for larval maturation and pupation. In the event that evidence of desiccation appears, bolts are split and larvae are removed and placed on diet in a 28 ml container to maintain a high humidity level (>90% RH) to ensure successful molting and development. These individuals will also be chilled and the adults will continue to feed on willow. Rearing in willow bolts that develop epicormic sprouting and continue to transpire (Figure 2) will support chemical ecology research to determine if ALB that are fed on a natural food source for their entire life cycle will produce volatiles different from those fed on artificial diet.

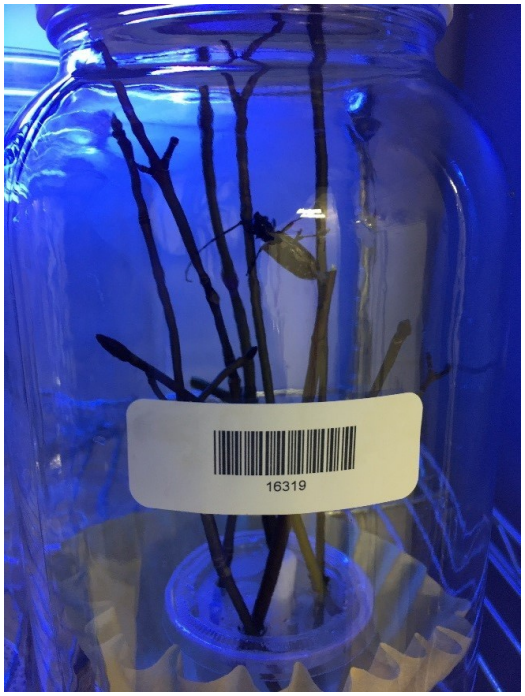

Figure 1. Adult female ALB under full spectrum lighting, supporting chemical ecology research.

Figure 2. *Salix* (willow) bolts with epicormic shoots under full spectrum lighting.

Figure 3. Wood bolts drilled and infested with ALB larvae for a biocontrol project. These will be used as “sentinel logs” (wood bolts infested with ALB larvae and placed in field cages to sample natural enemies) in 2018.

Corn earworm and old world bollworm rearing methods and supported projects

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Introduction

The corn earworm, CEW, *Helicoverpa zea*, and the old world bollworm, OWB, *Helicoverpa armigera*, are destructive to many important crops such as corn, cotton, tomatoes, soybean, and tobacco, among others. CEW is native and widespread throughout the U.S., but OWB is not yet established within the continental U.S. It is established in Puerto Rico, and has been recently detected in the U.S. [1] in traps baited with CEW pheromone. Because trapping surveys for OWB are hampered by the morphological similarity between adult OWB and CEW and lack of a specific lure for OWB, colonies of both species are being reared to support research on species-specific lure development. The maintenance of these colonies will also support research on improved rearing methods as well as molecular diagnostics of both species, their hybrids, and OWB source populations.

OWB Colony Rearing

In 2016 we were unsuccessful in establishing a colony using eggs and pupae from the APHIS supported colony (origin: Brazil) at the University of Puerto Rico. An OWB colony was successfully established in March, 2017 with 21 pairs of pupae (origin: Portugal) from the colony maintained at the Public University of Navarra, (PUN), Spain. We plan to establish additional colonies of OWB from other origins in 2018. Our rearing protocol was adapted from a method used at the PUN insectary.

The colony is held in a walk-in environmental chamber at 25°C, 65% RH, and a 16:8 light-day cycle. Several rearing variables were tested to optimize the methods:

- Larval rearing container type/size/air flow
- Larval rearing density
- Diet amount
- Diet replacement frequency
- Adult mating-cage design and adult density
- Egg-laying substrates
- Use of chilling to delay egg hatch and pupal development

An early attempt to rear OWB on Ward's Stonefly *Heliothis* Diet (Ward's Science, Rochester, NY) was abandoned due to poor larval performance and was replaced with gypsy moth diet, which improved growth and survival. To confirm that gypsy moth diet is suitable for OWB production, colony parameters measured over five generations were compared with those reported in the literature for other acceptable diets using agar and tapioca as gelling agents [2].

Insects on the agar and tapioca diets had been reared in individual vials, in contrast to the Otis Laboratory group-rearing system. The Otis gypsy moth diet, which is gelled with agar, is comparable to the other diets and produces slightly heavier pupae (Table 1).

Table 1. Comparison of OWB survival and pupa weight reared for five generations on gypsy moth diet at Otis Laboratory and on two diets reported in Abbasi et al. [2]. Abassi et al. tested four replicates of 25 individually-reared larvae per diet per generation. Otis Laboratory tested 20 to 50 replicates of multiple-reared larvae per generation.

Generation	% Pupation (Mean ± SEM*)			Pupal weight (g) (Mean ± SEM)		
	Agar diet (Abassi)	Tapioca diet (Abassi)	GM diet (Otis)	Agar diet (Abassi)	Tapioca diet (Abassi)	GM diet (Otis)
1	76.0 ± 2.6	79.0 ± 3.8	33.2 ± 4.8	0.37 ± 0.01	0.37 ± 0.02	0.32 ± 0.01
2	62.0 ± 3.2	69.0 ± 3.5	79.2 ± 2.5	0.34 ± 0.01	0.31 ± 0.01	0.35 ± 0.01
3	34.0 ± 2.8	51.0 ± 2.7	70.7 ± 2.3	0.33 ± 0.02	0.31 ± 0.02	0.35 ± 0.01
4	49.0 ± 3.8	46.5 ± 3.5	51.3 ± 3.1	0.33 ± 0.01	0.33 ± 0.02	0.35 ± 0.01
5	58.0 ± 4.0	69.0 ± 3.2	72.5 ± 3.4	0.34 ± 0.03	0.32 ± 0.02	0.35 ± 0.01

*Male and female pupa weights are similar and were pooled.

The OWB life cycle is completed in approximately one month. Eggs laid on paper towels are introduced into 600 ml plastic containers with a poured layer of Otis gypsy moth diet (pg 60) at the top (Figure 1A), and typically hatch after three days. In an effort to account for mortality due to early larval cannibalism, an abundance of eggs are infested onto the diet. After they reach the 3rd instar (~11 days later), 25 to 30 larvae are transferred into larger plastic containers (1,100 ml) with an arch of plastic scaffolding. Chunks of gypsy moth diet are placed on the scaffolding and vermiculite is used as a pupation substrate (Figure 1B). The diet is replaced about twice a week to prevent depletion, drying, and microbial growth. Pupae are collected from the vermiculite, counted, and sexed. Pupae tolerate 10°C for up to 14 days to delay development, if needed. Oviposition cages (46 cm long, 13 cm wide, 33 cm high) (Figure 1C) were developed jointly by Otis Laboratory and BioQuip (Rancho Dominguez, CA) for rearing European grapevine moth, with textured paper towel selected as the egg substrate.

The cage maximizes vertical surfaces, because we observed they are preferred over horizontal surfaces for egg-laying by OWB (CEW prefers a horizontal surface). The optimum density of adults to promote mating and the desired egg production was determined to be 60 pairs per cage. Pupae are introduced into the cage provisioned with 10% honey-water in cups with dental wicks, and egg substrate on removable frames. At peak egg laying (8-9 days later), the paper towels are replaced and the eggs are stored at 4°C as a backup. Egg substrates are harvested again after 24 hours to produce uniform-aged eggs for the colony. We found that eggs can be stored in a refrigerator (4°C) for up to nine days, if needed. Colony health is monitored by recording survival of 3rd instar larvae to the pupa stage, estimating egg numbers from counts on stamped grids (representing 20% of the egg-sheet surface area), and by recording weights of 20 pupae of each sex (Table 2). No significant differences in weight were observed between male and female pupae.

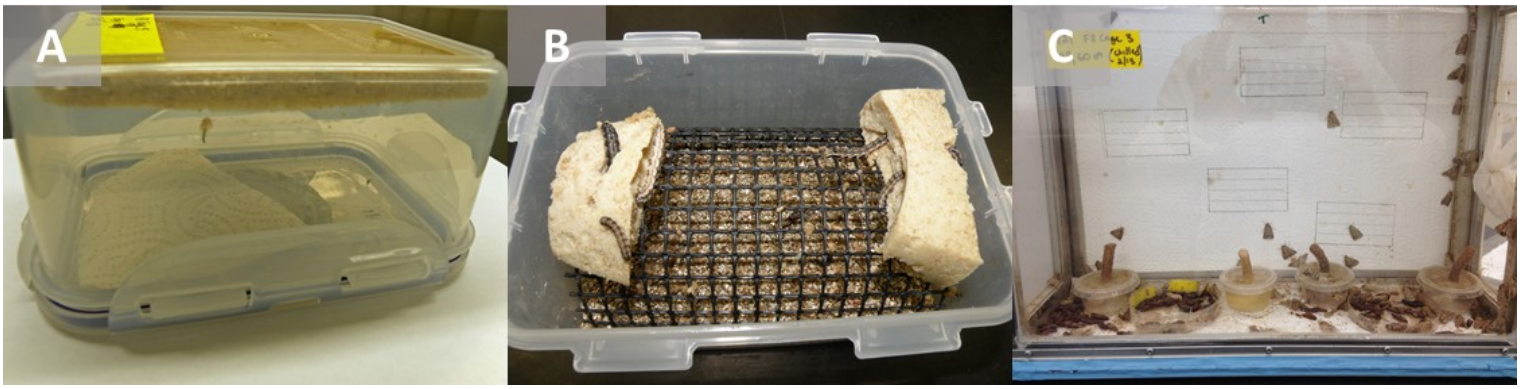

Figure 1. Old world bollworm rearing. A) Hatching and early-instar OWB in rearing box with gypsy moth diet. B) Later-instar OWB in rearing boxes with plastic scaffolding, GM diet chunks, and vermiculite as pupation substrate. C) OWB egg cages with paper-towel sides as egg substrate.

Table 2. OWB colony production in 2017.

Generation	Survival from L3 to pupa (%)	No. pupae produced	Pupal weight* (g) (Mean ± SEM)	No. of cages	Eggs/cage/24 hour estimate (Mean ± SEM)
P (Spain)	—	—	0.310 ± 0.006	2	2,000 ± 1,725
F1	33	199	0.316 ± 0.008	2	7,715
F2	78	732	0.347 ± 0.006	7	8,440 ± 2,530
F3	70	912	0.347 ± 0.009	6	10,768 ± 2,325
F4	51	624	0.349 ± 0.007	3	11,937 ± 3,007
F5	71	621	0.350 ± 0.006	4	10,605 ± 2,093
F6	56	967	0.349 ± 0.004	5	10,443 ± 258
F7	56	525	0.319 ± 0.006	4	6,483 ± 1,348

*Male and female weights were similar and therefore pooled.

For sanitization, all plastic boxes are hand-washed with dish soap or an industrial dish washer and then soaked in a 5% bleach solution before reuse. Mating cages are wiped with 70% ethanol. The paper towels used for egg collection and the vermiculite used for pupation are both autoclaved at 120°C on a 60 minute dry cycle before use. All insect work, particularly when involving diet, is performed in a laminar-flow cabinet to reduce contamination.

CEW Colony Rearing

The Otis Laboratory CEW colony was established in May, 2016 from 1,000 eggs purchased from Benzon Research Inc. (Carlisle, PA). The CEW life cycle takes approximately one month and is similar to that of OWB. Colony eggs are hatched in containers (Figure 2A) with the standard Otis gypsy moth diet. Second instar larvae are transferred to individual cells on well plates (Figure 2B) with Ward's Stonefly *Heliothis* Diet. After pupation, 20 to 24 randomly selected pupae are placed into each mating cage (Figure 2C), with a sheet of paper towel egg substrate at the top.

Attempts were made to rear multiple CEW in a container to increase efficiency. Rearing in groups is not considered feasible because the larvae are highly cannibalistic. We attempted to rear 40 larvae in containers identical to those used for OWB. However, these attempts were unsuccessful; a maximum of five survived to pupation in each container. We will continue to attempt group rearing, with the goal of reducing larval encounters through lower larval density, the addition of various materials to provide refugia, and the addition of feeding sites.

OWB and CEW Production for Research

CEW and OWB moths were provided to Otis chemical ecologist, Allard Cossé, to support development of species-specific lures. Adult OWB were also provided to the APHIS Science and Technology (S&T) Fort Collins Laboratory and to the APHIS S&T Miami Laboratory's cooperative project at University of Puerto Rico for characterization of global populations and other molecular studies.

OWB and CEW of opposite sexes were caged as individual pairs and in groups in various containers to produce hybrids for the Fort Collins Laboratory for molecular studies. Approximately 1/3 of mating pairs could not decouple after mating, presumably due to incompatibility of their genitalia. The majority of eggs laid were infertile, but viable eggs and offspring were produced from a single cage holding 13 male CEW and 11 female OWB. The cage was a horizontal, clear, Plexiglas cylinder (13 cm diameter, 20 cm long) with paper towels provided on the ends as vertical egg substrates. Several hundred hybrid larvae were reared to adult. Of those, some were provided to Allard Cossé for pheromone research, while the majority were sent on dry ice to the Fort Collins Laboratory for molecular studies and storage. Crossing attempts between female CEW and male OWB are ongoing.

References

1. Old World Bollworm [Internet]. USDA APHIS; c2018 [cited 2018 Feb 16]. Hungry Pests. Available from: https://www.aphis.usda.gov/aphis/resources/pests-diseases/hungry-pests/the-threat/old-world-bollworm/owb
2. Abbasi BH, Ahmed A, Khalique F, Ayub N, Liu HJ, Kazmi SAR, et al. Rearing the cotton bollworm, *Helicoverpa armigera*, on a tapioca-based artificial diet. J Insect Science. 2007 May 23; 7(35). Available online: insectscience.org/7.53

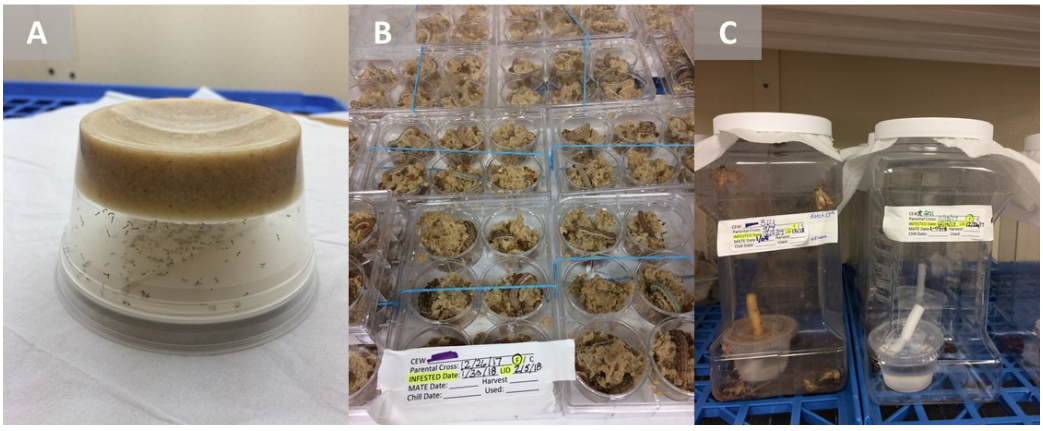

Figure 2. Corn earworm rearing. A) Hatch and early-instar CEW rearing cup with gypsy moth diet. B) Late-instar CEW larvae in individual wells with Ward's Stonefly *Heliothis* Diet. C) CEW mating cages with paper towel lid as egg substrate.

Effects of tetracycline on development of old world bollworm

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Introduction

A colony of old world bollworm, OWB, *Helicoverpa armigera*, is being reared at the Otis Insect Containment Facility at Otis Laboratory. Gypsy moth diet was found to be suitable for rearing OWB and is currently being used. It contains methyl paraben and sorbic acid that are added to control fungal growth. However, during the early phase of OWB rearing development at Otis, the diet in some rearing boxes developed a slimy, pink, malodorous layer that was suspected to be caused by bacterial growth. The deterioration of the diet required it to be replaced with fresh diet more frequently than is normally required for depletion or desiccation. Samples of the growth layer were sent to the APHIS Beltsville Laboratory for diagnostic analysis, where the microbes were confirmed to be bacteria, mainly *Bacillus* spp. We suspected that the sources of contamination included unsterilized pupation and oviposition substrates (vermiculite and paper towels, respectively) and eliminated the problem by improving our sanitation protocols. However, because microbial challenges may arise in the future if new rearing systems are proposed for OWB, we began testing the effects of antimicrobial compounds, focusing on identifying compounds and concentrations that do not have adverse developmental effects on OWB. We report on a preliminary study to test the effects of two levels of the antibiotic compound tetracycline on development and survival of OWB. We chose tetracycline because it is commonly used in insect diets to control bacteria. The 0.04% and 0.08% concentrations tested are within the range demonstrated to be safe levels for insects [1].

Methods

The rearing method developed for OWB (pg 53) was followed with one minor difference—instead of minimizing the chance of microbial growth, we attempted to encourage it by not replacing the diet. The growth of bacteria provided an opportunity to test the efficacy of the selected concentrations of tetracycline to control contamination in the diet.

Experimental batches of diet were prepared in a blender. The diet ingredients and tested levels of tetracycline are shown in Table 1. Before rearing experiments, all substrates and utensils were sterilized by autoclaving or wiping with 70% ethanol. Eggs were provisioned with diet containing 0%, 0.04%, and 0.08% tetracycline and reared to 3rd instar in boxes containing poured diet. Upon reaching 3rd instar, larvae were transferred into each rearing box with the appropriate diet treatment. The number of replicates (rearing boxes) per treatment varied from two to four, depending on availability of larvae. The date of first pupation, number of pupae, and weights of 10 to 15 randomly selected pupae per sex and per treatment were recorded. The parental larvae were hatched from colony eggs, and the adults reared from them were caged (15 pairs per treatment) for egg production to produce an F_1 generation. Offspring were reared on the same diet treatment as the parents and monitored and analyzed as described above.

Table 1. Gypsy moth diet ingredients and tested amounts of tetracycline.

Ingredient	Amount (g)
Wheat germ	120
Casein	25
Wesson Salt Mix	8
Sorbic acid	2
Methyl paraben	1
USDA Vitamin Premix	10
Agar	15
Water	800
Total grams of diet	**981**
Tetracycline 0.04%	**0.40**
Tetracycline 0.08%	**0.80**

Results and Discussion

This preliminary study showed no consistent adverse effects from the tested levels of tetracycline on several OWB fitness parameters (Figure 1). Weights of male and female pupae were not statistically different (t-test, $\alpha = 0.05$) and were therefore pooled. Parental-generation pupae reared in diet containing tetracycline weighed significantly less than pupae reared in control diet (ANOVA, followed by the Tukey-Kramer HSD test on the means, $\alpha = 0.05$). However, difference in pupa weight was not significant in the F_1 generation when compared to controls and the trend even appeared to contradict results in the parental cohort, therefore more replications are needed to draw concrete conclusions. Survival from 3rd instar to pupa was not significantly affected by tetracycline, although it did appear to have a negative effect in the parental generation. Developmental rate (egg to 1st pupation) was 18 days in all rearing boxes and therefore did not appear to be affected by treatment or generation.

Microbial growth was not observed in any diets from the time of 3rd instar infestation through the start of pupation (eight days), so no conclusion can be drawn from this study on the efficacy of tetracycline to control *Bacillus* spp. in gypsy moth diet. Furthermore, microbial growth was not observed in colony diet (without tetracycline) since sanitation procedures were improved.

We continue to maintain the OWB colony with the enhanced sanitation protocols, soaking all cage materials in 5% sodium hypochlorite (bleach) solution, dry-autoclaving the paper-towel oviposition substrate and the vermiculite at 120°C for 60 minutes, and handling diet and insects under a laminar-flow hood. Although this study was preliminary, no consistent effect was found from tetracycline on OWB development rate, pupa weight, or survival to the pupa stage. More replication is needed to confirm the results, but tetracycline remains among one of the potential antimicrobial compounds for OWB rearing in the event of future bacterial problems.

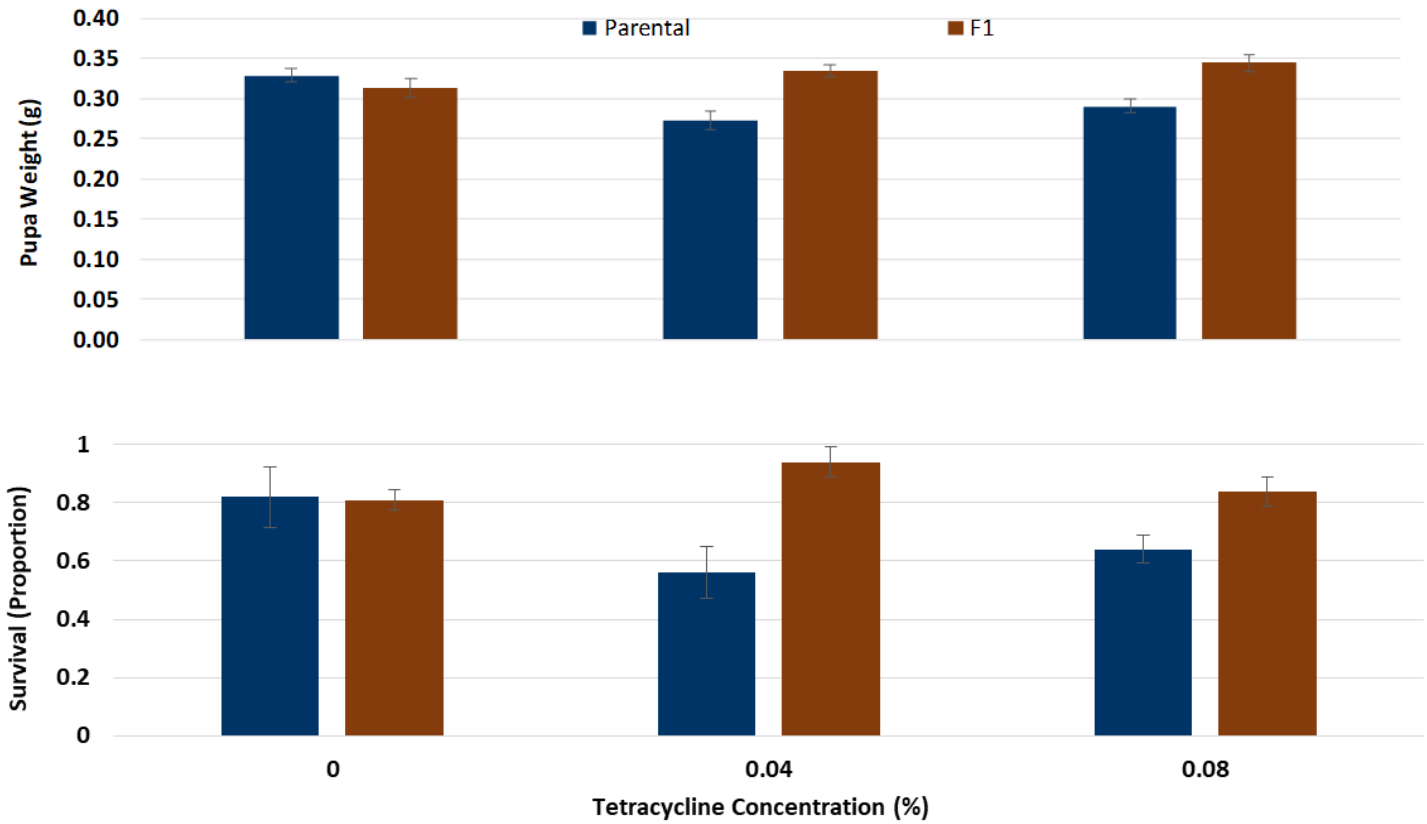

Figure 1. OWB pupa weight (top) and survival from 3rd instar to pupa (bottom) when reared over two generations on gypsy moth diet with two concentrations of tetracycline and a control. Data are presented as mean ± S.E.M.

Reference

1. Cohen AC. Insect Diets: Science and Technology. 2nd ed. Boca Raton: CRC Press; 2015.

Postharvest irradiation treatment for quarantine control of European grapevine moth

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Work was completed in 2017 to determine the minimum dose of radiation required to kill eggs and larvae of the European grape vine moth, EGVM, *Lobesia botrana*, as a postharvest treatment for grape berries and berry-like fruits. Effects of radiation on pupae were also studied. This report summarizes some of the findings published in 2018 [1] and provides additional data not included in the paper.

Effects of gamma irradiation on egg, larval, and pupal development and survival were studied over a period of several years at Otis Laboratory. Eggs, neonates, 3rd and 5th instars, and early- and late-stage pupae were irradiated at target doses of 50, 100, 150, or 200 Gy or left untreated as controls in replicated factorial experiments. Survival to the adult stage was recorded. Tolerance to radiation (survival to adult) generally rose with increasing age and developmental stage. A dose of 150 Gy prevented treated eggs and larvae from surviving to the adult stage. Pupae were more tolerant to radiation than larvae. Furthermore, mature pupae were more tolerant than young pupae, emerging as adults at rates of 90% and 70%, respectively, after exposure to 200 Gy. Large-scale validation tests were conducted where thousands of mature larvae were exposed to radiation in diet and in grapes. A dose of 150 Gy applied to 5th instar larvae in diet prevented any larvae from surviving to the adult stage; however, when 5th instar larvae were irradiated in table grapes, a small percentage (0.1%) survived. The next dose trial exposed larvae in grapes to a range of 200 to 250 Gy and resulted in complete mortality. A dose of 250 Gy was, therefore, reported as the minimum required to prevent movement of live eggs and larvae in grape commodities.

When these validation tests were performed, data were also gathered on the distribution of mature larvae in and around the fruit they were infesting. In nature, EGVM larvae feed on the interior and exterior of fruit, typically moving away to pupate in bark or soil. Care was taken, therefore, to conduct validation tests on larvae both inside and on the surfaces of grape berries.

Fifty mature larvae per replicate were confined with bunches of red table grapes for two to three days in 473 ml paper cans to allow them to settle before they were exposed to radiation.

Paired controls for each treated can were also made with 10 mature larvae each confined on grapes. Immediately following irradiation, treated and control cans were examined to exclude pupae from the larval treatment analysis, and larvae were transferred to rearing boxes with diet for evaluation of survival to the adult stage. Several replicates were randomly selected to record the location of the insects in and around the fruit.

Larvae were found inside berries, between berries, and on the outside of bunches (Figure 1), satisfying the aim of validating the treatment on larvae distributed in typical places within the commodity. Those that pupated were mostly on the outside of bunches, although about 19% pupated among berries within bunches, and one pupa was found inside a berry. The proportions of life stages in each location were similar regardless of treatment or larval density.

To kill EGVM pupae, doses higher than 250 Gy are required [1]. Because the incidence of pupation associated with fruit is unknown and cannot be ruled out, we studied whether irradiation of pupae at doses between 250 and 400 Gy can sterilize adults or prevent their ability to establish a population if they escape. The experimental dose was capped at 400 Gy because higher doses, needed to penetrate and deliver 400 Gy to the core of boxes of fresh produce, may negatively affect fruit quality. The most likely scenario with shipped commodities in regions free of EGVM is that adult moths would emerge and mate with each other in quarantined warehouses where treatment occurs. Therefore, the lowest dose to induce full sterility, or to induce partial sterility of parent moths that induces full sterility in the offspring, would eliminate the risk that escaped moths could establish new populations. Partial sterility, the production of viable but sterile offspring, is inducible in moths by irradiation. We irradiated mature pupae (6-7 days old) with 245, 295, and 325 Gy and mated them in groups with unirradiated partners.

At 295 Gy, females exhibited reduced fertility, which was also apparent in their offspring. A dose of 325 Gy, however, fully sterilized the females and resulted in few adult offspring by males. Although fertility trials made with these scarce offspring were limited, daughters of males irradiated with 325 Gy, produced few eggs (an estimated 1.5/female/day), all of which failed to hatch, while sons also sired few eggs (1.3/female/day), of which 7% hatched.

Compared with control data of 7.3 eggs/female/day and 94% hatch, these results suggest very low reproductive capacity by moths exposed as mature pupae to 325 Gy and mated to fertile partners. This year we plan to mate adults irradiated as pupae with similarly irradiated partners, rather than with unirradiated partners, and expect that 325 Gy or even lower doses will result in full sterility either by the treated moths or their offspring.

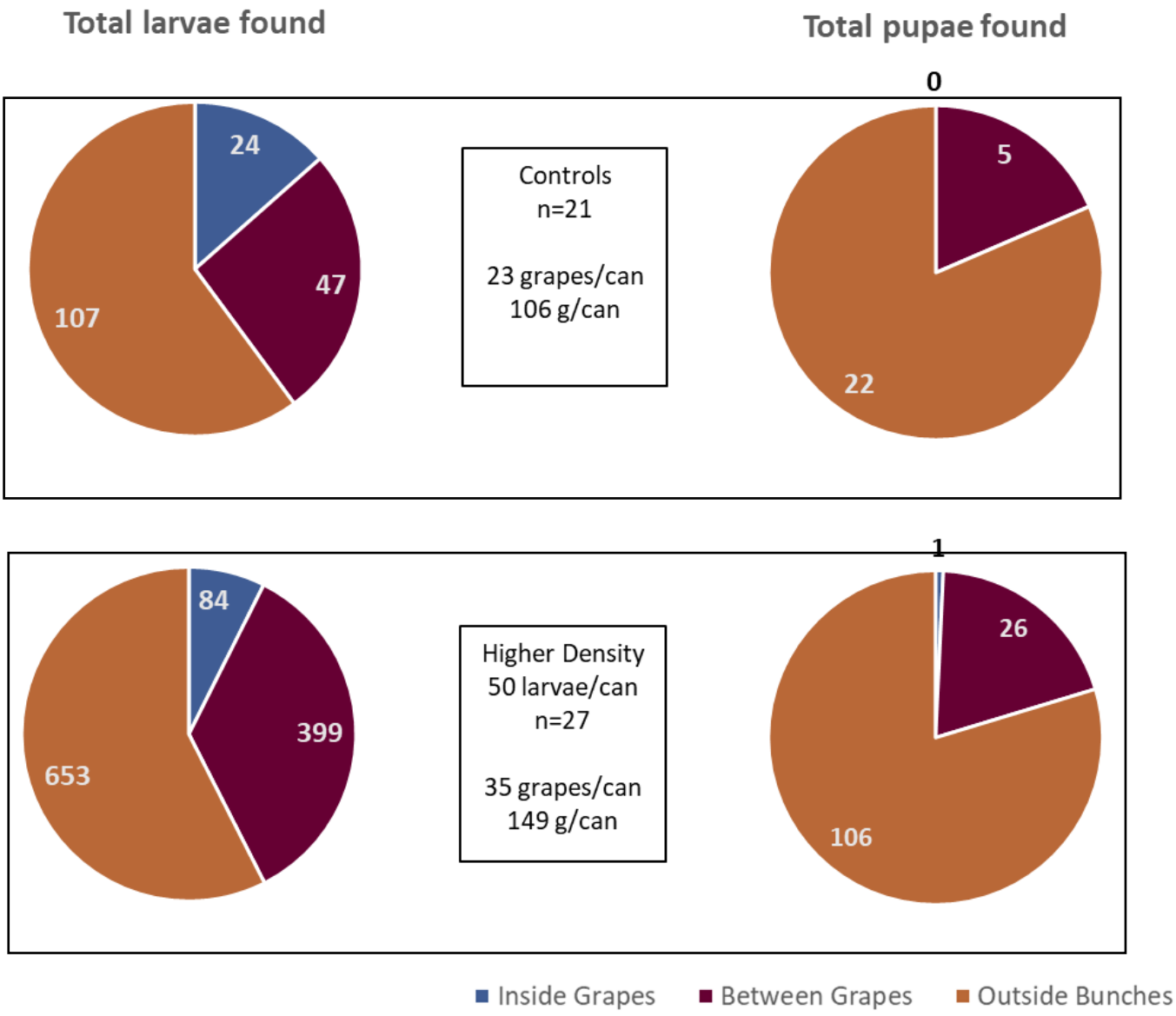

Figure 1. Life stages and locations of EGVM found two to three days after mature larvae were confined with grapes in 473 ml paper cans. Tests were made at two levels of larval and grape densities. Top: controls with 10 larvae. Bottom: treated cans with 50 larvae per can.

Published work

1. Nadel H, Follett PA, Perry CL, Mack RG. Postharvest irradiation treatment for quarantine control of the invasive *Lobesia botrana* (Lepidoptera: Tortricidae). J. Econ. Entomol. 2018;111(1):127-134. Available online at doi: 10.1093/jee/tox317

Gypsy Moth Rearing Facility activities for 2017

Susan Lane[1], Hannah Nadel[1], Chris McCallum[1] and Hannah Landers[1]

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Summary FY 2017 support

During fiscal year 2017, the Otis Gypsy Moth Rearing Facility (OGMRF) produced European gypsy moth, EGM, *Lymantria dispar dispar*, of all life stages for both in-house colony and production purposes. The European gypsy moth (EGM) colony has been maintained for 75 generations at Otis Laboratory. The EGM strain we rear is known as the New Jersey Standard Strain (NJSS). This strain originated from egg masses collected in New Jersey approximately 40 years ago. Each week a new cohort is established, producing about 1,000 egg masses. The production of our EGM colony is utilized for research as well as domestic and foreign outreach and training.

Over the past fiscal year we produced over 50,000 egg masses; approximately 68% were cycled back into the colony and the remainder were sent to researchers in the U.S., Canada and Europe. In addition to eggs, other EGM life stages were distributed to 15 U.S. and six foreign institutions for various research projects (Table 1).

Approximately 50,000 pupae were reared to support Gypsy Moth Slow the Spread Foundation's research on mating disruption. Over 14,000 egg masses were provided to private industry (Sylvar Technologies, New Brunswick, Canada) to assist in their establishment of a year-round colony and support production of a multiple nucleopolyhedrosis virus (MNPV) for EGM population control. About 300 marked adult males were supplied for quality assurance of pest-survey trappers in Washington State. Fifteen F1-sterile egg masses were provided to California Department of Food & Agriculture to act as indicators of gypsy moth hatching in the Santa Cruz area, where one Asian gypsy moth adult was detected (pg. 83). These are just a few examples of the use of the EGM colony in 2017. Recurring and new orders for 2018 are in process.

Table 1. Number of EGM life stages and specimens provided in FY17 to U.S. and foreign institutions, and purpose for the insects.

Egg Masses	Larvae	Male Pupa	Female Pupae	Riker Mounts	Specimens	Purpose
—	—	—	—	308	300	Training – Outreach
14,265	—	—	—	—	—	Colony establishment and NPV production
1,496	—	—	—	—	—	Research – Biocontrol/Pathology
50	300	—	—	—	—	Research – Larval dispersal distance
—	—	43,920	3,950	—	—	Research – Mating disruption
122	1,800	14,350	2,800	—	—	Research – Invasion risk, ecology, population dynamics, climatic effects, host-plant interactions
105	400	—	—	—	—	Teaching – Academic
16,038	**2,500**	**58,270**	**6,750**	**308**	**300**	**Totals**

Challenges Faced by OGMRF in FY 2017

During fiscal year 2017 the OGMRF was faced with two challenges that had the potential to impact the colony production and rearing protocols. Several cohorts had an increase in the number of pupae with physical deformities and an overall slower developmental growth rate in both the larval and pupal stages. This resulted in cohorts needing additional rearing time to fully develop, which disrupted the standard rearing schedule. By reviewing our past colony records we traced the potential source of the problem to a new lot of wheat germ, the main component of the in-house B-4 High Wheat-Germ Diet. Test samples of wheat germ (Honeyville Grain, Inc., Honeyville, UT) were sent to a private lab (AGC Labs USA of Oxnard, CA) for mycotoxin contamination and residue testing for a panel of pesticides. However, aflatoxins were not detected in any sample. Although Malathion residues were detected, they were present in a lower amount than in our control wheat germ sample. Therefore we do not believe aflatoxins to be the cause of the slower development. Although we did not determine the cause of the problem, we consequently instituted a new control measure for testing incoming wheat germ lots to prevent potential negative developmental effects to the colony. In addition, as an added precaution we are purchasing smaller quantities to minimize waste in case the wheat germ does not meet our standards and must be discarded.

An additional challenge for the OGMRF in 2017 was that production of the paper tabbed lid (DS306) that has been used in the rearing protocol for 30 years was discontinued by the manufacturer (Dart Container Corp., of Mason, Michigan). Without a suitable replacement, we would have been faced with a potential retooling of equipment and an overhaul of our existing semi-automated operations. The food service industry replaced the lid with double-coated product that completely seals the paperboard, preventing the air exchange required for our insects (Figure 1). We were able to locate a vendor (J.I.T. Manufacturing and Packaging Inc., of Paterson, New Jersey) who stocked paperboard lids with a single-sided coat of 0.024 mm Polarshield Mill Wax. The suitability of lid sizes was tested for fit on our 6 oz. rearing cups, as well as the lid's effect on larval hatch and development. A diameter of 3.5619" was found to be optimum. Finding and working with this new vendor avoided expenditures of time and funding on redesigning our rearing operations.

Product and protocol changes are generally tested over two generations before they are accepted and fully integrated into the rearing system, a process that spans about 18-months. Our staff works diligently to rear a high-quality insect strain year-round, responding to issues that develop in the process and maintaining our high quality standards.

Figure 1. Example of the paper lid used to cap the gypsy moth diet cups.

Dispersal of spotted lanternfly in a tree of heaven forest habitat

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Background

The newly invasive spotted lanternfly, SLF, *Lycorma delicatula,* is reproducing rapidly and spreading throughout eastern Pennsylvania and beyond. Its primary host is tree of heaven, *Ailanthus altissima*, however, it attacks over 70 species of woody plants and causes damage to a number of economically important host plants such as grape, hops, and walnut. Crucial to the eradication effort of this generalist pest is an understanding of its basic biology, including its dispersal in search of food, mating, and oviposition. While human aided transportation likely plays a major role in SLF movement over large distances, it is equally important to understand its natural movement, as that is what would be exploited when implementing traps using attractive semiochemicals.

Methods

We designed a mark-release-recapture experiment using fluorescent powder to mark SLF and measure their movement in an *Ailanthus* stand within the Pennsylvania quarantine zone. For each instar, individual nymphs were collected, marked with one of three colors of fluorescent powder, and released at three designated sites within the *Ailanthus* forest (Figure 1). Web-Cote sticky bands were placed on 33 trees at various distances from the release trees (~5-50 m) and baited with methyl salicylate lures developed at Otis Laboratory.

Figure 1. The release of SLF nymphs marked with fluorescent powder.

Bands were visually inspected for recaptures the day after each release. Subsequently sticky bands were collected and replaced approximately every week. After some time, the fluorescent dye became more difficult to see, so all sticky bands were examined in the laboratory under a UV light, revealing marked SLF that were otherwise difficult to see (Figure 2).

Figure 2. Images showing A) recaptured marked 4th instar SLF, B) sticky band under normal light, and C) the same sticky band under UV light revealing marked SLF.

Published studies have documented that SLF nymphs only walk up trees, fall, and walk up again; they never walk down trees. Consequently, they are only captured on the bottom edges of the sticky bands. Therefore, bands on the release trees were placed below the point of release.

Results and conclusions

In total, over 6,000 SLF were marked and released, of which 342 were recaptured, and 43,085 unmarked SLF were captured on the sticky bands between May and September, 2017. As predicted, all SLF nymphs were captured at the bottoms of the bands as they walked up the trees (Figure 2A).

Adults were also caught in the middle of the bands, suggesting they may have been flying when captured. Recapture rates were much higher (~10%) for 1st and 2nd instars than for 3rd instars, 4th instars, and adults (~1-2%), suggesting a decrease in trap efficacy with older SLF (Figure 3). The majority of the recaptured 1st, 2nd, and 3rd instar SLF were found on their release tree. However, starting with 3rd instars, there was an increase in distance travelled away from the release tree.

Roughly half of 4th instars and adults were recaptured a sizeable distance away from the release tree, suggesting a greater tendency to disperse at those stages. No SLF were recaptured beyond 28 meters despite the majority of traps being 28 to 50 meters from the release points. The maximum recapture distance for adult SLF was 24.3 meters. These data suggest that SLF do not move far if their preferred host, *Ailanthus altissima*, is readily available.

	Released	Recaptured	%Recaptured
1st	1140	117	10.3%
2nd	1895	176	9.3%
3rd	1555	24	1.6%
4th	887	14	1.6%
Adult	598	11	1.8%

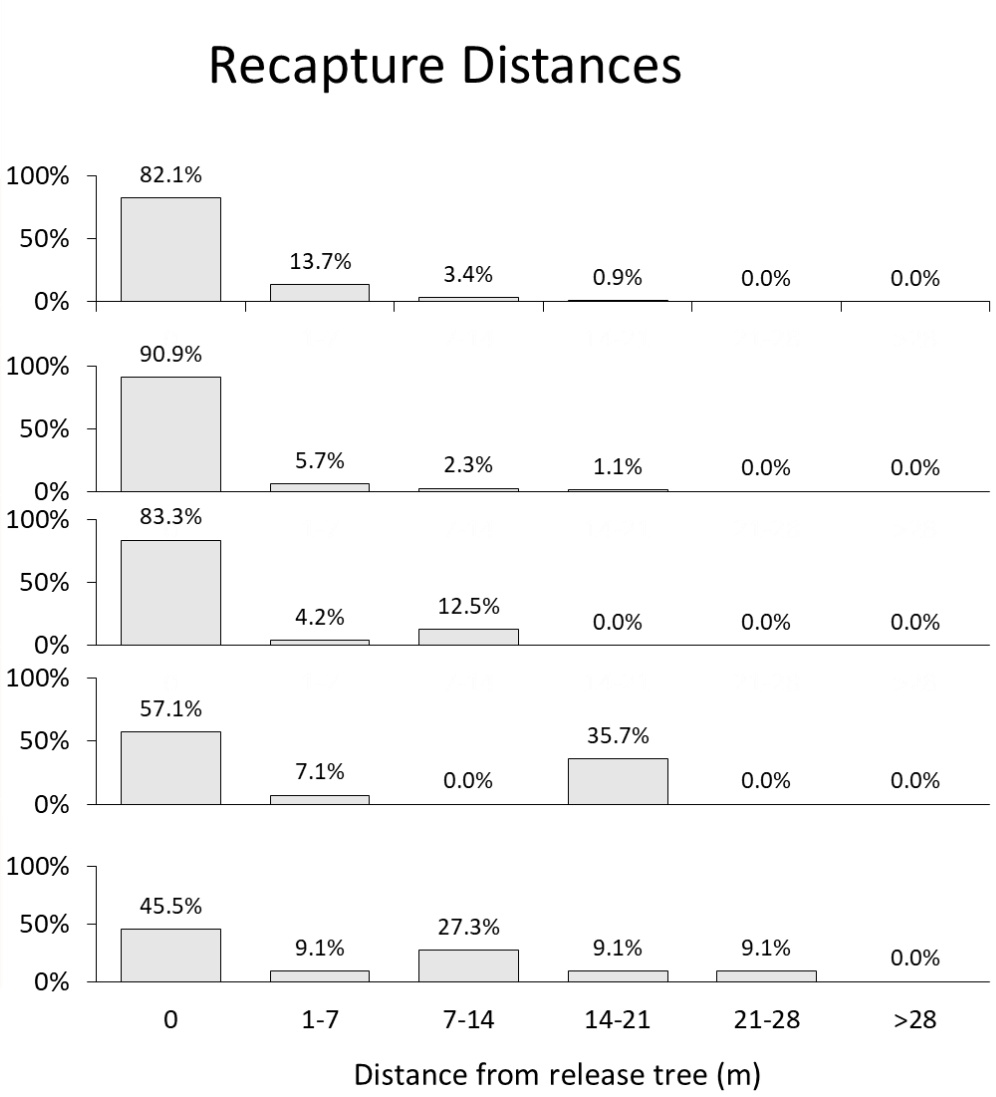

Figure 3. Summary of dispersal data for each instar. The table on the left describes the number released, recaptured, and the resulting percentage. The graph on the right describes how far from the release tree the recaptured SLF were found.

Discovery of host plant volatiles that elicit antennal responses and attraction in spotted lanternfly

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[2]Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

Introduction

The spotted lanternfly, SLF, *Lycorma delicatula*, is an invasive generalist phloem feeder from China. Its primary host is tree of heaven, *Ailanthus altissima*, which originates from China and was brought to the U.S. in 1784 and has since become a ubiquitous weed tree. Notably SLF feeds on and causes damage to agricultural crops such as grape and walnut, in addition to apple, oak, maple, willow, cherry, and over 70 other species. The spotted lanternfly was discovered in eastern Pennsylvania in 2014 and has now spread from one county to more than 14 counties and has been found in some neighboring states. Current eradication efforts are hampered by the lack of a highly attractive lure and an effective trap. To improve upon previously developed methyl salicylate lures, we collected volatiles from six different host plants and developed a gas chromatography coupled with electroantennographic detection (GC-EAD) technique to screen volatiles for electrophysiological activity.

Electrophysiology

In order to identify specific semiochemicals from volatile collections of host plants, GC-EAD methodology was developed, which addressed the unique structure of the spotted lanternfly antennae. The head was removed, and the apical arista and basal bulb of one antenna were removed with fine forceps. A recording electrode was brought into contact with the resulting cavity at the distal end of the antenna (Figure 1). The reference electrode was placed into the base of the head, allowing the recording of electrical responses from the unusual antenna shape of the spotted lanternfly. Recordings were made from 3rd instar, 4th instar, and adult spotted lanternflies, as they became available from the field.

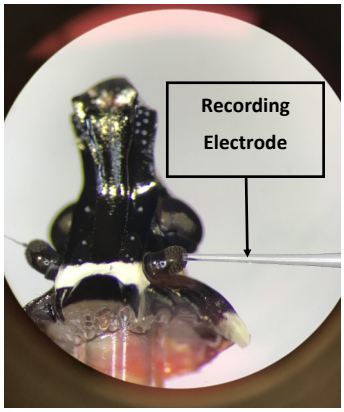

Figure 1. Antennal preparation of a 3rd instar spotted lanternfly for use in GC-EAD.

Volatile collections

A push-pull vacuum pump system was used to trap volatiles from a variety of host plants. In the case of *Ailanthus*, this included profile comparisons of damaged and undamaged bark, and collections made from actively feeding SLF vs. *Ailanthus* excluded from SLF. These volatiles were then analyzed by gas-chromatography coupled with mass-spectrometry (GC-MS) and GC-EAD (Figure 2). We surveyed volatiles from six different host plants using GC-EAD (Table 1), and found that antennal responses were elicited from 39 unique compounds found in these host plant volatiles. Of those 39 unique compounds, we have chemically identified 19 so far.

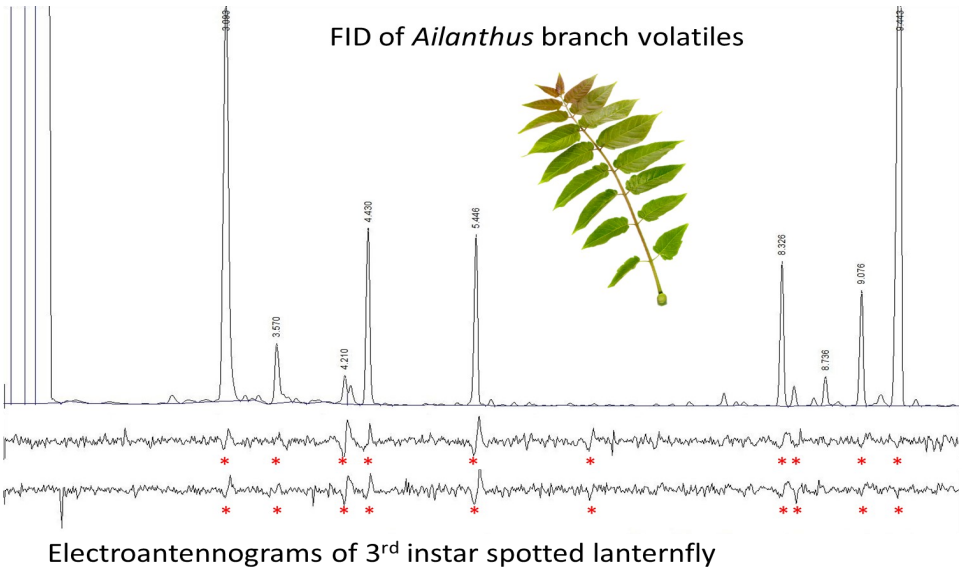

Figure 2. An example of two GC-EAD antennal responses to volatile profiles of *Ailanthus* branches using a flame ionization detector (FID). Red asterisks indicate repeatable antennal responses.

Behavioral bioassays

Dual choice olfactometers were used to measure attraction to antennally active compounds or odors from host plant material (Figure 3). Antennally active compounds were tested singly and in blends of up to nine compounds at ratios attempting to reproduce naturally occurring ratios.

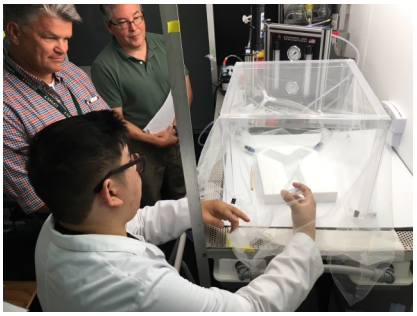

Figure 3. Isaiah Canlas demonstrates the use of a Y-plate dual choice olfactometer to measure the attraction of spotted lanternfly to a synthetic compound identified from host plant volatiles.

Volatiles to be tested for attraction (either synthetic compounds or plant material) were placed upwind of one arm of the Y-plate, while the control side held either a blank control or a competing compound, blend, or plant. An individual nymph or adult was then released into the downwind end of the Y-plate and allowed three minutes to respond by walking upwind and choosing one of the two upwind arms of the Y-plate. Each odor comparison was replicated on average 30 times using different individual insects.

Using this methodology, we conducted 70 different comparisons of individual volatile compounds, blends, or plants to different stages of spotted lanternfly in the summer of 2017. SLF were significantly attracted to *Ailanthus*, chinaberry, wild grape, and hops, when compared to a blank control. When comparing one plant against another, *Ailanthus* was still the most preferred (Figure 4). We also found that damaged *Ailanthus* produced compounds that were not present in undamaged *Ailanthus,* and a trend was seen in preference of damaged over undamaged *Ailanthus* volatiles.

Summary

We have developed a technique to record spotted lanternfly antennal electrophysiological responses. Using this technique, we were able to reveal 39 antennally active compounds in six different host plant species, illustrating the polyphagous nature of spotted lanternfly. Of those compounds, we have chemically identified 19; the rest are still being identified. We have tested numerous plant odors, compounds, and blends for attraction in the dual choice olfactometer and have identified at least 10 compounds that are attractive to some degree. Some of these compounds were not commercially available and had to be synthesized or isolated from essential oils. Work to identify the remaining compounds and to develop a lure that is more attractive than methyl salicylate is ongoing.

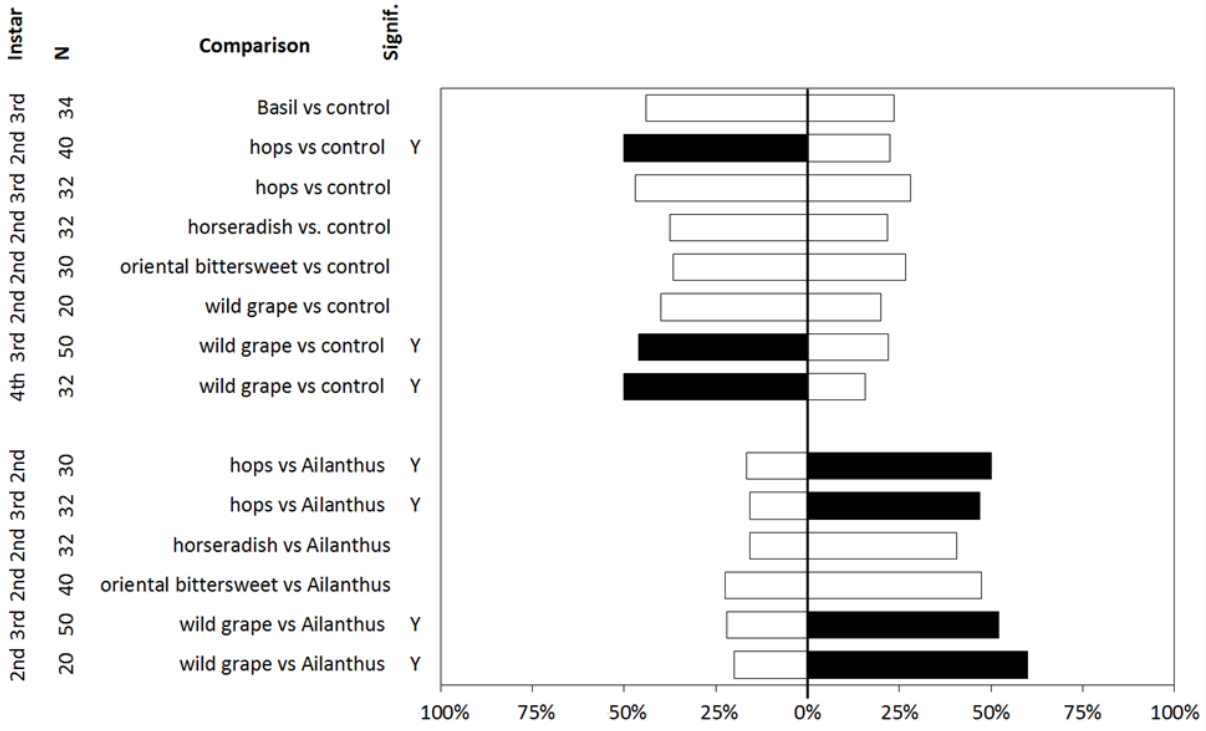

Figure 4. SLF responses to known and potential host volatiles in a dual choice olfactometer. While attractive on their own, hops and wild grape do not outcompete *Ailanthus* when compared head to head.

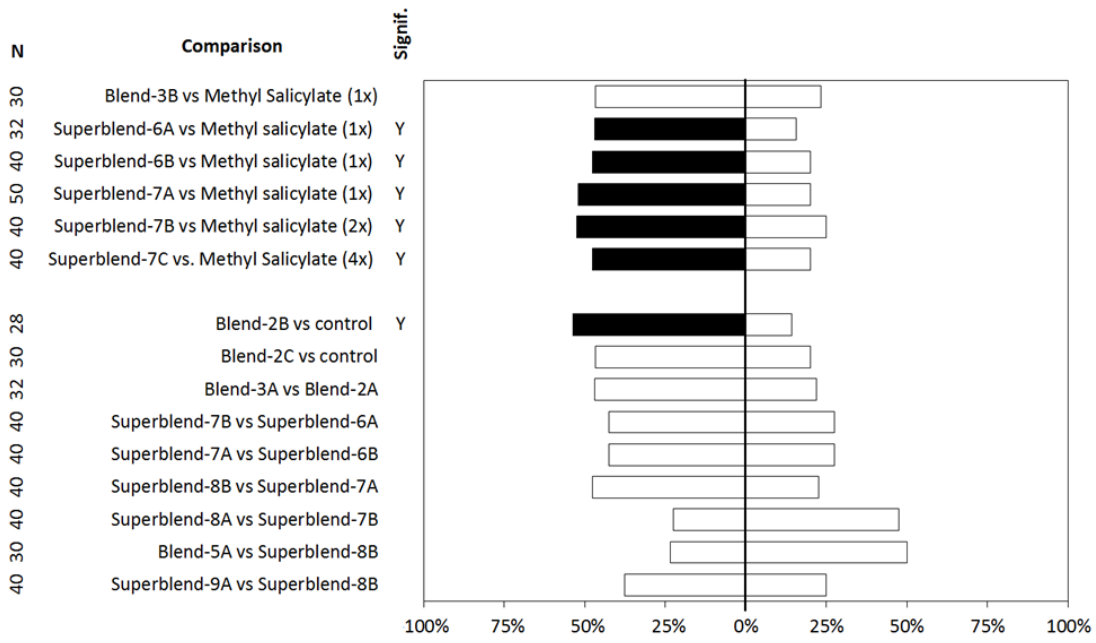

Figure 5. Summary table describing blends of synthetic compounds tested in the dual choice olfactometer.

Table 1. Summary of volatile collections and GC-EAD screening.

Source of volatiles	N (volatile	Electrophysiology			
		3rd instar	4th instar	♀ Adult	♂ Adult
Ailanthus branch	11	x	x	—	—
Ailanthus branch + SLF	4	—	x	—	—
Ailanthus trunk	5	x	x	—	—
Ailanthus trunk + SLF	8	—	x	x	—
Undamaged *Ailanthus* bark	1	—	x	—	—
Damaged *Ailanthus* bark	1	—	—	—	—
Black walnut branch	1	—	—	x	—
Black walnut branch + SLF	1	—	—	x	—
Black walnuts	1	—	—	x	—
Hops vine (greenhouse)	2	—	x	—	—
Oriental bittersweet	1	—	—	—	—
Oriental bittersweet + SLF	1	—	—	—	—
Chinaberry (greenhouse)	2	—	x	x	—
Spicebush with honeydew	1	—	x	x	—
Adult SLF (30 mixed sex)	1	—	—	—	—
Ylang ylang oil	N/A	—	x	—	—
SLF extracts	4	—	—	x	x

Development of traps and lures for improved detection of spotted lanternfly

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Introduction

The spotted lanternfly, *Lycorma delicatula*, is a newly invasive pest from China. It is a polyphagous phloem feeder that attacks over 70 species of woody host plants, but has a strong preference for tree of heaven, *Ailanthus altissima*, which is also invasive in the U.S. However, they are known to be pests of grape and walnut, and damage has been reported on a number of other important agricultural plants such as hops, apple, alfalfa, horseradish, and basil. They also pose a regulatory problem for the timber industry due to their placement of overwintering egg masses on trees. Their rapid reproductive rate results in large populations which overcome host plants by overfeeding and depositing copious amounts of honeydew, promoting the growth of sooty mold on leaves which blocks photosynthesis and ultimately causes host plants to wither or die. In addition, these dense populations have started to incur costs for businesses where the insects enter and contaminate facilities or machinery used to process food, medical supplies, and other commodities with low tolerance for contamination.

Eradication and suppression efforts effectively reduce populations using insecticide-treated *Ailanthus* trap trees. However, for these efforts to work, early detection is key to delineating and targeting the population. Currently, detection is conducted using visual inspections, community reporting, and sticky bands wrapped around *Ailanthus* trees. An attractive lure along with improved trapping technology are needed to improve detection capabilities.

Methods

In 2015 and 2016 we discovered three kairomones derived from *Ailanthus* and grape that were attractive in laboratory dual choice olfactometers. The three kairomones are methyl salicylate, (*Z*)-3-hexenol, and (*E,E*)-α-farnesene. Field studies testing these compounds revealed they had some attraction in the field as well, but more development into lures was needed. In the summer of 2017 we conducted additional field studies in China and Pennsylvania to build upon work done in previous years towards development of a lure for spotted lanternfly. Working with several companies, we developed and tested lures containing each of the three discovered compounds and field tested them.

Lure release rates were measured and compared at Otis Laboratory under standardized laboratory conditions for lures that were field tested. Methyl salicylate was tested using Alpha Scents pipette bulbs, Sino Green black hearts, Hercon laminated squares, and Alpha Scents high release lures. Scentry pouches were used to test (*Z*)-3-hexenol, while Sino Green LDPE bottles were used to test (*E,E*)-α-farnesene (Figure 1). All lures were designed to last a minimum of two weeks in the field, which is the frequency that sticky bands are changed in the Pennsylvania survey.

Because tree effects were difficult to overcome in the Beijing site, the tests of the Sino Green (*E,E*)-α-farnesene lures and the Alpha Scents high release methyl salicylate lures were conducted using a mark-release-recapture approach in order to ensure that the population being sampled started off with an even distribution. Adults in Beijing were captured, marked with fluorescent powder, and released between banded tree pairs—one with a lure and one without.

In addition we conducted tests to improve trap performance. A study we performed at the end of 2016 found that Web-Cote sticky bands caught 30 times more adults than the Korea Beneficial Insect Company (KBIL) brown paper sticky bands, which were previously the best known trap. In the summer of 2017, we followed up on this study by comparing the different sticky bands with both 1st and 2nd instar nymphs in China.

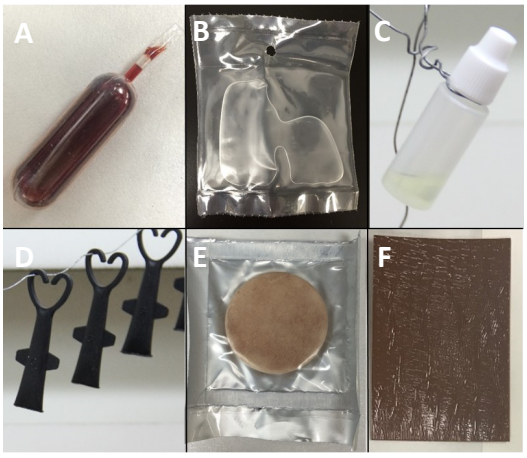

Figure 1. Types of lures used in field studies: A) Alpha Scents pipette bulb, B) Scentry pouch, C) Sino Green LDPE bottle, D) Sino Green black heart, E) Alpha Scents high release, and F) Hercon laminated square.

Results

In a chinaberry plantation in Anhui, China, we compared the KBIL sticky bands to the new Web-Cote sticky bands for 1st and 2nd instars, with and without two Hercon lures, respectively. The new Web-Cote traps outperformed the KBIL traps (Figure 2).

We then tested 3rd instars in the same plantation to methyl salicylate lures in three commercial formulations and release rates: Hercon squares (x2), Sino Green black hearts (x2), and Alpha Scents pipettes (x1). The laboratory release rates of each of these lure treatments were roughly 46 mg per day, 39 mg per day, and 17 mg per day, respectively. The results show a significant dose-type response. We captured significantly more spotted lanternfly with the methyl salicylate lures than controls (Figure 3).

The (*Z*)-3-hexenol lures and (*E,E*)-α-farnesene lures were not significantly different from controls. However, the Alpha Scents high release methyl salicylate lures caught significantly more marked (and unmarked) adults than controls (Figure 4).

Conclusion

Our studies demonstrate that the Web-Cote sticky bands catch approximately twice the number of spotted lanternfly nymphs, and about 30 times the number of adults than the KBIL bands, which were previously used. Working with companies to develop lures for laboratory and field testing, we found that the high release methyl salicylate lures captured three to five times more spotted lanternflies than control sticky bands without lures.

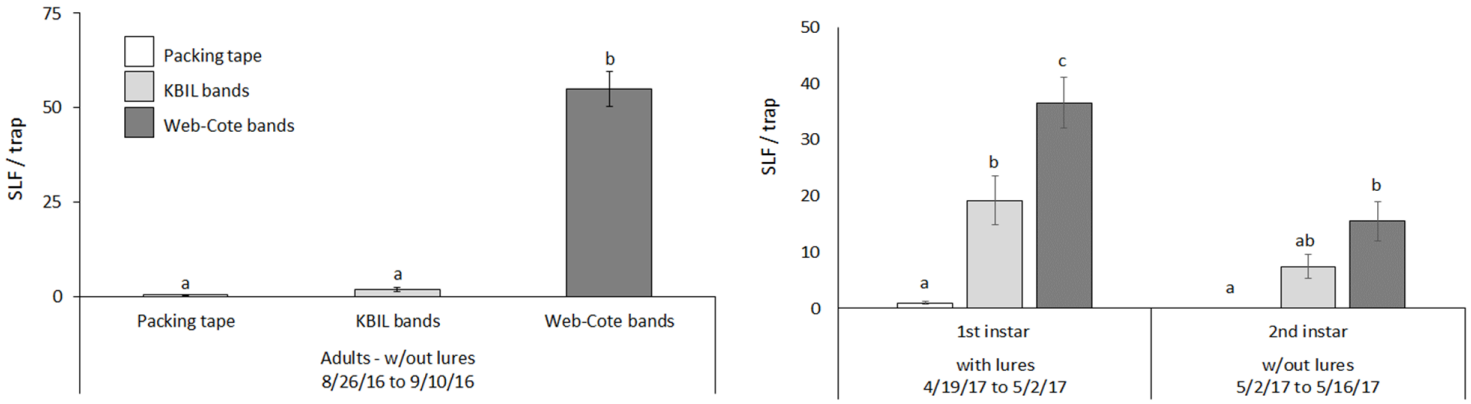

Figure 2. Web-Cote sticky bands caught significantly more adults (left) and nymphs (right) than KBIL sticky bands or packing tape.

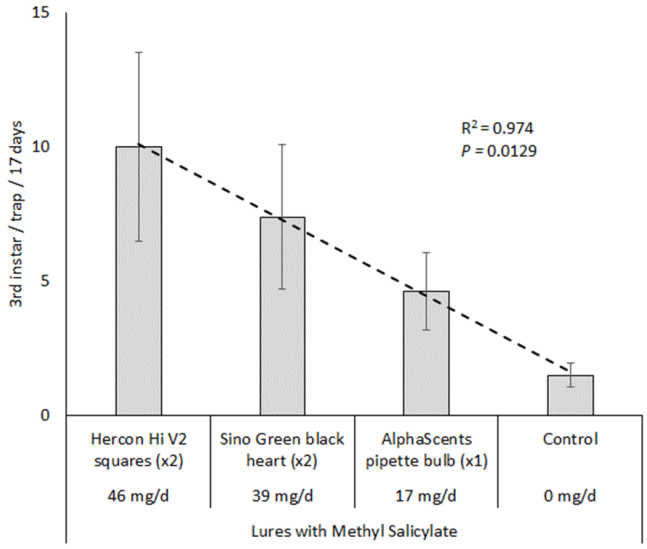

Figure 3. Mean number (±SE) of 3rd instar *L. delicatula* captured per trap from May 16 to June 2, 2017 in Anhui, China, comparing response to lures containing methyl salicylate. A linear regression model showed a significant correlation between mean trap capture and release rate of lures used.

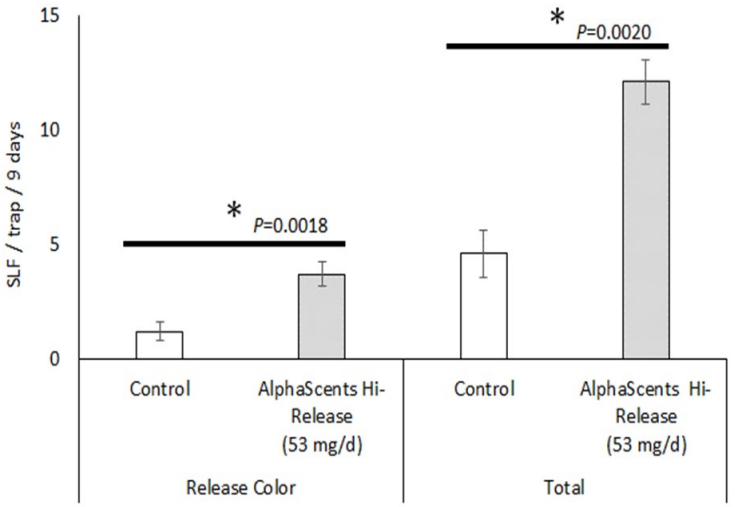

Figure 4. Twelve adult *L. delicatula* were marked and released halfway between each pair of banded *A. altissima* trees, one baited with a methyl salicylate lure and the other with no lure (control). Significantly more marked adults (left) and total adults (right) were captured on trees with lures than control trees (paired T-test, N = 10).

Host suitability studies for spotted lanternfly

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Introduction

Spotted lanternfly, SLF, *Lycorma delicatula*, are a newly invasive pest discovered in Pennsylvania in 2014, having originated from China. They have been observed to feed on a broad range of over 70 host plants, but there is evidence to suggest that some host plants are more preferred or more suitable for SLF development than others. Their abundance on various hosts in the field change as they develop, and their preferred host, tree of heaven, *Ailanthus altissima*, appears to be essential at certain life stages. We hypothesized that at some point in their life cycle, they may require *Ailanthus* in order to complete development. The eradication strategy currently employed in Pennsylvania hinges on this notion—the majority of *Ailanthus* trees are removed, and the remaining "trap" trees are treated with systemic insecticide. This has been shown to drastically reduce the population of spotted lanternfly on and around treated properties. However, if they are able to utilize other hosts to complete development in the absence of *Ailanthus*, the eradication strategy will likely need to be modified.

In the summer of 2016, Dr. Greg Setliff at Kutztown University placed ten insect sleeves on branches of each of seven species: black birch, sugar maple, spicebush, sassafras, tulip tree, grape, and *Ailanthus*. Inside each sleeve they placed 10 wild-caught SLF adults, and sleeves were checked every four days. They observed complete mortality by day 12 on all species except grape and *Ailanthus*, which both still had some SLF survival by day 20 in all replicates. These findings prompted us to study the capacity of spotted lanternfly to survive and develop from 1st instar nymph to adult on various hosts to determine if they can indeed develop fully in the absence of *Ailanthus.*

Methods

Inspired by the above study, we conducted a number of host suitability studies both in the field in Pennsylvania and China and in Otis Laboratory Insect Containment Facility. Field studies consisted of sleeves on branches, each with 10 spotted lanternfly nymphs on plant species suspected of being suitable hosts (Figure 1). Cages in the laboratory received five nymphs. A complete listing of all the tests conducted is listed in Table 1. Attempts were made to start each sleeve or cage with 1st instar spotted lanternflies and track their survival and development. However, some host species were added to the study later in the summer after 1st instar nymphs were no longer available. Additionally, since all spotted lanternflies used in the study were field caught, we could not control for possible field exposure to insecticides prior to capture. In fact, towards the end of the summer, we discovered that some of the nymphs captured as 2nd, 3rd, or 4th instars had been collected near an insecticide-treated "trap tree". Therefore sleeves were removed from the analysis.

Figure 1. Insect sleeve for rearing SLF on a black walnut branch.

Results

We found that not all hosts are equally suitable for survival and development of spotted lanternfly. Furthermore, we identified three new hosts, in addition to *Ailanthus altissima*, that were capable of supporting spotted lanternfly development from 1st instar to adult: hops, black walnut, and chinaberry (Figure 2). In our study, grape was able to support spotted lanternfly survival to 4th instar, but they did not develop to the adult stage. All host suitability findings are summarized in Table 2. No other species in our study supported development from 1st instar to adult. Longevity was similar on silver maple, black walnut, grape, chinaberry, *Ailanthus*, and hops (Figure 3), even though silver maple and grape were not suitable for development past 3rd and 4th instar, respectively. Therefore, although numerous plants may be suitable for nymphs to feed and survive for a time, nutritional requirements for successful development and advancement to other stages are not met by those hosts.

Conclusions

In addition to their preferred host, *Ailanthus altissima*, we identified three new hosts that are suitable for sustaining spotted lanternfly development from 1st instar to adult: hops, black walnut, and chinaberry. Surprisingly, spotted lanternfly on *Ailanthus* did not develop as well as on hops or chinaberry. Chinaberry is only present in the southern U.S., but hops and black walnut are present in Pennsylvania and many other states. We observed significant damage on all of these species, in which branches fed upon by spotted lanternfly were depleted. Although they did not develop into adults on other hosts, spotted lanternfly nymphs were able to molt two or more times on grape, silver maple, Virginia Creeper, and weeping willow. Black birch, spice bush, and blueberry all appeared to attract large numbers of spotted lanternfly nymphs for feeding; however, they were not able to survive long or develop well on those host plants. More research is planned to continue studying these and other plants for host suitability in 2018, starting with 1st instars for hosts where that was not possible during our first study, and with better controls over exposure to insecticide in sites where nymphs are collected.

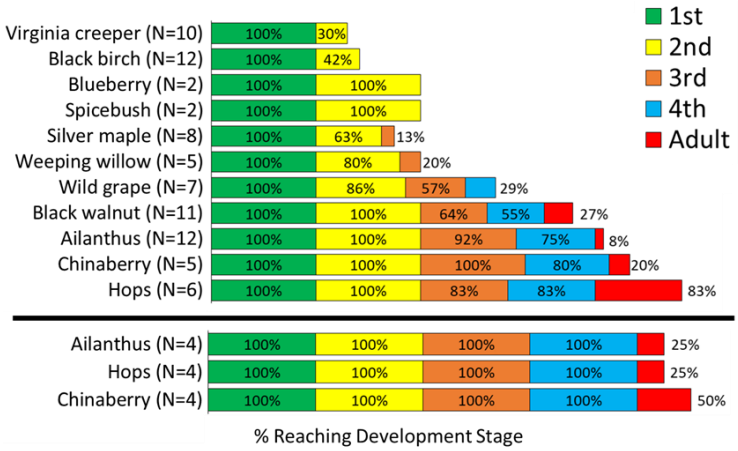

Figure 2. Mean percent of field sleeves (above line) and laboratory cages (below line), with different host plants, in which 1st instar SLF reached each subsequent stage. Each field sleeve was started with 10 first instar SLF and laboratory cages with five 1st instar SLF. Host plants with sleeves were tested in Berks County, Pennsylvania except for five sleeves on *Ailanthus* and five on chinaberry which were tested in Anhui Province in China. Blueberry and spicebush sleeves were started with late 1st instar nymphs.

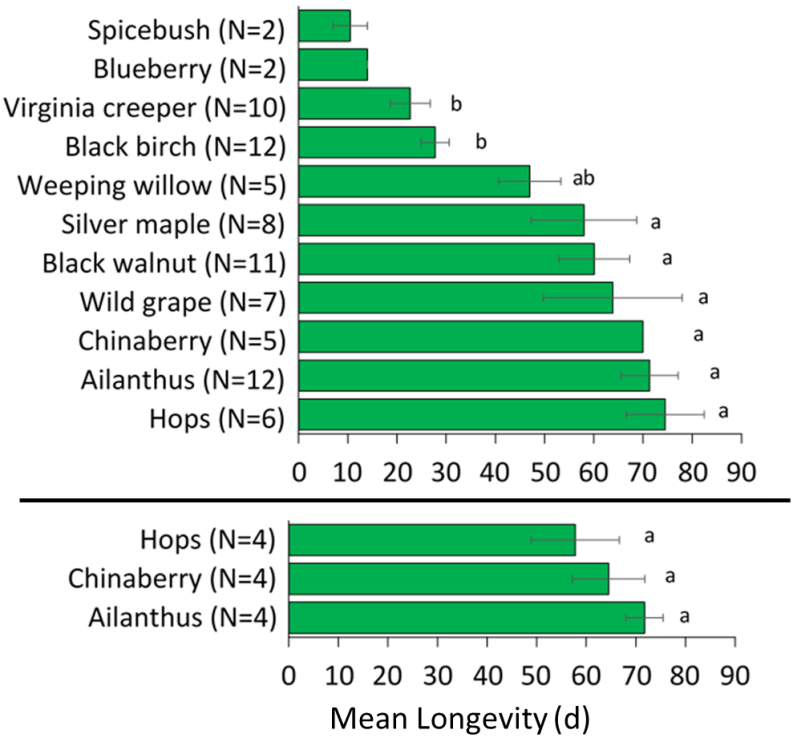

Figure 3. Longevity in sleeves starting with 1st instar SLF. Mean number of days that sleeves or cages contained living SLF with different host plants in the field (above line) or laboratory (below line). Each was started with 10 first instar SLF and checked at least weekly for survival. Host plants with sleeves were tested in Berks County in Pennsylvania except for five sleeves on *Ailanthus* and five on chinaberry which were tested in Anhui Province in China. Blueberry and spicebush sleeves were started towards the end of the 1st instar stage and were not included in the statistical analysis due to low N.

Table 1. Number of sleeves or cages with conditions described for each host suitability test and host plant species. In total, 16 species were evaluated for host suitability in 14 families. **A**: Ten 1st instars per field sleeve per branch; **B**: Field sleeves as in Test B but started with ten 2nd, 3rd, or 4th, or 3-7 adult SLF; **C**: Five 1st instars per plant cutting per cage in the laboratory; **D**: Cages as in Test D but started with five 2nd, 3rd, 4th, or adult SLF; **E**: Ten 2nd instars per potted plant per laboratory cage.

Plants tested for SLF host suitability			N for each test					Total No. SLF Tested
Family	Scientific Name	Common name	A	B	C	D	E	
Betulaceae	*Betula nigra*	Black birch	12	5	—	—	—	270
Brassicaceae	*Armoracia rusticana*	Horseradish	—	—	—	—	2	10
Cannabaceae	*Humulus lupulus*	Hops	6	18	4	7	—	242
Celastraceae	*Celastrus orbiculatus*	Oriental bittersweet	—	18	—	14	—	250
Ericaceae	*Vaccinium corymbosum*	Highbush blueberry	2	12	—	—	—	140
Juglandaceae	*Juglans cinerea*	Butternut	—	2	—	—	—	10
Juglandaceae	*Juglans nigra*	Black walnut	11	18	—	—	—	291
Lamiaceae	*Ocimum basilicum*	Basil	—	—	—	—	1	10
Lauraceae	*Lindera benzoin*	Spicebush	2	13	—	—	—	250
Meliaceae	*Melia azedarach*	Chinaberry	5	—	4	5	—	95
Rosaceae	*Rosa* sp.	Wild rose	—	—	—	3	—	15
Salicaceae	*Salix babylonica*	Weeping willow	5	4	—	—	—	74
Sapindaceae	*Acer saccharinum*	Silver maple	8	3	—	—	—	110
Simaroubaceae	*Ailanthus altissima*	Tree of heaven	12	14	4	11	—	435
Vitaceae	*Parthenocissus quinquefolia*	Virginia creeper	10	9	1	—	1	190
Vitaceae	*Vitis vinifera*	Grape	7	13	1	—	—	282

Table 2. Summary of results of all host suitability studies combined.

Scientific Name	Common name	Presumed feeding? (survived >7d)	Honeydew observed?	Sooty mold observed?	>1 stage advance observed?	Developed from first instar to adult exclusively on host?
Ailanthus altissima	Tree of heaven	YES	YES	YES	YES	**YES**
Melia azedarach	Chinaberry	YES	YES	YES	YES	**YES**
Humulus lupulus	Hops	YES	YES	YES	YES	**YES**
Juglans nigra	Black walnut	YES	YES	YES	YES	**YES**
Vitis vinifera	Grape	YES	YES	YES	YES	NO
Acer saccharinum	Silver maple	YES	YES	YES	YES	NO
Salix babylonica	Weeping willow	YES	YES	YES	YES	NO
Parthenocissus quinquefolia	Virginia creeper	YES	YES	YES	YES	NO
Betula nigra	Black birch	YES	YES	YES	NO	NO
Celastrus orbiculatus	Oriental bittersweet	YES	YES	YES	NO	*n.t.
***Rosa* sp.**	Wild rose	YES	YES	YES	NO	NO
Juglans cinerea	Butternut	YES	YES	YES	*n.t.	*n.t.
Vaccinium cyanococcus	Blueberry	YES	YES	NO	NO	NO
Armoracia rusticana	Horseradish	YES	NO	NO	NO	*n.t.
Lindera benzoin	Spicebush	NO	NO	NO	NO	*n.t.

*n.t. = not tested because study commenced too late in the season

Update: Improving detection tools for emerald ash borer by calibrating catch to population density

Joseph A. Francese[1], Everett G. Booth[1] and Emily Franzen[2]

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Since 2015 we have been conducting a project to determine if emerald ash borer, EAB, *Agrilus planipennis*, trap catch in green multi-funnel traps can be calibrated to population density. The ability to calibrate EAB catch to population density would provide another tool for the current biological control effort by assisting in targeting sites for future parasitoid releases. To begin this multi-state, multi-year study, four plots containing 13 traps in a 1 ha. grid each were originally set up in Ohio (n = 2), Massachusetts (n = 1), and New Hampshire (n = 1). The study was expanded in 2016 to include two new plots in MA. Additionally, the original Ohio plot had to be replaced with a new plot in Ohio, as nearly all of the ash trees had died. In order to compare results of the 13 trap grid site to a broader more "real world" survey trap deployment, nine single trap sites were set up in OH (n = 2) and MA (n = 7).

Figure 1. Everett Booth felling an ash tree designated for peeling in Dalton, MA.

In the winter and early spring of 2017, we completed our second year of felling (Figure 1) and peeling trees from the trapping plots. Trees to be sampled were felled and then cut into 1 m sections (Figure 2). Twelve trees were sampled in each of the six 13-trap plots and five of the single-trap plots for a total of 132 trees (1,759 logs).

In 2017, we completed our third year of trapping EAB in the original plots, and our second year in the 2016 plots. Three new plots (one 13-trap and two 1-trap) were deployed in OH to replace sites that could no longer support the study due to extensive host death by EAB colonization. We also conducted end of summer tree crown class assessments to observe beetle damage in survey plots.

Figure 2. Logs separated by plot and ready for peeling at the Bethel Field Station.

Findings from 2017 peeling include:

- *Atanycolus cappaertii* pupae were found in multiple EAB galleries at one plot in OH
- Three EAB larvae infested with *Tetrastichus planipennisi* were found in the NH plot; these larvae were found approximately three miles from the nearest release site
- Woodpeckers had a 57.5% success rate (287 successful attacks) finding EAB in host trees in the NH site; no other site had a woodpecker success rate higher than 3%

We are continuing to compile data. A third year of felling and peeling of ash trees in plots has begun as of January, 2018. Statistical analysis of the trapping, peeling, and stand assessments is currently underway. With the addition of the data collected from this winter and spring survey, the data set should be much more complete and will help to solidify general trends that have already begun to appear. Trapping continuing through the 2018 and 2019 field seasons will further support the applicability of current findings and trends.

Developing general woodborer survey techniques though collaborative projects

Joseph A. Francese[1], Jon Sweeney[2], Jeremy D. Allison[3] and Sven-Erik Spichiger[4]

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As part of our overarching goal to develop and improve survey tools for potential invasive woodborers, we are currently working with several institutions in the U.S. and Canada on ongoing Farm Bill funded projects. Two of these projects are detailed here.

Improved tools and trapping methods for surveillance of exotic bark- and wood-boring beetles

Since 2014, our lab has been a part of an international, multiagency project to develop and improve the suite of lures, traps, and deployment of survey methods for surveillance and early detection of exotic bark and wood-boring beetles in North America. This project was administered through a Farm Bill project awarded to Natural Resources Canada (Canadian Forest Service). While there are several studies related to this project being conducted world-wide, two of the studies that have been conducted with the assistance of Otis Laboratory staff.

In 2017, we tested the effect of trap color and trap height (understory and mid-upper canopy) on detection of cerambycids and buprestids in both New Brunswick, Canada and Massachusetts. Four trap color treatments were tested using 12-funnel Fluon treated multifunnel traps:

- Green only
- Purple only
- Top 6 funnels and lid were green while the bottom 6 funnels were purple
- Top 6 funnels and lid were purple while the bottom 6 funnels were green

Traps were hung at two different heights: a) in the mid-upper canopy and b) at approximately 2 m from the ground.

From 2016 to 2017 a trapping study was conducted in New Brunswick and Ontario (Canada), and MA and Louisiana (U.S.) to determine if trap capture on black multifunnel traps baited with a general cerambycid lure mixture could be improved by attaching a colored LED light strip to the middle of the trap (Figure 1).

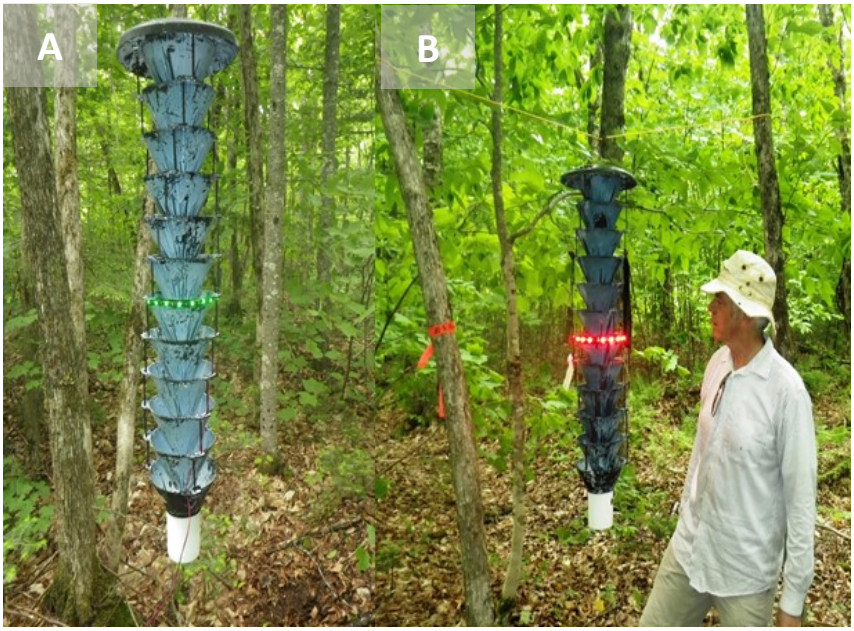

Figure 1. Fluoned, black multifunnel trap with an LED light strip fitted to the 7th funnel of a 12-unit trap. A) Trap with a green light strip. B) Jon Sweeney (Natural Resources Canada) checking a trap fitted with a red light strip at a site in Kingsclear, NB, Canada.

Light strips were powered by 12 volt marine batteries that were charged by solar panels. Five colors of LED light sources (blue, green, red, white, and yellow) were tested against an unilit control. A total of 920 cerambycids (34 species), 793 curculionids (19 species), and 57 buprestids (11 species) have been identified from the samples collected in this light trapping study.

Identification support

Since 2014, samples collected by our lab have been sent to the Pennsylvania Department of Agriculture (PDA) Entomology Survey. As part of a cooperative agreement with PDA all trap collection samples are cataloged and input into PA Plants—PDA's password-protected online database. Once entered, all samples are then sent to the PDA Identification Laboratory for processing. In 2017 this cooperative agreement supported trapping samples from multiple projects including:

- By-catch samples from velvet longhorned beetle, VLB, *Trichoferus campestris*, pheromone identification trapping studies conducted in Utah by Utah Department of Food and Agriculture and in New York by New York State Department Agricultural Markets
- General woodborer survey projects conducted in MA by Otis staff (including those described above)
- General woodborer survey projects conducted by U.S. Forest Service personnel at Pineville, Louisiana (as described above)

Prior to shipment to PDA, all samples are pre-sorted to remove leaves and other debris as well as gypsy moths that are collected in high numbers during the adult flight period in MA (Figure 2). In 2016 a total of 3,397 trap samples containing 66,007 specimens were processed through the Identification Laboratory via this agreement. Sample data is provided through an Excel file containing all of the taxonomic information for each sample/specimen. As of December, 2017 1,683 samples containing 16,148 specimens have been processed from 2017 samples; this represents just a fraction of what was sent in and identification is ongoing.

Figure 2. Gypsy moth by-catch found in most traps deployed in Otis Laboratory trapping studies conducted in southeastern Massachusetts.

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

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

We recently isolated and identified a novel variant of the conserved 2,3-alkanediol/hydroxyketone chemical structure from headspace volatiles of males only. In earlier field bioassays, this compound, which we call trichoferone, attracted significantly more adult beetles than did commercially available high-release ethanol lures or solvent control. In 2017, we conducted a dose-response study in an attempt to determine the optimal dose of trichoferone for monitoring. Adult VLB appear to respond to traps baited with trichoferone in a dose-dependent manner (Figure 1).

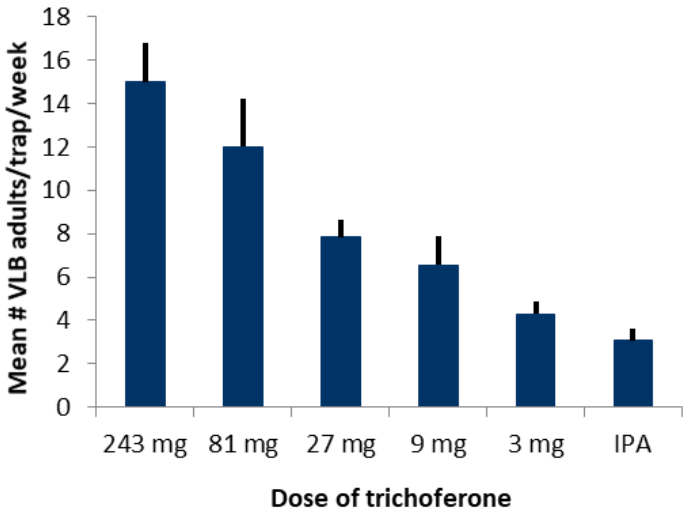

Figure 1. Adult VLB appear to respond to traps baited with trichoferone in a dose-dependent manner, although it is not yet apparent which dose is optimal for monitoring due to the short duration of 2017 bioassays.

However, the optimal dose could not be determined due to low sample size, resulting from the limited duration of the bioassays. We also tested the response of adult VLB to canopy and understory traps baited with trichoferone versus ethanol-only lures.

As part of a separate study, we deployed traps baited with trichoferone in East Fork State Park in Clermont County, Ohio and subsequently collected two adult VLB (Figure 2). This detection represents a new county record, and the first time that VLB adults have been collected in a natural (non-urban or industrial) location in OH.

Figure 2. Two VLB adults captured in trichoferone baited traps in East Fork State Park, Bethel, OH.

Ten traps baited with 50 mg trichoferone lures were also deployed in White Plains, New York in an urban residential and commercial area. A total of 27 VLB adults were caught in eight out of 10 deployed traps. One of the traps that did not catch a beetle was a lower counterpart (hung at 1.5 m) to a mid-canopy (5-8 m above the ground) trap supporting our earlier conclusion that height plays a role in trap capture. The mid-canopy trap caught one beetle. This trapping assay has expanded the known infested area in White Plains beyond what was thought to be established in 2017. The results of our work will assist state and U.S. federal personnel as well as land managers in developing monitoring surveys for VLB.

Modifying wood-boring beetle survey tools with commercially-available solar powered LEDs

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[1]Otis Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA

Invasive wood-boring beetles can pose a serious threat to native trees and can greatly alter forest health. Early detection of these species plays a crucial role in implementing control measures. Several factors have been shown to be important in the detection of wood-boring beetles including: 1) trap shape, 2) trap color, 3) beetle and host-produced volatiles, and 4) UV light.

While light trapping has been shown to be very successful in detecting some species, the added cost, additional maintenance, and electrical limitations have made light trapping a less commonly used option in larger program wide woodborer surveys. In an effort to improve survey detection tools, we designed light traps that do not require large external batteries, making them more cost efficient and easier to maintain. The traps were created by modifying existing intercept panel traps to combine all four of the aforementioned attraction properties and fit with weatherproof self-contained solar powered LED light units. Void of wires and cumbersome external batteries, traps were able to be safely deployed in the upper tree canopy, where traditional light traps are unable to be set.

In 2017, ten replicates with four traps each were set throughout mixed forest types in Freetown Fall River State Forest, Bristol County, Massachusetts. Twenty of the traps were modified (Figure 1) with two eight-diode LED solar sensor light units (Litom model 82080) set to the “bright” setting and attached to the top component of the trap, facing opposite sides.

Lights were controlled via an internal photo sensor that shut them off during daylight hours. Traps were set in matched pairs at 20 different locations with one trap suspended in the mid-canopy (5-8 m above the ground) and one deployed at ground level on a 1.5 m PVC pole. General cerambycid lures were used as bait and collection cups were filled with 250 to 300 ml propylene glycol as the killing agent. Traps were checked on a weekly basis, and all contents were collected, sorted, and shipped to the Pennsylvania Department of Agriculture for identification. All cerambycids were identified to the species level.

During the eight week field season (June to July) a total of 2,489 individual cerambycids were caught. As expected, significantly more cerambycids were caught in the traps within the upper canopy as compared to the traps placed on poles ($df = 1$, $F = 18.077$, $p < 0.001$). Light did not have a significant effect on the number of individual cerambycids caught per trap ($df = 1$, $F = 2.277$, $p = 0.131$. However, of the 59 different species of cerambycids detected, 88% of species types were captured in light traps and only 69% were detected by unlit traps.

These preliminary results suggest that while the addition of the LED lights that were tested didn’t increase the total number of wood-boring beetles caught within the treatment, it did improve the overall diversity of species detected. These results suggest that the basic premise of adding the solar powered LED lights does widen the scope of wood-boring beetles detected. With further work investigating different wavelengths and brighter, longer-lasting LED options, we expect to further improve the effective range of the traps.

Figure 1. Intercept panel trap modified and fit with solar powered LED lights. Light has been circled in red.

Field testing novel commercially available trap designs for wood-boring beetles

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A variety of trap designs have been used for detecting bark- and wood-boring beetles. Among these, Lindgren multi-funnel and the intercept panel trap are both widely used and effective. In 2017, Synergy Semiochemicals Corporation developed a hybrid multi-trap system that incorporates aspects of both funnel and panel trap designs by using varying sizes of funnels in combination with corrugated plastic intercept panel traps. Comprised of interchangeable parts, these traps allow users to construct three very different trap configurations from the same trapping system.

In 2017, we conducted a field trial comparing the three new trapping configurations to standard 12 multi-funnel Lindgren traps. In an ongoing effort to improve program detection and monitoring tools, these trap designs were investigated for their long-term durability, maintenance feasibility, and ability to detect native wood-boring beetles.

In total, 12 trap lines were set up in Massachusetts (n = 7) by Otis staff, and in Pennsylvania (n = 5) by Pennsylvania Department of Agriculture (PDA) staff. Each trap line consisted of eight different traps. These traps included each of the following four trap types (Figure 1) in both green and black:

- 12 multifunnel trap as a control
- Synergy Multitrap 5 funnel trap to serve as a direct alternative to the multifunnel trap; the funnel walls were steeper than the multifunnel trap
- Synergy Multitrap with a larger Allison Collar fitted at the bottom to capture larger beetles that may bounce away from the main trap body
- Synergy Multitrap panel trap; a three panel trap fitted with a funnel of the same color below it

All traps were coated with a 50% solution of Fluon before being deployed in the mid-canopy (5-8 m above the ground), and collection cups were filled with propylene glycol as the killing agent. Traps were maintained and checked on a weekly basis for the duration of the summer. Trap design features and flaws were noted throughout the season. At the end of the trapping season, all MA samples were pre-sorted, processed, and shipped to PDA for species identification.

Samples are still currently being processed and identified. The new multi-trap design showed promise as an alternative to the currently used intercept panel and multi-funnel traps. The main funnels and Allison funnel collars were durable.

Additionally, cups detached and reattached more easily and seemed less prone to damage than standard multifunnel trap cups. However, there were several design issues that could pose potential problems in a survey setting including:

- Connectors were a weak point causing funnels to separate, which could lead to sample loss
- Allison Collar funnel often captured large amounts of falling plant material and rainwater, causing the collection cup to overfill, and making presorting samples more time intensive
- Corrugated plastic panels did not hold up to wind and weather
- The rigid construction of the connectors prevented the traps from easily collapsing into themselves, therefore the traps require more storage space

A list of these observations has been provided to the manufacturer by the groups working with these new traps. At this time no recommendations can be made on these novel traps as currently constructed.

Figure 1. Traps used in a field test comparing three novel trap designs to a control standard multifunnel trap A) standard multifunnel trap, B) Synergy Multitrap 5 funnel trap, C) Synergy Multitrap with Allison Collar fitted at the bottom, and D) Synergy Multitrap panel trap.

Research conducted at Bethel Field Station in 2017

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Introduction

During the 2017 fiscal year, the Bethel Field Station conducted several field and laboratory studies on invasive wood-boring insects. Bethel staff supported researchers from the Otis Laboratory, Xavier University, Purdue University, and Cornell University in collecting and processing data for studies on Asian longhorned beetle, ALB, *Anoplophora glabripennis*, and emerald ash borer, EAB, *Agrilus planipennis*. In addition, generic woodborer trapping assays were conducted within the ALB quarantine to assess wood-boring beetle community composition and attempt to quantify the effect of host tree removal on native woodborer populations.

Asian longhorned beetle research

Field station staff assisted with multiple tree removals to collect damage for ongoing dendrochronology and life history data collection. Samples were processed in the laboratory to provide data for modelling and to better inform the USDA's survey program (Figure 1, 2).

ALB adults were used in laboratory bioassays to assess attraction to allyl isothiocyanate (ITC), a potential plant volatile lure. ALB was suspected to be responsive to this chemical due to the presence of a gene coding for an allyl ITC proteinase, myrosinase. However, no significant attraction was found in Y-tube bioassays. Headspace volatiles from ALB fed on leaves of different host species were also collected to determine if there are any differences in volatile composition. Analysis is ongoing.

Dr. Ann Hajek of Cornell University used East Fork State Park to test the effects of weather on the efficacy of a new fungal pathogen for ALB, *Metarhizium brunneum*. Wood veneer pieces treated with microsclerotia and growth medium were deployed in the state park and collected once a month. Veneer pieces were sent back to Cornell University for testing with ALB in colony. Cornell personnel will continue to examine the efficacy of *Metarhizium brunneum.*

Other woodborer research

Emerald ash borer grid traps were collected weekly and staff assisted in felling, hauling, and debarking ash logs (pg 72). New grids were set up in the beginning of the season and maintained throughout the spring and summer.

Field station staff also conducted generic cerambycid beetle trapping assays to assess cerambycid community composition. Traps were located inside and outside of the ALB quarantine to determine if cerambycid species composition or abundance were affected by the removal of host trees. In total 2,406 individuals from 57 species of cerambycid were collected during the study. There were no significant differences between the ALB quarantine and non-quarantine locations.

Figure 1. ALB damage on a red maple felled for dendrochronology research.

Figure 2. ALB exit hole in a small branch.

Identifying and isolating attractants for *Bactrocera minax*

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Chinese citrus fly, *Bactrocera minax* (Figure 1) is a destructive citrus pest in many groves in South Central China. With increased global trade of citrus from China to different parts of the world there is a possibility that fresh infested citrus fruits will reach the U.S. *Bactrocera minax* completes one generation per year and the species is oligophagous, exclusively feeding on citrus fruits. The pupal stage can last from 150 to almost 200 days and a cold temperature threshold is required to break pupal diapause.

Figure 1. Male and female *Bactrocera minax* adults on citrus foliage.

Currently there are insufficient data on effective lures and survey tools for early detection of *B. minax*, which is especially important in the case of detection in the U.S. The present study addresses this lack of knowledge by investigating possible pheromone and host odor attractants. This ongoing work is a collaboration between APHIS PPQ, the NSF Center for Integrated Pest Management at North Carolina State University, and scientists from the Guangdong Institute of Applied Biological Resources in China.

Chemical ecology studies

In 2017 the Otis Laboratory received approximately 700 pupae collected by our collaborators in Jishou, Hunan province of China. These pupae are being kept in moist sand under different environmental conditions to stimulate the emergence of adult flies from diapause. Emergence of flies is ongoing; several adult flies have emerged and are being kept as virgin insects for volatile collection studies for the detection of possible pheromone compounds and electrophysiological studies. Preliminary collection data suggest that adult *B. minax* produce compounds that are similar in structure to known *Bactrocera* pheromone compounds, which will be the focus of further studies. Dr. Aruna Manrakhan, from Citrus Research International in South Africa, supplied the Otis Laboratory with a shipment of uninfested, immature sweet orange fruits, *Citrus sinensis*, that were collected near Komatipoort, South Africa. The collected fruits were used for the collection of Chinese citrus fly-host odor (Figure 2). In an ongoing study, the collected host odor and adult-produced volatiles are being tested for biological activity using a gas chromatograph-coupled-electroantennal detector (GC-EAD). This technique will be used to pin-point compounds for further study of effective lures for *B. minax* and possible behavioral work in the future.

Figure 2. Collection of volatile compounds emitted from immature sweet oranges.

Diorhabda sp. trap and lure validation

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Introduction

The introduction of the exotic invasive saltcedar weedy trees, *Tamarix* spp., into riparian areas in the western U.S. has caused ecosystem damage, such as water loss due to transpiration, decreased habitat quality for wildlife and native plant species, increased soil salinity, and increased fire risk. Efforts to control saltcedar through conventional means such as herbicides or physical removal have been expensive and of limited success. For this reason, a multi-state biological control program was initiated to release the leaf beetle, *Diorhabda* sp. [1,2]. Both the adult and larva stage of *Diorhabda* sp. feed on the saltcedar to complete defoliation. Monitoring the dispersal of adult *Diorhabda* beetles has been successfully demonstrated with yellow sticky traps baited with pheromone and/or green leaf volatiles in saltcedar stands in riparian areas in Nevada and Utah [3,4]. These trap and lure combinations were able to detect male and female beetles in both foliated and defoliated stands of saltcedar. In addition, beetles could be trapped in areas several kilometers away from known populations of *Diorhabda*, in areas without any visual evidence of the presence of larvae and/or beetles. The green leaf volatiles are attractive throughout the adult season, while the pheromone is only attractive until the beetles go into photoperiod induced reproductive diapause. The pheromone is attractive at much lower dosages than the green leaf volatiles, however pheromone formulations are currently not commercially available. One of the attractive green leaf volatiles, 3Z-hexenol, is available as a commercial formulation that releases at approximately 50 mg per day for at least six weeks.

This study evaluated trapping of *Diorhabda* sp. beetles with three different trap types, with and without a 3Z-hexenol lure at two locations along the Rio Grande River in New Mexico during May through July of 2017.

Methods

- Study locations: New Mexico; Caballo Lake State Park (Cab), Albuquerque Riverside Trial (ABQ)
- Lure types: (Z)-3-hexen-1-ol pouch releasing at approximately 50 mg per day, longevity approximately six to nine weeks
- Trap types: Yellow Hotmelt sticky boards (HM), Yellow Tanglefoot sticky boards (TF), Yellow Unitraps (Uni)
- Treatments: six, baited vs un-baited
- Replicates: seven blocks of six treatments, total traps = 42
- Field design: Traps were placed at chest height in accessible *Tamarix* spp. stands, trap counts were made on an approximate two week basis, and traps were replaced when needed
- Statistical analysis: Presence or absence (yes/no) of *Diorhabda* beetles by trap type, lure presence, and location was analyzed by contingency table analysis (*Courtesy of Miriam Cooperband*)

Results and discussion

Visual inspections at the start of the experiment showed feeding damage on *Tamarix* and the presence of larvae at the Caballo site (Figure 1), indicating that the overwintering population had already emerged, mated, and laid eggs. However, the feeding damage was very spotty, mostly restricted to a few branches on widely spaced saltcedar, indicating a relatively low population density. Initial visual inspections at the Albuquerque site showed no feeding damage at all, and *Diorhabda* life-stages were not observed. This location had no previous reports of the presence of *Diorhabda* (personal communication PPQ field staff).

Figure 1. *Diorhabda* larvae (circled in red) and *Tamarix* feeding damage at Caballo Lake, New Mexico.

Table 1 lists the total number of beetles trapped at the two locations with the three different trap types with and without the 3Z-hexenol lure. At both sites the HM traps with lures caught significantly ($p < 0.001$) more beetles than either the TF or Uni baited trap types. Table 2 lists the total overall trap catch at approximately two week intervals for the two locations. At the ABQ site the majority of the beetles were trapped towards the end of July whereas at the Cab site beetles were detected throughout the trapping period. The yellow hotmelt sticky board trap in combination with the 3Z-hexenol lure is an effective tool for the detection of *Diorhabda* beetles in *Tamarix* shrubs at relatively low population densities.

Table 1. Total number of beetles trapped at two sites, Caballo Lake (**Cab**) and Albuquerque Riverside Trail (**ABQ**), using three different trap types: Yellow Hotmelt sticky boards (**HM**), Yellow Tanglefoot sticky boards (**TF**), Yellow Unitraps (**Uni**), baited with (**y**) and without (**n**) 3Z-hexenol during May through July, 2017.

Sum of Beetles Trapped	Column Labels		
Row Labels	n	y	Grand Total
ABQ	**72**	**84**	**156**
HM	71	84	155
TF	1	0	1
Uni	0	0	0
Cab	**16**	**39**	**55**
HM	9	38	47
TF	2	1	3
Uni	5	0	5
Grand Total	**88**	**123**	**211**

Table 2. Total number of beetles trapped at different time intervals during May through July, 2017 at Caballo Lake (**Cab**) and Albuquerque Riverside Trail (**ABQ**).

Row Labels	Sum of Beetles Trapped
ABQ	**156**
5/16/2017	9
5/31/2017	0
6/12/2017	1
6/29/2017	0
7/10/2017	22
7/24/2017	124
Cab	**55**
5/10/2017	1
5/25/2017	0
6/1/2017	25
6/13/2017	9
6/27/2017	1
7/21/2017	19
Grand Total	**211**

References

1. Deloach CJ. Prospects for biological control of saltcedar (*Tamarix* spp.) in riparian habitats of the southwestern United States., in E. S. Delfosse (ed). Proc. 7th Int. Symp. Biol ControlWeeds, Rome, Italy, 1988, Instituto Sperimentale per la Patologia Vegetale, MAF, Rome. 1989:307-314.
2. Deloach CJ, Carruthers R, Dudley T, Eberts D, Kazmer D, Knutson A, et al. First results for control of saltcedar (*Tamarix* spp.) in the open field in the western United States. 11th Int. Symp. Biol. Control Weeds, Canberra, Australia, 2004.
3. Cossé AA, Bartelt RJ, Zilkowski BW, Bean DW, Petroski RJ. The aggregation pheromone of *Diorhabda elongata*, a biological control agent of saltcedar (*Tamarix* spp.): Identification of two behaviorally active components. J. Chem. Ecol. 2005;31(3):657-670.
4. Cossé AA, Bartelt RJ, Zilkowski BW, Bean DW, Andress ER. Behaviorally active green leaf volatiles for monitoring the leaf beetle *Diorhabda elongata*, a biocontrol agent of saltcedar, *Tamarix* spp. J. Chem. Ecol. 2006;32:2695-2708.

Otis Chemical Ecology and CAPS Lure Support

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The Cooperative Agricultural Pest Survey (CAPS) program is tasked with providing a survey profile of exotic plant pests in the U.S. deemed to be of regulatory significance to USDA APHIS PPQ, State Departments of Agriculture, tribal governments, and other cooperators through early detection and surveillance activities. Otis Laboratory's Chemical Ecology group supports the CAPS program efforts by supplying program stakeholders with specialized insect lure formulations on an annual basis. In addition to lure production Otis' CAPS support work also includes quality control of insect attractant chemicals, formulation, release analysis (Figure 1), and field efficacy. Approximately 106,900 lures were produced and analyzed in 2017. Lure efficacy testing was conducted for the European grapevine moth, *Lobesia botrana*, eradication program, European gypsy moth, *Lymantria dispar dispar* and Asian gypsy moth, *L. d. asiatica*, as well as individual research projects of Otis research collaborators.

Examples of lure support provided to collaborators include:

- Lure formulations and evaluation of the sex pheromone of the sweet potato vine borer, *Omphisa anastomosalis*, in collaboration with Dr. Grant McQuate (USDA ARS, Hilo, Hawaii)
- Repellent/lure development support [1] for the coffee berry borer, *Hypothenemus hampei*, in collaboration with Dr. Fernando Vega (USDA ARS, Beltsville, Maryland)
- Isolation and purification of attractants from tree of heaven, *Ailanthus altissima*, for the spotted lanternfly, *Lycorma delicatula*, in collaboration with Dr. Miriam Cooperband (Otis Laboratory)

Figure 1. Collection of volatile materials from insect lure formulations

Reference

1. Vega FE, Simpkins A, Miranda J, Harnly JM, Infante F, Castillo A, Wakarchuk D, Cossé A. A Potential Repellent Against the Coffee Berry Borer (Coleoptera: Curculionidae: Scolytinae). J. Insect Sci. 2017 Nov;17(6):122. doi: 10.1093/jisesa/iex095

Update: 2017 port and domestic Gypsy Moth Molecular Diagnostics survey

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Introduction

The Gypsy Moth Molecular Diagnostics Survey serves as a means for detecting the possible introduction of Asian gypsy moth, AGM, *Lymantria dispar asiatica/japonica*, in the U.S. Additionally, the survey functions to monitor the spread of European gypsy moth, EGM, *L. d. dispar*, outside of and along the edge of the established federal EGM quarantine (Figure 1).

Standard Gypsy Moth Diagnostic Assay

Traps containing specimens suspected of being gypsy moth are sent to Otis Laboratory, which serves as the confirmatory laboratory for molecular gypsy moth detections. The Standard Gypsy Moth Diagnostic Assay is utilized to molecularly identify AGM and EGM. DNA extracted from a specimen is amplified using polymerase chain reaction (PCR).

For this assay, two genetic markers are analyzed: the gypsy moth specific nuclear FS1 marker [1] and a mitochondrial marker [2]. The final identification for each specimen is made by interpreting the combined outcomes for each of these markers. This particular assay is capable of defining a specimen as either EGM or AGM. A specimen is determined to be unknown if DNA fails to amplify for either of the two genetic markers. This could be due to poor specimen condition, which leads to degraded DNA. However, since the FS1 marker is specific to gypsy moth, failure of this assay could be due to the fact that the specimen is not gypsy moth. For specimens of regulatory significance that result in an unknown identification, DNA barcoding is used to attempt to identify the specimen.

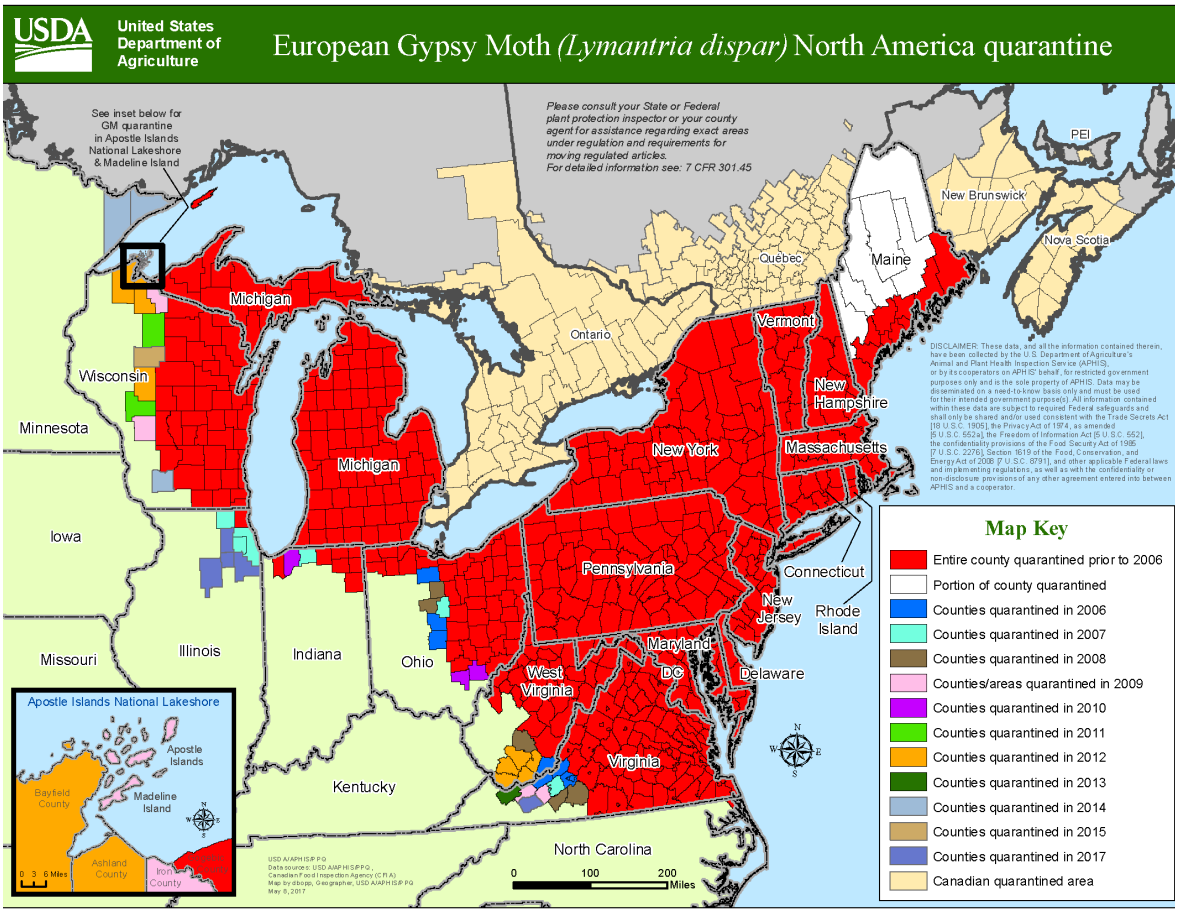

Figure 1. 2017 European Gypsy Moth North America quarantine.

DNA barcoding

DNA barcoding is based on the genetic COI marker. This section of mitochondrial DNA is effective in distinguishing animals of different species as it is highly conserved and species specific. The technique relies on obtaining the unknown specimen's COI DNA sequence and comparing it to sequences of known species that are available in public databases, GenBank and BOLD Systems. Contrary to the standard assay, this method can identify a specimen even if it is a species other than gypsy moth. There are cases, however, when the barcode obtained for a specimen does not match any records available in the public databases. In this instance, the identity of the specimen remains unknown and is categorized as inconclusive.

2017 AGM Survey results

In 2017, a total of 4,255 specimens from 27 states were submitted for molecular analysis (Figure 1). Only a single AGM detection was confirmed in Santa Cruz, CA. Additionally, seven egg masses intercepted from five U.S. ports were analyzed; six were identified as a species other than gypsy moth, while one remains unknown due to DNA sequencing failure. The Gypsy Moth Molecular Diagnostic Survey will continue to support the Gypsy Moth Program. In the coming survey year, a specimen priority system will be implemented, allowing us to streamline the reporting of diagnostic results.

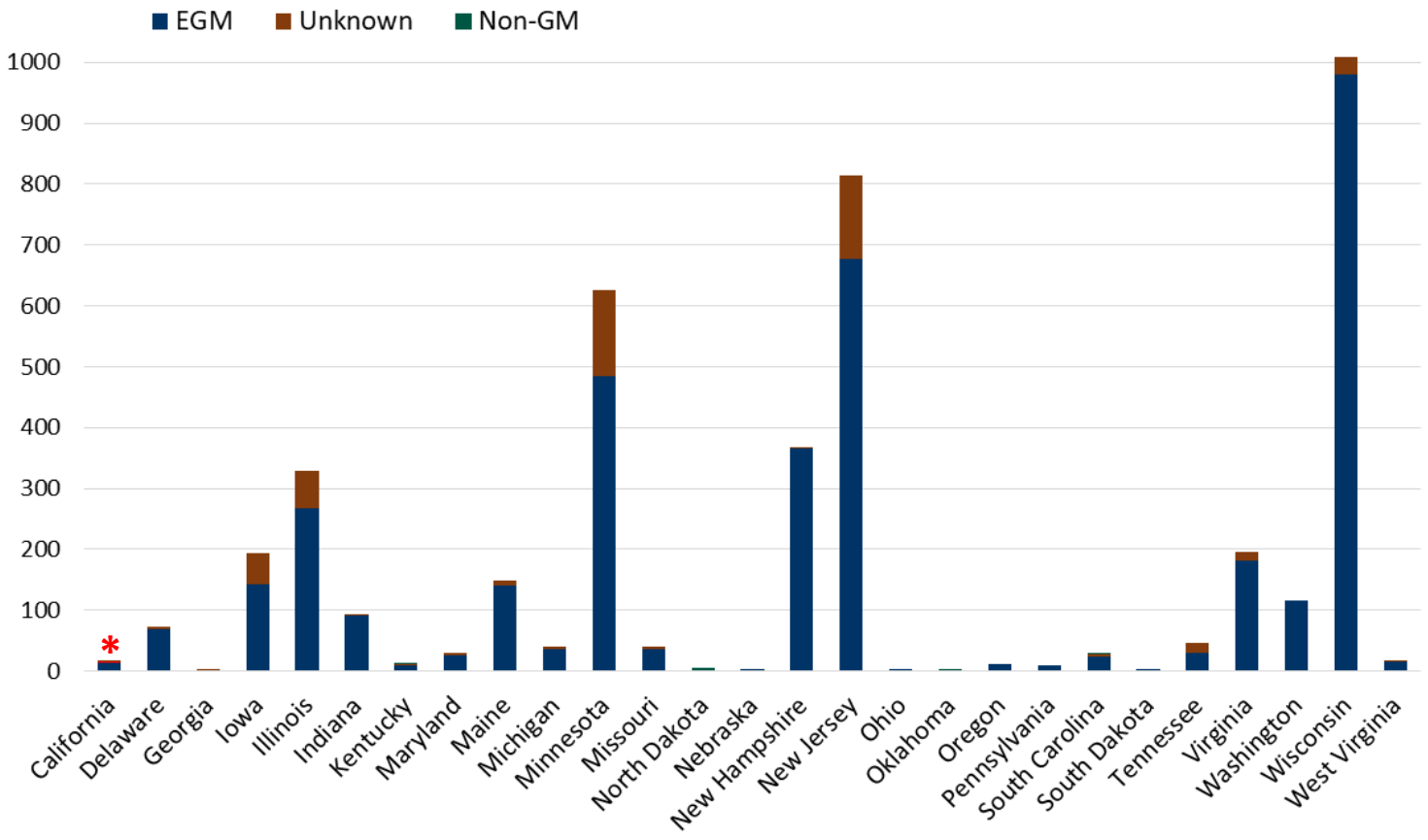

Figure 2. In 2017 4,255 specimens from 27 states were submitted for molecular analysis. The red asterisk indicates a single AGM detection from Santa Cruz, CA.

References

1. Garner KJ, Slavicek JM. Identification and characterization of a RAPD-PCR marker for distinguishing Asian and North American gypsy moths. Insect Molecular Biology. 1996;5(2):81-91.
2. Bogdanowicz SM, Wallner WE, Bell J, Odell TM, Harrison RG. Asian gypsy moth *(Lepidoptera: Lymantriidae*) in North America: Evidence from molecular data. Ann. Entomol. Soc. Am. 1993;86:710-715.

Assigning Asian gypsy moth intercepted in the United States to their geographic origins

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Introduction

Gypsy moth, *Lymantria dispar*, is a polyphagous pest that was introduced to the U.S. in the late 1860s from a European population now known as *Lymantria dispar dispar,* EGM. The U.S. EGM infestation has caused billions of dollars in losses for the forest industry and significant damage to urban forest [1]. In recent decades, Asian populations of this moth, Asian gypsy moth, AGM, *L. d. asiatica/japonica*, have also been introduced into the U.S. through the movement of international cargo [2]. While AGM has not established in the U.S., it would pose a greater threat than EGM due to its wider larval host range and strong female flight capability. Tracing the geographic origin of invasive AGM provides critical information to adjust pest management strategies between the U.S. and its Asian trading partners [3].

Methods and results

A total of 1,075 adult moths and eggs were sequenced for 55 nuclear and five mitochondrial loci generated through next-generation sequencing. The specimens analyzed consisted of 986 adults collected from wild populations, 84 AGM eggs intercepted at U.S. ports between 2014 and 2015, and five AGM males caught in pheromone traps around U.S. cities. Previous analysis of the 60 loci showed substantial genetic differentiation between EGM and AGM as well as within AGM, which provides the basis for assigning intercepted AGM to their original population. We conducted statistical analysis, discriminant analysis of principal components [4] (DAPC) using wild AGM samples as a reference to predict the geographic origin of intercepted AGM.

Three major genetic groups were obtained through DAPC: 1) most parts of China, 2) Japan, and 3) a mixture zone composed of northeastern China, Russian Far East, and the Korean peninsula (Figure 1).

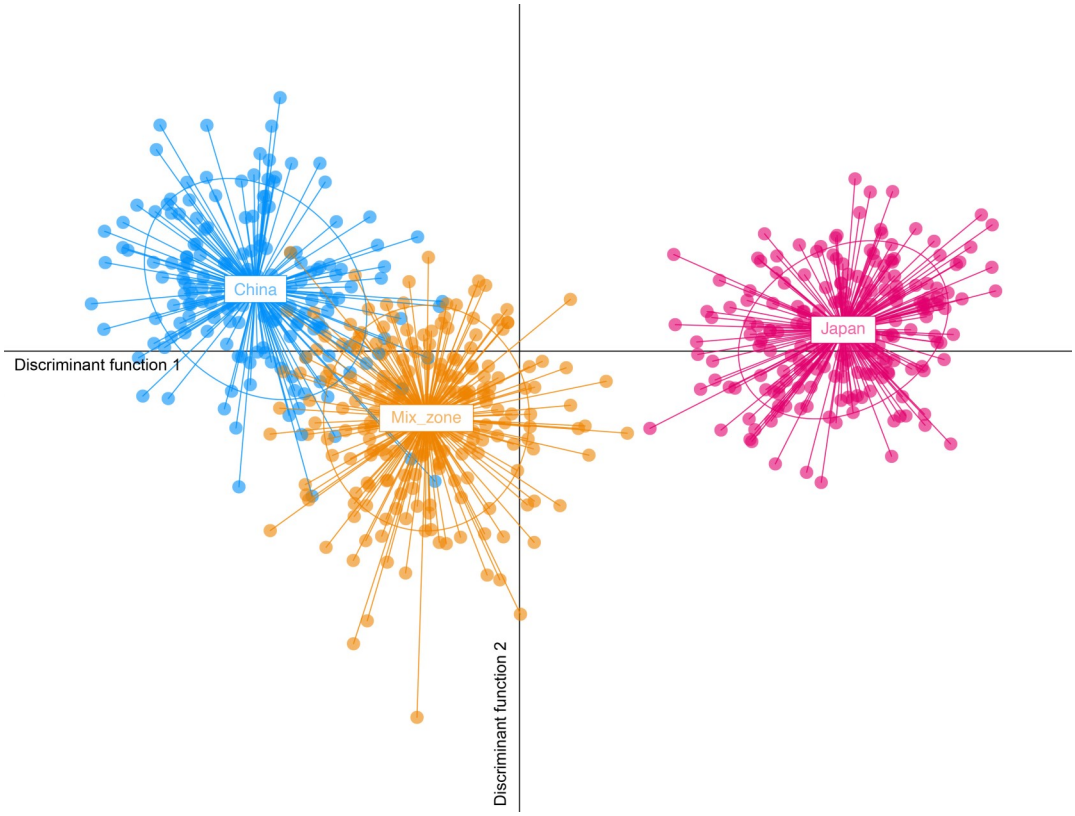

Figure 1. Genetic differentiation between three AGM groups based on the 60-loci dataset.

The third group is a genetic mixture between the Chinese and Japanese populations. Of the 84 individual AGM eggs, posterior group membership probability assigned 53 eggs to the mixture zone population (all posterior probabilities, or PP > 0.9) and 22 back to the Japanese population (all PP > 0.99). DAPC further predicted that one adult moth caught in North Charleston, South Carolina (2014), one in Portland, Oregon (2015), and two in Washington State (Kent and Tacoma, 2015) may belong to the mixture zone population. However, a second male moth from Tacoma, WA (2015)was assigned to the Japanese population with high confidence. Surprisingly, no AGM were assigned to the main Chinese population (Figure 2).

Because multiple eggs were sampled from intercepted egg masses, which are expected to have identical group assignment, they serve as excellent data points for cross validation of the assay itself. The assay is considered inconclusive if DAPC assigns individual eggs sampled from the same egg mass to different groups with high corresponding posterior probabilities. The 84 eggs in this study represent 26 different egg masses, 24 of which had between two and eight eggs sampled. Among those 24 egg masses, 23 have identical or congruent assignments between their individual eggs. Only one egg mass intercepted in Portland, Oregon yields an inconclusive assignment, in which one egg is grouped with the mixture zone population (PP = 0.999) and the other three eggs are assigned to the Japanese population (all PP = 1).

Conclusion

We developed a genetic assay capable of assigning AGM of unknown origin, regardless of life stage, back to its origin population in Asia with a relatively high success rate. This will help the Gypsy Moth Program identify the source of intercepted AGM and the possible routes of introduction, and support the development of effective management strategies

References

1. Bradshaw CJA, Leroy B, Bellard C, Roiz D, Albert C, Fournier A. Massive yet grossly underestimated global costs of invasive insects. Nature Communications. 2016; 7: 12986. doi:10.1038/ncomms12986
2. Animal and Plant Health Inspection Service (APHIS). Pest alert: Asian gypsy moth. 2016. https://www.aphis.usda.gov/publications/plant_health/content/printable_version/fs_phasiangm.pdf.
3. Picq A, Keena M, Havill N, Stewart D, Pouliot E, Boyle B, et al. Assessing the potential of genotyping-by-sequencing-derived single nucleotide polymorphisms to identify the geographic origins of intercepted gypsy moth (Lymantria dispar) specimens: a proof-of-concept study. Evol Appl. 2017; 00: 1–15.
4. Jombart T, Devillard S, Balloux F. Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. BMC Genetics. 2010; 11(1): 1–15.

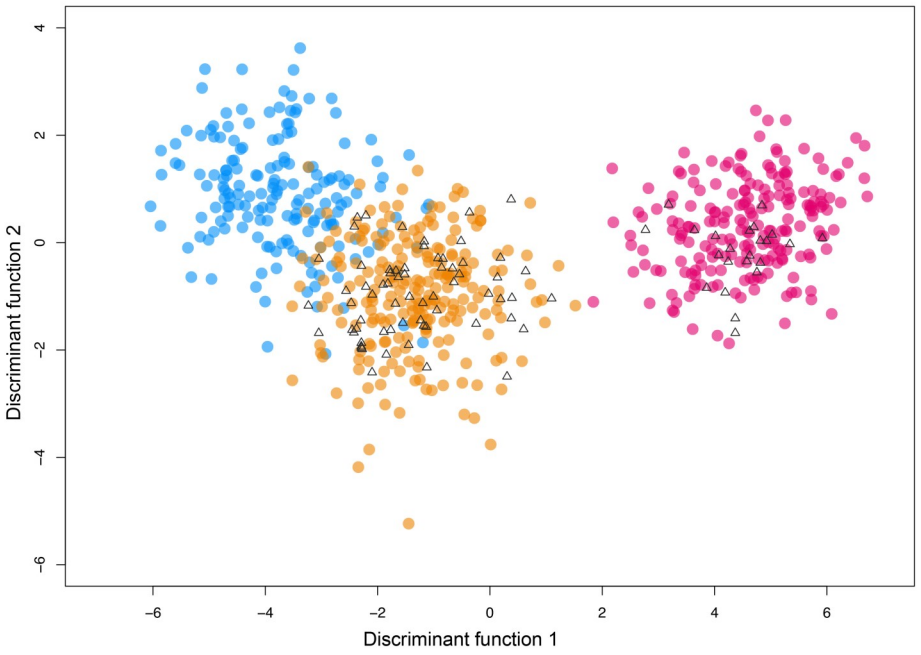

Figure 2. DAPC analysis of intercepted AGM (empty triangles) overlaid on three AGM groups.

Genetic evidence for natural hybridization between introduced Asian gypsy moth and European gypsy moth in the United States

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Introduction

The European subspecies of gypsy moth, EGM, *Lymantria dispar dispar*, was introduced to the U. S. in the late 1860's and is a significant defoliator of trees and shrubs. Asian subspecies of this moth, AGM, *L. d. asiatica* and *L. d. japonica*, have been detected in the Pacific Northwest region as well as other coastal and inland cities since the 1990's [1,2]. Because AGM are considered a greater threat compared to EGM, prevention of its establishment in North America is critical. Meanwhile, studies have shown that dispersal-promoting traits such as female flight capability can be partially inherited by offspring when AGM hybridize with EGM [3]. Therefore, in addition to closely monitoring AGM introduction, it is necessary to screen for potential natural hybrids in places where AGM has been detected alongside existing EGM populations.

Methods and results

The current assay is based on the 55 nuclear loci and five mitochondrial loci developed for identification of geographic origin of intercepted AGM. Our objective is to test whether those loci can distinguish hybrid moths from pure AGM and EGM.

Since there are no confirmed natural AGM x EGM hybrids in wild populations, we created lab hybrids by crossing the Japanese colony (J) with the New Jersey colony (N) housed in the Otis Insect Containment Facility. A total of 249 F1 progeny were randomly selected and sequenced for the 60 loci. For representatives of pure AGM and EGM, 11 adults from the Japanese colony and 14 from the New Jersey colony were sequenced. Because laboratory-reared colonies are often products of the founder effect [4] and may display biased genetic composition, data was further supplemented with wild populations of AGM (Russian Far East and South Korea) and EGM (Massachusetts and Connecticut).

We performed discriminant analysis of principal components (DAPC) [5]. To validate the diagnostic power of the dataset, all pure AGM, EGM, and 199 hybrids were used as a *"training data set"* to fit the algorithm for the analysis, while 50 hybrids were set aside as an unknown "*testing data set*". As expected, DAPC clearly separated the F1 hybrids from either AGM or EGM on the first discriminant function (Figure 1). A priori assignment and posterior assignment were a 100% match for the *training data set*, suggesting strong diagnostic power. Additionally, the validation test correctly predicted all 50 moths from the *testing data set* as hybrids between AGM and EGM.

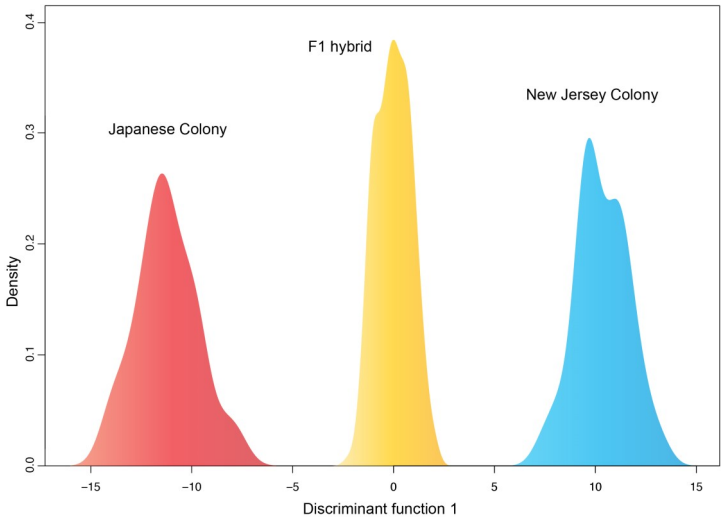

Figure 1. Histogram of first discriminant function scores for AGM, EGM, and lab-reared F1 hybrids.

Thirty-seven male gypsy moths trapped in Oregon and Washington from the 2014 and 2015 flight seasons were sequenced. In both states, AGM males have been detected before, therefore there is a possibility that they may have produced hybrids with the local EGM population (Figure 2). The analysis identified four pure AGM, 27 pure EGM, and four hybrids (F1) with high confidence (all posterior probabilities = 1). Those hybrids were originally detected as EGM when analyzed with the Standard Gypsy Moth Diagnostic Assay (pg.83).

Two additional moths were found to have intermediate scores between those inferred F1 and pure EGM. One possible explanation is that they could be the progeny of hybrid moths back-crossing with EGM (e.g., F2 generation).

Conclusion

The DAPC assay can successfully identify hybrids between AGM and EGM and supplements the Standard Gypsy Moth Diagnostic Assay by providing a powerful tool to monitor AGM introduction, allowing the agency to detect early signs of AGM establishment and potential gene flow into EGM.

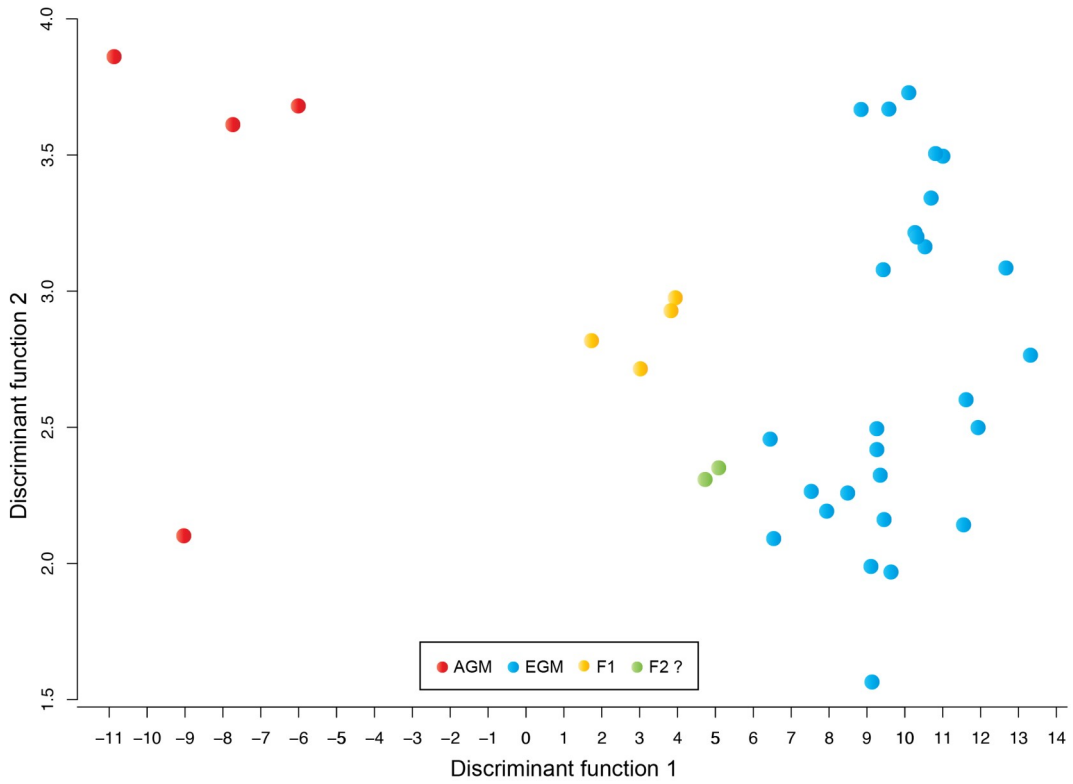

Figure 2. DAPC prediction of 37 male moths trapped in Oregon and Washington between 2014 and 2015.

References

1. Bogdanowicz SM, Wallner WE, Bell J, Odell TM, Harrison RG. Asian gypsy moths (Lepidoptera: Lymantriidae) in North America: evidence from molecular data. Annals of the Entomology Society of America. 1993;86:710-715.
2. Animal and Plant Health Inspection Service (APHIS) 2016. Pest alert: Asian gypsy moth. https://www.aphis.usda.gov/publications/plant_health/content/printable_version/fs_phasiangm.pdf.
3. Keena MA, Grinberg PS, Wallner WE. Inheritance of female flight in *Lymantria dispar* (Lepidoptera: Lymantriidae). Environmental Entomology. 2007;36(2):484-494.
4. Picq A, Keena M, Havill N, Stewart D, Pouliot E, Boyle B, et al. Assessing the potential of genotyping-by-sequencing-derived single nucleotide polymorphisms to identify the geographic origins of intercepted gypsy moth (*Lymantria dispar*) specimens: a proof-of-concept study. Evol. Appl. 2017;00:1-15.
5. Jombart T, Devillard S, Balloux F. Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. BMC Genetics. 2010;11(1):1-15.

Characterization of female gypsy moth flight capability in F2 generation hybrids

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Introduction

Despite the long establishment of European gypsy moth, *Lymantria dispar dispar*, in North America, introductions of the Asian subspecies, *L. d. asiatica* and *L. d. japonica*, must be viewed with concern. Unlike European female gypsy moths, Asian females are capable of powered flight (i.e., sustained ascending flight). This ability may accelerate the spread of populations if they are established, confounding attempts to eradicate gypsy moth in a given area. This ecologically important trait also has the potential for introgression—movement of a gene from one species into the gene pool of another—into the North American population through hybridization between subspecies [1]. Results have shown that female flight capability is most likely controlled under a polygenic effect—when multiple genes interact additively to influence a phenotypic trait—and varies continuously across the species native geographic range from Asia to Europe [2,3]. This study aims to better understand the genetic basis of female flight capability in gypsy moth.

Methods and results

In order to map female flight capability to its genomic components, we first produced an F1 generation by crossing the pure Japanese and pure New Jersey colonies, which are maintained at the Otis Insect Containment Facility and represent the flying female Asian subspecies and the non-flying female European subspecies, respectively. A total of 16 mating pairs were set up and reciprocally crossed between the parental colonies (Figure 1).

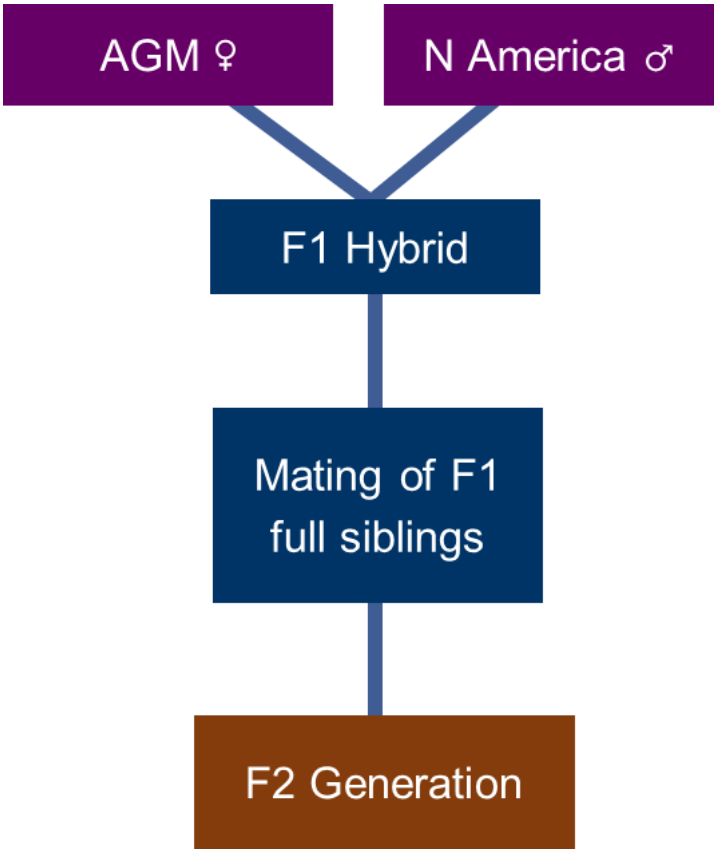

Figure 1. The reciprocal cross between the pure Japanese and pure New Jersey gypsy moth colonies.

Because only a fraction of females from the Japanese colony can fly (Hannah Nadel, unpublished), Japanese female moths were selected through a bucket test—only females that demonstrated flight capability were used for crossing (Figure 2). The produced F1 generation were reared to adults and mated with their full siblings to produce the F2 generation. During this process, it was observed that the cross between Japanese males and New Jersey females resulted in all male progenies, some of which had a female body size but male-like antennae. This phenomenon is termed "intersexuality" and was documented and studied by Richard Goldschmidt [4].

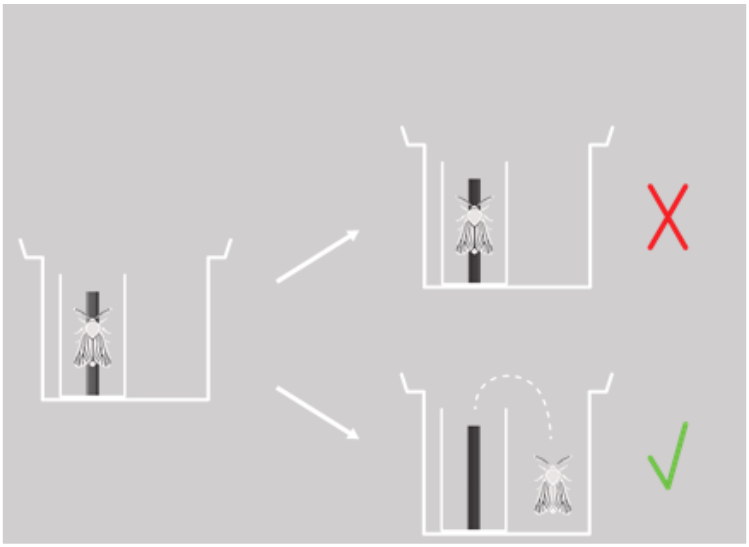

Figure 2. Bucket test for Japanese female moths. Only moths that flew out of the inner bucket were selected for subsequent mating.

Mating intersex moths, if successfully emerged from their pupal case, with normal moths produced no egg masses. Therefore, we only retained the lineage descended from the cross between Japanese males and New Jersey females.

After 14 months of rearing, two separate F2 populations were obtained, named H and K, and comprised of 240 females and 161 females, respectively. In order to quantitatively measure flight capability, which is reflected through thoracic muscle strength, the flip test developed by Melody Keena [1] was adopted and the original scoring categories were modified as follows:

1. Back flip on first attempt (i.e., instant flipper)
2. Back flip between two and 15 attempts
3. Side flip (rolls over on its side)
4. No flip during first 15 attempts
5. No attempts to flip, lies on back with legs moving
6. No attempts to flip, lies on back motionless

Each female moth was tested 24 to 48 hours after eclosion by placing the moth flat on its back with forceps. Each test was videotaped for the purpose of scoring. Counts of each category for both populations are shown in Figure 3.

Thorax weight and body mass for each tested female was measured, under the rationale that more thorax muscle indicates stronger flight performance. Therefore, a positive correlation was expected between flip test scores and thorax weight as well as the ratio of thorax weight to total body weight [5] (e.g., instant flipper will have the heaviest thorax weight); however, such correlation was not apparent in ether comparison (Figures 4, 5).

Conclusion

Based on the current flip test scoring system, there is no consistent correlation between female flight capability and thorax weight. This result suggests that, instead of weight difference in thoracic muscle mass, other factors, such as differential energy generation in the thorax, may contribute to variation in female flight capability.

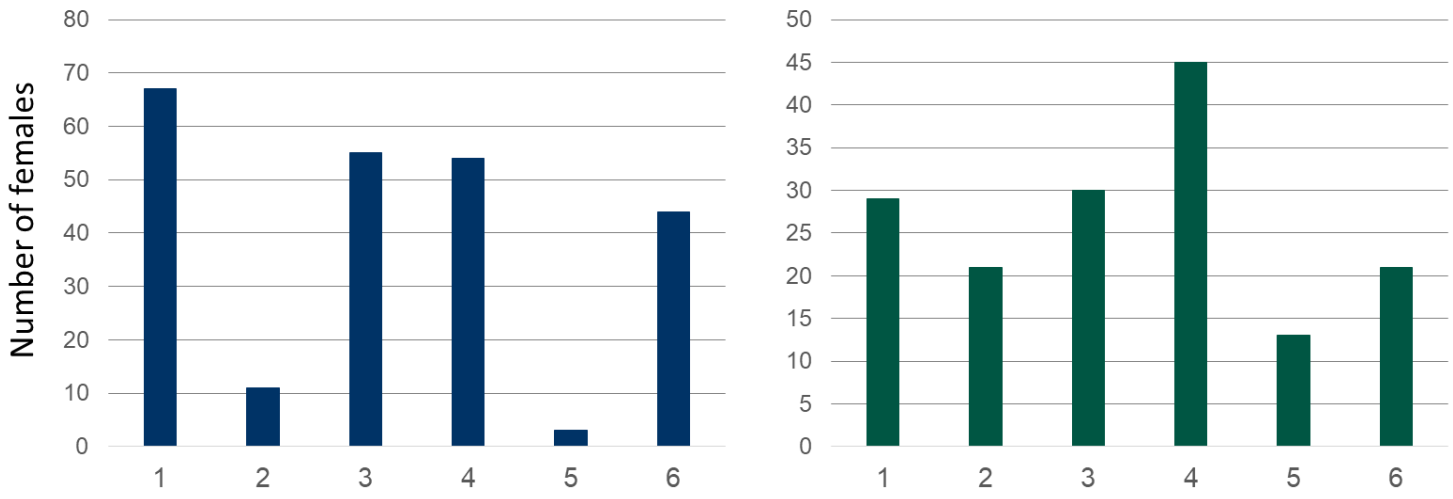

Figure 3. Flip test category counts for both F2 populations, H (blue) and K (green).

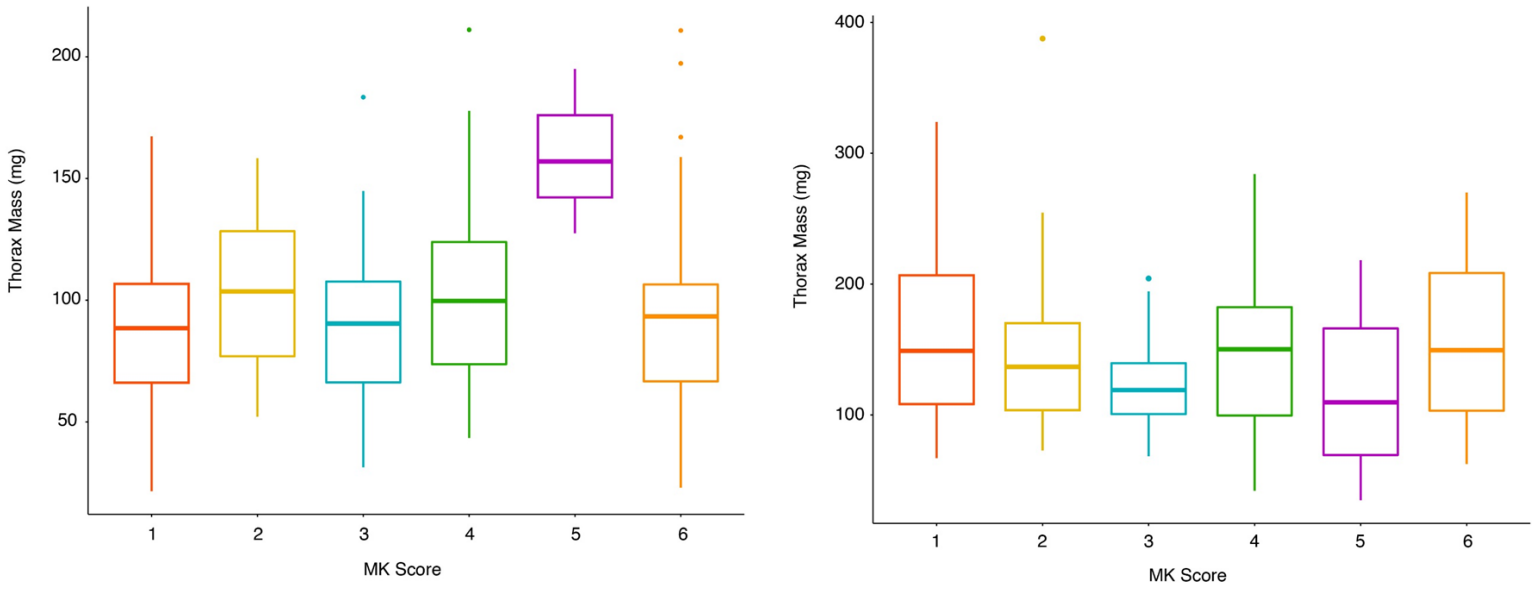

Figure 4. Box plots for thorax weight under each flip test category for both F2 populations H (left) and K (right).

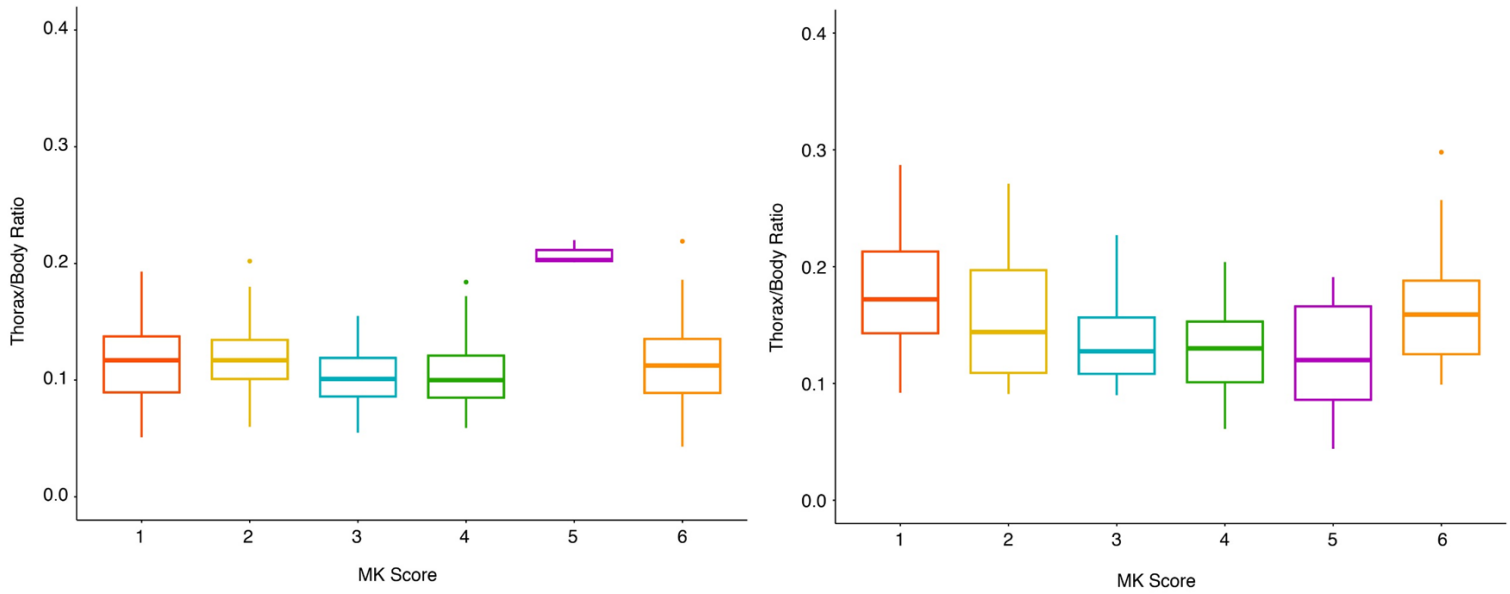

Figure 5. Box plots for ratio of thorax weight to total body weight under each flip test category for both F2 populations H (left) and K (right).

References

1. Keena MA, Grinberg PS, Wallner WE. Inheritance of female flight in *Lymantria dispar* (Lepidoptera: Lymantriidae). Environ Entomol. 2007;36(2):484-494.
2. Keena MA, Wallner WE, Grinberg PS, Carde RT. Female fight propensity and capability in *Lymantria dispar* (Lepidoptera: Lymantriidae) from Russia, North America, and their reciprocal F1 hybrids. Environ Entomol. 2001;30:380-387.
3. Reineke A, Zebitz CPW. Flight ability of gypsy moth females (*Lymantria dispar L.*) (Lep., Lymantriidae): a behavioural feature of characterizing moths from Asia? J. Appl. Entomol. 1998;122:307-310.
4. Goldschmidt R. Analysis of Intersexuality in the Gypsy-Moth. The Quarterly Review of Biology. 1976;51:25-44.
5. Shields KS, Hirth RT, Young RF, Keena MA. Comparison of thoracic musculature of Asian and North American gypsy moths, p. 78. In S.L.C. Fosbroke and K. W. Gottschalk (eds.), Proceedings of the USDA interagency gypsy moth research forum 1997. USDA, Radnor, PA.

Update: Genetic analysis of Asian longhorned beetle in North America

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Introduction

In order to further clarify genetic relationships between populations of Asian longhorned beetle, ALB, *Anoplophora glabripennis*, in North America, 1,606 base pair (bp) of mitochondrial fragments [1] were sequenced from beetles collected after 2008. In addition to 112 specimens from the three persisting populations (Bethel of Ohio, Worcester of Massachusetts, and the Long Island region of New York), we sequenced 45 historical samples including Boston (MA), Chicago (IL), Staten Island (NY) and a few miscellaneous beetles collected outside quarantine areas.

Methods and results

Re-sequencing historical samples allowed us to directly compare our sequencing results with those of Maureen Carter [1], which were generated from the same specimen. Multiple comparisons revealed at least three discrepancies consistently present between our data and Carter's published data, likely due to sequencing error. The discrepancies had led to a previous conclusion that the persisting populations are the result of new introductions because they did not match with any historical samples. However, when discrepant nucleotide sites were excluded, most haplotypes in persisting populations can be tracked back to historical haplotypes, limiting the likelihood that new introductions had occurred.

All 13 sampled Ohio ALB share the same haplotype (OH haplotype) identical to the eradicated Canadian population. The only other detection of this haplotype in the U.S. is from a dead beetle found inside a package of curtains at the Christmas Tree Shop in 2013 from Michigan. In Massachusetts, one common haplotype (MA haplotype) is shared by most beetles between Worcester and Boston. The MA haplotype has widely been found in historical populations across New York and Eastern New Jersey.

One of the beetles collected from the Boston infestation has a remarkably different haplotype, not closely related to any other North American ALB or published European ALB. One Worcester ALB is different from the MA haplotype by two bp.

All 84 specimens from three sites of New York (Farmingdale, Amityville, and Copiague) share a single haplotype (NY haplotype), which was found in various areas of New York and Chicago. In contrast to the current homogeneity, historical New York ALB had a much higher genetic diversity. It is likely that intensive eradication efforts wiped out most haplotypes. Presumably, only the current haplotype survived, by chance, and founded the new population. A Chicago ALB collected near Deerfield, after declared eradication, possesses the MA haplotype, suggesting a separate introduction. Distribution of North American ALB haplotypes is shown in Figure 1.

A further comparison was done on the three major North American haplotypes and ALB sampled from its native range in East Asia. Interestingly, these three haplotypes are also the top three most widespread haplotypes in China (Figure 2). The OH haplotype is common in eastern China along the coast; the MA haplotype is mainly found in northern China; and the NY haplotype is widespread in China both in the north and south part of its range.

Conclusion

Genetic relationships of ALB in North America were studied using mitochondrial DNA. Persisting populations are likely descendants of old infestations, but new introductions may also have occurred. Confirmation of new introductions through genetic analysis aids in policy decisions.

Reference

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

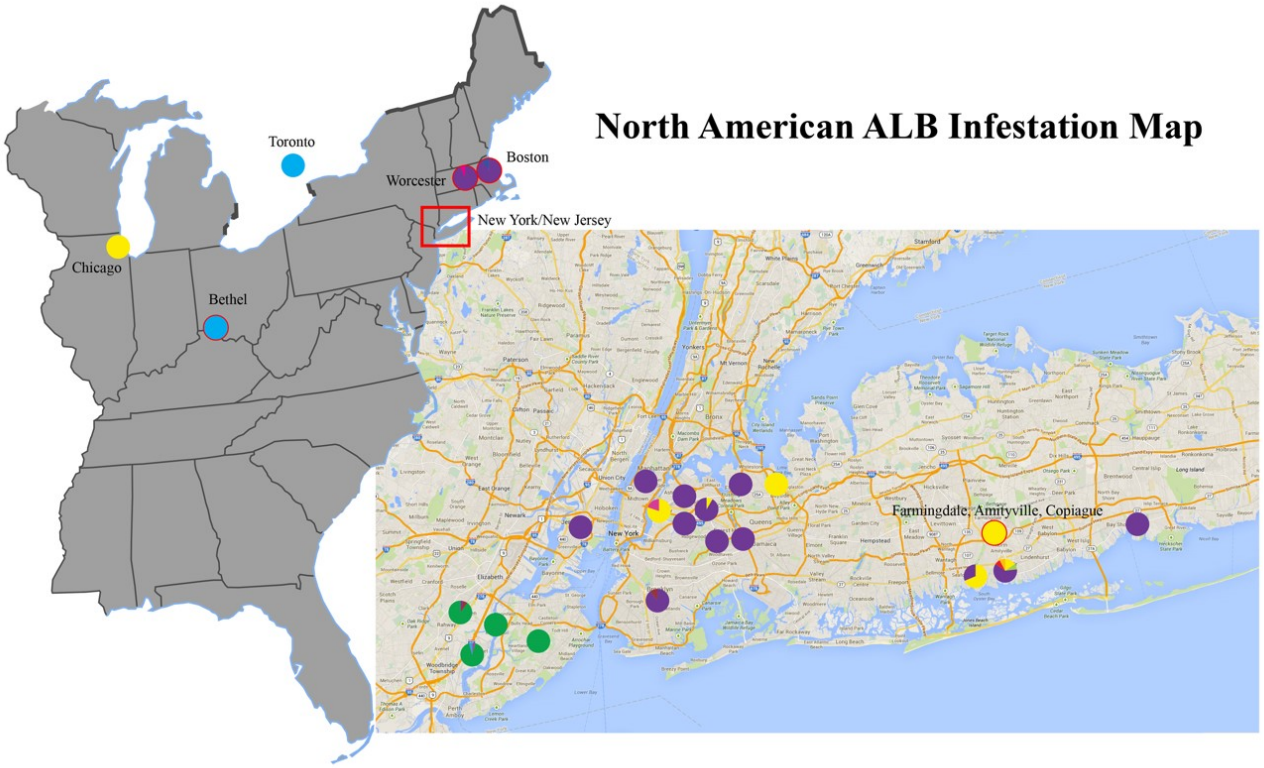

Figure 1. Distribution of ALB haplotypes in North America. Blue color is the OH haplotype; purple color is the MA haplotype; and yellow color is the NY haplotype.

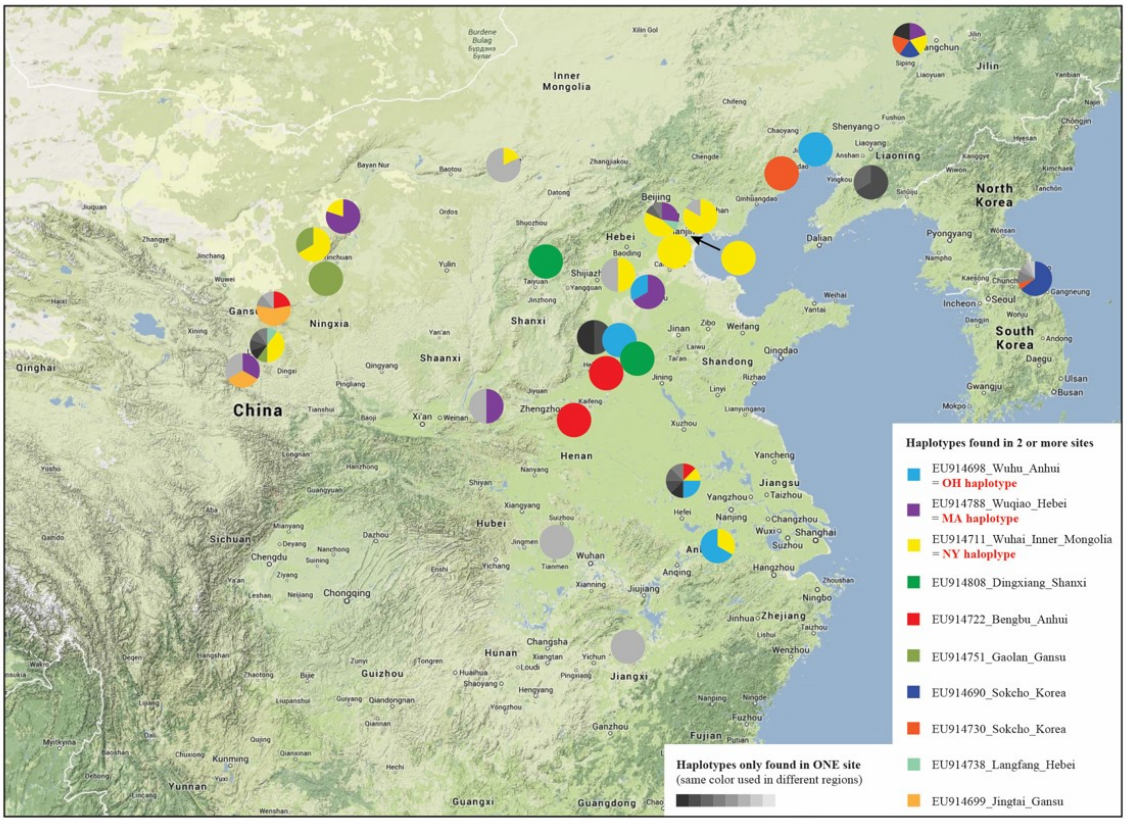

Figure 2. Distribution of ALB haplotypes in East Asia. Blue, purple, and yellow designate OH, MA, and NY haplotypes, respectively.

Update: Identification of wood-boring cerambycids, buprestids, and siricids intercepted in trade-associated solid wood packaging material

Sindhu Krishnankutty[1,3], Yunke Wu[1,2], Nevada Trepanowski[1], Kendra Vieira[1], Peter Reagel[4], Steven Lingafelter[5], Adam Taylor[6], Hannah Nadel[1], Scott Myers[1] and Ann Ray[3]

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Introduction

Live larvae of potentially destructive exotic wood borers are frequently intercepted at U.S. ports of entry in "treated" solid wood packaging material (SWPM). Intercepted larvae often remain unidentified beyond family level. As a result, valuable information is lost about risk pathways and trade routes for particular genera and species. In the *Otis Laboratory 2016 CPHST Laboratory Report*, we reported earlier results of this project, which was started in 2012 with the aim of identifying DNA barcode sequences and tissue vouchers of wood borers intercepted alive in SWPM; the data are made available to promote rapid species diagnosis from any life stage [1]. Project findings are being disseminated to federal decision makers and stakeholders to provide information on the SWPM pathway of entry by high-risk wood-boring species, areas that are at high risk of introduction, and species of high establishment potential in the U.S.

Identification of specimens

During 2017, we continued to rear intercepted larvae to the adult stage in the Otis Insect Containment Facility to allow identification by morphology. Additionally, all life stages that died in rearing as well as metamorphosed adults were DNA-barcoded.

From April, 2012 to September, 2017, we received 1,371 wood borers found in SWPM by Customs and Border Protection agriculture specialists. The number of participating U.S. ports increased from six to eight in 2014, and to eleven in 2015. Most of the intercepted specimens were cerambycids (81%), 14% were buprestids, and siricids, which were submitted starting in 2016, comprised 5% of specimens.

We obtained DNA barcode sequences for 923 specimens that included 208 adults reared from the larval stage. Of these, 601 were identified to genus or species by querying sequences in the Barcode of Life Data System (BOLD). Adult specimens were identified by both morphological and molecular methods. By September 2017, 32 cerambycid genera, seven buprestid genera, and three siricid genera were identified, including 47, nine, and three species of each woodborer family, respectively. Specimens remaining unidentified to genus or species comprised 56% of the total. Some of these are pending analysis. Some specimens couldn't be analyzed as they were too small in size, degraded, or died and desiccated during rearing. Some sequences remain unidentified as they lacked barcode matches in BOLD or had unresolved conflicting matches. A large proportion of unmatched sequences (54%) belong to specimens intercepted from Mexico; this likely reflects a historically lower priority for barcoding species that may already be present in both countries.

Identification of host wood

Pests submitted to Otis Laboratory for rearing were accompanied by 828 samples of host wood; of these infested wood samples, 483 were identified to genus. The most frequently intercepted wood genera are the softwoods *Pinu*s (47%) and *Picea* (19%), and the hardwood *Populus* (14%). These frequencies likely reflect the abundance of these wood genera in the SWPM manufacturing industry, but they may also reflect a high infestation rate in these hosts. These host genera harbor a high diversity of wood borer species, some of which utilize multiple hosts.

Expansion of DNA barcode databases for cerambycids

Efforts are being made to expand the public DNA barcoding databases by locating and sequencing identified specimens from museum collections. As of September 2017, DNA from 476 specimens of species not represented in DNA databases were sampled from the Smithsonian National Museum of Natural History and the National Autonomous University of Mexico. To date, DNA was extracted from 214 specimens, of which 121 were successfully sequenced.

Development of a risk map for *Trichoferus campestris,* velvet longhorned beetle

We are compiling port interception and domestic detection data on *Trichoferus campestris,* velvet longhorned beetle, to ultimately map the risk of its introduction and establishment in the U.S. *Trichoferus campestris* (Figure 1) is native to China, Japan, Korea, Mongolia, and Russia. It attacks dead wood and both healthy and slightly stressed living trees, and has a large host range comprising at least 40 genera of conifers, hardwoods, and herbaceous plants. In the U.S., it has been detected in several states. Several specimens have been collected recently over consecutive years in Utah, Illinois, and New York.

Figure 1. *Trichoferus campestris.*

The establishment risk of this beetle in other parts of the U.S. is high because of its frequent introduction through multiple pathways into urban areas and commodity distribution centers linked to U.S. ports, and due to its potential to inhabit natural and urban forests as well as agricultural lands (fruit orchards).

Forty-five separate interceptions of this species were recorded at 13 U.S. ports between June 10, 1997, and June 20, 2016. These records were obtained from the PestID database in the Agricultural Quarantine Activity System (AQAS). Thirty-seven (82%) of the interceptions were in SWPM. The majority of infested SWPM was associated with stone and metal products. Other pathways included passenger baggage and wood products (including furniture, a birdhouse, and decorative vine cuttings). Records exist of domestic detections of adult *T. campestris* in 14 states in both residential and commercial properties.

We are collaborating with analysts in the APHIS CPHST S&T Pest Epidemiology and Risk Analysis Laboratory to develop a pest risk map for *T. campestris* that integrates risks associated with several invasion correlates including trade, the SWPM pathway, and climate suitability. The map will enable informed prioritization of resources in detection surveys. The risk mapping methods developed in this case study will be applicable to other exotic wood boring species moving through the SWPM pathway.

Reference

1. Wu Y, Trepanowski N, Molongoski J, Reagel P, Lingafelter S, Nadel H et al. Identification of wood-boring beetles (Cerambycidae and Buprestidae) intercepted in trade-associated solid wood packaging material using DNA barcoding and morphology. Scientific Reports. 2017;7:40316. doi: 10.1038/srep40316

Genetic analysis of velvet longhorned beetle, *Trichoferus campestris*, in the United States

Yunke Wu[1, 2], Kendra Vieira[1], Sindhu Krishnankutty[1,3] and Hannah Nadel[1]

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Introduction

Similar to the Asian longhorned beetle and emerald ash borer, the velvet longhorned beetle, VLB, *Trichoferus campestris*, is another emerging invasive species that made its way from Asia to North America. Since its first official record in Quebec, Canada in 2002 and subsequent detection 2006 [1], this wood-boring pest has been intercepted dozens of times in solid wood packaging materials at U.S. ports of entry [2]. Domestic trapping studies have captured VLB in multiple states. Understanding the genetic relationships between U.S. detections and port interceptions can provide critical information to support the potential threat of VLB.

Methods and results

A total of 26 VLB specimens were collected from eight U.S. states and additionally 46 specimens were intercepted at five ports of entry (Figures 1, 2). The mitochondrial DNA barcoding region (658 base pairs) was sequenced for each specimen. We constructed a maximum-likelihood gene tree (Figure 3) that depicts relationships between those beetles. The gene tree is rooted using *Trichoferus fasciculatus* as an outgroup species—an organisms that serves as a reference group when determining the evolutionary relationships of the set of organisms under study.

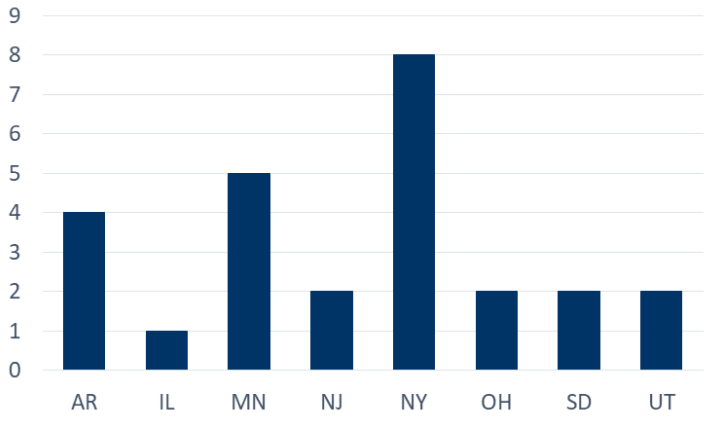

Figure 1. Number of VLB collected from eight U.S. states.

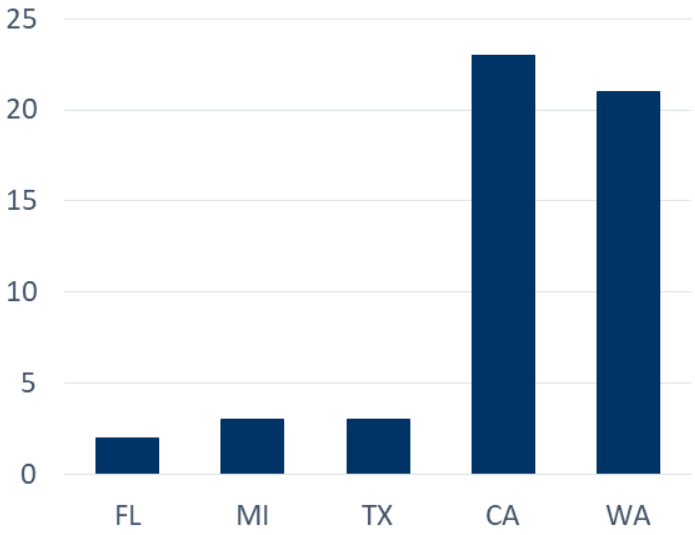

Figure 2. Number of VLB collected from five ports of entry.

Surprisingly, we found extensive genetic diversity both within and among the analyzed specimens. For example, uncorrected genetic distance between two Ohio specimens reaches 1.8%. Such genetic distances exceed the 1% sequence threshold for species limit in the widely recognized Barcode of Life Data System (BOLD) [3]. The eight VLB collected from White Plains, New York also have two very different haplotypes that differ by 1.5%. The result suggests that some of the analyzed samples could potentially represent cryptic species that have not been described. The observed genetic divergence in VLB sharply contrasts with that of Asian longhorned beetle, which has less than 1% divergence among all U.S. populations.

Similarly, such large genetic divergence is also observed among port-intercepted specimens, even when extracted from a single piece of wood. For example, four VLB larvae from the same host material were intercepted in California in 2013. One specimen's barcode sequence genetically differs from the other three (which are identical to each other) by 1.7% . The results further show that VLB from different states are not closely related, however some are identical to specimens intercepted at ports. This result would be consistent with independent introduction of VLB in those states through the pathway of solid wood packaging materials.

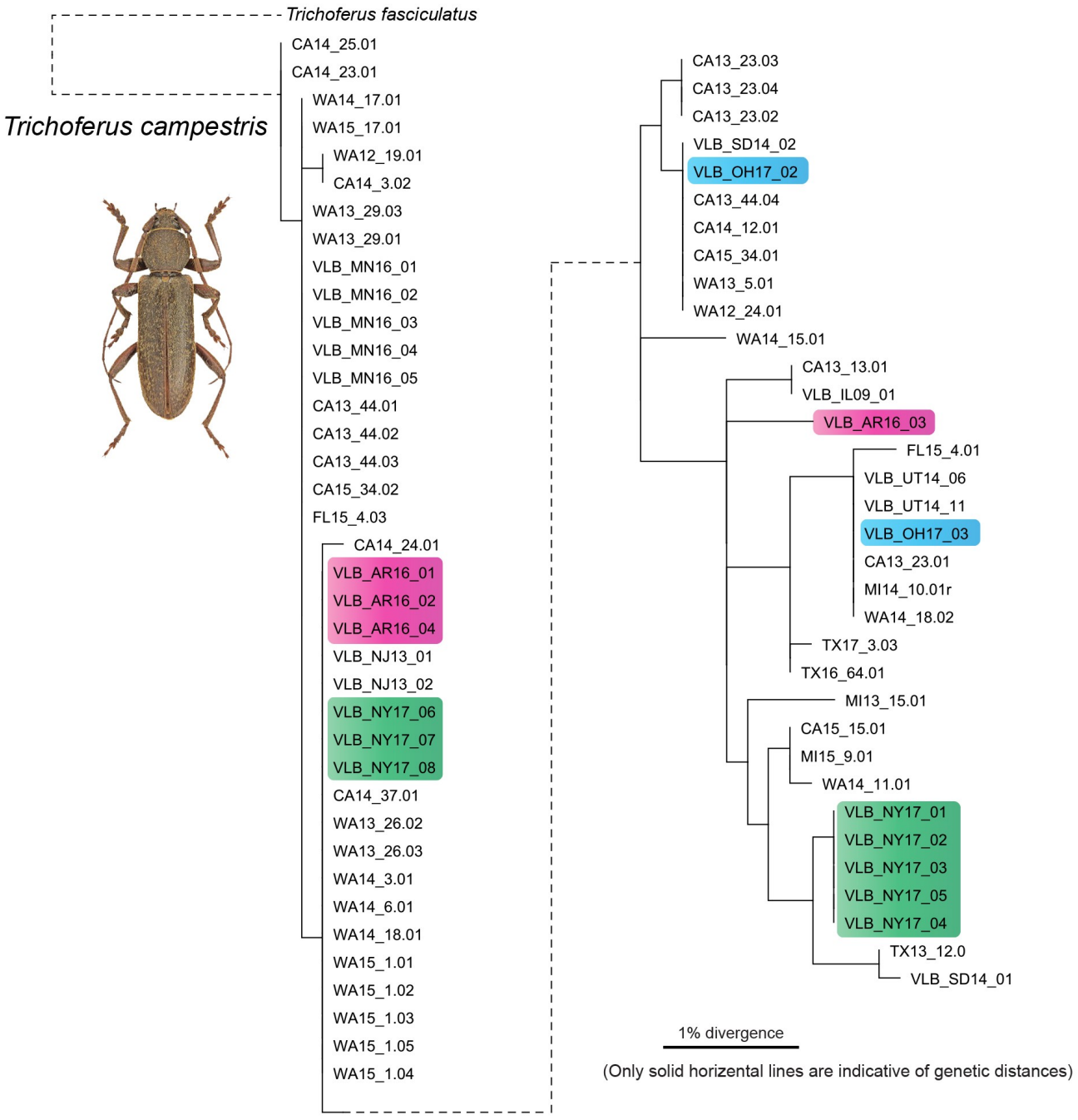

Figure 3. Maximum-likelihood tree based on mitochondrial barcoding sequences. The tree was folded along the dash line to fit the page space. Only solid horizontal lines are indicative of genetic divergence. Magenta: beetles from Arkansas. Green: beetles from New York. Blue: beetles from Ohio.

References

1. Grebennikov VV, Gill BD, Vigneault R. *Trichoferus campestris* (Faldermann) (Coleoptera: Cerambycidae), an Asian wood-boring beetle recorded in North America. Coleopterists Bulletin. 2010;64(1):13-20.
2. Wu Y, Trepanowski NF, Molongoski JJ, Reagel PF, Lingafelter SW, Nadel H, et al. Identification of wood-boring beetles (Cerambycidae and Buprestidae) intercepted in trade-associated solid wood packaging material using DNA barcoding and morphology. Scientific Reports. 2017;7:40316. doi: 10.1038/srep40316
3. Ratnasingham S, Hebert PDN. BOLD: the Barcode of Life Data System (www.barcodinglife.org). Mol Ecol Notes. 2007;7:355-364.

Evaluation of lighting preference to enhance trap catch of Asian citrus psyllid and coconut rhinoceros beetle, *Oryctes rhinoceros*

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Combating newly introduced pest species is one of the most significant biosecurity challenges facing U.S. agriculture today. Effective surveillance is critical for controlling the spread of pests and associated diseases. For example, the bacterial pathogen causing citrus greening is vectored by the Asian citrus psyllid, ACP, *Diaphorina citri*, and efforts to control the spread of disease in California and other citrus producing states include testing field caught adults for the pathogen.

Similarly, efforts are underway on the island of Oahu in Hawaii to control the recently introduced coconut rhinoceros beetle, CRB, *Oryctes rhinoceros*, which can result in significant damage to the crown and canopy of coconut palms.

There is an urgent need to improve upon monitoring tools for both ACP and CRB. We have developed a miniature LED-based lighting circuit to test lighting preferences to potentially improve the efficacy of trapping and surveillance efforts for these insects. The circuit can be programmed wirelessly through a custom Android application to turn on any of six LEDs (UV, blue, green, yellow, amber, and/or red) to create different modulation schemes and diurnal cycles based on ambient light.

These lighting circuits were used to test lighting perception/preferences of ACP and CRB. We found that illumination of custom traps for ACP with yellow resulted in the best trap yield compared to other colors, and are currently investigating effects of lighting modulation and diurnal cycles. Electroantennogram data for ACP indicates that adults appear to be most sensitive to 570 nm (within the yellow region of the spectrum). The currently used yellow sticky traps have a plateau peak of between 550 to 600 nm when measured using a hand held spectrophotometer.

Field test have shown UV light enhances pheromone traps. Our electroretinogram data for CRB suggest blue and green could also be attractive (Figure 1). In the coming year we will test blue and green LEDs on CRB traps.

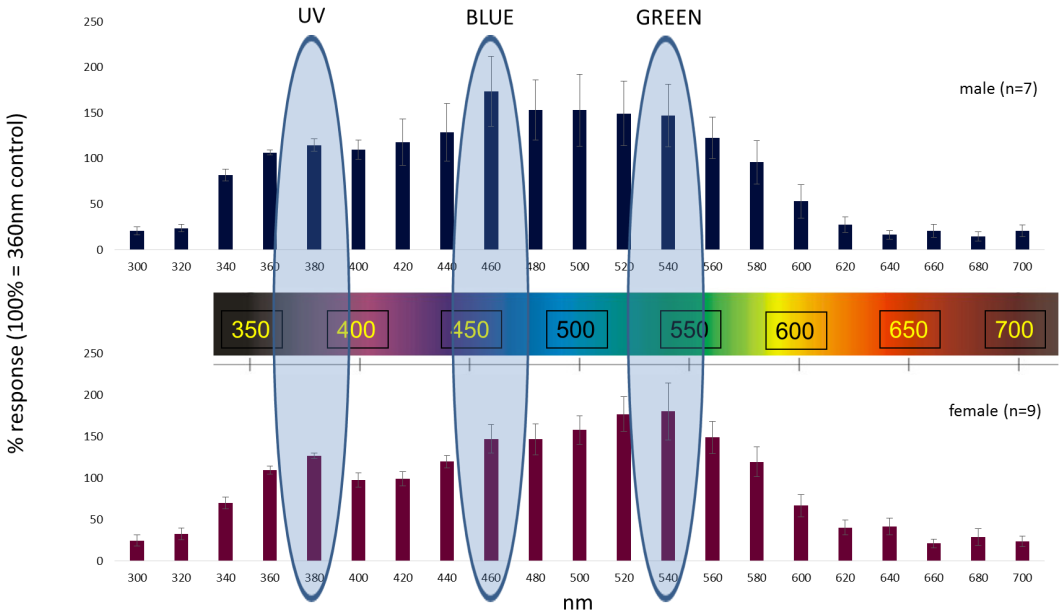

Figure 1. Retinal sensitivity of adult coconut rhinoceros beetle.

Update: Identification of "cryptic" female produced attractants for Asian longhorned beetle

Damon Crook[1], Allard Cossé[1], Carrie Crook[1], Joseph Francese[1], Miriam Cooperband[1] and Tappey Jones[2]

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[2]Department of Chemistry, Virginia Military Institute, Lexington, VA

In the last two years volatiles from Asian Longhorn Beetle, ALB, *Anoplophora glabripennis*, have been evaluated as candidates as sex pheromone components. In previous studies, male ALB have been shown to produce a three-component pheromone [1,2] which enhanced female attraction, but only over a short range. We have been testing the hypothesis put forward by Wickham et al. [3], that one or more of the female produced ALB pheromones is a precursor that undergoes abiotic oxidation to yield volatile pheromone components. Wickham et al. [3] identified three active components that caught more beetles than controls in trapping studies. The lure was believed to be missing some essential components so our main goal has been to identify them via gas chromatography coupled electroantennography detection (GC-EAD) and evaluate their activity using laboratory assays. In 2016 we identified two new components and in 2017 we identified three more.

Female body wash extracts were bubbled with ozone and then reduced with dimethyl sulfide to produce 16 aldehydes (nine of them being produced in trace amounts). GC-EAD recordings (Figure 1) revealed that both male (and female) antennae responded to seven of these aldehydes.

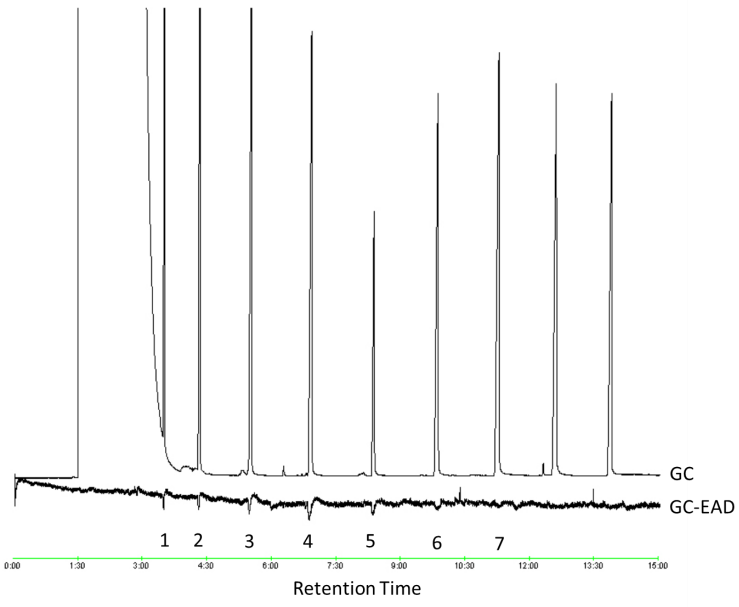

Figure 1. Male antennal responses to aldehyde standards.

Olfactometer behavioral assays (Figure 2) showed that males (but not females) were significantly attracted to the GC-EAD active seven-component aldehyde blend at two different dosages (75% attraction in each case). We hope to test this new blend of attractants in the field in 2018 with the aim of eventually improving current monitoring methods.

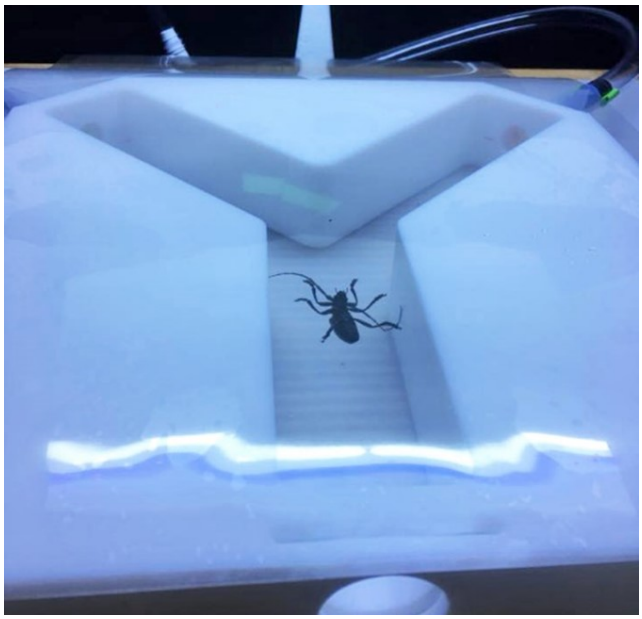

Figure 2. Olfactometer assay test of adult ALB to aldehyde components.

References

1. Zhang A, Oliver JE, Aldrich JR, Wang B, Mastro VC. Stimulatory beetle volatiles for the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky). Z Naturforsch C. 2002;57:553-558.
2. Crook DJ, Lance DR, VC Mastro. Identification of a potential third component of the male-produced pheromone of *Anoplophora glabripennis* and its effect on behavior. Journal of Chemical Ecology. 2014;40:1241-1250.
3. Wickham, JD, Xu, Z, Teale, SA. Evidence for a female-produced, long range pheromone pf *Anoplophora glabripennis* (Coleoptera: Cerambycidae). Insect Science. 2012;19:355-371.

Simple spread analysis of spotted lanternfly in Pennsylvania

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Summary

In order to support the spotted lanternfly, *Lycorma delicatula*, cross functional working group, and the USDA APHIS PPQ Plant Epidemiology and Risk Analysis Laboratory (PERAL), an analysis of potential spotted lanternfly spread for 2018 was conducted. This model was based on 2016 and 2017 SLF detection data. I estimated the mean radial expansion of spotted lanternfly in Pennsylvania based on the difference from the 2017 positive detection buffer to a 2016 positive detection buffer. The average expansion was approximately 26.5 km (16.5 mi).

Methods

I estimated annual radial spread of spotted lanternfly from 2016 to 2017 as a function of the displacement of annual positive detection buffers. I created buffers around positive detections for 2016 and for 2017 through the use of simple convex hulls, applying the tightest boundary to enclose the points. In order to focus primarily on natural spread (as opposed to human assisted spread) I removed three outlying detections in 2017 from the formation of the convex hulls (see most western and southeastern 2017 detections in Figure 1).

I measured the distance from these boundaries to the introduction point of the infestation (Figure 1) at transects radiating from the introduction point at 10° intervals [1]. At each of the 36 bearings, I subtracted the 2016 distance from the 2017 distance to get the annual displacement as an estimate of radial spread. I then applied the mean radial spread to the 2017 buffer to estimate a positive detection buffer for 2018 (yellow line in Figure 1).

Results

The mean estimated spread was 26.5 km (16.5 mi) and the maximum was 44.1 km (27.3 mi). In addition to estimating the potential 2018 buffer as displaced from the 2017 buffer by 26.5 km, I also applied the radial spread estimate to the three outlying detections in 2017 and joined those three buffers to the main estimated 2018 buffer (Figure 1). When combined with preferred host density, this buffer characterizes where 2018 surveys might be conducted in order to maximize efficiency.

Reference

1. Tobin PC, Liebhold AM, Anderson Roberts E. Comparison of methods for estimating the spread of a non-indigenous species. J Biogeogr. 2007;34:305-312. doi:10.1111/j.1365-2699.2006.01600.x

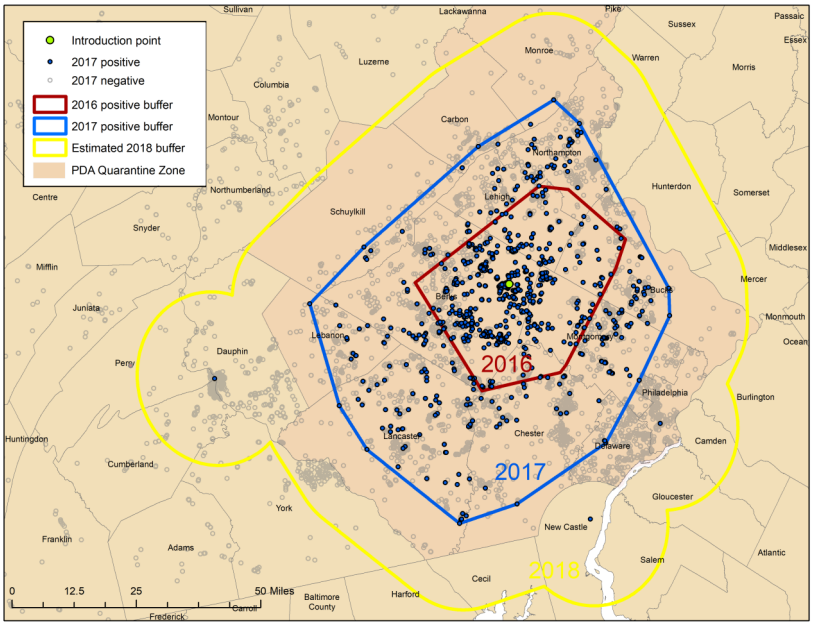

Figure 1. Potential spotted lanternfly, *Lycorma delicatula*, range expansion for 2018. PDA quarantine zone is Pennsylvania Department of Agriculture SLF quarantine zone as of 12/01/2017.

Gypsy moth regulated sawmill analysis

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Introduction

In 2014, APHIS PPQ's Gypsy Moth Program initiated a collaboration with state and industry representatives to formulate a new regulatory position regarding sawmills and the utilization of regulated logs received from within the quarantine area. The result of this collaboration was the development of a new analytical tool that provides the Gypsy Moth Program and its cooperators with transparent methodology for inferring sawmill compliance with regulatory policy by analyzing whether sawmills significantly contribute to elevated gypsy moth populations compared to the surrounding background population. Significant results from the analysis alerts program management to potential issues in the field, and allows program management and state cooperators to then decide on appropriate follow-up actions to ensure regulatory compliance.

Methods

Data: Regulated sawmill locations and 10 years (2007-2016) of gypsy moth trap data were obtained from the Slow the Spread Foundation. Trap counts that intersected treatment areas were removed from the analysis.

Statistical basis: The basis for the statistical analysis was Anselin's Local Indicator of Spatial Association (LISA), which is a measure of spatial autocorrelation. It was used to identify "hot spot" clusters of trap counts compared to the background population, or average trap count. Hot spots could be clusters of high values (HH) or low values (LL). The analysis also detected spatial outliers, which is useful in identifying a single trap with catch surrounded by traps with zero catches (which may be indicative of a new introduction). Outliers could consist of a high value surrounded by low values (HL) or a low value surrounded by high values (LH). The HH clusters and the HL outliers were of primary interest in the analysis.

Procedure: Sawmills were buffered by two km to represent their immediate environs affected by a pathways introduction of gypsy moth via transport and processing of regulated lumber. Sawmills that were within two km of one another were considered a single sawmill feature with a dissolved two km buffer. The background gypsy moth population was evaluated over a local environs created by a 24 km buffer around the mill, which also defined the extent of the LISA analysis.

Trap catch data within the local environs area for each mill were analyzed with neighboring traps defined by a 4 km, two year space-time window. Because multiple trap locations were tested, significance testing was adjusted for multiple comparisons by applying a false discovery rate. Significant HH or HL results within the immediate mill environs (i.e., 2 km buffer) indicated statistically elevated gypsy moth populations in proximity to the mill. Significant clusters were further analyzed to ensure that they were "spatially distinct" and separated by a sufficient distance from other clusters of matching years outside the mill's immediate environs. This ensured that the population cluster detected was not likely influenced by nearby dynamics outside the mill environs.

Results

For 2016, there were five sites of significantly elevated gypsy moth populations within sawmill immediate environs. In all cases, the results were single traps with catches of high value surrounded by traps with zero catch (HL outlier type). Three of the five sites were within proximity to the gypsy moth spread front (see Figure 1 for an example). The remaining two sites were in areas of low gypsy moth density.

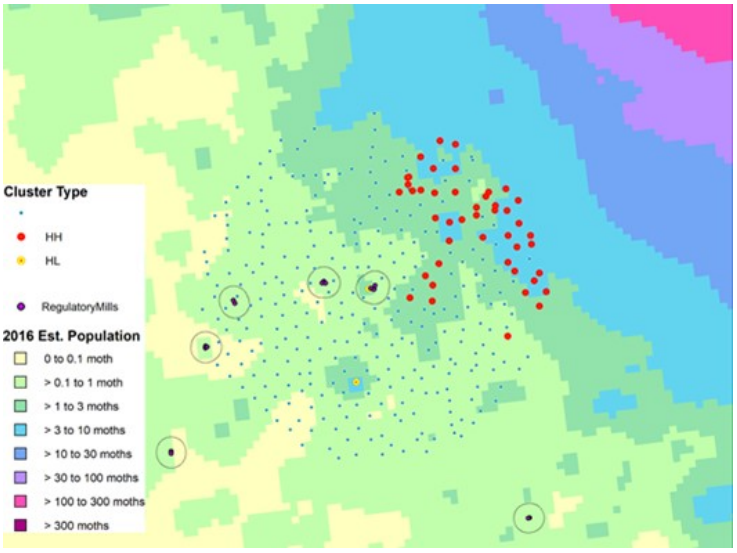

Figure 1. A significant HL outlier within a two km local sawmill environs (black circles). All sawmill environs shown encompass more than one regulatory sawmill. Also present are HH clusters in proximity to the gypsy moth population spread front and not associated with the sawmill.

Emerald ash borer national risk assessment

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Introduction

The emerald ash borer national risk assessment model provides an objective and transparent process for selecting *Agrilus planipennis,* EAB, emerald ash borer, trap locations by statistically modeling the detection likelihood of EAB as a function of environmental and anthropogenic covariates. The model was originally developed by the USDA Forest Service Forest Health Technology and Enterprise Team (FHTET) as a species distribution model using MaxEnt—a machine learning spatial modeling program. APHIS PPQ assumed responsibility for the model in 2016, and PPQ changed the modeling procedure to use an ensemble of statistical models in order to mitigate model uncertainty between statistical platforms. The MaxEnt model used only positive detections of EAB, but the current model uses positive and negative detection data, thus avoiding some major statistical assumptions regarding the data.

By guiding the deployment of traps, the EAB risk model benefits PPQ by increasing the likelihood of EAB detections outside the known infested area, increasing the capability of detecting EAB close to the date of a new attack, and finding locations that are best suited to implement biological control.

Methods and results

The modeling framework was a species distribution model conducted in the open-source Software for Assisted Habitat Modeling (SAHM) [1]. Species distribution models identify areas on the landscape with similar site characteristics to locations where the pest has already been found; these areas are estimated to have high likelihood of detecting the pest.

EAB detection data provided by APHIS PPQ and state cooperators included trap locations and positive detections (trap and visual) for 2011 through 2017.

Data were thinned so that all positive detections and all negative observations in the same year were at least 5 km apart in order to reduce spatial autocorrelation. Negative observations were delimited to within 60 km of positive detections so that the model would be trained only on geographic regions that the pest could reach based on its natural annual spread.

Site characteristics that were tested for relationships with EAB presence were a mix of environmental and anthropogenic factors: ash frequency, population density, distance from campgrounds, elevation, drainage index, household income, traffic volume, number of households with wood as primary fuel, topographic position index, distance from rest stops, four-year time lagged moisture deficit, and distance from any prior year positive detection.

The ensemble of models consisted of four statistical platforms: general linear model, random forest, multivariate adaptive regression spline, and boosted regression tree. The output from each was a nationwide map of detection probability. There were two model applications for each statistical platform: one using all site characteristics including distance from prior year positives (spread model) and one using all site characteristics except for distance from prior year positives (range model). A maximum overlay of the spread model and range model map outputs was computed for each statistical platform. The maps were then averaged across the statistical platforms to create the ensemble detection likelihood (Figure 1).

Recommended placement for traps for 2018 was determined by combining the detection likelihood with a host availability layer and a spatially balanced sample design. Each trap location was within a 1 km square grid cell, identified with a unique ID and also by the center point of the grid cell (latitude and longitude). Trap placement was limited to within 100 miles of the EAB quarantine zone (Figure 1).

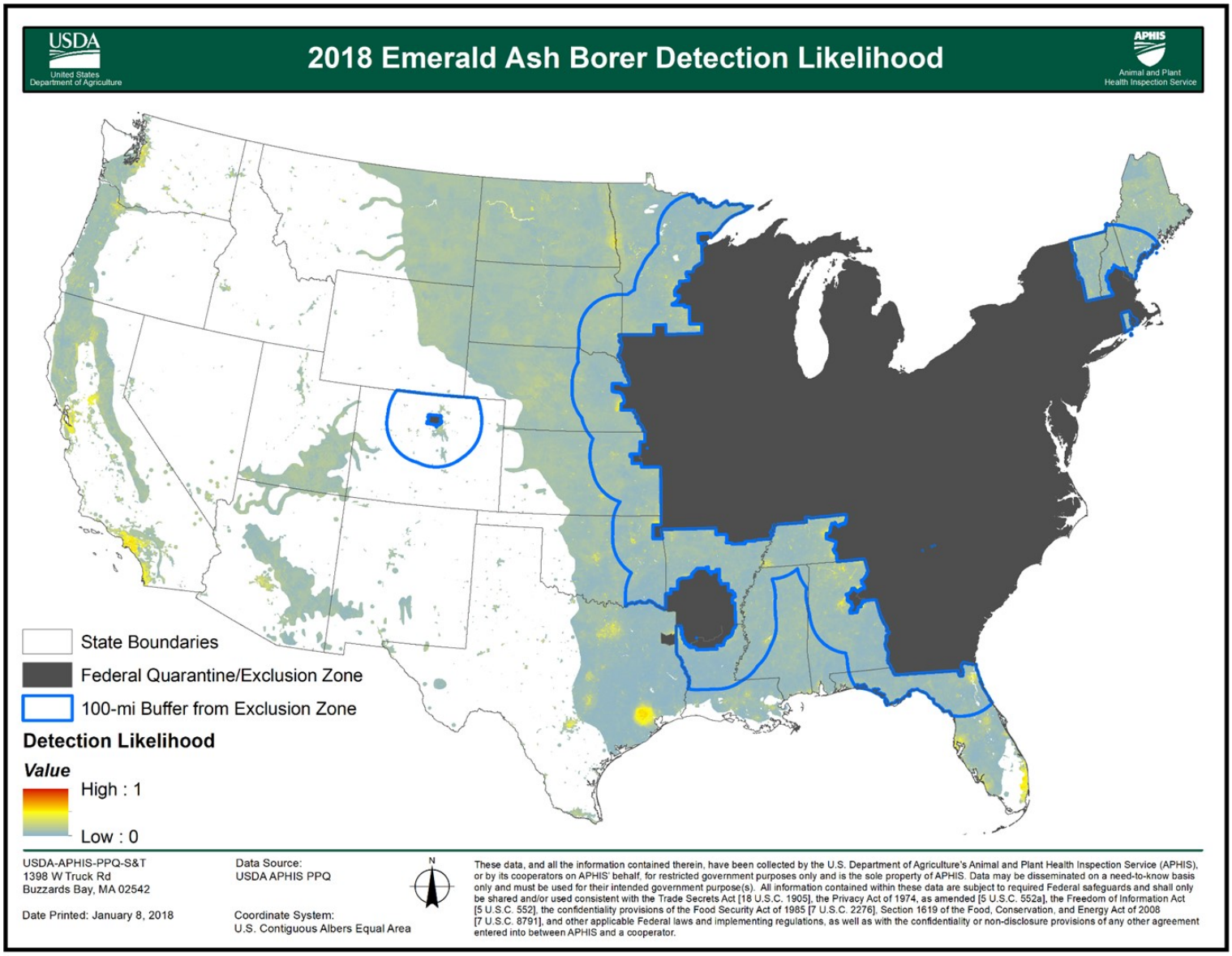

Figure 1. The 2018 emerald ash borer detection likelihood. Traps for 2018 were limited to within 100 miles of the federal EAB quarantine zone.

References

1. Morisette JT, Jarnevich CS, Holcombe TR, Talbert CB, Ignizio D, Talbert MK, Silva C, Koop D, Swanson A, Young NE. VisTrails SAHM: visualization and workflow management for species habitat modeling. Ecography. 2013;36:129-135. doi:10.1111/j.1600-0587.2012.07815.x

Update: Preferred host plant for rearing Asian citrus psyllid for mass production of *Tamarixia radiata*

Raju Pandey[1], Ruth Henderson[1], Gregory Simmons[2], Judith Herreid[3] and David Morgan[3]

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Asian citrus psyllid, ACP, *Diaphorina citri,* can feed, survive and multiply in many Rutaceous host plants. Various species of *Citrus*, and closely related species, such as Orange jasmine, *Murraya paniculata*, and curry leaf plant, *Bergera koenigii*, are widely used as host plants for rearing ACP and *Tamarixia radiata* in greenhouses. Curry leaf plant is the preferred plant host for raising ACP as a host for *T. radiata* in California. Seeds of curry leaf plants were collected from several sources. There were a wide range of variations among the curry leaf plants, but three of the most common phenotypes were identified based on leaf characteristics: 1) large leaf, 2) small leaf, and 3) dark leaf. Previously, we reported results of an experiment where the large leaf variety of curry leaf plant produced more *T. radiata* than the dark leaf or small leaf type plants. The California Department of Food and Agriculture (CDFA) plant production facility removed most of the small leaf variety of curry leaving the large leaf and dark leaf (Figure 1) as the predominant plant types being used for *T. radiata* production.

Figure 1. Dark leaf (left) and large leaf (right) varieties of curry leaf plant being used for *T. radiata* production.

Here, we report a follow-up study where *T. radiata* production data from CDFA greenhouses located within Calpoly University Pomona and Mt. Rubidoux Field Station site were analyzed. Information from the CDFA data archive was extracted for cages where the plant variety used was recorded. Data points from the cages where either large leaf or dark leaf variety was used were selected.

The number of *T. radiata* produced per cage for the two varieties was compared for each location as well as for combined sites by ANOVA procedures. *Tamarixia radiata* increase factor was calculated by dividing the total *T. radiata* collected by the number of *T. radiata* initially used for inoculation and analyzed by ANOVA procedures.

The Mt. Rubidoux facility has experienced technicians who have had an established production system for *T. radiata* since 2013; the Pomona site only started *T. radiata* production in late 2016. These factors might have contributed to higher *T. radiata* production per cage at the Mt. Rubidoux site than those at the Pomona site.

The large leaf cages outperformed the dark leaf cages at both Pomona and Mt. Rubidoux by 46% and 29% percent, respectively. When combined, the large leaf cages produced about 28% more *T. radiata* wasps than the dark leaf cages (Table 1).

The *T. radiata* increase factor (the number of progeny per parent used) was also higher for cages using large leaf plants. Increase factor was slightly higher at the Pomona site (17.8 for large leaf and 11.1 for dark leaf) when compared to the Mt. Rubidoux site (13.6 for large leaf and 9.8 for dark leaf). On average, *T. radiata* increase factor was around 16 on large leaf plants, compared to 10.2 on the dark leaf plants (Table 2). In a separate study, it was found that the dark leaf type plant produced fewer of the young flush leaves necessary for ACP egg laying compared to the large leaf variety. The new leaves remained suitable for ACP oviposition for longer on large leaf plants, which also might have helped to produce more ACP and thereby more *T. radiata*.

Using five to eight curry leaf and at least one *Citrus volkameriana* plant in a cage was a common practice adopted in the CDFA *T. radiata* mass production facility, with an assumption that *Citrus* plants would boost *T. radiata* production. Studies were conducted within CDFA greenhouses at the Mt. Rubidoux field station to evaluate the role of *Citrus* plants within a *T. radiata* production cage. Seven curry leaf plants were used as host plants in each cage. Cages were randomly assigned to one of three treatments: no *C. volkameriana* (control), one *C. volkameriana*, or two *C. volkameriana* plants per cage. Cages were inoculated with 300 ACP adults.

Citrus plants were removed from the cage just prior to inoculation with *T. radiata* so that each cage was left with seven curry leaf plants only. The study was repeated ten times. The number of adult *T. radiata* produced by each cage was recorded and analyzed by ANOVA procedures. The results showed that the highest mean number of *T. radiata* (1,129 wasps) was produced from the cages with no *C. volkameriana* followed by those with single *C. volkameriana* (981 wasps) and two *C. volkameriana* (841 wasps) plants; however, these differences were not statistically significant (Table 3).

Following these findings, CDFA has been working to replace the dark leaf plants by increasing the inventory of large leaf plants. CDFA has also stopped using *Citrus* within cages. Avoiding *Citrus*, a potential HLB/citrus greening disease carrier, will diminish any risk of accidental spread of HLB from the *T. radiata* rearing system. It will also simplify plant management in the greenhouses as only one type of plant needs to be grown.

Table 1. Comparison of *T. radiata* production on two varieties of curry leaf plants at CDFA production facilities.

Curry Variety	Pomona		Rubidoux		Combined	
	N	TR out	N	TR out	N	TR out
Large leaf	248	984	178	1,178	426	1,065
Dark leaf	158	673	332	911	490	834
	$F_{1,390}$, 34.9 $p < 0.001$		$F_{1,490}$, 13.5 $p < 0.001$		$F_{1,881}$, 39.3 $p < 0.001$	

Table 2. Comparison of *T. radiata* increase factor on two curry leaf varieties at CDFA production facilities.

Curry Variety	Pomona		Rubidoux		Combined	
	N	X-factor	N	X-factor	N	X-factor
Large leaf	248	17.8	178	13.6	426	16.0
Dark leaf	158	11.1	332	9.8	490	10.2
	$F_{1,390}$, 15.4 $p < 0.001$		$F_{1,490}$, 14.7 $p < 0.001$		$F_{1,881}$, 29.8 $p < 0.001$	

Table 3. Mean number of *T. radiata* wasps produced per cage over ten replicates with or without *C. volkameriana* as the host plant. $F_{2,18} = 1.32$, $p = 0.29$

No. of *C. volkameriana*	No. of curry leaf plants	*T. radiata* produced
0	7	1,129
1	7	981
2	7	841

Cold temperature storage of Asian citrus psyllid

Raju Pandey[1], Ruth Henderson[1] and Gregory Simmons[2]

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Asian citrus psyllid, ACP, *Diaphorina citri*, a vector of Huanglongbing, HLB, disease was first detected in Florida in 1998 and in California in 2008. Chemical pesticides and biological control agents are among the most important pest control tools used to directly suppress ACP populations. Research activities to explore the possibility of employing sterile insect technique (SIT) has received some attention. Our ability to keep the insect alive while away from its host plays a key role in the success of a potential SIT program. A preliminary study was conducted to identify the best temperature regime and storage environment for ACP adults. The two-factor study included:

- Four storage temperature regimes: refrigerator (35-42°F), incubator (64°F), wine chiller (49-52°F), and room temperature (74-78°F)
- Three moisture sources: 0.5 ml 2% agar gel, cotton ball soaked with 2 ml water, and no moisture (control)

Ten newly-emerged ACP adults were collected in a 40-dram vial provisioned with one of the moisture treatments. Vials were assigned to one of the temperature treatments. Daily mortality of the ACP adults was recorded. The study was repeated seven times. ACP mortality data after one, three, and seven days of storage was analyzed by ANOVA procedures.

Provision of moisture source was crucial for ACP survival. A significantly higher rate of ACP adult mortality was observed in the absence of a moisture source (control vials) under all storage environments ($F_{2,234} = 35.2$, $p < 0.001$). Mortality rate between the agar gel and cotton ball treatments was not significantly different (Table 1).

Mortality rates did not differ significantly between the storage within wine chiller and incubator. Similarly, mortality rates between the room temperature storage and refrigerator storage were not significantly different (Table 1). Storing ACP adults between 48 to 64°F led to significantly lower mortality when compared with the mortality rate at refrigeration (35-42°F) as well as at room temperature (74-78°F) (Figure 1).

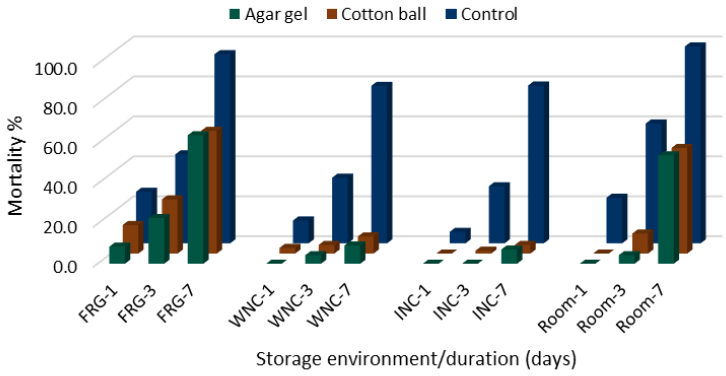

Figure 1. ACP adult mortality rates under various storage conditions, 2017. (FRG = Refrigerator; WNC = Wine chiller; INC = Incubator; Room = Room temperature).

Table 1. ACP adult mortality rates under various storage conditions, 2017.

Storage Environment	Storage Temperature	Storage Period (days)	ACP mortality		
			Agar gel	Cotton ball	Control
Refrigerator	35-42°F	1	8.6	14.3	25.8
		3	22.9	27.1	44.5
		7	64.3	61.4	94.7
Wine chiller	49-52°F	1	0.0	2.9	11.4
		3	4.3	4.3	32.9
		7	9.2	8.6	78.8
Incubator	64°F	1	0.0	0.0	5.7
		3	0.0	1.4	28.6
		7	7.1	4.3	79.0
Room	74-78°F	1	0.0	0.0	22.9
		3	4.3	10.0	60.0
		7	54.3	52.9	98.6
Environment (E) $F_{3,234} = 11.6$ ($p < 0.001$)		Temperature (T) $F_{2,234} = 35.2$ ($p < 0.001$)		E x T $F_{6,234} = 0.51$ ($p = 0.8$)	

Techniques for greenhouse production of *Tamarixia radiata*

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Asian citrus psyllid, ACP, *Diaphorina citri*, a vector of Huanglongbing disease, was first detected in Florida in 1998 and subsequently in California in 2008. The parasitoid *Tamarixia radiata* was imported from Pakistan as a biological control agent for ACP, and is now being mass produced for field releases in California. The ACP nymphs raised on curry leaf plants are used for *T. radiata* productions in California. Two studies were conducted at CDFA greenhouses at Mt. Rubidoux to streamline current *T. radiata* production practices.

ACP inoculation practice

Based on abdominal coloration, the adult ACP can be broadly divided into three morphotypes: gray-brown, blue-green, and orange. Because ACP eggs are orange in color, the orange colored adults were often considered "mature females" filled with eggs (Figure 1). Therefore, extra efforts were made to collect "*orange bellied*" (OB) ACP to assure that each cage setup received at least 100 of them. In this study, two treatments were compared: all 300 regular ACP per cage and 200 regular ACP along with 100 orange bellied ACP per cage.

Nine curry leaf plants were used as host plants in each cage. When the nymphs produced on these plants reached 3rd and 4th instar, 100 *Tamarixia radiata* adults were added in each cage. There were 39 cages set up for each of the treatments. The number of *T. radiata* produced in each cage was counted. Data were analyzed by ANOVA procedures.

The cages inoculated with 200 regular ACP and 100 orange bellied ACP produced an average of 665 *T. radiata* per cage, while the control cages inoculated with 300 regular ACP adults produced an average of 675 wasps. The *T. radiata* production from these two treatments was not statistically different (Table 1).

***Tamarixia radiata* inoculation rate**

Once the nymphs within the cage reach the desired stage (3rd and 4th instars), *T. radiata* adults are typically introduced into the cages in two batches of 50 (100 total wasps) without recording the sex ratio.

This study was conducted to explore if the practice can be improved by modifying the release strategy. Five treatments were evaluated: single release of 50 females, two split releases of 50 or 75 females and three split releases of 100 or 125 female wasps (along with a few male wasps) (Table 2).

Figure 1. ACP color morph regular (left) and orange bellied (right).

The first release was made when ACP nymphs reached 3rd or 4th instar and subsequent releases were made two days after the previous release. Two replications were set up each week for three consecutive weeks, leading to a total of six replications. *Tamarixia radiata* were collected and counted when they emerged. Increase factor was calculated by dividing the total *T. radiata* produced per cage by the number of total females used in inoculation. Data were analyzed by ANOVA procedures.

The largest numbers of *T. radiata* were produced when cages were inoculated with A) 50 females as a single release, B) 75 *T. radiata* females (split into releases of 25 and 50), and C) 50 *T. radiata* females (two releases of 25 each); however, these differences were not statistically significant (Table 2).

Inoculating cages with 100 or 125 females in three split releases led to production of significantly fewer *T. radiata* compared to the other treatments (Table 2). The low production in cages inoculated with a higher number of *T. radiata* females may be attributed to host feeding, resulting in fewer ACP nymphs available for parasitization.

Following the findings of these studies, the extra effort to collect orange belly ACP has stopped, saving time and labor without any adverse impact on *T. radiata* production. Similarly, the practice of *T. radiata* inoculation has also changed. The cages are being inoculated with either 50 to 75 adult females (along with a few males) usually through two split releases.

Table 1. Mean *T. radiata* production from cages inoculated with and without orange bellied ACP. $F_{1,60} = 0.1$, $p = 0.75$

Treatment	OB ACP	Reg. ACP	Total ACP	No. treatments	*T. radiata*
Orange belly (OB)	100	200	300	39	665
Regular ACP (Control)	0	300	300	39	675

Table 2. Effect of different *T. radiata* inoculation rates on its production.

T. radiata released			Total *T. radiata* used	*T. radiata* produced	Increase factor
First	Second	Third			
50	0	0	50	1,400 a	28.0 a
25	25	0	50	1,164 ab	23.3 b
25	50	0	75	1,352 a	18.0 c
25	50	25	100	919 bc	9.2 d
25	50	50	125	935 c	7.5 d
				$F_{4,14}$, 16.1 $p < 0.001$	$F_{4,14}$, 102.7 $p < 0.001$

Update: Evaluating *Citrus* varieties to optimize *Tamarixia radiata* production methods

Ruth Henderson[1], Raju Pandey[1] and Gregory Simmons[2]

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When mass-production of *Tamarixia radiata* in field cage insectaries began in 2013, the most readily available *Citrus* varieties were sweet oranges, lemons, and grapefruits in commercial orchards. When making initial comparisons, sweet oranges produced noticeably fewer *T. radiata* on average (Figure 1).

After 2015, sweet oranges were no longer used as field cage insectary hosts, with the exception of a few experimental cages. In 2016, testing of un-grafted rootstock varieties began using trees planted in the Cal Poly Pomona experimental plot three years earlier. Additionally, 2016 was also the year that split *T. radiata* inoculations based on host infestation level became the standard inoculation method. When all production data from previous years were used to compare between all host varieties being used, production of *T. radiata* in cages using rootstock varieties appeared to be much higher than in cages using even the best scion (grafted) varieties.

However, it was not clear whether this was due to rootstock varieties actually being better hosts for production, or if the difference was due to all rootstock cages benefitting from improved rearing practices, unlike the early field cages which all used scion varieties. Here, production data from 2016 and 2017 are compared for the two most productive scion varieties available (grapefruits and lemons), along with four rootstock varieties (macrophylla, rough lemon, sour orange, and volkameriana). Carrizo citrange and trifoliate, two rootstock varieties tested in 2016, were not favorable field cage hosts due to poor flush growth after pruning and are also no longer used. Although per cage production of *T. radiata* did not differ statistically between varieties (ANOVA; $p = 0.28$, $F = 0.34$), most of the rootstock varieties, most noticeably sour orange, produced more *T. radiata* per cage than the two scion varieties on average (Figure 1). When looking at how many *T. radiata* are produced in each harvest, however, this gap closes, and all hosts examined produce similar amounts (ANOVA; $p = 0.89$, $F = 0.34$) (Figure 2).

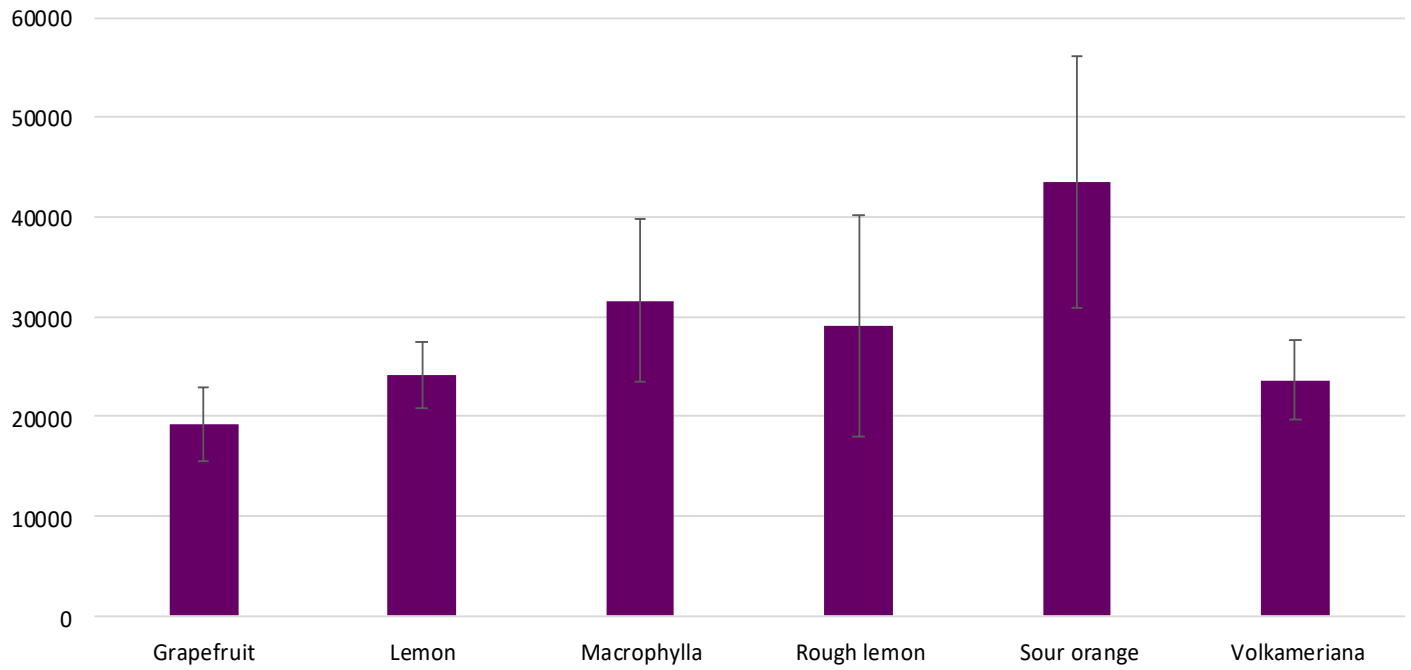

Figure 1. Average number of *T. radiata* produced per cage (total from all harvests) on two scion and four rootstock varieties. Error bars represent standard error.

The main difference between scion and rootstock varieties as hosts is the number of times the same plant can be re-harvested. While only 11% of grapefruit hosts were harvested a second time, 100% of sour orange were harvested twice, and 75% were harvested a third time (Figure 3). Rootstocks, bred for vigorous growth, appear to be better suited for multiple consecutive harvests—in which new growth is infested by ACP and then clipped off repeatedly—than scion varieties.

Our observation has been that although overpopulation by ACP can sometimes kill newly re-gown flush between harvests in rootstock field cages, the plants do continue to grow new flush, whereas flush production seems to decrease more noticeably after harvests on scion varieties. While scion varieties are still valuable hosts for *T. radiata* production, especially as they are more widely available as mature trees, rootstock varieties show great promise for the long term efficiency of field cage insectaries.

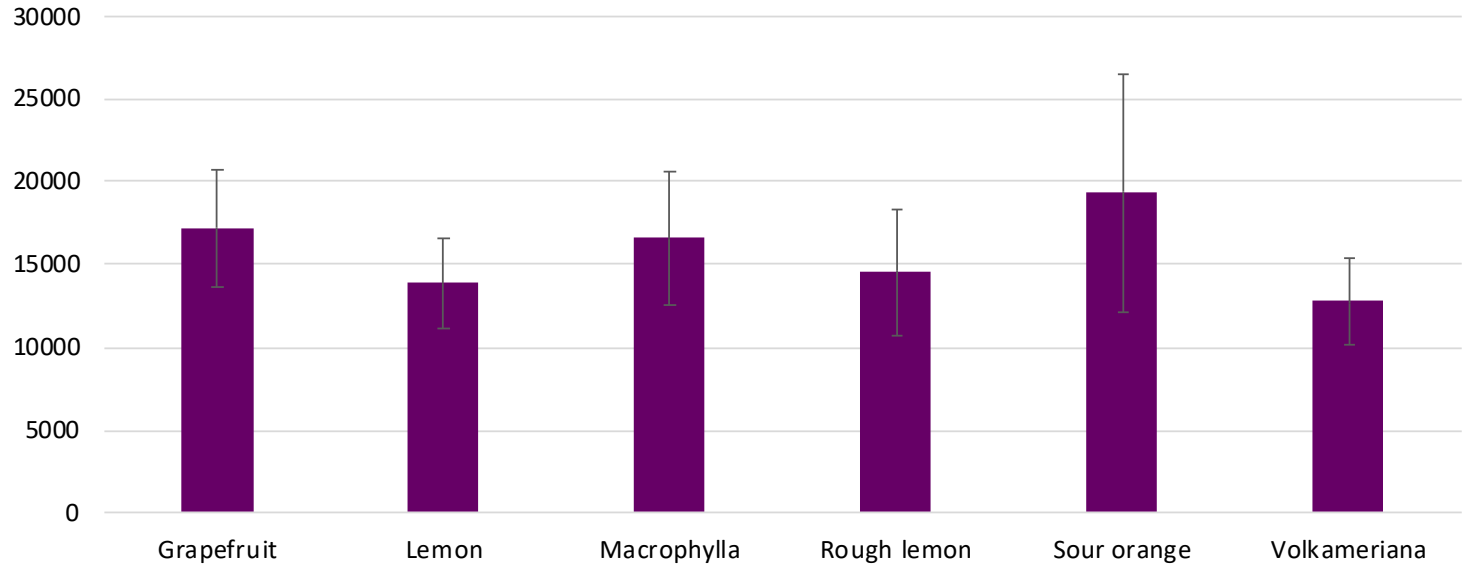

Figure 2. Average number of *T. radiata* produced per harvest on two scion and four rootstock varieties. Error bars represent standard error.

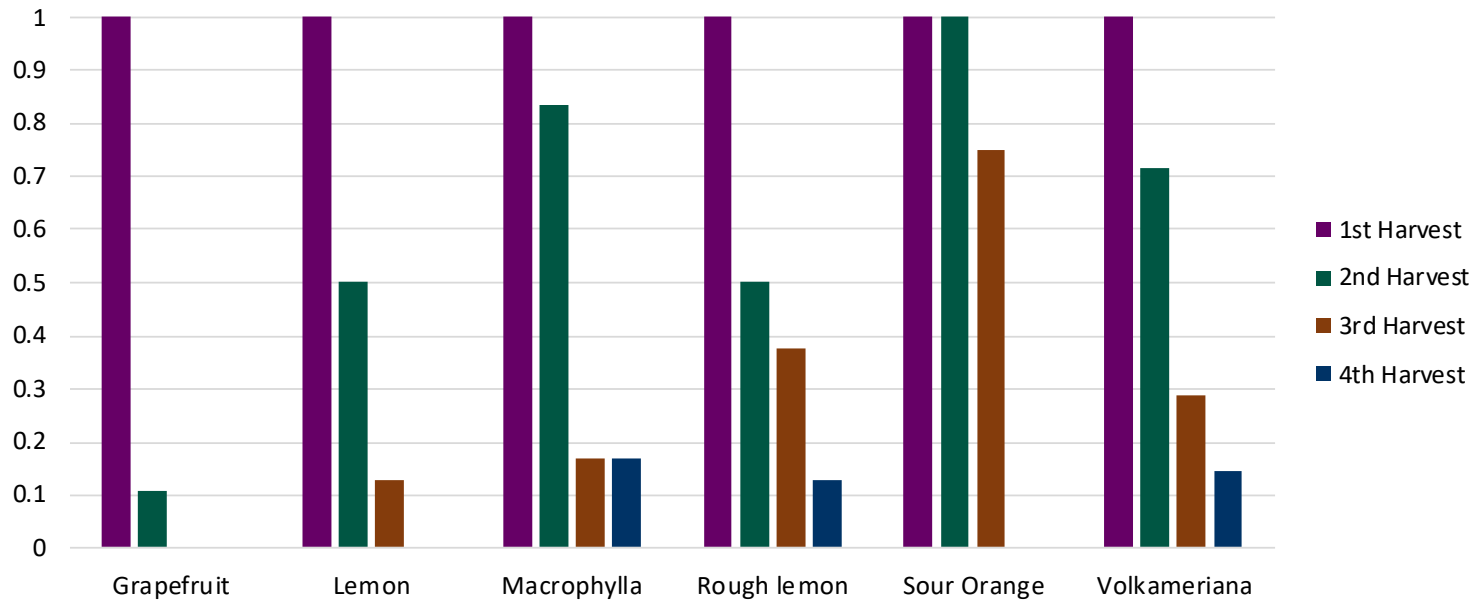

Figure 3. The percent of cages that underwent 1st, 2nd, 3rd, and 4th consecutive harvests when using different *Citrus* varieties.

Development of the sterile insect technique as a control tactic for Asian citrus psyllid

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The Asian citrus psyllid, ACP, *Diaphorina citri*, is well established in large citrus production areas of Southern California. It is a serious citrus pest because it vectors the bacterium *Candidatus Liberibacter asiaticus* [1] which is responsible for Huanglongbing disease, HLB [2,4]. The area-wide application of pesticide treatments for ACP is the current strategy for managing the threat of disease establishment in commercial citrus orchards. In ACP infested areas outside of commercial citrus production areas this control strategy is impractical, both because of the size of the area needing treatment and concerns over the wide-spread use of pesticides in urban areas. In these areas biological control approaches are considered the only options. Long-term and sustainable area-wide management of ACP will require additional tools to develop effective control strategies [3]. Development of the Sterile Insect Technique (SIT) for ACP would add an additional tool that could be effective in eradicating new infestations outside of current quarantine areas and would provide sustainable methods for long-term management of ACP in commercial and urban areas.

The main objectives of this project were to determine the radiation dose for ACP that is sufficient to induce sterility when mating wild and irradiated ACP and to test the longevity and mating competitiveness of irradiated ACP in the laboratory.

The secondary objective was to develop methods for collection, handling, storage and irradiation of ACP.

In 2017 the Stouthamer Laboratory from the University of California Riverside (UCR) performed several laboratory experiments to test the sterility and competitiveness of irradiated ACP. The ACP were shipped under a California Department of Food and Agriculture plant pest movement permit for irradiation at the USDA APHIS PPQ CPHST Laboratory in Salinas, California. A total of 17,472 ACP were received for irradiation.

Inducing sterility in ACP

Prior to irradiating ACP, we conducted multiple dosimetry studies on two Rad-Source™ X-Ray irradiators to ensure the desired dose would be uniformly administered. A RadCal Ion Chamber with calibration based on a NIST (National Institute of Standards and Technology) traceable standard was used to verify the absorbed doses of ACP within the plastic vials used for the assays.

Our first assays sought to determine the sterilizing dose required to induce a reduction in ACP fertility, using irradiated mixed groups of males and females. We tested irradiation doses ranging from 0 to 150 Gy. We found the egg hatch was reduced to near 0% hatch at a dose of 70 Gy (Figure 1).

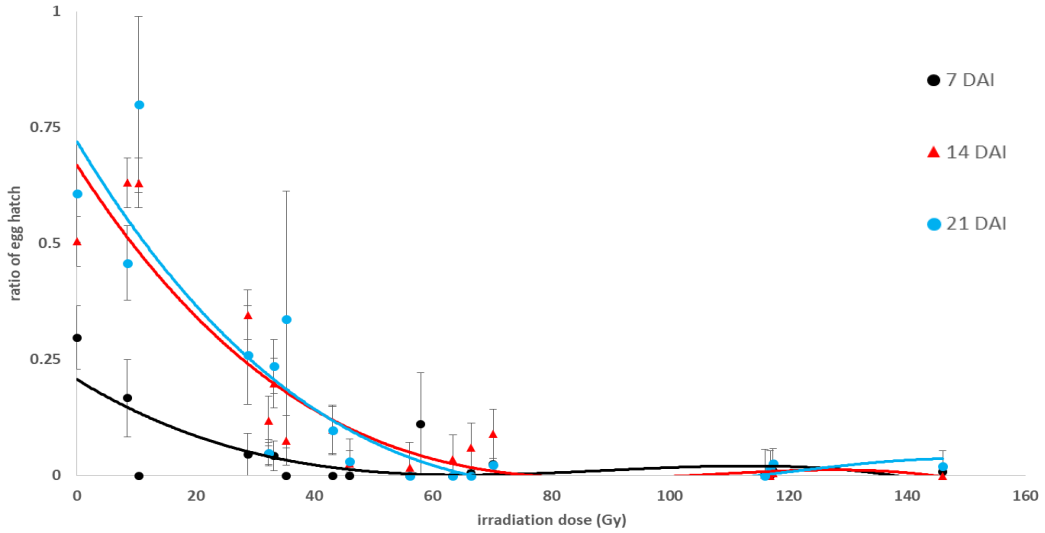

Figure 1. Ratio of eggs hatched to eggs laid by ACP females irradiated at different dosages (from 8.5 up to 146 Gy) and non-irradiated both on *M. koenigii* seedlings during different observation periods, indicated by days after irradiation (DAI). Plant exposure time to ACP was always seven days.

We also found that the mortality of the ACP adults 24 hours after being shipped and irradiated never exceeded 18% for any irradiation dosage.

For the next set of assays, we determined the single sex sterilizing dose for both females and males when crossed with fertile ACP of the opposite sex. Only the data for the irradiated female crosses are reported here. For the irradiated (I) females, we crossed them with untreated (U) males (I♀ x U♂) irradiated at a range of doses from 40 to 100 Gy. Single pair ACP couples were set up in glass vials containing *Murraya koenigii* seedlings.

For these crosses, we did not find any differences in oviposition between irradiation treatments, however, there were significant differences in the percentage of hatch between the irradiated and control treatments (Figure 2). Only a few eggs were able to hatch in the 40 Gy irradiation treatment in the first days of oviposition (Figure 2).

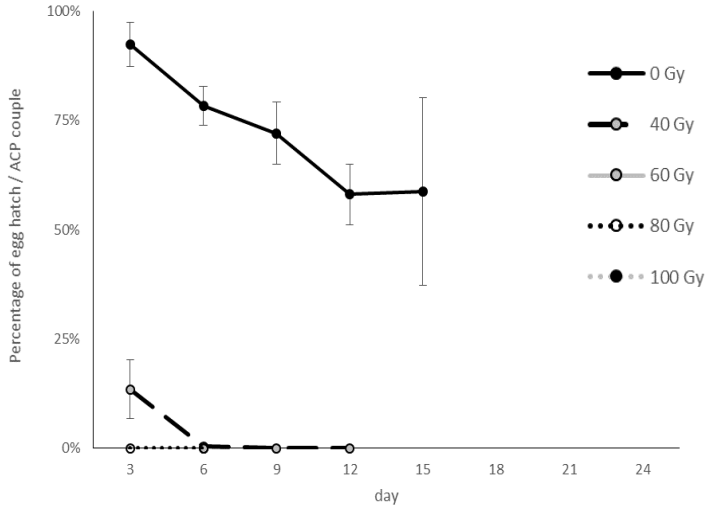

Figure 2. Average of the percentage of egg hatch from ACP females irradiated at different dosages (40 Gy, 60 Gy, 80 Gy, and 100 Gy) and non-irradiated (0 GY), every three days throughout the lifespan of the couple. Couples were individualized on *M. koenigii* seedling.

Survival of irradiated ACP

The Stouthamer Laboratory also performed some experiments to obtain the survival curves and longevity of the ACP males and females irradiated at different dosages. There were significant differences in the survival of the females irradiated at 60 and 100 Gy when compared with the control females. However, there were no significant differences in the survival of the males irradiated (40-100 Gy) compared with the control males.

Methods to increase ACP survivorship

We also developed some methodology to handle the ACP before and after being irradiated. In order to optimize the survival of the ACP adults in the shipments, some simulations were performed with different inserts in the shipping vial (vermiculite: perlite, wet filter paper, plastic filaments and *M. koenigii* leaf). The differences between treatments were not significant and the simplest treatment (a wet filter paper hold with the lid on the top of the vial) was selected to ship the ACP adults.

Another methodology that was optimized by the Stouthamer Laboratory was obtaining ACP virgin adults during rearing. This is difficult because adults can mate in the first 24 hours after emergence and it is not possible to visually sex the nymphs at any stage. Therefore, it was necessary to isolate the ACP individuals at the nymph stage and sex them after emergence. We prepared a full protocol to conduct the isolation and increase the adult survivorship during the shipments.

References

1. Bové JM. Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. Journal of plant pathology. 2006 Mar;1:7-37.
2. Halbert SE, Manjunath KL. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. Florida entomologist. 2004 Sep;87(3):330-53.
3. Hall DG, Gottwald TR. Pest management practices aimed at curtailing citrus huanglongbing disease. Outlooks on Pest Management. 2011 Aug 1;22(4):189.
4. Hung TH, Hung SC, Chen CN, Hsu MH, Su HJ. Detection by PCR of *Candidatus Liberibacter asiaticus*, the bacterium causing citrus huanglongbing in vector psyllids: application to the study of vector–pathogen relationships. Plant Pathology. 2004 Feb 1;53(1):96-102.

Phenology and classical biological control of Asian citrus psyllid in Arizona and preliminary evaluation of ACP predators

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Introduction

The Asian citrus psyllid, ACP, *Diaphorina citri*, is one of the most significant threats to citrus production worldwide, largely due to its role as a vector of the pathogens (*Candidatus Liberbacter* spp.) associated with Huanglongbing disease, HLB. Areas where both ACP and HLB have become widespread, such as Brazil and Florida, have suffered dramatic economic losses via reduced fruit yield and quality, extensive tree mortality, and increased production costs. Until early detection methods or disease treatments become available, HLB management hinges on limiting vector populations [1]. *Tamarixia radiata*, a parasitoid of ACP, was introduced from its native area (Punjab, Pakistan) in 2011 and has been mass reared since then at the Insectary & Quarantine Facility at the University of California Riverside (UCR) in order to develop a biological control program against this pest in Southern California and Arizona [2]. More recently, there has been interest in either amplifying the impact of resident predatory insects or employing inundative releases of commercially available predators into ACP hotspots to further suppress psyllid populations. This could provide an additional method for ACP biocontrol that may be of particular value in residential buffer areas around HLB detections where pesticide applications may be limited. Three resident predators are being evaluated:

1. *Diomus pumilio,* the longblack ladybird, a small coccinellid beetle that appears to be a specific psyllid predator, originally introduced from Australia against the Acacia psyllid in the 1970's and established in California [3,4]
2. *Chrysoperla comanche*, the green lacewings, a resident generalist predator that also feeds on ACP in citrus orchards [4]
3. *Chrysoperla rufilabris*, which is a commercially available lacewing

Production of *T. radiata*

To prevent the loss of genetic variants that may prove essential to the establishment of *T. radiata* in the field, rearing is carried out by keeping 16 distinct small populations that were collected from different sites in Pakistan.

Individuals from these populations are then placed into a mixing cage and allowed to mate randomly [5], and the "hybrids" that result from this mass cross-mating are allocated to partners for field releases (e.g., USDA) and to provide starter material to mass-rearing startup operations (e.g., California Department of Food Agriculture, Citrus Research Board, Foothill Agricultural Research Facility insectaries). In 2017, a total of 666,136 wasps were produced at UCR and allocated to partners as shown in Figure 1.

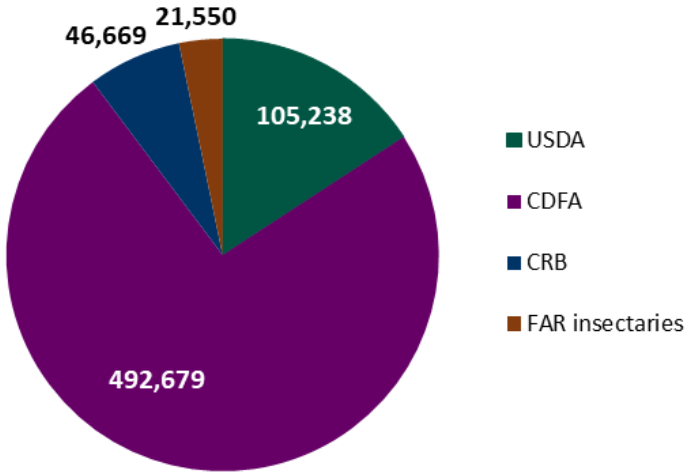

Figure 1. Allocation of *T. radiata* mass reared at UCR to partners in 2017.

Biological control of ACP in Arizona

About 1,600 wasps are shipped from UCR to USDA-APHIS in Yuma, Arizona every week using FedEx's First Overnight service at a temperature between 60 to 64°F in eight plastic tubes containing 200 wasps. Mortality of wasps during shipments is approximately 1 to 3%. These wasps are then released on 32 trees in four zones (three in Yuma County, one in Mohave County), resulting in one release per month per zone. After release, eight additional trees in each zone are visually surveyed to check ACP density and parasitization of ACP by *T. radiata*. Furthermore, 64 yellow sticky traps are used to catch ACP adults for determining the sex distribution. Mean monthly number of eggs, small and large nymphs, and adult ACP per tree were used to determine cumulative insect days (i.e., the cumulative count of each ACP stage per tree per day over sampled dates) to estimate the ACP population load. Currently five citrus varieties are being surveyed: grapefruit, lemon, lime, orange, and tangelo.

Data collected from 2015 in Yuma County and from 2016 in Mohave County from trapping and visual surveys have proven useful to reconstruct the phenology of ACP in the Arizonan desert. Based on visual survey and trapping data, we have determined that in both 2015 and 2016 ACP populations build-up as early as February and the exponential increase of the ACP population stops around mid-June [160 Julian Days (JD)]. The extremely hot desert weather coupled with lack of new flushes is very likely the cause of the end of exponential increase seen in June. ACP was recorded in higher numbers in 2016 in both counties, in particular in Yuma County. This could be explained by an exceptional storm on September 8, 2015, where almost two inches of rain fell at a time of the year when it usually does not rain at all. The data collected showed no effect of the host citrus species on the increase of the ACP populations. However, these results may be an artifact of the current survey protocol, which does not currently follow flushing and thus cannot account for the differences in flushing patterns among varieties. Average parasitism rate of ACP by *T. radiata* reached a peak of 33.3% on 2016 at 195 JD, whereas no parasitism was recorded in 2017 (Table 1). This result may be an artifact of the current release and survey protocol. By surveying trees that are not the specific trees where *T. radiata* are released, and therefore may not be infested by ACP, the impact of the parasitoid where ACP is present could result in an underestimation. Regarding the dispersal of *T. radiata*, the parasitoid has been recovered at least once at 23 of the 32 survey sites, indicating that the parasitoid is able to move at least over the short distances between release and survey trees.

Evaluation of predatory insects as an additional element of ACP biocontrol

In the summer of 2017 laboratory colonies were established for *D. pumilio* and *C. comanche*; these predators are reared on ACP eggs infesting curry leaf plants, and on frozen *Ephestia kuehniella* eggs, respectively.

Table 1. Parasitization of ACP by *T. radiata* in Arizona.

Year	Zone	Maximum average parasitism rate	Julian Day
2015	1	11.1%	**225**
	2	—	—
	3	—	—
	4	16.7%	**174**
2016	1	25.0%	**143**
	2	33.3%	**195**
	3	1.1%	**151**
	4	—	—

During late summer and fall, 2017 two preliminary field cage trials were carried out at UCR's Agricultural Operations experimental field. In the first trial, *D. pumilio* was released in six cages that were assigned to two treatments (1 or 5 beetles per ACP colony) with the goal of evaluating the effect of the predators on reducing the size of ACP colonies. This trial showed that the beetle is more effective in ACP colonies with larger proportions of eggs, and that five beetles per colony have a stronger effect than one beetle per colony. In the second trial, the three predators were tested simultaneously. All predators were released at a density of five per ACP colony (beetles as adults and lacewings as 1st instar larvae). *Chrysoperla comanche* was released in four cages, *C. rufilabris* and *D. pumilio* in two cages each, and the remaining two cages were used as controls. All predators were released when ACP colonies were mostly at the egg stage, and flushes were heavily infested. After 10 days the flushes were scored as alive or dead, and the number of ACP nymphs was recorded on the flushes determined to be alive. Nearly all flushes in the control cages were dead, given the high density of ACP feeding. Whereas in the cages where predators were released, there was a higher proportion of flushes alive as predators had reduced the density of ACP. *Chrysoperla comanche* was overall the most effective predator.

Work in progress

Greenhouse experiments are being prepared to further evaluate the predators. Ways to improve current release and survey protocols in Arizona are being discussed. In particular, the following are being considered:

1. The use of yellow sticky traps and DNA sequencing to not only count and sex ACP adults, but also to check dispersal of *T. radiata* to confirm ACP origin
2. The use of steel hoop to determine the presence or absence of flush growth during visual surveys by conducting four hoop samples per tree

References

1. Grafton-Cardwell EE, Stelinski LL, Stansly PA. Biology and management of Asian citrus psyllid, vector of the Huanglongbing pathogens. Annu Rev Entomol. 2013;58(1):413-32.
2. Hoddle MS, Hoddle CD. Classical biological control of Asian citrus psyllid with *Tamarixia radiata* in urban Southern California. Citrograph. 2013 March/April;4(2):52-8.
3. Dreistadt SH, Hagen KS. Classical biological control of the acacia psyllid, *Acizzia uncatoides* (Homoptera: Psyllidae), and predator-prey-plant interactions in the San Francisco Bay area. Biol Control. 1994;4:319-27.
4. Goldmann A, Stouthamer R. Identification and characterization of two candidate predator species for ACP biocontrol. Citrograph. 2017 Fall;8(4):52-6.
5. Soper A, Hare J, Withman T, Manzo D, Stouthamer R. Preserving genetic variability in mass-rearing *Tamarixia radiata* to control ACP. Citrograph. 2014 Fall;5(4):58-63.

Producing *Tamarixia radiata* for classical biological control of Asian citrus psyllid

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Field Cage Production of *Tamarixia radiata*

In 2013, efforts by the Citrus Research Board (CRB) biological control group to mass-produce *Tamarixia radiata* in large field cage insectaries began as a rapid, low-cost means to provide agents to the Southern California Asian citrus psyllid, ACP, *Diaphorina citri*, classical biological control program. Each year, production of *T. radiata* in field cages has increased. Whereas less than 120,000 wasps were produced in 2013, over 860,000 were produced in 2017 (Figure 1).

Over 800,000 wasps were provided to CDFA for releases in Southern California, and an additional 14,600 for biological control efforts in Yuma, Arizona. Remaining wasps were used as additional starter material for field cages or were used in small-scale laboratory experiments. Overall, the program produced over three million *T. radiata*, over 20% of which were produced by the CRB program (Table 1).

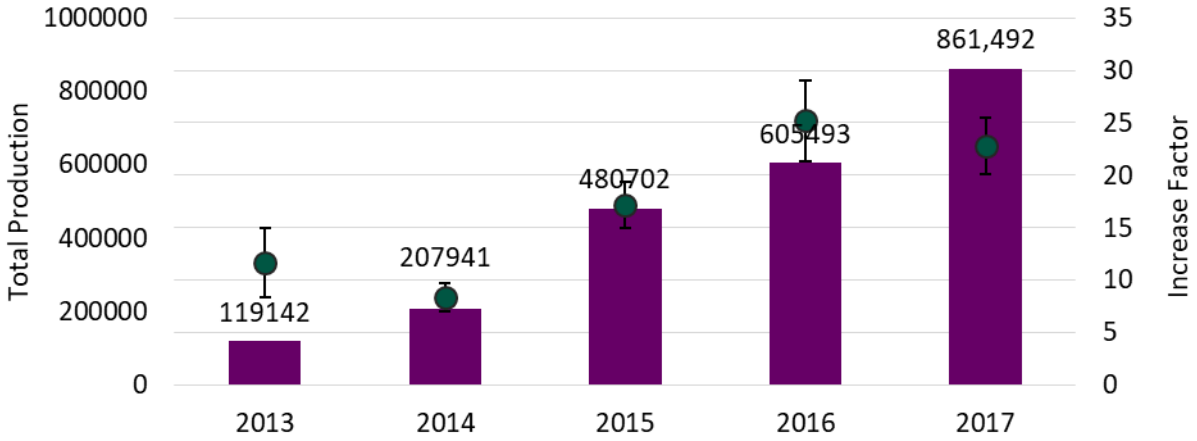

Figure 1. Yearly total *Tamarixia radiata* production (columns) and average increase factor (dots) in field cage insectaries. Error bars represent standard error.

Table 1. Yearly *Tamarixia radiata* production by cooperating organizations. Note: program contributions from Foothill Agricultural Research Facility Corona California, a private insectary, ended in April, 2017.

Produced by:	2013	2014	2015	2016	2017	Total
CRB Lab/GH	—	32,515	30,195	1,526	17,335	**81,571**
CRB Field Cages	119,142	207,941	480,702	605,493	861,492	**2,274,770**
FAR	—	137,524	265,961	147,850	205,383	**756,718**
UCR	161,057	296,881	165,445	523,015	650,748	**1,797,146**
CDFA	60,626	963,373	1,355,240	990,290	2,045,350	**5,414,879**
Total Produced	340,825	1,638,234	2,297,543	2,268,174	3,780,308	**10,325,084**

Thirty-two cages were constructed for *T. radiata* production between March and October, 2017 to be harvested one or more times. Additionally, four cages constructed in 2016 were re-harvested in early spring for a total of 54 harvests in 2017. Fourteen cages were set up using rootstock varieties, planted in an experimental plot at Cal Poly Pomona. Eight cages were set up using grafted lemon varieties at the same field site. Another eight cages were built in a commercial grapefruit orchard near Mentone, CA. Two experimental cages at the Cal Poly Pomona site used curry leaf and two used Valencia oranges. On average, each field cage produced just over 23,000 *T. radiata*, with the most productive cage of the season producing 69,652 wasps over the course of two harvests (Table 2). On average, the per cage increase factor (number of wasps produced per wasp used in inoculation) was 22.8 (Figure 1). Production of *T. radiata* varied seasonally, with peak production coinciding with the hottest days in August (Figure 2). To continually improve the efficiency of field cage insectaries, we use best-known practices for selecting host plants, inoculating cages with insects, and maintaining insect populations within field cages.

Production data from past seasons have shown that among citrus varieties tested, lemons and grapefruits are the most productive scion-type hosts, and rough lemon, volkameriana, sour orange, and macrophylla are good hosts among ungrafted rootstock varieties. As such, these varieties are selected for use in field cage insectaries whenever an experiment does not call for another selection. We also continue to inoculate at a rate of one *T. radiata* per 50 nymphs and to split *T. radiata* inoculations into an early release on 2nd and 3rd instar nymphs and a main release on 3rd and 4th instar nymphs in order to maximize parasitism. One new field cage practice that we have begun to use is applying one light spray of 1% Dawn dish soap in water solution to rootstock host plants after harvests. This kills some, but not all of the remaining adult ACP in the field cage and prevents overcrowding of nymphs on post-harvest flush growth, which sometimes kills flush and reduces *T. radiata* production in second or third harvests.

Table 2. Annual production figures for field cage rearing of *Tamarixia radiata*.

Year	No. cages	No. harvests	Avg. TR per cage (±SE)	Max. TR per cage	Avg. increase factor (±SE)	Max. increase factor	Total TR produced
2013	13	18	9,164(2,908)	32,418	11.6(3.3)	36.0	**119,142**
2014	35	39	5,830 (970)	22,520	8.4(1.3)	28.9	**207,941**
2015	46	58	10,411(1,308)	47,588	17.2(2.2)	66.5	**480,702**
2016	31	47	19,532(3,580)	82,730	25.2(3.9)	78.0	**605,493**
2017	32	54	23,738(2,682)	69,652	22.8(2.7)	77.8	**861,492**

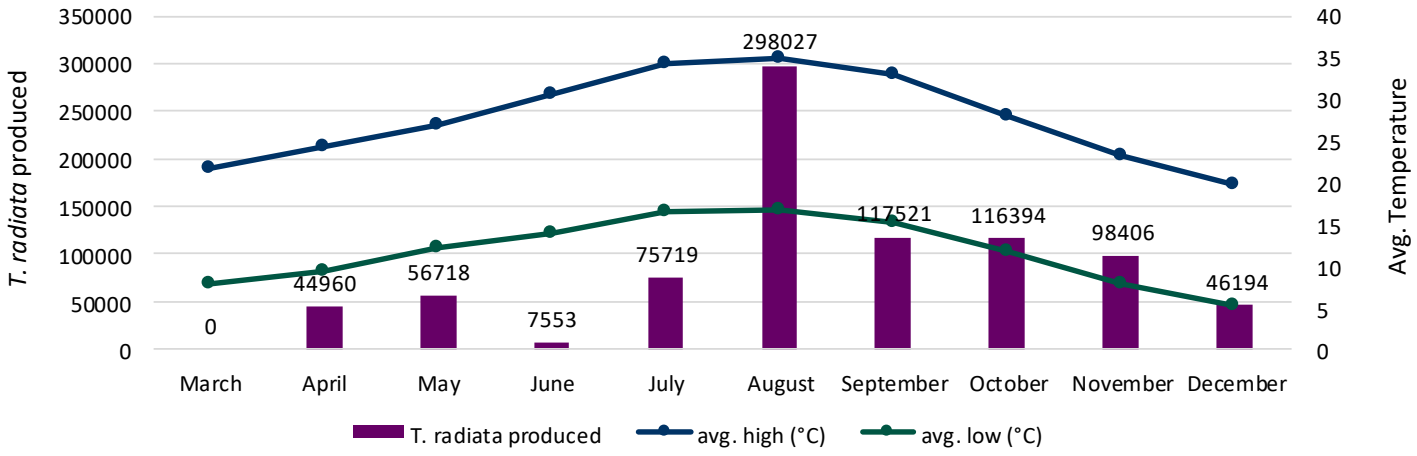

Figure 2. Monthly production of *Tamarixia radiata* from field cages (columns) and monthly average high and low temperature (°C) in Riverside, CA (lines; data from www.usclimatedata.com).

Developing collection methods for mass-reared *Tamarixia radiata*

Under current practices, newly-emerged *T. radiata* are hand-collected from emergence cages using a vacuum pump powered aspirator. This is a time-consuming and labor-intensive process. In CDFA greenhouse production, this labor has been reduced by using a passive collection method in which a plastic jar is attached to an opening at the top of a production cage. The cage is then covered with a shroud, made from black plastic bags, with a hole on top for the jar to pass though. This leaves the uncovered jar as the only source of light in the cage, prompting wasps to walk upward into the jars, which can then be removed and capped.

A similar method is currently under development for collection of *T. radiata* from field cage emergence cages. Two 70" by 72" black vinyl shower curtains enclose a three-shelf metal rack (Figure 3), blocking light (Figure 4). Three rectangular BugDorm insect cages modified with affixed jar-attachments are placed on the shelves.

Figure 3. A rack of BugDorms with curtains open to let in light and promote emergence.

A circle of mesh fabric in the jar-attachment opening blocks the passage of adult ACP while allowing the smaller *T. radiata* to pass through (Figure 5). This saves additional labor as ACP often are aspirated by accident while collecting *T. radiata* and must be removed from collection vials by hand. The natural light provided by windows inside a building is less intense than the sunlight inside a greenhouse, but the addition of artificial light can further encourage phototaxis. Small LED grow lights emitting light in the 660 nm, 630 nm, and 460 nm wavelengths will be tested.

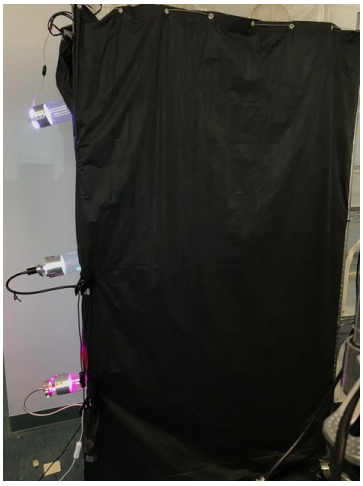

Figure 4. Emergence rack with curtains closed and with additional external lights to induce *Tamarixia radiata* movement into collection jars.

The efficiency of this collection method is currently being tested using curry leaf clippings from CDFA dorms. These clippings are usually collected from multiple production cages by CDFA workers after approximately 12 days of *T. radiata* emergence and pooled in separate emergence dorms to make space for new cages. During testing, the curtains will be closed, and the lights turned on for one-hour intervals. Immediately after removal of jars, the curtains will be opened and the remaining *T. radiata* in each dorm will be collected via aspirator. The number of *T. radiata* collected in jars and the number collected by aspirator will be used to compare the percent of *T. radiata* in each dorm that were lured into the jars for passive collection.

Figure 5. Jar attachment point with selective ACP-blocking mesh.

Light brown apple moth colony production

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The light brown apple moth, LBAM, *Epiphyas postvittana* colony is maintained at the Salinas Field Station. This colony supports multiple research projects. LBAM colony is reared under conditions of 22 ± 2°C, 60% humidity and 16:8 hour light-dark cycle. Three larval trays and two mating cages are produced per week, unless more are required for a specific experiment. As larvae LBAM is fed pink bollworm diet and as adults the moths are fed a 7.5% sucrose solution in mating cages. As part of continuing efforts to improve LBAM production, baking liners (i.e., "cupcake cups") have been recently implemented as a pupal substrate. Studies in 2016 showed that pupae were easier to collect from coffee filters than from the diet, though only 40% more moths pupated outside of the diet when using coffee filters. However, additional substrate comparison studies showed that moths appear to prefer baking liners, as 64% pupate outside the diet when using baking liners under optimal conditions (Figure 1).

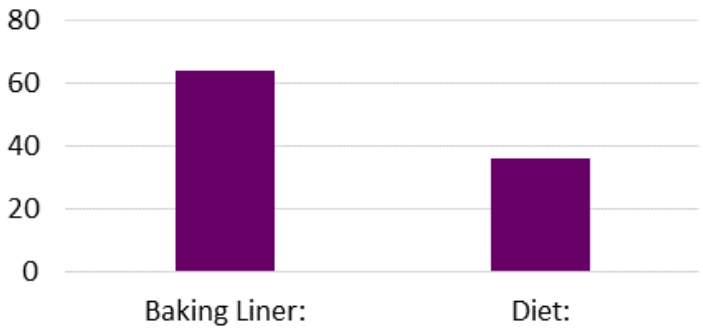

Figure 1. Comparing LBAM pupal yields on baking liner vs. diet.

This may be due to selective pressure from primarily choosing pupae from the baking liners to set up rearing cages, and the wax paper texture of the baking liners being more similar to the moths' natural habitat (leaves). Total yield per tray averaged over 800 pupae—300 in diet and 500 in baking liners.

In an eight week study in Salinas, California, CPHST tested the effectiveness of three kairomone lures against two different formulations of pheromone lures used for monitoring LBAM. Six treatments were used (3 kairomone, 2 pheromone, 1 acetic acid, and 1 blank control) in five replication blocks around the Crop Improvement and Protection Research Unit ARS station in Salinas, California.

Within each of the five blocks, seven large red plastic delta traps were placed 10 meters apart in shrubs and trees in designated positions. Each week the traps were rotated to the consecutive position with their sticky bases replaced if LBAM were caught within the week prior or if they were dirty. Collected bases were brought back to the laboratory to be identified for morphology and gender. The recorded data showed that traps containing kairomone lures (A, B, and C) caught noticeably less LBAM than those containing pheromone lures (D and E) (Figure 2). The data also showed that the 4-component pheromone lure was significantly more effective than the 2-component pheromone lure at attracting LBAM. Interestingly, the kairomone lures caught a proportionately larger number of female moths (Figure 3), which may be worth investigating in an effort to develop a female LBAM lure in future studies. Weekly pheromone trapping will continue in 2018.

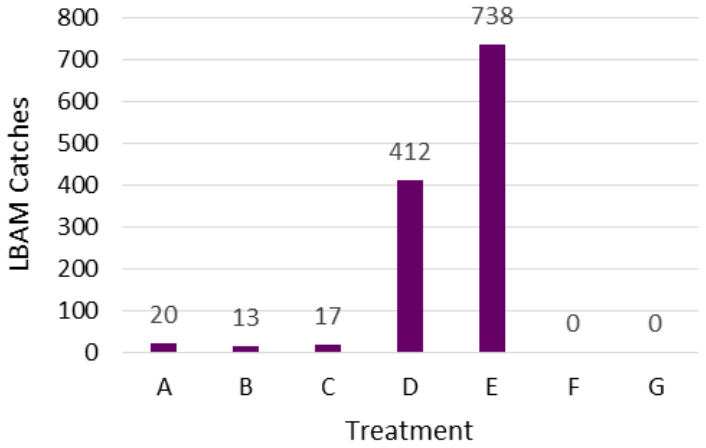

Figure 2. Total LBAM catches per lure treatment.

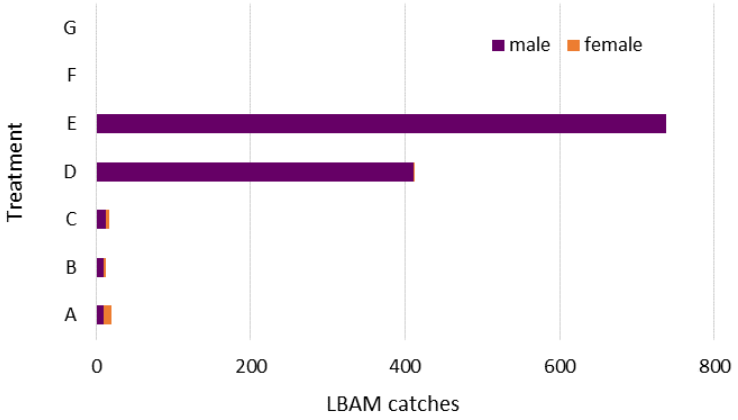

Figure 3. Total male and female LBAM catches per treatment.

2017 development of regulatory nursery treatment protocols for light brown apple moth

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Introduction

Nursery production and shipping was identified as a significant risk pathway for the spread of the invasive light brown apple moth, LBAM, *Epiphyas postvittana*. Due to this risk, California Department of Food And Agriculture (CDFA) regulates wholesale ornamental plant nurseries within an interior regulated area [1]. Nurseries within the regulated area are subjected to monthly inspections and every shipment outside of the regulated area must be inspected prior to treatment, treated with an approved insecticide [2], and inspected again prior to shipment. This regulatory program has resulted in significant costs to nursery operators along the central California coast. It also strains the resources in the offices of county agricultural commissioners that are in charge of inspections. This project aims to monitor the dynamics of light brown apple moth in ornamental plants, measure the control provided by parasitoids in urban settings, and to aid in the development of a more cost-effective regulatory program for nurseries.

Monitoring

Larval densities of LBAM were monitored in different locations all along the California coast. These surveys were part of different monitoring efforts and focused mainly on irrigated ornamental plants in urban areas, where LBAM appears to be more prevalent. In general, these surveys provided evidence that larval abundance was higher in early summer in most coastal regions, presumably due to vigorous growth after the winter rain; however larval levels were found to drop substantially by late summer (Figure 1). The surveys also showed that LBAM had not spread to regions in California where summer temperatures were high, as predicted by results from our species distribution models.

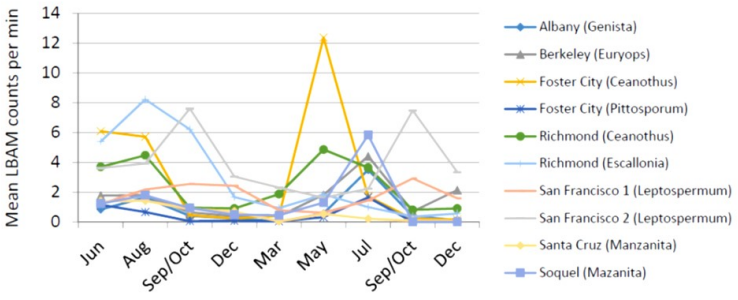

Figure 1. LBAM larval densities in non-pruned sites, San Francisco and Monterey Bay areas, from June, 2016 to December, 2017.

In fact, LBAM was mostly confined to coastal towns that experience additional cooling in the summer from the marine fog layer. Consequently, it seems unlikely that larval populations would be able to establish in the Central Valley or in other southern states that do not experience summer fog as a cooling influence.

Parasitism

In those surveys, when numbers were abundant, larvae and pupae were transferred to a laboratory to finish the rearing process and to obtain an estimate of the prevalence of parasitism in the population. In general, rates of parasitism oscillated between 25% and 49% during the year in 13 different locations sampled in northern California. Parasitism was higher in late summer, and the dominant parasitoid species are *Meteorus ictericus* and *Enytus eureka* in the larval stage, and *Pediobius ni* in the pupal stage. It's also worth noting that 56% of the cocoons of *E. eureka* were hyperparasitized by either *Gelis* spp. or *Scambus decorus*. LBAM continues to be heavily attacked by resident parasitoids at the original points of introduction in the San Francisco and Monterey Bay areas, despite declining levels of larval abundance. Data from others parts of the state showed that other parasitoids can also attack the larvae and pupae of LBAM.

Bioassays

New experimental work started at Salinas in June, 2017 to continue developing treatments for ornamental nursery stock. The three nursery plants that were used are favored LBAM hosts and are representative of plants with open and closed canopy architectures and leaf sizes. The main goals were:

- To evaluate the control power of existing CDFA LBAM Program approved pesticides
- To develop recommendations to target existing infestations of eggs
- To evaluate residual protective effects and duration of different treatments to determine the length of time and degree of control that can be expected for materials used for regulatory treatment

The efficacy experiments have shown that all active ingredients recommended by the CDFA for LBAM control in eggs and larvae have a high degree of efficacy.

Lambda-cyhalothrin (Scimitar) appears to be the most effective material, with complete control of neonate larvae and a fast knock-down effect, followed closely by spinosad (Intrepid), which also provides good control against LBAM. The other two insecticides tested thus far also offer good larval control, but their action is not as effective as lambda-cyhalothrin and spinosad.

In terms of residual effect, the results are very similar, with lambda-cyhalothrin providing protection for up to two weeks. The other materials showed similar residual effect and the level of protection did not change significantly in the tested interval (Figure 2).

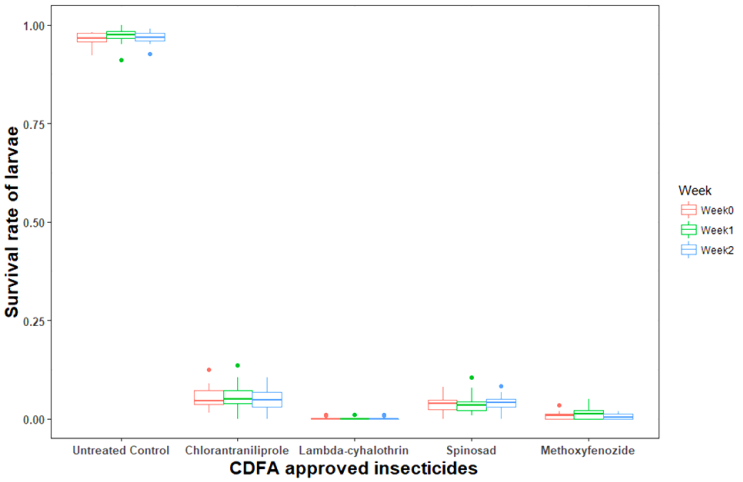

Figure 2. Results from residue experiment. Efficacy of CDFA-approved pesticides against *Epiphyas postvittana* measured as survival rate of first instar larvae divided by the total number of eggs during two continuous weeks. W0 = one day after treatment, W1 = one week after treatment, W2 = two weeks after treatment.

Egg visualization

One of the most difficult aspects of working with tortricid moths is obtaining accurate egg counts. Egg masses are small, difficult to see with the naked eye, and packed with eggs. In order to facilitate counting under the microscope, we have developed a protocol that allows us to preserve the egg masses and obtain reproducible data (Figure 3).

This is done by placing a drop of 5% methylene blue on the egg mass while still on the leaf and letting it sit for 20 seconds. We wash the dye out with distilled water and gently dry the egg mass with a paper towel. After, we cover the egg mass with clear scotch tape and remove it from the leaf. The egg mass can then be taped on white paper or on a microscope slide for photographing and preservation (Figure 3A). Once pictures are obtained, these can be enhanced using photo editing software (Figure 3B). The egg masses are counted on the computer screen, using the editing tools to facilitate counting. For example, different colored dots are used to count eclosed and uneclosed eggs (Figure 3C). This protocol is important because it allows for the preservation of egg masses if needed for re-analysis and facilitates the counting process.

References

1. Light Brown Apple Moth (LBAM). Map of quarantine boundaries. February 16, 2018. https://maps.cdfa.ca.gov/QuarantineBoundaries/LBAM/Layout_PQM_Northern_Area.pdf
2. Light Brown Apple Moth (LBAM). Approved pesticide treatments. February 16, 2018. http://phpps.cdfa.ca.gov/PE/InteriorExclusion/PDF/LBAMApprovedTreatments.pdf

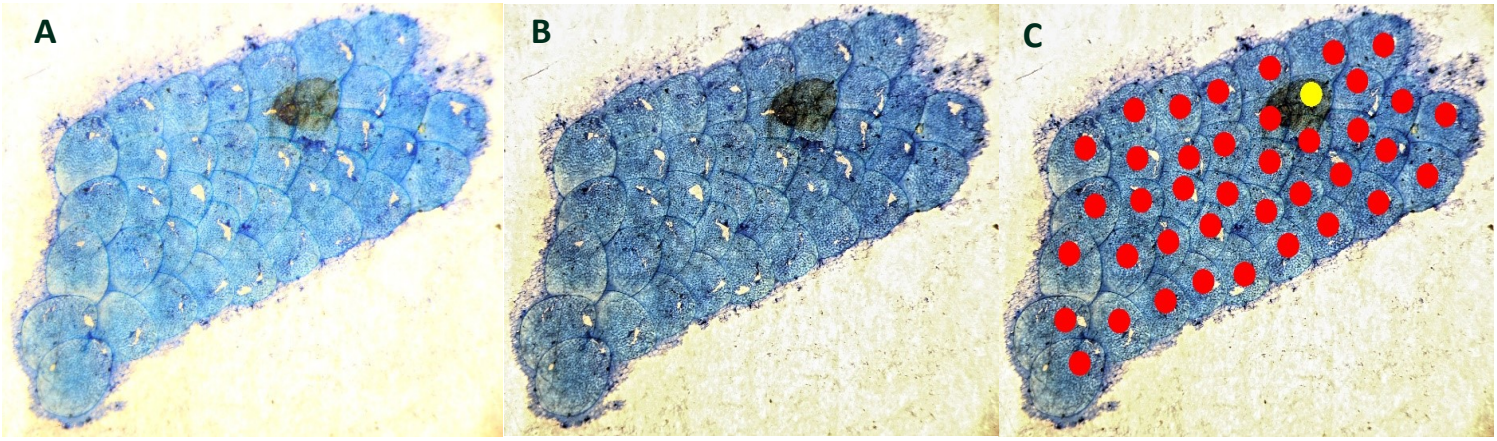

Figure 3. Example of egg mass analysis. A) Raw picture of egg mas. B) Edited picture of egg mass. C) Edited picture of egg mass with egg counts. Red dots represent eclosed eggs and yellow dots represent uneclosed eggs.

Reconstructing the invasion dynamics of European grapevine moth in California: insights from a successful eradication

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The ecological and economic consequences of biological invasions highlight the need for both increased understanding of the factors governing invasions and effective management strategies for mitigating invader spread and impacts. To this end, successfully eradicated invasions are ideal opportunities to better understand the factors governing invasions as well as refine management strategies should the same, or similar, organisms invade again. Quantitative methods such as geospatial analyses and niche-based/habitat suitability modeling can be especially useful in characterizing factors governing invasions and have previously been applied in investigations on plant [1,2], aquatic invertebrate [3], amphibian [4], and insect [5] invasions. Here we use geospatial analyses and habitat suitability modeling to reconstruct the invasion dynamics of an important vineyard pest, the European grapevine moth, EGVM, *Lobesia botrana*, in Napa County, California. All results are primarily interpreted in the context of improving detection, monitoring, and management strategies for EGVM should it again invade Napa.

Objectives

1. Quantify spatiotemporal patterns in EGVM occurrence in Napa County, CA
2. Characterize landscape, climatic, and anthropogenic factors associated with EGVM occurrence in Napa
3. Leverage understanding of factors associated with EGVM occurrence to generate predictions of habitat suitability for EGVM in Napa

Methods

A geodatabase of 2010 to 2014 EGVM trapping records and detections was used to reconstruct EGVM invasion dynamics in Napa County. Prior to analysis, all yearly trapping data were checked for identical georeferenced records; duplicate records were removed from subsequent analyses. Throughout all analyses, we considered whether a given trap recorded any EGVM captures (i.e., presence or absence) rather than number of EGVM caught per trap.

Analyses were, unless otherwise noted, restricted to 2010 EGVM detections as detections in other study years were lower in number by orders of magnitude. A suite of geospatial analyses were used to both quantify spatial autocorrelation (SAC) and identify statistical hotspots among 2010 EGVM detections. Regression-based and machine-learning approaches were used in conjunction with habitat suitability and ensemble modeling methods to identify factors associated with EGVM occurrence, quantify factor-EGVM relationships, and predict habitat suitability of EGVM in Napa.

Results

A total of 1,297 traps captured at least one male EGVM in Napa in 2010. The number of traps recording male EGVM captures in Napa decreased in the following years to 64 in 2011, 20 in 2012, 31 in 2013, and one in 2014. EGVM was officially declared eradicated two years later. A considerable amount of SAC existed among 2010 traps recording EGVM detections. At a Napa-wide scale, SAC was detected at scales of up to approximately 10 km (Figure 1). Significant autocorrelation at such large distances may be indicative of long distance spread by EGVM, likely as a result of human-assisted movement. Imposing a 1 km^2 grid over Napa County and aggregating all 2010 traps recording EGVM captures into these grid cells identified one cell that contained the greatest number of EGVM detections (n = 27). At this more local scale we detected significant SAC at distances of less than 50 m (Figure 1). This pattern is consistent with very localized movement by EGVM, which notably occurs at distances well within the 500 m treatment zones around EGVM detections. We also observed substantial spatiotemporal heterogeneity in the location and size of hotspots in the 2010 to 2013 EGVM occurrences (Figure 2). In general, EGVM hotspots tended to occur in central and western Napa; some of these hotspots were consistent in location across study years. The habitat suitability modeling was used to understand why EGVM hotspots occurred where they did.

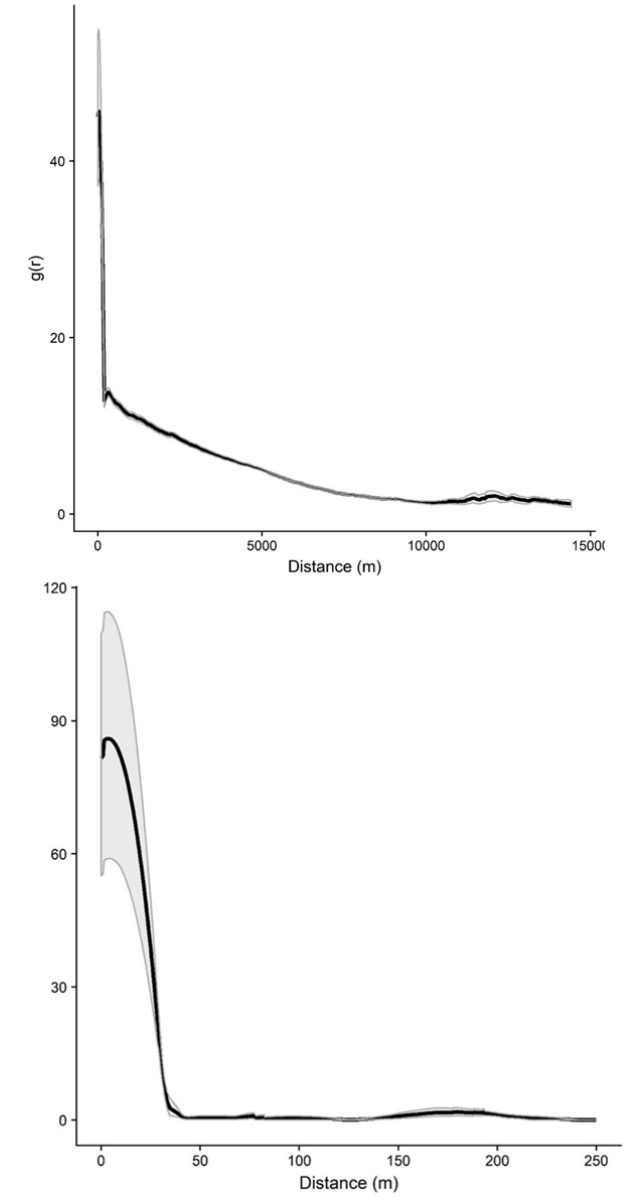

Figure 1. The magnitude and spatial scale of spatial autocorrelation (SAC) among 2010 traps recording EGVM captures quantified at Napa-wide (top panel) and 1 km^2 cell (bottom panel) scales.

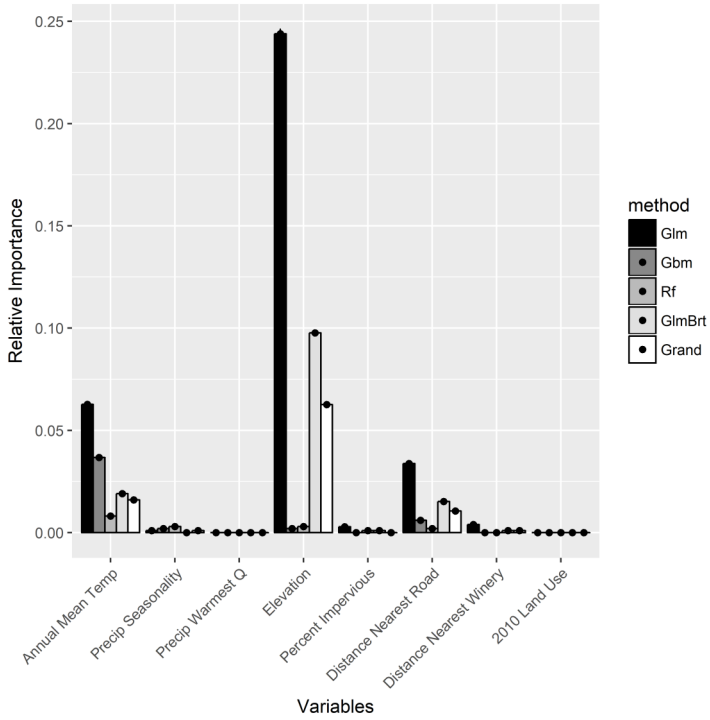

Figure 3. The mean relative importance of the selected landscape, climatic, and anthropogenic predictors for each method of generating ensemble predictions of habitat suitability for EGVM in Napa County, CA. Error bars represent the standard error associated with the mean relative importance of each predictor variable across model replicates included in the represented ensemble predictions.

Table 1. Summary of ensemble method performance in generating predictions of habitat suitability for EGVM in Napa County, CA.

Performance metric	BRT	GLM	RF	GLM + BRT ensemble	Grand ensemble
ROC	0.848	0.84	0.831	0.847	0.855
TSS	0.575	0.564	0.503	0.572	0.588

All methods of predicting habitat suitability for EGVM in Napa performed well (ROC > 0.8; Table 1), though the boosted regression tree and generalized linear modeling methods out-performed the random forest algorithm. An ensemble model generated using five replicate predictions from both boosted regression and generalized linear modeling methods also performed well in predicting habitat suitability (ROC = 0.847). A grand ensemble prediction across all model replicates (n = 15, 5 for each modeling method) was the best performing prediction (ROC = 0.855, TSS = 0.588). The relative importance of our landscape, climatic, and anthropogenic predictors of interest varied among modeling methods (Figure 3).

However, elevation, annual mean temperature, and distance to nearest road were the most important predictors across all methods of generating ensemble predictions. The fitted responses from our grand ensemble prediction indicate these predictor variables are negatively associated with the probability of EGVM occurrence. Conversely, precipitation of the warmest quarter and 2010 land use were the least important predictors of interest across all ensemble predictions. These results suggest that both environmental context and anthropogenic effects may explain why some locations are more favorable for EGVM than other locations.

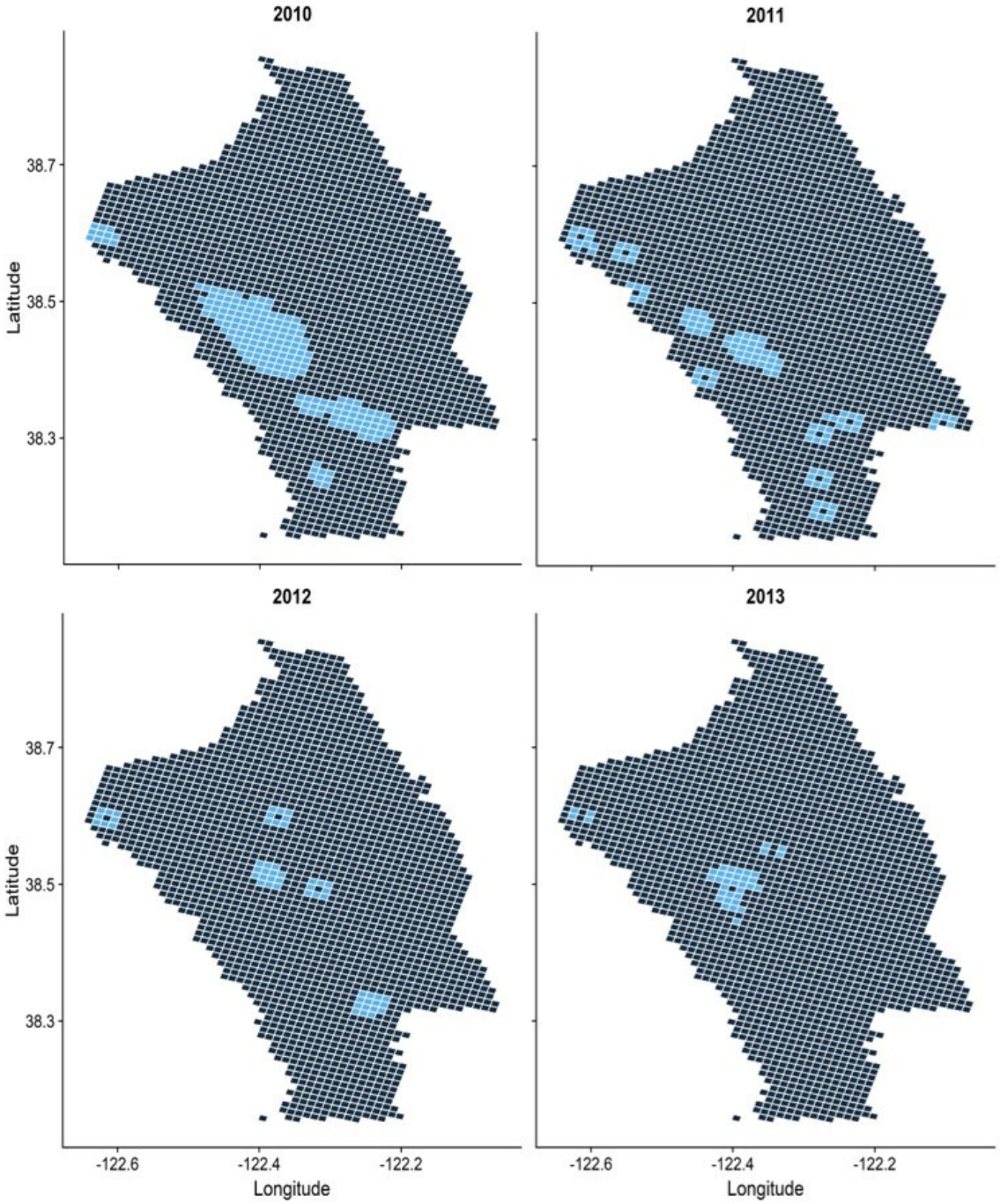

Figure 3. Statistical hotspots for 2010 to 2013 EGVM occurrences identified using a local Getis-Ord statistic. This Getis-Ord statistic calculates a Z score for each 1 km^2 grid cell that can be used as a diagnostic tool and transformed into probabilities; light blue grid cells represent hotspots where the transformed Getis-Ord Z score is a probability equal to or greater than 95%.

References

1. Thuiller W, Richardson DM, PYŠEK P, Midgley GF, Hughes GO, Rouget M. Niche-based modelling as a tool for predicting the risk of alien plant invasions at a global scale. Global Change Biology. 2005 Dec 1;11(12):2234-50.
2. Vicente JR, Pereira HM, Randin CF, Goncalves J, Lomba A, Alves P, et al. Environment and dispersal paths override life strategies and residence time in determining regional patterns of invasion by alien plants. Perspectives in Plant Ecology, Evolution and Systematics. 2014 Feb 20; 16(1): 1-0.
3. Herborg LM, Jerde CL, Lodge DM, Ruiz GM, MacIsaac HJ. Predicting invasion risk using measures of introduction effort and environmental niche models. Ecological applications. 2007 Apr 1;17(3):663-74.
4. Ficetola GF, Thuiller W, Miaud C. Prediction and validation of the potential global distribution of a problematic alien invasive species—the American bullfrog. Diversity and distributions. 2007 Jul 1;13(4):476-85.
5. Hartley S, Harris R, Lester PJ. Quantifying uncertainty in the potential distribution of an invasive species: climate and the Argentine ant. Ecology Letters. 2006 Sep 1;9(9):1068-79.

2017 Publications

Otis Laboratory members are indicated in bold

- Arthur FH, Ghimire MN, **Myers SW**, Phillips TW. Evaluation of Pyrethroid Insecticides and Insect Growth Regulators Applied to Different Surfaces for Control of *Trogoderma granarium* (Coleoptera: Dermestidae) the Khapra Beetle. Journal of Economic Entomology. 2018:612-9.

- Audley J, Klingeman WE, Mayfield A, **Myers SW**, Taylor A. Walnut Twig Beetle (Coleoptera: Curculionidae: Scolytinae) Colonization of Eastern Black Walnut Nursery Trees. Journal of Insect Science. 2017;17(3).

- Audley J, Mayfield AE, 3rd, **Myers SW**, Taylor A, Klingeman WE, 3rd. Phytosanitation Methods Influence Posttreatment Colonization of *Juglans nigra* Logs by *Pityophthorus juglandis* (Coleoptera: Curculionidae: Scolytinae). Journal of Economic Entomology. 2016;109(1):213-21.

- Chen Z, White MS, **Mack R**. Evaluating vacuum and steam process on hardwood veneer logs for export. European Journal of Wood and Wood Products. 2016;75(6):911-8.

- **Cooperband MF**, **Cossé AA**, Jones TH, Carrillo D, Cleary K, **Canlas I**, Stouthamer R. Pheromones of three ambrosia beetles in the *Euwallacea fornicatus* species complex: ratios and preferences. PeerJ. 2017;5:e3957.

- **Cossé AA**, **Cooperband MF**, Zilkowski BW, Jones TH, Stouthamer R, Carrillo D. Physiology of the invasive ambrosia beetle *Euwallacea fornicatus* in response to four stereoisomers of p-menth-2-en-1-ol, their synthesis and absolute configurations. Pheromones and Other Semiochemicals in Integrated Production. 2017;126:112-3.

- Dodge C, Coolidge J, **Cooperband MF**, **Cossé AA**, Carrillo D, Stouthamer R. Quercivorol as a lure for the polyphagous and Kuroshio shot hole borers, *Euwallacea* spp. nr. *fornicatus* (Coleoptera: Scolytinae), vectors of Fusarium dieback. PeerJ. 2017;5:e3656.

- Dohino T, Hallman GJ, Grout TG, Clarke AR, Follett PA, Cugala DR, Minh TD, Murdita W, Hernandez E, Pereira R, **Myers SW.** Phytosanitary Treatments Against *Bactrocera dorsalis* (Diptera: Tephritidae): Current Situation and Future Prospects. Journal Economic Entomology. 2017;110(1):67-79.

- Fang J, Qian L, Xu M, Yang X, **Wang B,** An Y. The complete nucleotide sequence of the mitochondrial genome of the Asian longhorn beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae). Mitochondrial DNA Part A. 2016;27(5):3299-300.

- Ghimire MN, **Myers SW**, Arthur FH, Phillips TW. Susceptibility of *Trogoderma granarium* Everts and *Trogoderma inclusum* LeConte (Coleoptera: Dermestidae) to residual contact insecticides. Journal of Stored Products Research. 2017;72:75-82.

- Hallman GJ, Maset BA, Martínez EIC, Cáceres Barrios CE, Vreysen MJB, **Myers SW**, Wornoayporn V. Phytosanitary Cold Treatment Against *Anastrepha grandis* (Diptera: Tephritidae). Florida Entomologist. 2017;100(1):29-31.

- Kononov A, Ustyantsev K, **Wang B**, **Mastro VC,** Fet V, Blinov A, Baranchikov Y. Genetic diversity among eight *Dendrolimus* species in Eurasia (Lepidoptera: Lasiocampidae) inferred from mitochondrial COI and COII, and nuclear ITS2 markers. BMC genetics. 2016;17(3):157.

- Limbu S, Keena M, Chen F, Cook G, **Nadel H**, Hoover K. Effects of Temperature on Development of *Lymantria dispar asiatica* and *Lymantria dispar japonica* (Lepidoptera: Erebidae). Environmental Entomology. 2017;46(4):1012-23.

Otis Laboratory members are indicated in bold

- Lopez VM, Hoddle MS, **Francese JA**, **Lance DR**, Ray AM. Assessing Flight Potential of the Invasive Asian Longhorned Beetle (Coleoptera: Cerambycidae) With Computerized Flight Mills. J Econ Entomol. 2017;110(3):1070-7.

- McInnis DO, Hendrichs J, Shelly T, Barr N, Hoffman K, Rodriguez R, **Lance DR**, Bloem K, Suckling DM, Enkerlin W, Gomes P, Tan KH. Can Polyphagous Invasive Tephritid Pest Populations Escape Detection for Years Under Favorable Climatic and Host Conditions? American Entomologist. 2017;63(2):89-99.

- Menocal O, Cruz LF, Kendra PE, Crane JH, **Cooperband MF**, Ploetz RC, et al. *Xyleborus bispinatus* Reared on Artificial Media in the Presence or Absence of the Laurel Wilt Pathogen (Raffaelea lauricola). Insects. 2018;9(1).

- **Cooperband MF**, **Cossé AA,** Stouthamer R, Carrillo D, Tappey J. Attractants of ambrosia beetles in the *Euwallacea fornicatus* species complex. Pheromones and Other Semiochemicals in Integrated Production. 2017;126:89-92.

- **Myers SW**. Justification to support railroad tie production and preservative treatment process as a mitigating measure to prevent the spread of emerald ash borer, *Agrilus planipennis*. Otis Laboratory USDA APHIS PPQ S&T CPHST. 2017.

- **Nadel H**, Follett PA, Perry CL, **Mack R**. Postharvest Irradiation Treatment for Quarantine Control of the Invasive *Lobesia botrana* (Lepidoptera: Tortricidae). J Econ Entomol. 2017.

- Short DPG, O'Donnell K, Stajich JE, Hulcr J, Kijimoto T, Berger MC, Macias AM, Spahr EJ, Bateman CC, Eskalen A, Lynch SC, Cognato AI, **Cooperband MF**, Kasson MT. PCR Multiplexes Discriminate Fusarium Symbionts of Invasive *Euwallacea* Ambrosia Beetles that Inflict Damage on Numerous Tree Species Throughout the United States. Plant Disease. 2017;101(1):233-40.

- Stouthamer R, Rugman-Jones P, Thu PQ, Eskalen A, Thibault T, Hulcr J, Wang LJ, Jordal BH, Chen C, **Cooperband MF**, Lin C, Kamata N, Lu S, Masuya H, Mendel Z, Rabaglia R, Sangusansub S, Shih H, Sittichaya W, Zong S. Tracing the origin of a cryptic invader: phylogeography of the *Euwallacea fornicatus* (Coleoptera: Curculionidae: Scolytinae) species complex. Agricultural and Forest Entomology. 2017.

- Streifel MA, Tobin PC, Hunt L, **Nadel H**, **Molongoski JJ**, Aukema BH. Landscape-Level Patterns of Elevated FS1 Asian Allele Frequencies in Populations of Gypsy Moth (Lepidoptera: Erebidae) at a Northern U.S. Boundary. Environ Entomol. 2017;46 (2):403-12.

- Shelly TE, **Lance DR**, Tan KH, Suckling DM, Bloem K, Enkerlin W, Hoffman K, Barr N, Rodríguez R, Gomes PJ, Hendrich J. Issues in Entomology: Tephritid Pest Populations American Entomologist. 2017.

- Uzunovic A, Mukherjee A, Elder P, **Myers SW**. Rapid Screening of Sulfuryl Fluoride as a Potential Phytosanitary Treatment for a Broad Selection of Fungi Relevant to Forestry. Forest Products Journal. 2017;67(1-2):4-12.

- **Williams DW,** Hajek AE. Biological control of *Sirex noctilio* (Hymenoptera: Siricidae) in the northeastern United States using an exotic parasitic nematode. Biological Control. 2017;107:77-86.

- **Wu Y, Trepanowski NF**, **Molongoski JJ**, Reagel PF, Lingafelter SW, **Nadel H**, **Myers SW**, Ray AM. Identification of wood-boring beetles (Cerambycidae and Buprestidae) intercepted in trade-associated solid wood packaging material using DNA barcoding and morphology. Sci Rep. 2017;7:40316.

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