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Accomplishments 2016

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Center for Plant Health Science and Technology

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It was designed, formatted, and produced by Nevada Trepanowski, for the Otis Laboratory.

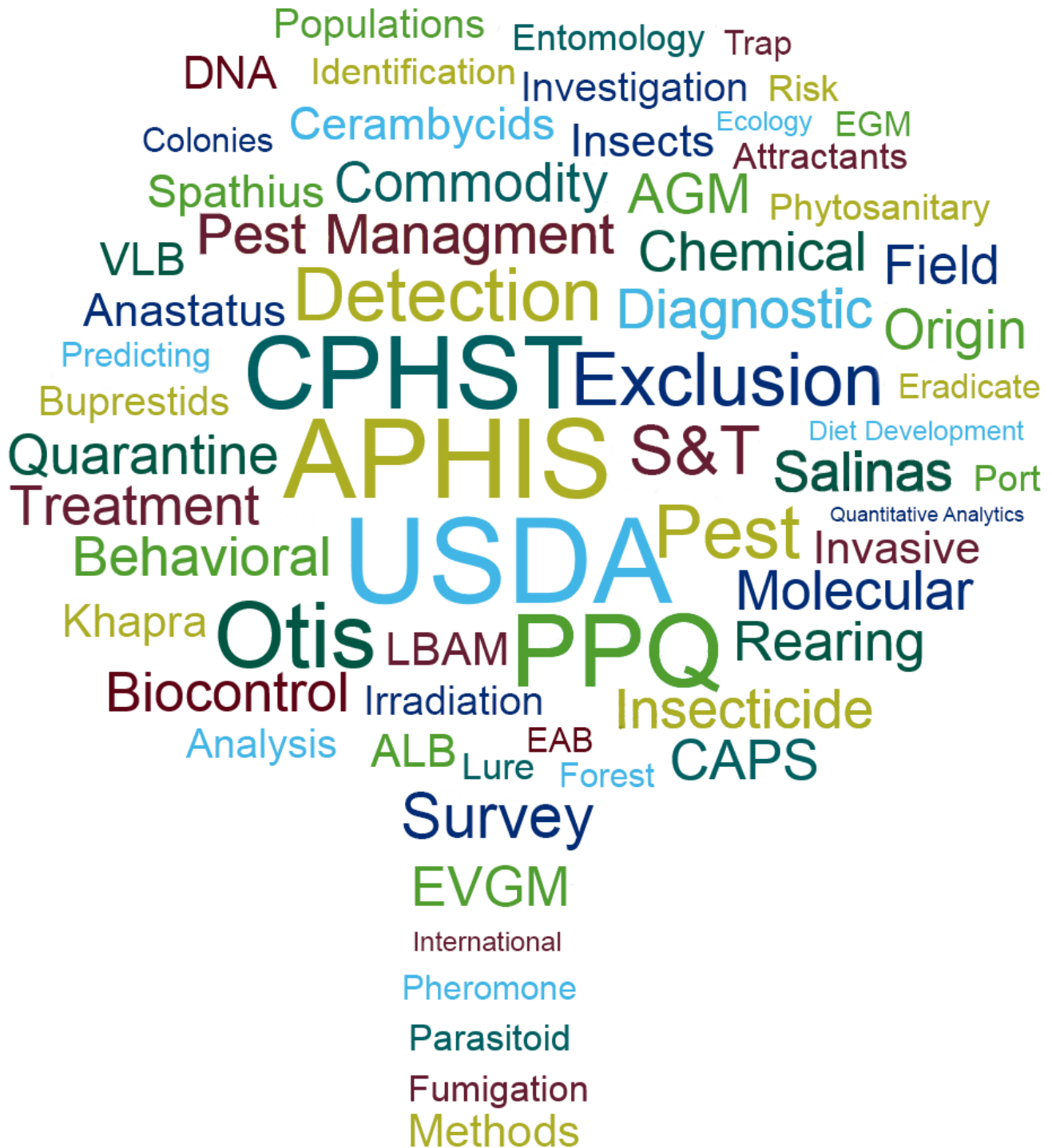
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OTIS LABORATORY

ACCOMPLISHMENTS 2016



2016 OTIS LABORATORY ANNUAL REPORT INTRODUCTION

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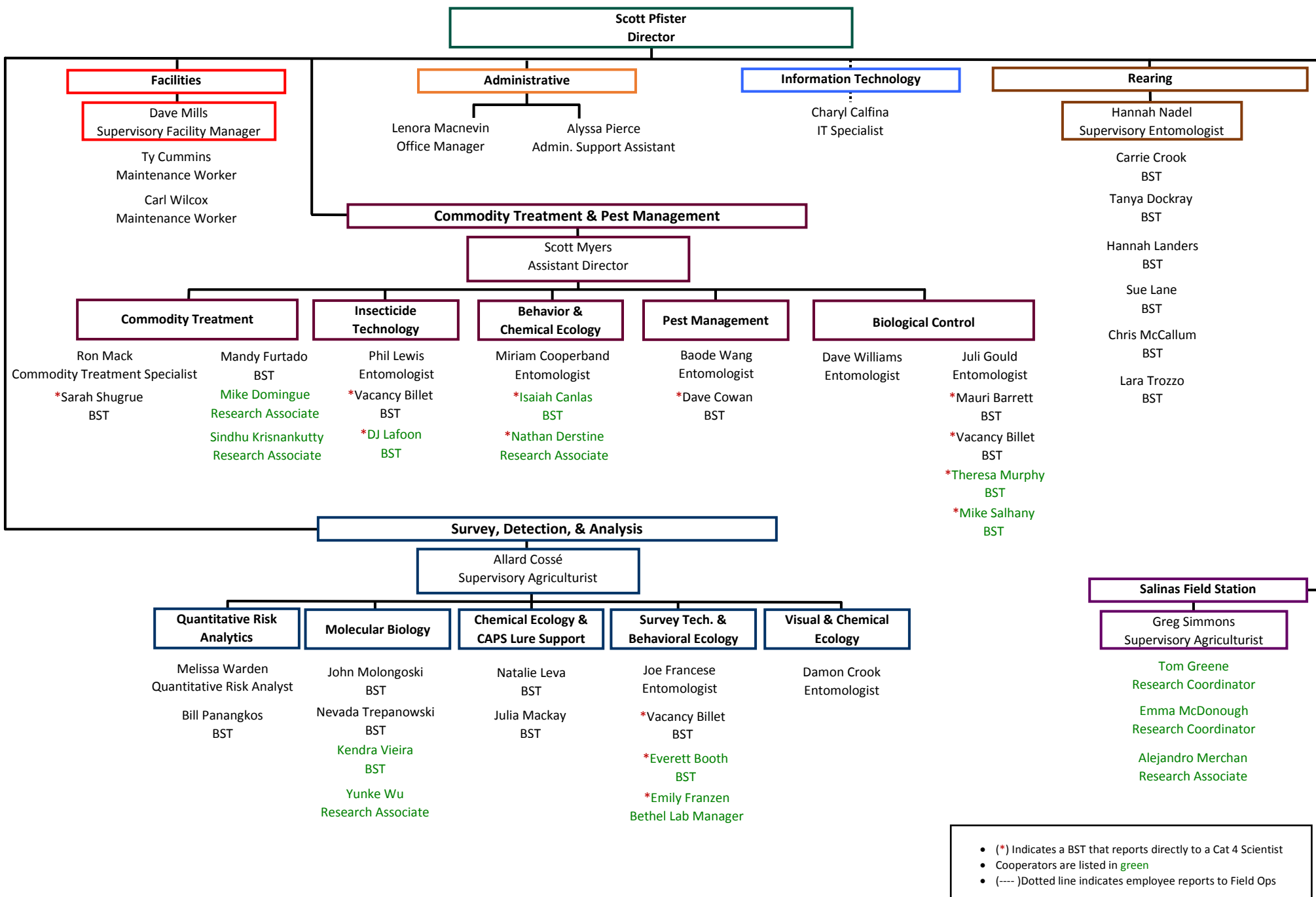
The Otis Laboratory is part of the United States Department of Agriculture (USDA), Animal and Plant Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), Center for Plant Health Science & Technology (CPHST). PPQ's mission is to safeguard America's agricultural and natural resources against the entry, establishment, and spread of economically and environmentally significant plant pests. The Otis Laboratory is one of several in the U.S. that provides scientific and technical support to PPQ programs through the development and transfer of technology and information. The staff includes approximately 50 scientists, technicians, support personnel and cooperators. The facility is housed on an 11-acre campus with approximately 35,000 square feet of laboratories, offices, conference space, a workshop, and insect rearing areas. The insect containment facility currently holds colonies of 13 quarantined insects and 3 biological control agents, which allows for the development of rearing and control methods of invasive pests. Staff also work out of satellite facilities in Bethel, Ohio and Salinas, California.



The Otis Laboratory started in the 1960s, in support of USDA's gypsy moth programs. Since then Otis has expanded into a multi-functional laboratory with projects that include: developing methods for eliminating plant pests in produce and packaging materials that are imported into the U.S., detecting and monitoring pest populations through the development of traps and lures, supporting pest programs with molecular biology and quantitative risk analytics, and developing methods for pest control including behavioral and biological controls as well as insecticidal methods. This work is carried out through cooperative working relationships with scientists at universities, government agencies, private organizations and in industries across the U.S. and in many foreign countries. The research areas, and the organizational structure of the laboratory are broken out into four main functional groups which include: commodity treatment and pest management, survey detection and analysis, insect rearing and the Salinas, California Field Station.

The Salinas Field Station coordinates and conducts scientific support activities for light brown apple moth (LBAM) and European grapevine moth (EGVM). The station develops control and detection methods for these pests and provides technical analysis of program data to assist our stakeholders to maintain export markets of affected commodities. The station also supports development of mass-rearing strategies for biocontrol agents of the Asian citrus psyllid.

This annual report provides an overview of the methods development work that occurred at the Otis Laboratory during fiscal year 2016. The work is presented in abstract format and organized by the labs four functional groups. Each abstract provides a summary of the work and relevant results. In instances where the work has led to a publication, scientific report, or other forms of documentation a reference page with appropriate links is provided. A separate guide cataloging the abstracts by insect order is also provided.



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

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Order: Hemiptera

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<i>Diaphorina citri</i>	Psyllidae	83, 84, 86, 87, 88, 90, 92

Order: Coleoptera

Scientific name	Family	Pages
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Order: Hymenoptera

Scientific name	Family	Pages
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<i>Tamarixia radiata</i>	Eulophidae	83, 84, 86, 87, 88, 90, 92
<i>Tetrastichus planipennis</i>	Eulophidae	20, 21, 22, 23
<i>Anastatus orientalis</i>	Eupelmidae	25

Kairomones of the invasive spotted lanternfly

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Background

The newly invasive spotted lanternfly (SLF), *Lycorma delicatula*, is a univoltine phloem feeder. Its primary host is *Ailanthus altissima*, but it has more than 65 hosts and causes serious damage to grape and hops. Plants wilt from heavy feeding and copious amounts of honeydew cause sooty mold, which blocks photosynthesis. Survey and detection tools are desperately needed.

SLF were field collected and reared in the Otis Laboratory Insect Containment Facility. Volatile collections from *Ailanthus*, grape, and chinaberry were analyzed by gas chromatography coupled mass spectrometry (GC-MS) and results were compared to the literature. Several compounds of interest were selected based on abundance or unique presence in *Ailanthus*. Compounds were field tested in Pennsylvania and in laboratory behavioral bioassays (Figure 1). Field tests were subsequently conducted in China. Lure release rates were compared and correlated to results in the field. So far, three attractive kairomones have been identified and trap technology has improved.

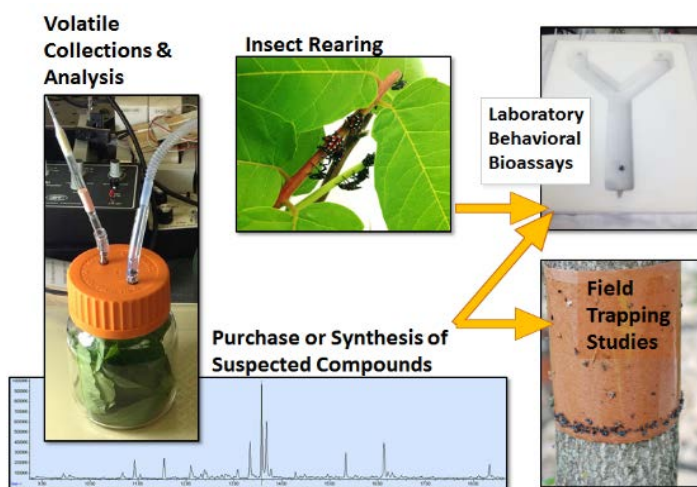


Figure 1. Technical approach for discovery of kairomones for spotted lanternfly.

Behavioral bioassays

Small and large Y-tube bioassays were used to test nymphs and adults for attraction to compounds in the laboratory. Odors were placed in one upwind arm of the bioassay, the other arm contained the control. Seven odors were tested and three (an alcohol, an ester, and a sesquiterpene) were highly attractive to one or more stages of SLF (Figure 2). Higher doses reduced attraction. Spearmint oil, which was reportedly attractive in the literature, was not attractive.

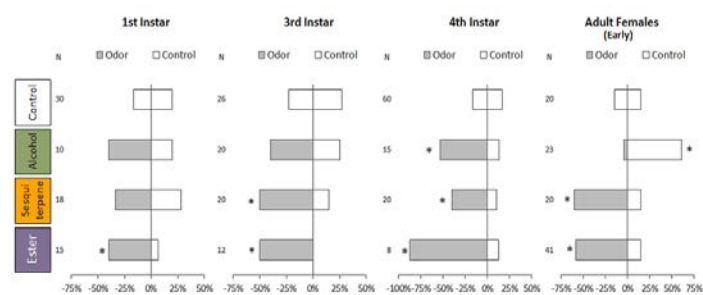


Figure 2. Y-tube responses to three kairomones found to be attractive to SLF. Two compounds, the ester and the alcohol are commercially available. The sesquiterpene had to be synthesized.

Highlights from PA field studies

A 2015 field study in PA, targeting 1st and 2nd instars, tested five odors on brown sticky bands from Korea, unbaited controls, and unbaited clear sticky bands (by Alpha Scents). The odors formulated into lures by Alpha Scents contained: 1) a spearmint oil, 2) an acetate from *Ailanthus*, 3) an alcohol from *Ailanthus*, and 4) a blend of the alcohol, acetate, and a sesquiterpene (B) from *Ailanthus* (ratio 2:1:1), which is used for brown marmorated stink bug. The study was replicated at ten field sites. The alcohol caught the most SLF, but there were no significant differences due to variation in the quarantine zone (Figure 3).

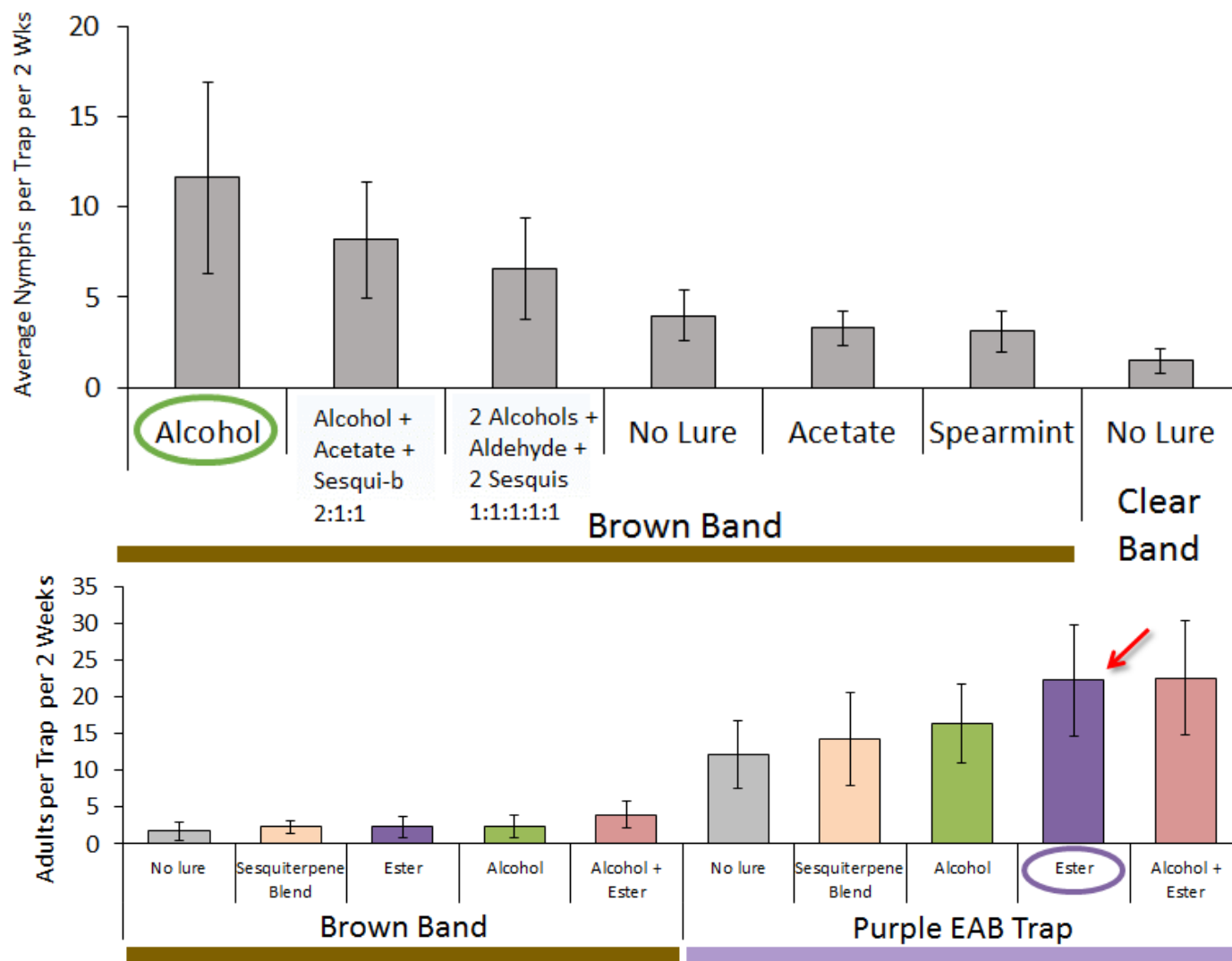


Figure 3. First set of field results in Pennsylvania targeting 1st and 2nd instar nymphs (top) and adults (bottom) with various plant-associated compounds.

Highlights from PA field studies (continued)

Korean brown sticky bands and purple prism traps wrapped around tree trunks (Figure 4) were tested on adults. Five odors were tested: the alcohol from *Ailanthus*, the ester from *Ailanthus*, alcohol + ester (1:1), a commercial sesquiterpene blend, and blank controls. The purple prism traps caught more SLF adults than brown sticky bands. The alcohol + ester and the ester alone caught the most SLF adults (Figure 3).

Highlights from China field studies

Twenty field studies were conducted on all SLF stages in three locations in China in 2016. Two of those studies are highlighted here. Ester lures were tested at three doses (control, 1/2x, 1x, and 2x). The high dose caught significantly more SLF than controls (Figure 5A). Three sticky bands were compared for adult SLF, and Web-Cote's sticky band captured 30 times more adult SLF than the Korean sticky bands (Figure 5B). Phenology for SLF in PA and China was recorded (Figure 6).



Figure 4. Purple prism traps, typically used against EAB, were wrapped around trees in order to test attraction of adults to various odors because the Korean brown sticky bands were ineffective at trapping adults.

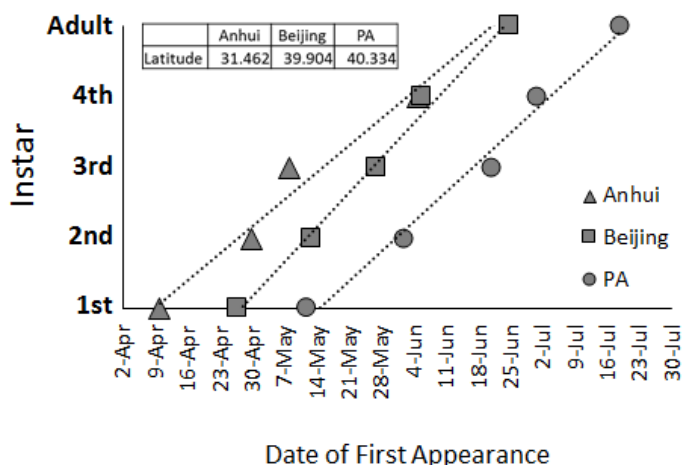


Figure 6. Phenology of SLF in 2016 developing in the field at three different latitudes in Anhui, China, Beijing, China, and Pennsylvania, USA.

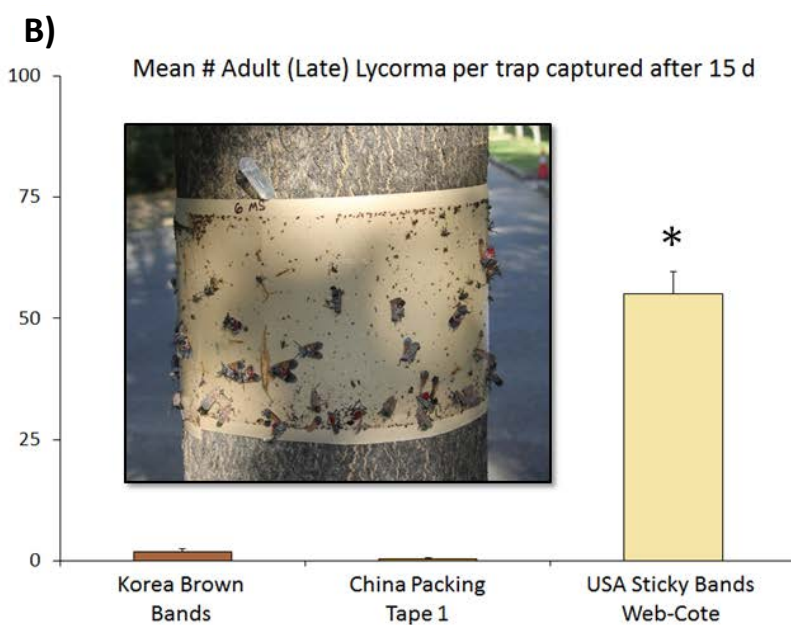
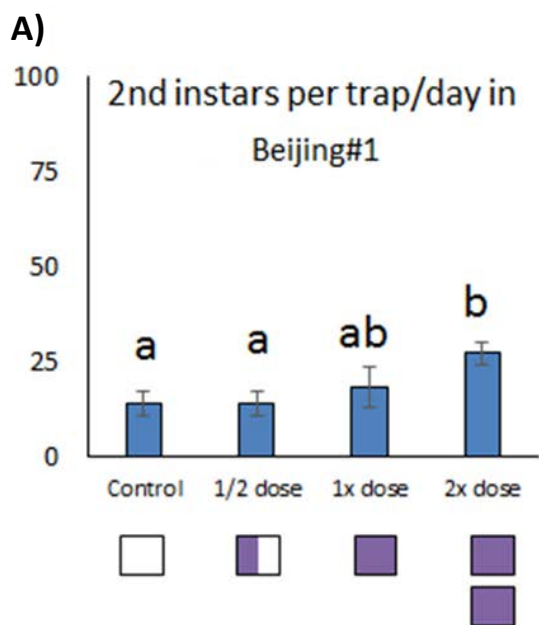


Figure 5. Results of two field tests in China. A) Dose response study showed that the ester was attractive at the highest dose. B) Trap technology study showed a 30-fold improvement in trap catch of adult SLF using Web-Cote sticky bands.

Pheromones of three cryptic species of the *Euwallacea fornicatus* species complex

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Three members of the *Euwallacea fornicatus* species complex, undescribed cryptic species of ambrosia beetles, have become invasive in the United States (Carrillo et al. 2016; Stouthamer et al. 2017). Polyphagous shot hole borer (PSHB), *Euwallacea* sp. #1, is invasive in areas surrounding Los Angeles, California, tea shot hole borer (TSHB), *Euwallacea* sp. #2, is invasive in Hawaii and Florida, and Kuroshio shot hole borer (KSHB), *Euwallacea* sp. #5, is invasive in areas surrounding San Diego, CA. They each attack a broad range of host trees and plants and infect them with their associated *Fusarium* species of symbiotic ambrosia fungus. It is necessary to develop attractive lures for survey and detection. Laboratory rearing capabilities were developed using a sawdust-based artificial diet where they excavate and raise their young inside galleries lined with *Fusarium* (Cooperband et al. 2016). In order to study them in the context of searching for attractants and in searching for a possible pheromone, odors were collected from six sources and compared: a) beetles, b) galleries, c) diet + fungus + beetles, d) diet + fungus, e) diet f) control. Volatile profiles were analyzed and examined for GC peaks that uniquely appeared in certain treatments. Two ketones were present only when beetles were present, and were particularly abundant inside the galleries (Figure 1).

Both ketones were found in odors from both sexes. These two ketones were found in all three species, but each species had a different ratio of the two ketones (Figure 2). Synthetic blends were formulated to match natural ratios for each species for behavioral testing. Female beetles of each species were highly attracted in a Y-tube to the synthetic ratio that matched their natural ratio, and they were highly repelled by the ratios matching those of the other two species (Figure 3).

Males responded in a similar manner. In addition to the two ketones, quercivorol was found in volatile collections associated with the fungus, not the beetles. Quercivorol was found to be attractive to all three species (Figure 4). The discovery of the two ketones, and the highly sensitive specificity to the correct ratios, supports the notion that these three beetles are separate species, even though they are morphologically identical. The finding that quercivorol was not associated with the beetles, but rather the fungal symbiont, and that it was attractive to all three species, supports the notion that it is a fungal kairomone. Quercivorol is now being used in commercial lures for survey and detection work (Carrillo et al. 2015), and other compounds are still under investigation as potential attractants.

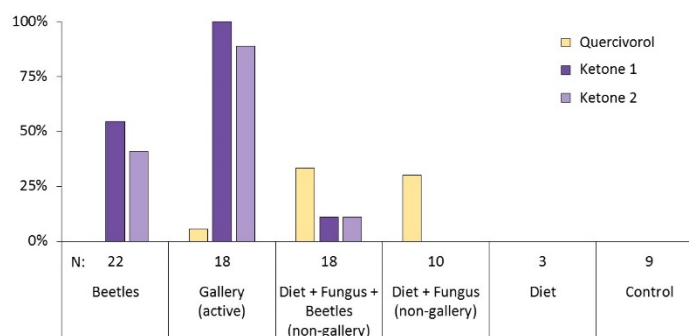


Figure 1. Presence of the two ketones was found in odors only with beetles present, but not the diet or the fungus. Presence of quercivorol was found in odors only when fungus was present.

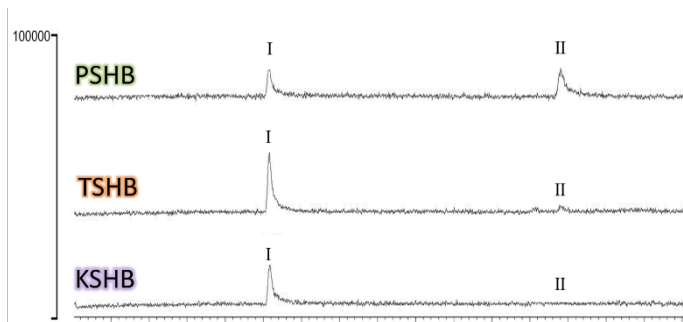


Figure 2. Ratios of the two ketones were found to be different for each of the three cryptic *Euwallacea* species, polyphagous shot hole borer (PSHB), tea shot hole borer (TSHB), and Kuroshio shot hole borer (KSHB).

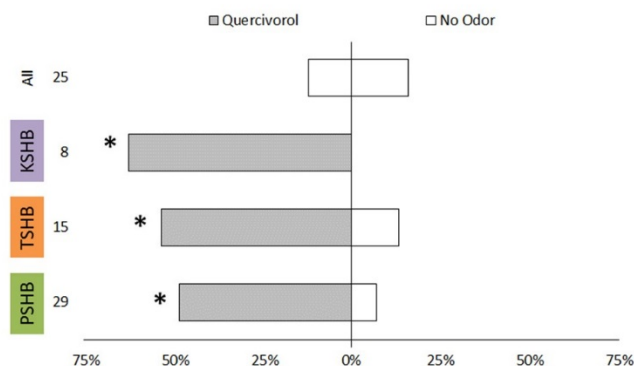


Figure 4. Responses of female beetles to synthetic quercivorol, which was attractive to all three species: polyphagous shot hole borer (PSHB), tea shot hole borer (TSHB), and Kuroshio shot hole borer (KSHB).

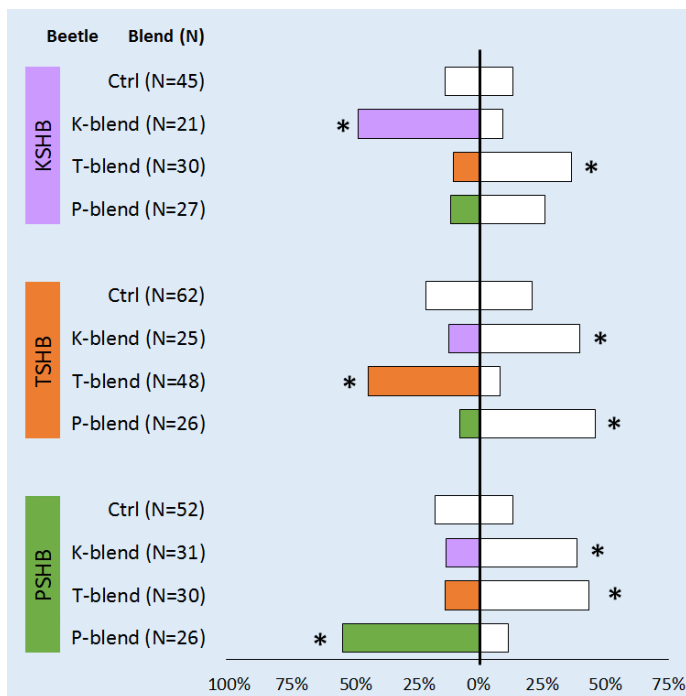


Figure 3. Responses of female beetles in three cryptic species of *Euwallacea*, polyphagous shot hole borer (PSHB), tea shot hole borer (TSHB), and Kuroshio shot hole borer (KSHB), to synthetic blends of two ketones at three ratios, resembling the ratios found in each species (P-, T-, and K-blend).

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Response of *Euwallacea fornicatus* sp. #1 to four stereoisomers of their fungal kairomone quercivorol

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Three cryptic and undescribed ambrosia beetle species in the *Euwallacea fornicatus* species complex have invaded the United States, and each carries a symbiotic *Fusarium* fungus which infects trees and causes Fusarium dieback. The polyphagous shot hole borer (PSHB), *Euwallacea* sp. #1, is invasive in the Los Angeles, California area and attacks over 300 plant species. PSHB can cause serious damage to avocado (Figure 1) and many native and protected plant species. The tea shot hole borer (TSHB), *Euwallacea* sp. #2, is invasive in Hawaii and Florida, and the Kuroshio shot hole borer (KSHB), *Euwallacea* sp. #5, is invasive in San Diego County, CA. These latter two species are less studied but appear to have similar host ranges and cause similar damage. A strong attractant is needed to develop survey and detection tools. We developed laboratory rearing capabilities on a sawdust-based artificial diet using sawdust from a preferred host, boxelder. To explore attractants, initial still-air Petri dish bioassays compared attraction of PSHB to odors from either diet, diet + fungus, or diet + fungus + beetles. Beetles were attracted to odors from the latter two which both contained fungus odors. Analysis of volatiles revealed the presence of (1S,4R)-menth-2-en-1-ol (quercivorol) in diet + fungus odors, but not in diet alone. Quercivorol has four stereoisomers, all of which were present in commercial quercivorol lures, but their behavioral function was not known. The four stereoisomers were individually synthesized, enabling behavioral testing on each isomer. Subsequent behavioral bioassays were conducted on responses to the four stereoisomers of *p*-menth-2-en-1-ol to evaluate the most attractive enantiomers, in order to improve lures containing these compounds.

The (1R,4S)-menth-2-en-1-ol isomer, and quercivorol (the 1S,4R isomer), both E isomers, were attractive. The two Z isomers (1R,4R and 1S,4S) were not attractive when tested alone, and when the two Z isomers were combined they became a repellent. The Z isomers also removed attraction when combined with either of the attractive E isomers. To optimize attraction, lures should be formulated to maximize the (1R,4S) and (1S,4R) isomers, and minimize the (1R,4R) and (1S,4S) isomers.



Figure 1. *Fusarium* dieback symptoms in avocado.

Evaluating three members of the *Euwallacea fornicatus* species complex for reproductive compatibility

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Three isolated invasive populations of ambrosia beetles, *Euwallacea fornicatus*, were found to differ considerably based on molecular data in recent research by colleagues. Based on these molecular findings, they were considered separate cryptic species. Recent introductions of these genotypes occurred in Miami-Dade County, Florida, Los Angeles County, California, and San Diego County, California. From a regulatory and control perspective, questions surfaced with regard to their species status, as they are morphologically indistinguishable. The two invasions in California have now started to overlap geographically. We developed laboratory rearing capabilities (Figure 1) and conducted crossing experiments in our insect containment facility to determine whether they are reproductively isolated or able to hybridize. Three experiments were conducted to determine if the different types are capable of interbreeding, and if so, whether they could produce viable offspring. The first experiment examined crosses between the polyphagous shot hole borer (PSHB), *Euwallacea* sp. #1, from Los Angeles County and the tea shot hole borer (TSHB), *Euwallacea* sp. #2, from Miami-Dade County. A second experiment was conducted between the PSHB and the Kuroshio shot hole borer (KSHB), *Euwallacea* sp. #3, from San Diego.

The third experiment evaluated crosses between TSHB and KSHB. Because they are haplo-diploid, initial crosses produce hybrid females and pure haploid males with the maternal genotype. Therefore, several generations were monitored, allowing natural sibling-mating to occur, where resulting offspring would include hybrid males as well. We found that all three cryptic species were able to hybridize to a varying degree initially. PSHB x TSHB viability diminished after several generations, whereas PSHB x KSHB, after an initial, major loss in viability, recovered after several generations. TSHB x KSHB appears to be the least viable combination with the lowest fecundity or fitness after crossing, although the study is ongoing. These results generally align with recent molecular findings that they are three separate cryptic species. However, this demonstrates a small chance of hybridization may be possible when their geographical ranges overlap, particularly in southern California. In their native range, all three species were found to coexist in some areas, so hybridization will probably be unlikely. However, transmission of fungal symbionts between species may be possible and deems further investigation.

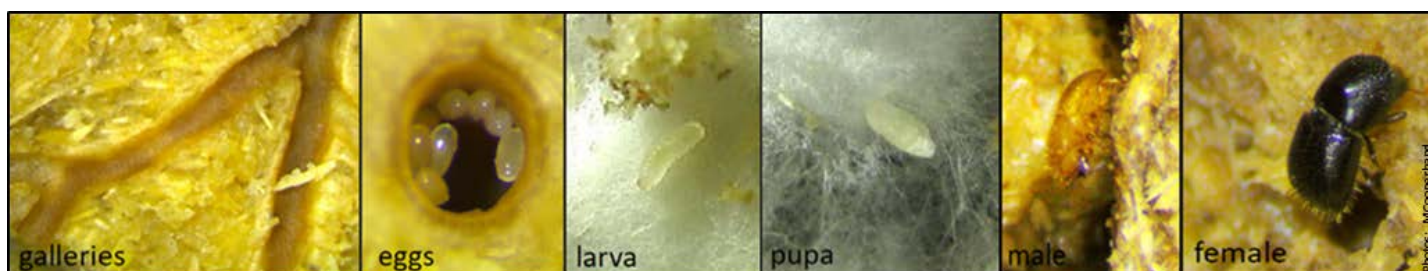


Figure 1. *Euwallacea fornicatus* species #1 (PSHB) developing in artificial diet.

Exploration for parasitoids of Asian longhorned beetle and citrus longhorned beetle in South Korea

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Asian longhorned beetle (ALB), *Anoplophora glabripennis*, is under eradication in the U.S., and citrus longhorned beetle (CLB), *Anoplophora chinensis*, continues to be intercepted at our ports. Treatment options, such as tree injections, are limited in natural wooded areas. Natural enemies that evolved where their hosts are not abundant can be quite effective at locating and attacking hosts at low density. In the U.S., parasitoids could be useful in managing beetles that have escaped programmatic control, thus supplementing and complementing the current eradication effort. Exploration for natural enemies is needed in parts of East Asia where ALB seems to be controlled by natural factors.

Our goal was to seek and identify parasitoids attacking ALB and CLB in locations where parasitoids may be partly responsible for reducing pest population density. South Korea has large national parks with forests containing maple and abundant riparian habitats that support both *Anoplophora* species at low densities.

Because ALB is difficult to find at low density, our approach was to use lab-infested logs (sentinel logs) containing ALB or CLB eggs and larvae to attract parasitoids. We found both species in the northern sites, whereas we found CLB primarily farther south. Our northern base of operations was the Korean National Arboretum (KNA), which has a plantation of *Acer tegmentosum*. This species is a favored host of both beetles, and the stand was heavily infested by ALB. The first step in producing sentinel logs was to collect adult beetles. We placed them in cages made from plastic bins (Figure 1). We provided the adults with maple twigs as food and bolts of host wood as oviposition substrate. The hosts were *Acer tegmentosum* and *Aesculus turbinata* (Japanese horse chestnut). After 5-9 days, the logs had many egg pits. We suspended them in the *A. tegmentosum* stand. We hung some at the ground surface to attract CLB parasitoids, and the remainders higher up to attract ALB parasitoids (Figure 2).



Figure 1. Breeding cages with sentinel logs ready for oviposition by *Anoplophora* species.



Figure 2. ALB adult emergence cage and sentinel logs in a mesh cage at the KNA site.

We suspended the logs inside cylindrical mesh cages to give free access to parasitoids but prevent woodpecker predation. We recovered numerous ALB eggs and first instars in 2016. We peeled the bark, extracted the eggs and larvae, and placed them on ALB diet. Four parasitoid species were identified. The eulophid egg endoparasitoids included *Aprostocetus anoplophorae* on CLB and *Aprostocetus fukutai* on ALB (Figure 3). The braconid larval ectoparasitoids were *Spathius ibarakius* on CLB and ALB (Figure 4) and *Leluthia honshuensis* on ALB (Figure 5). The egg endoparasitoids could be useful as biological control agents because of their host specificity and high rates of parasitism. Two CLB larvae were attacked by a newly described species of *Spathius*. This encourages us because other *Spathius* species have been used as biological control agents for the management of emerald ash borer in the U.S.



Figure 3. Pupae of *Aprostocetus fukutai* with host egg shell at top left.



Figure 5. Adult of *Leluthia honshuensis*.



Figure 4. Adult *Spathius ibarakius*.

Development of a field insectary to propagate emerald ash borer parasitoids

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Field insectaries were first developed in 2014 to investigate the feasibility of rearing large numbers of emerald ash borer (EAB), *Agrilus planipennis*, and its parasitoids under ambient conditions without losses to woodpecker predation. The principal objectives of the experiments in 2016 were as follows: to estimate the intrinsic reproductive rate of a parasitoid (that is, the maximum increase in numbers over a generation when protected from predation) and to use the insectary system to disperse parasitoids spatially ahead of the EAB infestation front. We constructed field insectary cages from 40-gallon plastic barrels, with port holes for introducing water, honey, and parasitoids and for ventilation (Figure 1). We located study sites in Concord, New Hampshire, and Dalton, Massachusetts, and carried out experiments on two dates (mid-June and mid-August). We placed four barrels at each date-site. The setup for each date-site was as follows: four trees were felled at each site and bolts were cut to length to fit in the barrels. Trees were selected by their apparent high stress levels as indicated by their thinning crowns. Seven infested bolts were then selected at random and placed in each barrel with the bottom ends in 500 ml plastic cans filled with water (to prevent desiccation).

The feeding tubes were filled with water and honey (Figure 1), and the barrel lid was fastened. One barrel at each date-site was used as a control, with no parasitoids added. This served to provide an indication of parasitism that might already be present at the site. The remaining three barrels received varying numbers of parasitoid males and females. *Tetrastichus planipennis* was chosen as the parasitoid because of its apparent effectiveness in attacking EAB and its good record of establishment. Wasps were obtained from the APHIS rearing facility in Brighton, Michigan. After three weeks exposure, we returned the barrels to the Otis Laboratory and peeled the bolts. Although barrels at the New Hampshire site were vandalized, bolts were removed and peeled anyway. We found few EAB larvae and none were parasitized. The barrels at Dalton survived intact, but again, the trees had too few insects to test our hypotheses. Nevertheless, we have confidence that this method will be useful in mass rearing with a little more understanding of phenology and fine tuning of host and parasitoid numbers, or perhaps, with a different parasitoid species.

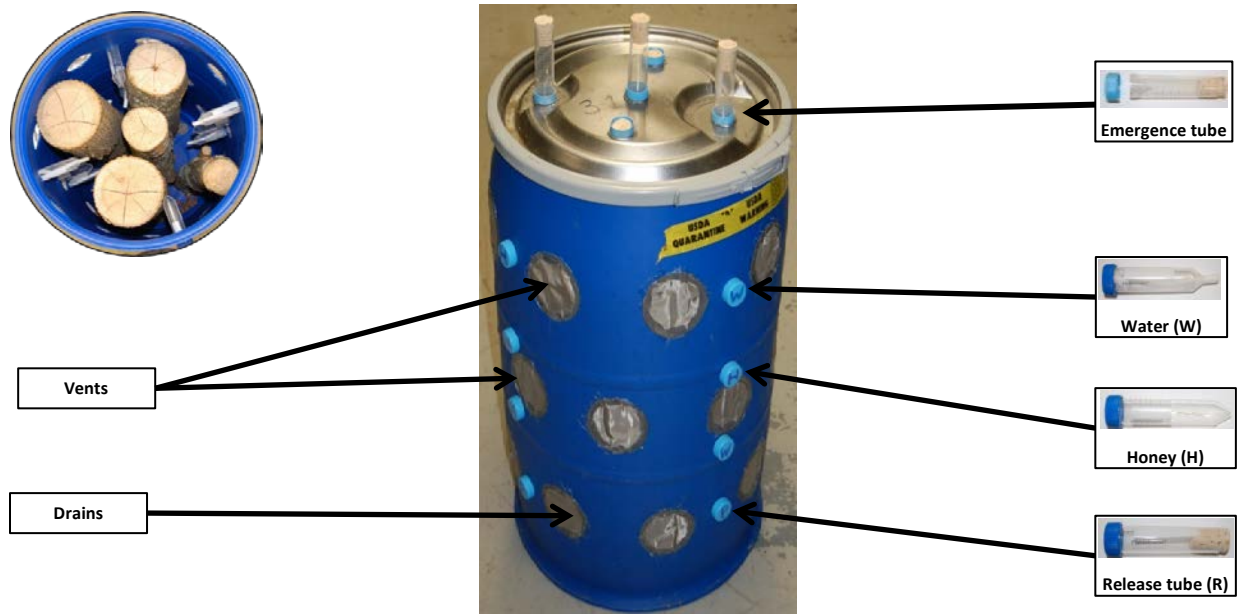


Figure 1. Insectary barrel with modular port inserts.

Phenology of emerald ash borer and its parasitoids in the northern and southern United States

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It is difficult and expensive to rear parasitoids of insects that live under the bark of trees, such as emerald ash borer (EAB), *Agrilus planipennis*. Therefore, it is an ineffective use of resources to release parasitoids where the probability of establishment is low. We currently know that one EAB larval parasitoid, *Spathius agrili* (Figure 1), can overwinter in the northern U.S., but populations do not appear to persist after a few years. One possible explanation for the lack of establishment is that *S. agrili* was collected from Tianjin, China, which is near the southern limit of the EAB distribution in China, where the climate is comparable to the central U.S. rather than the northern U.S. This deviation in climatic conditions may lead to an asynchrony between the emergence of adult *S. agrili* and availability of the mature EAB larvae that they attack, or the final generation of *S. agrili* may emerge too late in the season for their progeny to properly overwinter. EAB can take either one or two years to develop, depending on factors such as climate, within-tree densities and/or tree health. Whether or not populations are experiencing a one or two year life cycle affects the availability of larvae throughout the summer. A second EAB larval parasitoid, *Tetrastichus planipennis* (Figure 2), has established well in the northern U.S., however recent monitoring of southern releases has not documented establishment there. A third larval parasitoid, *Spathius galinae*, was collected from Russia, and the climate in its native range is more similar to the northern U.S. *Spathius galinae* has a long ovipositor, like its congener *S. agrili*, allowing it to attack EAB in large trees with thick bark; the presence of *S. galinae* would complement that of *T. planipennis*, which is not capable of attacking such trees. All three parasitoid species have multiple generations per year, and whether or not they can establish depends on having appropriately sized hosts available when they emerge in the spring and then again for all succeeding generations. Determining the phenology of the three larval parasitoids of EAB and how well synchronized they are with the availability of mature EAB larvae in northern and southern climate zones is a critical step in selecting the best suited parasitoid for release. Researchers used a combination of field and lab (e.g., growth chamber) studies to answer these important questions.

Preliminary findings indicate EAB populations in Tennessee exhibit a typical one-year life-cycle, with the majority of the EAB larvae overwintering as J-larvae in chambers, which are not available to the *S. galinae* or *T. planipennis* adults that emerge in the spring. *Spathius agrili* emerges after EAB has a chance to mate and produce offspring, although to date only one *S. agrili* has been recovered in yellow pan traps in Tennessee. In New York, both *S. agrili* and *S. galinae* have a fall generation, however only *S. galinae* produces the darker overwintering cocoons and successfully emerges the following spring. Based on these initial findings we expect that *S. galinae* and *T. planipennis* would establish in the north, while only *S. agrili*, which emerges well after EAB, has the ability to establish in the south.

Figure 1. *Spathius agrili*.Figure 2. *Tetrastichus planipennis*.

Dispersal of *Tetrastichus planipennis*, a parasitoid of emerald ash borer

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Anecdotal evidence indicates that *Tetrastichus planipennis*, a parasitoid attacking larval emerald ash borer (EAB), *Agrilus planipennis*, disperses well following release. We calculated the minimum dispersal rate for parasitoid recoveries and found that in over half of the recorded instances *T. planipennis* dispersed over 2 km in a single year, with several examples over 5 km per year (Figure 1). We conducted a study along a linear path of ash in upstate New York so that we could quantify parasitoid dispersal when it occurred in only two directions, as opposed to dispersal in all directions. Parasitoids were released at three locations along a 15 km path in 2013, and yellow pan traps were placed every 250 m along the path. Parasitoids were first recovered in 2014. At the north end of the ash path, where EAB density was highest, the number of traps that collected *T. planipennis* and the number of insects per trap were higher than further south. In areas where the density of EAB was lower, the parasitoids moved three times as far (1.6 km versus 0.5 km) to find their hosts.

Sampling in 2015 showed that as populations of EAB moved to the south, parasitoid populations followed the trajectory of their hosts. Because of dying trees in the north, we shifted our sampling to the south in 2016. The distribution of *T. planipennis* recoveries continued to fill in the gaps, and when a small satellite population of EAB emerged 5 km south of the generally infested area, *T. planipennis* was found there as well. To investigate parasitoid movement off the linear ash path we placed traps parallel to the path approximately 2 km to the east and west of the path. We recovered *T. planipennis* in 17 of these 40 traps, and some of these positive traps were on solitary ash trees in the middle of a field. We conclude that *T. planipennis* is well suited to grow in numbers when its host density is high and to disperse long distances to find hosts when host population densities are lower.

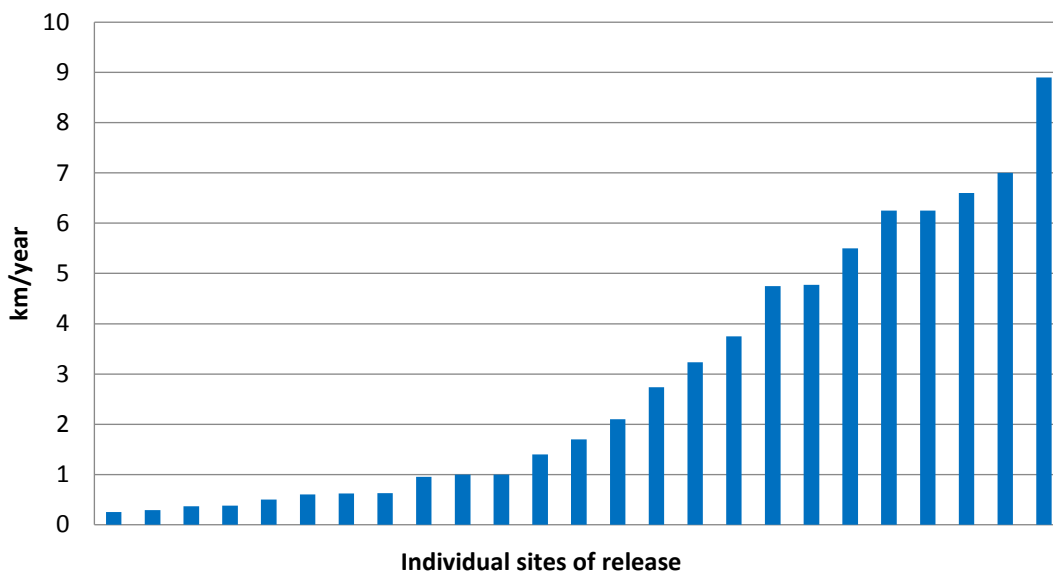


Figure 1. Minimum dispersal rate for populations of *T. planipennis* at 25 sites over a 1 year period.

Interaction of woodpeckers and parasitoids attacking emerald ash borer

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Emerald ash borer (EAB), *Agrilus planipennis*, is considered one of the most destructive forest pests to ever reach North America. Management of EAB is focused on biological control through the introduction of larval parasitoids, such as *Tetrastichus planipennisi* and *Spathius agrili*. In many ash stands infested with EAB, even stands where EAB populations have collapsed, predation by woodpeckers can be considerable, averaging around 40%. Woodpeckers and larval parasitoids both prefer to attack late instar larvae. Therefore, there is a high potential for woodpeckers to attack EAB that are parasitized. Predation on such individuals would not add to EAB mortality but would instead remove parasitoids from the population. This study was designed to investigate whether woodpeckers prefer, avoid, or are neutral to the presence of parasitoids when foraging on EAB. If woodpeckers avoid consuming parasitized EAB, then overall mortality of EAB will be enhanced and this knowledge will allow us to better interpret the results of life-table studies. This research involved caging parasitoids on EAB infested trees to induce parasitism, and then woodpeckers were either excluded or allowed access to parasitized EAB over the winter, when most predation occurs.

Research in 2016 found that on a per tree basis, parasitism was not significantly different if woodpeckers were excluded or not, suggesting that woodpeckers were not avoiding parasitized EAB on the tree. If woodpeckers avoided parasitized EAB one would expect parasitism levels to increase in areas exposed to woodpeckers when compared to areas where woodpeckers were excluded, and on a per tree basis this was not observed. However, woodpecker predation was significantly lower on trees containing parasitoids (Figure 1), suggesting that they may have left to forage on a different tree after encountering parasitized EAB. Woodpeckers feed by sticking their barbed tongues into the holes they drill through the bark and pulling out the EAB. If an EAB larva still contains small *T. planipennisi* larvae, this action is still possible. However, once *T. planipennisi* burst from the EAB or after *S. agrili* spin cocoons, the woodpeckers would not be able to find an acceptable food source. Figure 2 shows evidence of woodpecker activity that failed to provide sustenance.

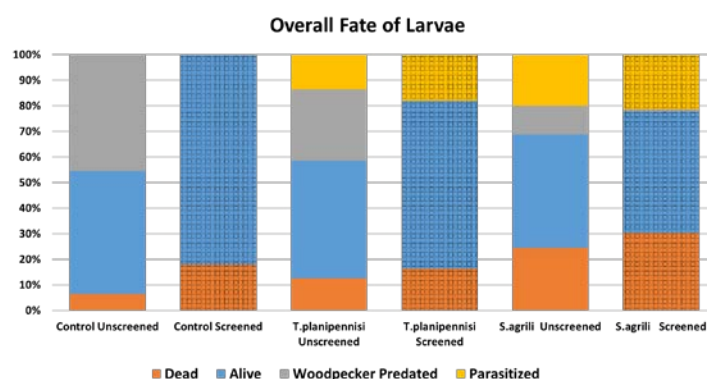


Figure 1. Fate of EAB larvae with and without parasitoid and/or woodpecker exposure. Bars with hash marks indicate trees with screening to exclude woodpeckers.

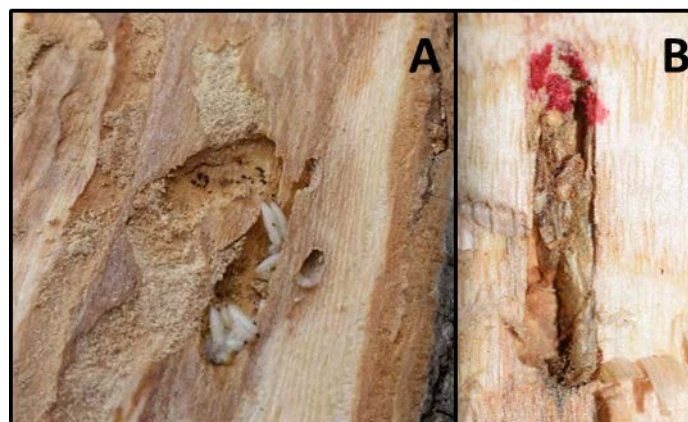


Figure 2. EAB galleries attacked by woodpeckers that did not consume the parasitoids inside. A) *Tetrastichus planipennisi*. B) *Spathius agrili*.

Biological control of Asian longhorned beetle

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Exploration for natural enemies of Asian longhorned beetle (ALB), *Anoplophora glabripennis*, continued in China in 2016. Collections were made in Beijing, Gansu, Hunchun, and Shanghai. In areas where ALB density was low, sentinel logs containing eggs or young ALB larvae were deployed to attract parasitic wasps. Ten parasitic wasps and one parasitic beetle were found attacking ALB. In general, parasitism levels were 10% or less, however parasitism of up to 17% was found for the parasitic beetle, *Dastarcus helophoroides* (Figures 1 and 2). *Dastarcus helophoroides* was imported to the Otis Laboratory and was successfully reared in the insect containment facility. The first step in determining if a natural enemy is sufficiently safe to release as a biocontrol agent is to conduct host specificity testing to see if any non-target native species are at risk. We used green funnel traps and black intercept traps baited with lures to attract cerambycids, as well as light traps to collect nocturnal flying species. We collected 24 species of native cerambycids; we reared 543 adults and these adults laid 1,802 eggs.

A number of methods were utilized to encourage the beetle females to lay eggs, and we then reared non-target larvae in both natural logs and on artificial diet. A total of 12 species were reared and used for testing. When the non-target cerambycid larvae were mature, we tested them alongside ALB (positive control) to determine if *D. helophoroides* would attack the larvae and successfully develop. Parasitism on cerambycid hosts in pine was quite low, however parasitism of non-target larvae in hardwoods was actually greater than parasitism of ALB. We conclude that *D. helophoroides* is not sufficiently host specific to be considered as a biocontrol agent of ALB in the United States.



Figure 1. Adult *Dastarcus helophoroides* are nocturnal and hide during the day.



Figure 2. Larvae of *Dastarcus helophoroides* that have consumed an ALB larva.

Spotted lanternfly egg mass storage study and a life cycle study for a newly discovered egg parasitoid, *Anastatus orientalis*

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Introduction

Spotted lanternfly (SLF), *Lycorma delicatula*, is a sap-sucking insect native to China, India and Vietnam, that was first reported in Berks County, Pennsylvania in 2014 (Figure 1). SLF has a wide host range, and has the potential to impact grapes, fruit trees, and hardwood species. Despite extensive eradication efforts, this pest has continued to spread, therefore we have started to examine potential biological control agents from China. Research is being conducted to develop a rearing procedure for SLF and a newly discovered SLF egg parasitoid, *Anastatus orientalis* (Figure 2). Shipments of SLF egg masses from China suspected of containing *A. orientalis* were used to:

- 1) Determine the optimal 5°C chill period to produce the highest egg hatch
- 2) Rear *A. orientalis* and examine its life cycle and potential for host specificity on SLF egg masses in the U.S.
- 3) Calculate percent parasitism of SLF egg masses
- 4) Test *A. orientalis* oviposition on field-collected egg masses from Pennsylvania

Egg hatch and parasitism

The Chinese egg masses were collected in November, 2015 and April, 2016. Overall, percent egg hatch and percent parasitism were much higher in eggs collected in April compared to November (Figure 3). Egg masses were stored in 5°C for 99-142 days. The number of emerged SLF decreased as the storage time increased. Dissection of unhatched eggs revealed desiccated nymphs, suggesting that as chill period of the eggs increases, the likelihood of viable nymphs decreases. This is valuable information for mass rearing and research because it demonstrates that while egg hatch can occur without the induction of winter temperature, egg hatch is higher after winter temperatures are simulated. Additionally, this has highlighted the need to develop proper chill conditions to ensure desiccation does not increase over time.

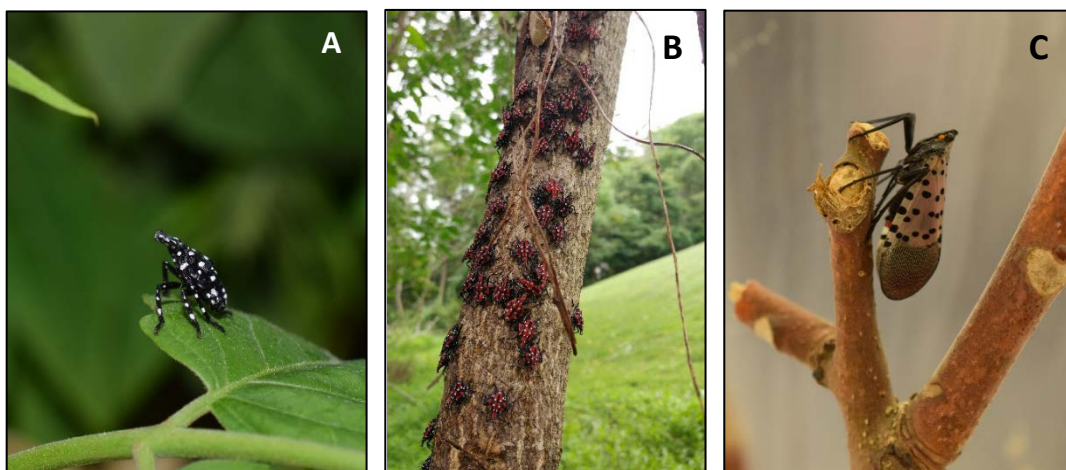


Figure 1. A) Otis lab-reared first instar SLF. B) Berks county fourth instar SLF population. C) Otis lab-reared SLF adult female.

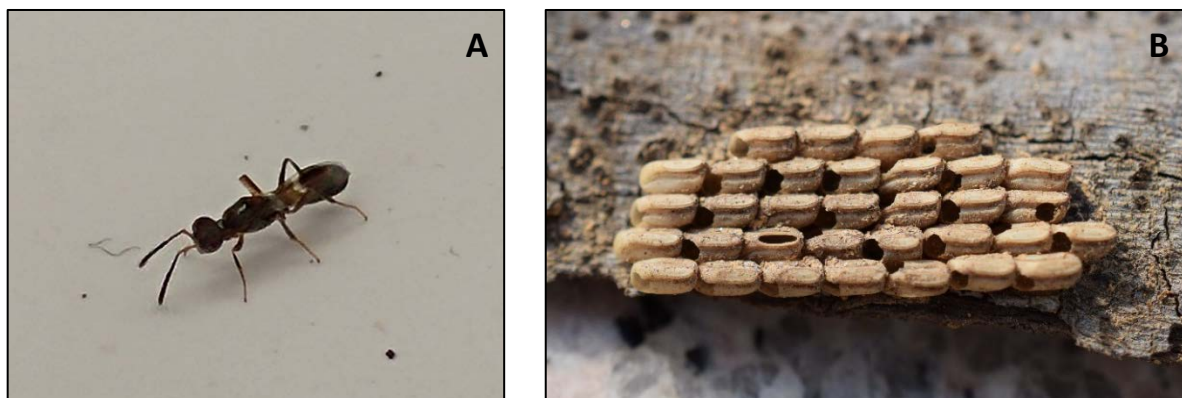


Figure 2. A) *Anastatus orientalis*. B) *Lycorma delicatula* egg mass with round *A. orientalis* emergence holes.

Anastatus orientalis parasitism

Very low parasitism was found in SLF eggs collected in November and an average of 6% parasitism was found in the April collection (Figure 3). Parasitoids that emerged from the April egg masses were continually given new Pennsylvania collected eggs every two weeks throughout their life cycle. There was a spike in the parasitism rate for the first lab exposed egg masses (54%). When those same parasitoids were given a second and third egg mass to determine if continued parasitism was possible, the parasitism rates more closely matched those of natural populations (Table 1). These results demonstrate that offspring of field collected *A. orientalis* can achieve high parasitism in a laboratory setting and are able to continually parasitize egg masses if egg masses are present, despite the low levels of natural parasitism observed in the parent field caught generation.

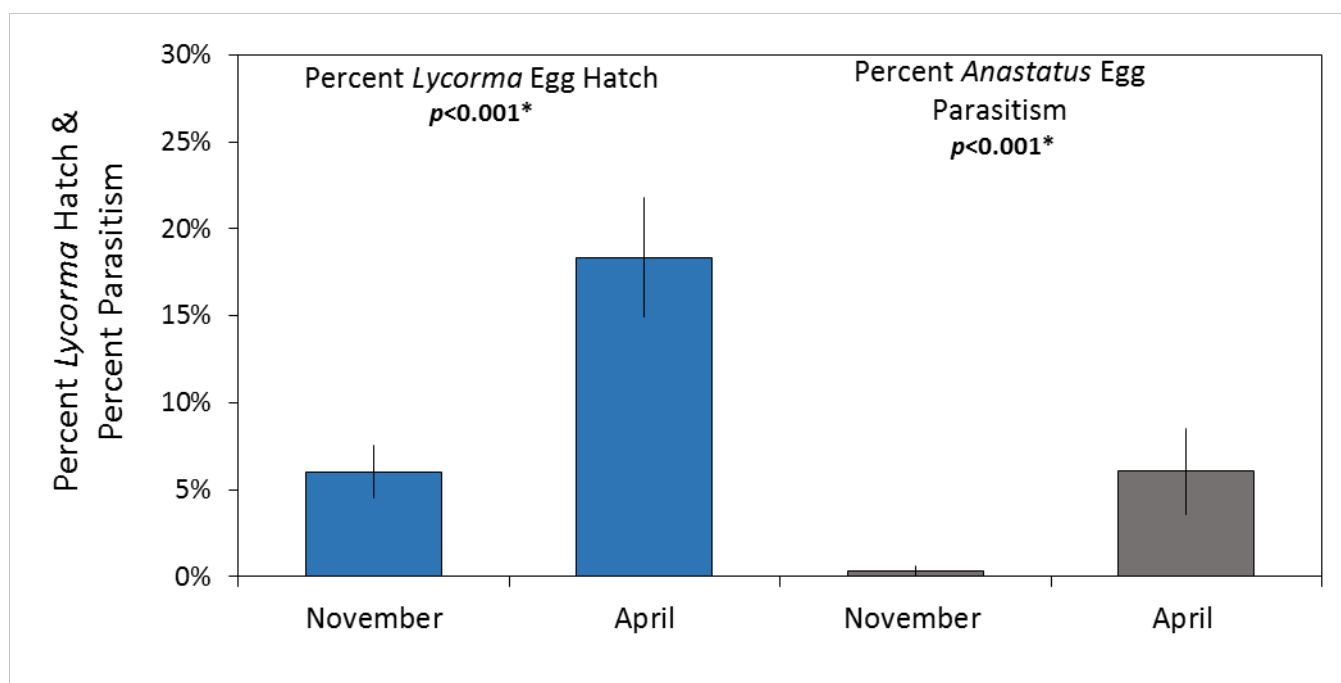


Figure 3. Percent SLF egg hatch and percent parasitism for eggs sent from China in November and April. There was a significant difference ($p < 0.001$) in hatch and parasitism in November compared to April.

Table 1. Percent parasitism, total *A. orientalis* adults, and total *A. orientalis* larvae (found during dissections) per *L. delicatula* egg mass. These parameters are shown for the original Chinese egg masses from November and April, two rounds of lab-induced parasitism (round 1 and round 2), and the first lab generation parasitism.

	Percent parasitism		Number of <i>A. orientalis</i> adults per egg mass		Number of <i>A. orientalis</i> larvae per egg mass	
Natural parasitism (China-Nov)	0%	(SE±0)	0.1	(SE±0.12)	0	(SE±0)
Natural parasitism (China-April)	6%	(SE±2)	2.2	(SE±0.88)	1.8	(SE±.25)
Lab tested parasitism round 1	53%	(SE±7)	22.3	(SE±2.77)	1.8	(SE±0.96)
Lab tested parasitism round 2	7%	(SE±2)	2.3	(SE±0.83)	9.4	(SE±2.5)
First lab generation parasitism	10%	(SE±5)	4.0	(SE±1.5)	6.0	(SE±4.5)

Life cycle and development of spotted lanternfly and *A. orientalis* parasitoid

SLF nymphs emerged after 16 days and 9 days at 25°C, for the November and April egg masses, respectively. *Anastatus orientalis* emerged after approximately 7 days at 25°C. Therefore, it was common for *A. orientalis* to emerge before SLF. However, this was not always the case, at times the two insects emerged simultaneously.

A biological control agent needs to be host specific and therefore should not be able to survive on an alternate host. *Anastatus orientalis* has the ability to continually parasitize eggs if they are present. This is concerning because SLF eggs are not present throughout the summer and if the parasitoid's life cycle requires continued generations, this may be indicative of the potential to parasitize an alternate host. However, egg dissections have indicated that the parasitoids may enter a summer diapause. If *A. orientalis* does diapause during the summer and only emerges in May and September when SLF eggs are present, this may suggest a synchronized life cycle between the two insects. This synchrony has been observed in some provinces in China.

Conclusion

The findings have allowed us to better rear SLF from the egg stage, by simulating a winter chill period to optimize egg hatch. Continued research is required to better understand the life cycle of SLF and *A. orientalis*. Rearing studies for all stages of SLF will continue. Next year the parasitoid will be reared using climate conditions of the current SLF infestation in Berks County, Pennsylvania. The potential for continued parasitism by *A. orientalis* will continue to be examined to determine if alternate hosts are required for summer survival or if the summer diapause will lead to synchrony with SLF.

A new method to characterize risk of chipping using Asian longhorned beetle and surrogate insects

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Introductions of Asian longhorned beetle (ALB), *Anoplophora glabripennis*, in the United States and elsewhere have resulted in expensive, long-term eradication programs to eliminate this pest from the local landscape. At the core of these programs are aggressive mitigation measures that aim to make host material safe for deregulation and movement. Commercial chipping of host material infested with ALB was examined at the request of the USDA-APHIS ALB program, with emphasis on assessing the risk of ALB life stage survivorship post-chipping. Current regulation suggests that host material should be chipped to less than 1 inch in two dimensions before being removed from the regulated area. Initial experiments were designed to evaluate survivorship in relation to increasing chip size by manipulating anvil (gap) settings on disc and drum style chippers. Using red maple bolts that were artificially seeded with surrogate larvae (tenebrionids, *Zophobas morio*), as well as naturally infested ALB material, we found 100% mortality of all life stages at every chipper setting (Figure 1). When anvil settings were increased from the factory settings to their maximum width, chip size for both styles of chippers increased marginally from 1 inch in two dimensions to 1.25 inches in two dimensions. However, even at the larger 1.25 inches in two dimensions, mortality was still 100%. Additional experiments characterized the mode of action of chipping by isolating discrete components of the chipping process. Results indicate that chipping is in fact a combination treatment that involves the obvious cutting action of knives along with associated physical forces inside the chipping chamber. The implications of this finding are important in assessing risk, and directly impact decisions on the regulations of chip movement for such uses as landscaping and fuel for cogeneration plants.



Figure 1. A) Artificially seeding logs with surrogate larvae. B) Loading drum/disc chipper with ALB infested maple logs.

Comparative study of radio-frequency and microwave heating for phytosanitary treatment of wood

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Wood packaging material is frequently made from lower-grade raw wood that may not have undergone sufficient levels of processing to reduce pest risk, and has been identified as a major pathway for the introduction and spread of quarantine pests. Dielectric heating through radio-frequency (RF) or microwave (MW) heating for phytosanitary treatment of wood packaging material was recently accepted as an alternative to methyl bromide fumigation and conventional heating. These technologies were evaluated and compared to assess treatment time, depth of electromagnetic wave (EMW) penetration, and heating uniformity. White oak, *Quercus alba*, wood cants (48 cm long with cross-section dimensions ranging from 10x10 cm² to 25x25 cm²) were heated in a 19 MHz RF or 2.45 GHz MW laboratory oven using an equivalent heating power (3.4 kW). In each specimen, temperature was measured at different depths (distance from the upper face). Specimens were held in the treatment chamber for 2 minutes after the target temperature of 60°C was reached throughout the profile of the specimen to ensure compliance with the ISPM-15 treatment schedule. Thermal image analyses of treated specimens as well as theoretical depth of penetration for dielectric energy were explored. Wood specimens were also heated using RF at high power (9 to 11 kW) and results were compared with RF heating at 3.4 kW.

For wood with cross-section dimensions of 10x10 cm² to 15x15 cm², heating rates for RF and MW were relatively similar. However, above 15x15 cm², RF heating was more than 40% faster with greater heating uniformity than MW. The theoretical values derived for depth of penetration and thermal image analyses indicate that RF (19 MHz) penetrates wood more uniformly and is better suited than MW (2.45 GHz) for bulk volume treatments of wood.

To further develop the use of RF heat as a phytosanitary treatment for wood packaging material, multiple commercial scale RF ovens were investigated. These ovens are typically used for wood drying, so they did not provide enough energy to reach the necessary temperature for phytosanitary treatment. Therefore, a custom RF oven was built to the necessary specifications and it will be used for further experiments (Figure 1).



Figure 1. A custom-built radio-frequency oven, to be used in developing the phytosanitary treatment for wood packaging material.

Evaluation of vacuum and steam as a methyl bromide alternative for phytosanitary treatment of hardwood veneer logs

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There is an immediate need to develop and adopt new treatment technologies for eliminating insect pest and tree pathogens from veneer logs moved in trade. This is largely due to the current phase-out of methyl bromide fumigation and the uncertainty associated with the efficacy of potential alternatives. The combination of vacuum and steam has a proven and reliable record for commercially sanitizing a variety of commodities including cotton, spices, and textiles among others. An initial study was designed to evaluate basic parameters of vacuum and steam application on five high value hardwood veneer log species to determine feasibility of vacuum steam as a phytosanitary treatment. Relative heating rates to log center, damage and value loss assessment due to treatment, and overall energy used during treatment were recorded for logs treated individually in a flexible polymer chamber (Figure 1).

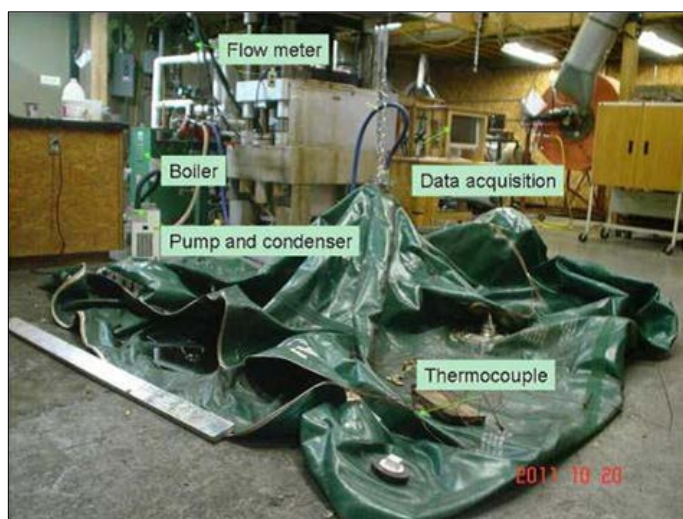


Figure 1. Flexible polymer chamber used for initial experiments on vacuum steam as a phytosanitary treatment for hardwood veneer logs.

At 200 mm Hg vacuum, time to reach 56°C for 30 minutes to core ranged from 17-29 hours, depending on density and log diameter. Damage varied by species, but veneer sawn from logs was largely unaffected in terms of yield and value (Figure 2). Energy used during treatments ranged from 54 to 205 kilowatt-hours (kWh) for individual logs. These results suggested that vacuum and steam had potential as a phytosanitary treatment for hardwood veneer logs, so field studies were conducted the following year.

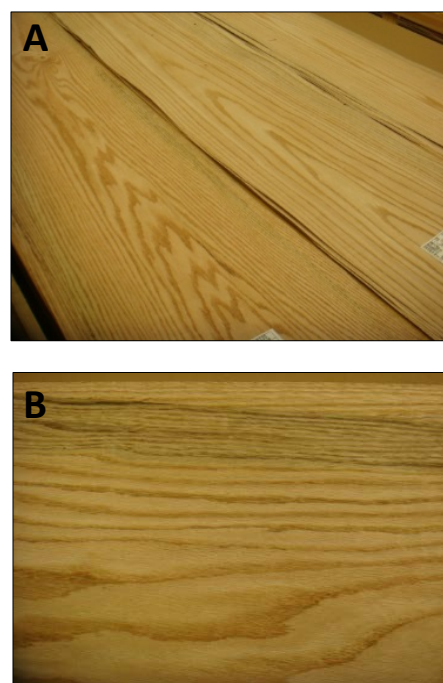


Figure 2. Veneer sawn from A) treated and B) untreated wood showed no difference in quality.

A portable chamber was designed to test vacuum and steam as an alternative for treatment of logs infested with oak wilt, *Ceratocystis fagacearum*, or thousand canker disease complex (TCD), *Geosmithia morbida*, and *Pityophthorus juglandis* (Figure 3).



Figure 3. The portable vacuum steam chamber being loaded with oak logs.

Both naturally infested and artificially inoculated oak logs were harvested and treated on site in Shakopee, Minnesota. Temperature inside the log and energy use were monitored during treatment. Isolations from the infested logs were taken before and after treatment, and plated on a selective medium to detect the presence of the oak wilt fungus (Figure 4).



Figure 4. Small wood chips were taken before and after treatment, and plated on a selective medium to detect the presence of pathogens.

The fungus was not isolated from any locations assayed in post-treatment logs, indicating successful treatment. Next, naturally infested and artificially inoculated TCD walnut logs were harvested and treated in Manheim, Pennsylvania. Again, temperature and energy use were monitored, and isolations were taken before and after treatment. One post-treatment isolation was positive for the TCD fungus, but sample contamination is suspected. Preliminary results suggest that vacuum and steam is a viable treatment for oak wilt and TCD. In the future, large-scale field studies will be conducted to determine a treatment schedule. Furthermore, this technology is being investigated as a potential treatment for other pathogens.

Identification and origin of infested wood packaging material

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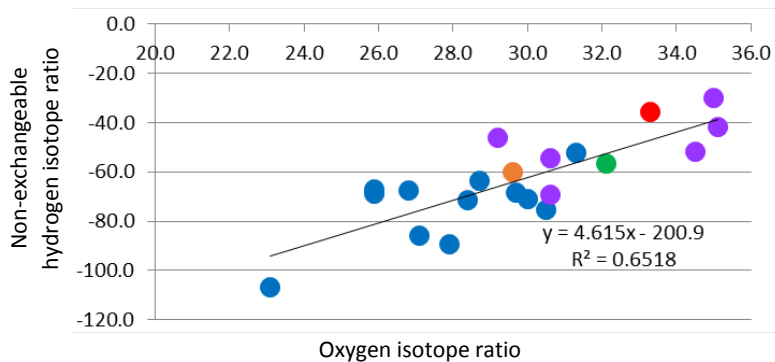
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Despite stringent phytosanitary regulations, solid wood packaging material (WPM) continues to act as an important pathway of introduction for exotic wood boring insects. Through an ongoing cooperative effort between U.S. Customs and Border Protection (CBP) and USDA Animal and Plant Health Inspection Service (APHIS), live woodborer larvae, in particular cerambycids, buprestids and siricids intercepted during agricultural inspections at U.S. ports, are sent to the containment facility at Otis Laboratory for rearing to the adult stage and for DNA barcoding. Data are used to develop risk maps for pest entry and establishment. In this study, we aimed to identify the most commonly intercepted, infested wood genera used for construction of WPM to understand the pathway by which pests might arrive in the U.S. Wood identification revealed *Pinus* (40%), *Picea* (19%), and *Populus* (20%) as the three most commonly infested WPM tree genera.

Because WPM may originate in areas outside the shipment origin, we explored the possibility of using stable isotope analysis to ultimately pinpoint the origin of the WPM. $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values were calculated for 20 pine WPM samples from five Eurasian countries using the Cellulose Extraction Method. *Pinus* was selected as it was the most commonly intercepted wood genus. Because precise wood origin is unknown, we used location of facilities that manufacture and treat WPM as a proxy for its origin; origin is, therefore, limited to country. $\delta^2\text{H}$ and $\delta^{18}\text{O}$ isotope profiles in the wood were significantly correlated to each other. The combined profile showed some discrimination among regions (Figure 1). $\delta^2\text{H}$ values accounted for 90% of the observed variation in geographical origins. If a global map of $\delta^2\text{H}$ isotope values is created from wood originating at precise locations, it may be possible to pinpoint the geographic origins of WPM samples by comparing the wood isotope values with isoscape, a predictive geographic model.



Map illustrating five Eurasian countries where treatment facilities of WPM samples are located

Figure 1. $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values in pine cellulose samples from WPM likely originating in Europe and Asia; origin data are from wood treatment (IPPC)* marks; cargo originating from Italy had an illegible IPPC mark, so Italian origin is assumed; *IPPC: International Plant Protection Convention. Each piece of WPM should be stamped with an IPPC mark denoting country of treatment, treatment facility, and type of treatment.

Evaluation of methyl bromide alternative fumigants for wood pathogens

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The movement of whole logs in international trade provides a potential introduction pathway for a number of important forest pest and pathogens. For this reason many countries require phytosanitary treatment of log imports. Log exporting countries have typically relied on fumigation with methyl bromide as it has been an inexpensive and efficacious treatment. However, increasing restrictions on the use of methyl bromide due to its status as an ozone depleting compound have created a need to find alternative treatments that are effective in eliminating exotic pest establishments via trade in wood products. Efficacy data for established phytosanitary fumigants have primarily focused on arthropods and nematodes, whereas limited information exists for plant pathogens and fungi relevant to forestry.

As part of a broader project to develop methyl bromide alternative fumigants, we developed a rapid screening process to evaluate efficacy and compare relative tolerance to fumigation treatments for a broad selection of relevant fungi under laboratory conditions. The fumigant sulfuryl fluoride was chosen for evaluation as it is currently labeled in the U.S. for use on logs and previous work has found it to be effective against wood-inhabiting insects, pinewood nematode, and oak wilt fungus. However, little is known about the efficacy of sulfuryl fluoride against a broader range of fungi.

We conducted sulfuryl fluoride fumigations in 10 liter chambers at six target concentrations (40-240 mg per liter) at 15°C and 20°C for 24-, 48-, and 72-hour exposure times against 23 fungal species represented with 35 isolates. Fungi were grown on sterilized barley grain and then distributed in felt-covered borosilicate glass tubes to allow uninhibited gas penetration during fumigation while minimizing the risk of fungal contamination (Figure 1).

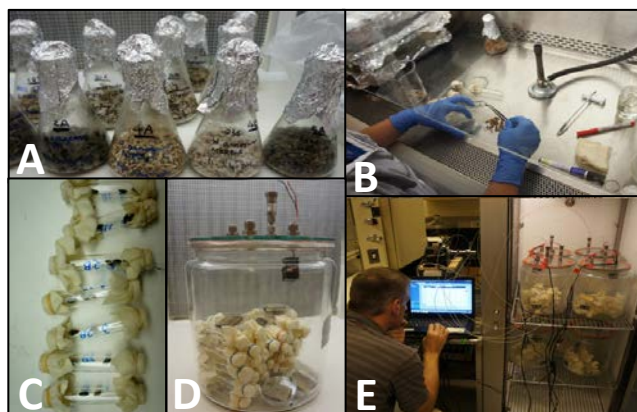


Figure 1. Test methodology and apparatus used to screen fungal isolates with sulfuryl fluoride. A) Colonized barley grains with individual fungi. B) Distribution of grains into individual tubes. C) Sealed tubes containing inoculated grain. D) 10-liter test jars filled with tubes containing different test isolates. E) Measuring of gas concentrations via gas chromatography.

This allowed simultaneous testing of numerous species and isolates, followed by 100 percent recovery of controls without contamination. Results found that sulfuryl fluoride is an effective fumigant for a broad range of fungi. Several fungi and isolates were found to consistently be among the most tolerant to the sulfuryl fluoride fumigant treatment. Among the most tolerant were *Geosmithia morbida*, the fungal component of thousand cankers disease in walnut and *Ceratocystis fagacearum*, the fungus responsible for oak wilt. See Uzunovic et al. 2017 for the complete list.

These sulfuryl fluoride tolerant species can serve as a benchmark for screening in treatments of logs and other wood products with the potential to carry fungi. Methods developed here will be useful in efficacy screening of other methyl bromide fumigant alternatives. Follow up studies are ongoing to assess fumigant penetration and efficacy in larger-scale trials using logs to demonstrate feasibility for the replacement of methyl bromide as a phytosanitary measure for international trade of log imports.

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Development of new treatment options for khapra beetle

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The khapra beetle, *Trogoderma granarium*, is a serious pest of stored products and is the only stored products pest with quarantine status in the United States. The larvae of *T. granarium* feed on a wide range of dry food products of plant and animal origin including cereals, processed grains and dried meats. New treatments are needed to replace older generation insecticide treatments facing label use and registration restrictions for the control and eradication of khapra beetle. We evaluated efficacy and residual activity of two pyrethroid (deltamethrin and β -cyfluthrin) and a pyrrole insecticide (chlorfenapyr) as well as two insect growth regulators (methoprene and pyriproxyfen) to provide data in support of their use for regulatory control of khapra beetle. Each of these products are labeled for food and feed mill application in the U.S.

The products were applied on a variety of surfaces including concrete, wood, painted-wood, vinyl flooring tile, and metal.

Small and large khapra beetle larvae and adults were tested in a series of experiments to evaluate differences in insecticide efficacy in a range of environments across khapra beetle active life stages. Khapra beetles with provision of a food source were exposed on the treated surfaces and residual assays were conducted up to 12 weeks post-treatment time. Results found that all three insecticide products provide a high level of control against khapra beetle larvae and adults (Figure 1). The adults from both laboratory and field populations were easily killed long after treatment (60+ days). On average, larval mortality of laboratory populations was higher than the field population across treatments, however, this difference was non-significant (Figure 2). Larvae were more tolerant to insecticides compared to adults and residual efficacy was reduced after 60 days (Figures 2 and 3). Although there were significant effects of insecticides, residual efficacy and insect populations on the mortality of khapra beetle larvae, most of the surviving larvae did not pupate and develop to the adult stage after they were transferred into new untreated arenas with additional food.

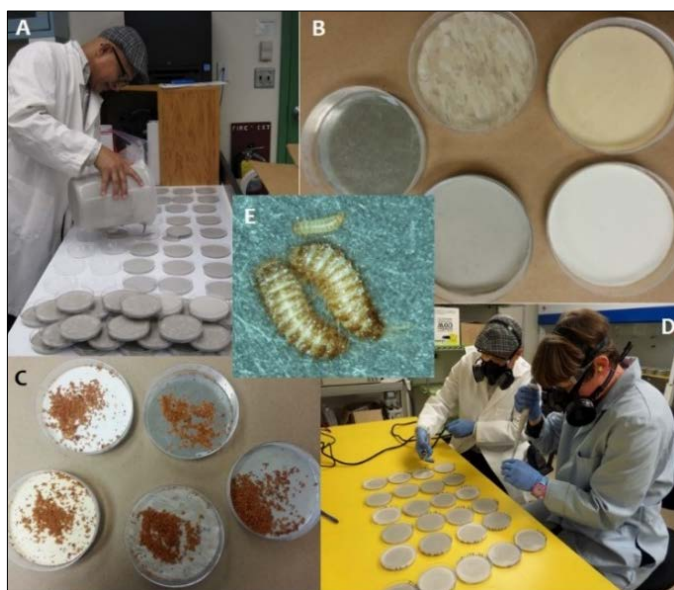


Figure 1. Small scale assays used to evaluate treatments. A) Preparing concrete arenas in petri dishes. B) Arenas with a variety of treatment substrates. C) Food provisions provided to larvae following treatments. D) Treatment applications made using airbrush. E) Small and large khapra beetle larvae.

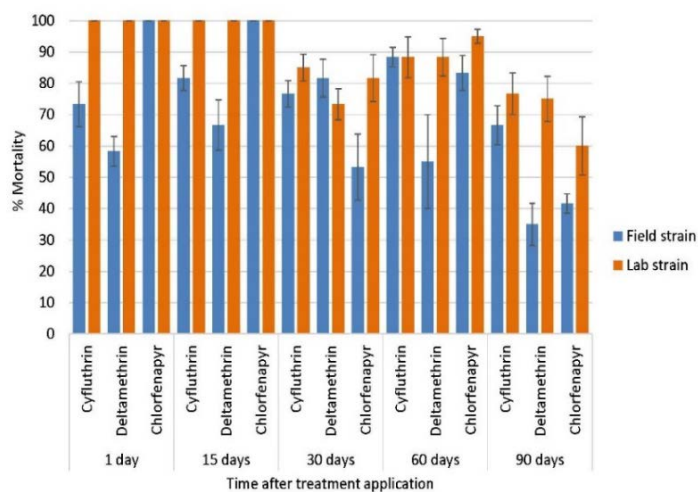


Figure 2. Mortality (mean \pm SE, $n=6$) of laboratory and field populations of khapra beetle larvae exposed to concrete arenas treated with β -cyfluthrin, deltamethrin, and chlorfenapyr at label rates.

Residual efficacy of the insect growth regulators (IGRs), methoprene and pyriproxyfen applied on wood, metal, and concrete was similarly evaluated using small and large khapra beetle larvae. In general, small larvae (Figure 4) were more susceptible than the large larvae. For both larval categories, consistently high larval mortality was obtained on the metal surface treated with IGRs, indicating greater residual persistence on metal compared to wood and concrete.

These experiments show that larvae of khapra beetle are more tolerant than adults to the three insecticides evaluated. Longer exposure times were required to produce complete mortality of larvae compared to adults. Adults were controlled up to three months post application. Additionally, the three insecticides outperformed the IGRs, providing a higher level of control and greater residual efficacy. These results will provide new treatment options for control and eradication of khapra beetle in a variety of commercial and residential environments.

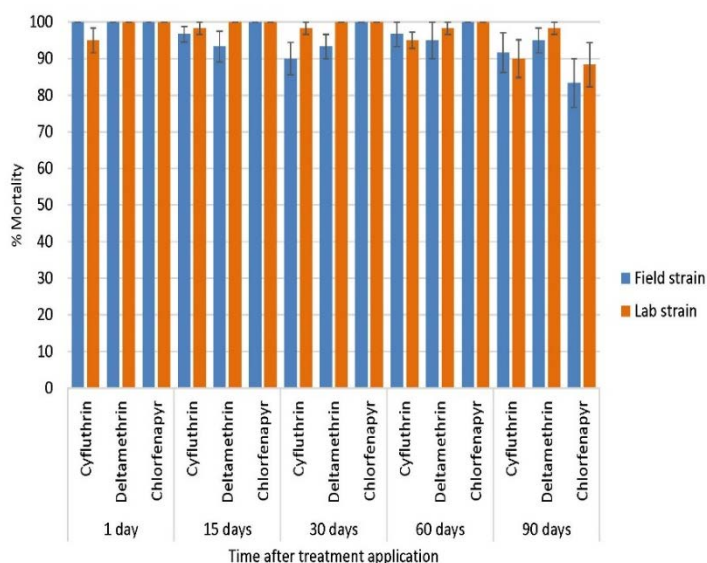


Figure 3. Mortality (mean ± SE, n=6) of laboratory and field populations of khapra beetle adults exposed to concrete arenas treated with β-cyfluthrin, deltamethrin, and phantom at label rates.

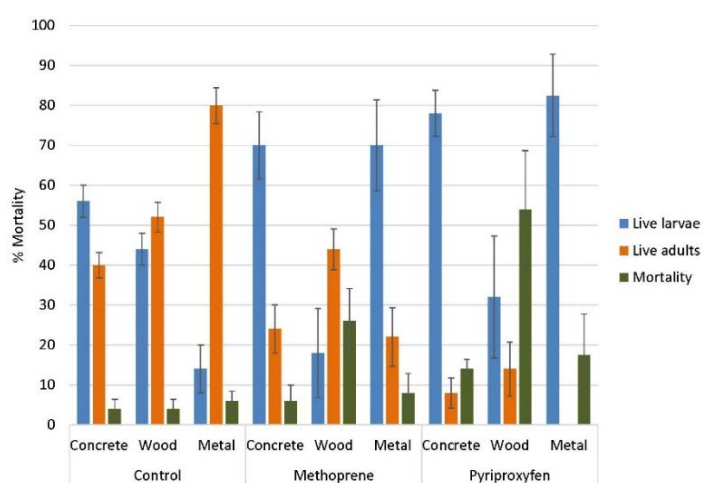


Figure 4. Residual activity of the insect growth regulators methoprene and pyriproxyfen 30 days post treatment on small khapra beetle larvae in assays with three surface materials. Assessments were made 30 days after exposure.

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Harmonizing phytosanitary cold treatments for *Bactrocera* speciesScott W. Myers¹, Elena Cancio-Martinez², Guy J. Hallman², Emily A. Fontenot² and Marc J.B. Vreysen²¹Otis Laboratory, USDA-APHIS-PPQ-S&T-CPHST, Buzzards Bay, MA²Insect Pest Control Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, IAEA, Vienna, Austria

International trade in citrus, tropical fruits and other horticultural commodities that are fruit fly hosts often relies on phytosanitary cold temperature treatment to prevent the introduction and establishment of exotic fruit flies. Cold treatments have been used for decades as a safe, reliable and non-chemical phytosanitary treatment. However, the vast number of economically important species of Tephritid fruit flies has resulted in a wide variety of treatment schedules among trading partners. To assess whether a single (i.e., generic) cold treatment could be developed that would control a broad group of fruit flies, small scale experiments were conducted using laboratory reared cultures. The relative cold treatment tolerance across the economically important Tephritid fruit flies, *Bactrocera carambolae*, *B. correcta*, *B. cucurbitae*, four populations of *B. dorsalis*, *B. zonata*, and *B. tryoni*, eggs (*in vitro*) and larvae (in infested fruit or on carrot diet) were compared in cold treatments at $2.0 \pm 0.2^\circ\text{C}$ for selected durations.

Probit regression models, used to establish the hierarchy of cold resistance, found 3rd instar larvae reared on carrot diet to be the most cold tolerant, followed by 3rd instar larvae reared in orange, while eggs tested *in vitro* were the least tolerant (Table 1). The relative ranking of cold tolerance in larvae reared in carrot diet and those reared in orange was very similar for four of the six species. This suggests that treatment in diet may prove useful in rapidly ranking species and populations for relative cold tolerance or in development treatments for poor hosts. Differences in mortality responses of 3rd instar larvae reared in oranges across populations of *B. dorsalis* were observed only at sub-efficacious levels of control. The majority of *Bactrocera* species responded the same at the high levels of control demanded of phytosanitary treatments, which indicated that cold treatments would be similarly effective across the species and populations tested. *Bactrocera cucurbitae* was found to be the most cold tolerant of all the species tested, suggesting that cold treatments that are efficacious against *B. cucurbitae* will also be efficacious against the other species tested.

Table 1. Probit model estimates and 95% fiducial limits of days cold treatment at $2.0 \pm 0.2^\circ\text{C}$ required to produce 99.9% and 99.99682% mortality for eggs, larvae in fruit and larvae treated in diet bags. Within each column, values followed by different letters were significantly different based on lethal dose ratio tests ($\alpha=0.05$).

	Egg		Navel Orange		Diet	
	99.90%	99.99682%	99.90%	99.99682%	99.90%	99.99682%
<i>B. zonata</i>	5.72 (5.21, 6.36) C	7.37 (6.69, 8.23) C	4.70 (4.38, 5.08) D	5.64 (5.24, 6.11) D	9.46 (8.95, 10.05) D	11.30 (10.66, 12.05) D
<i>B. dorsalis</i>	7.25 (7.00, 7.52) B	9.25 (8.92, 9.61) B	6.88 (6.70, 7.07) C	8.31 (8.09, 8.54) C	11.09 (10.80, 11.40) BC	13.25 (12.89, 13.64) BC
<i>B. correcta</i>	7.11 (6.60, 7.74) B	9.05 (8.37, 9.88) B	8.38 (8.06, 8.75) B	9.86 (9.46, 10.31) B	10.35 (9.75, 11.05) CD	12.42 (11.67, 13.29) CD
<i>B. carambolae</i>	7.39 (6.96, 7.89) B	9.30 (8.74, 9.96) B	6.97 (6.79, 7.15) C	8.21 (8.00, 8.44) C	11.44 (11.14, 11.78) B	13.47 (13.09, 13.89) BC
<i>B. tryoni</i>	7.29 (6.87, 7.78) B	9.09 (8.54, 9.73) B	10.97 (10.59, 11.39) A	12.93 (12.46, 13.47) A	11.69 (11.26, 12.16) B	13.86 (13.33, 14.43) B
<i>B. cucurbitae</i>	10.65 (10.15, 11.21) A	13.18 (12.54, 13.91) A	10.93 (10.52, 11.39) A	12.64 (12.13, 13.22) A	18.09 (17.29, 19.00) A	21.41 (20.41, 22.55) A

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Targeted treatments for Asian longhorned beetle management within low-risk sites

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Populations of Asian longhorned beetle (ALB), *Anoplophora glabripennis*, have been under a control effort through an APHIS eradication program since 1998 and in the last few years populations in Chicago, New Jersey, Boston and portions of New York have been successfully eliminated. Eradication activities are multi-faceted and include visual survey and delimitation, infested tree removal for population reduction, and chemical treatment of at-risk trees to prevent population expansion and growth.

Treatment of trees with a systemic insecticide (imidacloprid) has been a valuable tool for many years and is used to protect trees from ALB infestations within urban areas. At-risk trees in the vicinity of known infestation sites have been treated either by direct tree injection or soil injection (Figure 1). The most recent discovery of ALB populations in Bethel, Ohio and Worcester, Massachusetts have presented new challenges to the eradication effort. These infestations are large and are located in rural and heavily wooded areas whereas prior control efforts were primarily conducted within urban and city park settings.

With beetle populations now present in contiguous wooded habitats, chemical treatment of at-risk trees is cost prohibitive due to numerous woodlots, high tree densities and the large quarantine area (>150 square miles). Eradication resources are focused on removing host trees, even when risk of infestation is low.

In order to investigate if chemical treatments can be a viable tool for the Ohio and Massachusetts infestation, a targeted treatment study is being undertaken. Previously, chemical treatments by the ALB program have extended up to 400 meters (¼ mile) from the core of an infested area. However, in this study visually uninfested host trees will be treated out to a 20 meter radius from an infested tree (Figure 2). This reduction in treatment area is projected to reduce both treatment and personnel cost. Treatments will be conducted for three years via trunk injection of host trees using standard treatment protocols beginning in 2016. Study sites will be inspected regularly for signs of ALB infestation. Woodlots selected for this study have at least three of the following key attributes: near the edge of the quarantine boundary; removed trees had been recently infested; previously contained multiple infested trees; numerous host trees present within the 20 meter distance from the infested tree(s).



Figure 1. Tree injection equipment used in the ALB eradication program.

Two distinct approaches are being used to investigate the suitability of adopting these targeted treatments as a control method for the ALB eradication program. Chemical treatments applied during the spring will target all of the host trees within the 20 meter circumscribed areas, and will be compared to control areas where no treatments were applied. The second approach in the fall, will involve applying insecticide to 50% of the host trees present within the 20 meter radius. Trees will be randomly selected from the inventory list such that half of the total tree diameter is treated in those plots. The two treatment seasons were selected to coincide with the existing operational schedule of the ALB program.

Treatment effectiveness will be gauged by comparing the two approaches to each other as well as to the control sites. The fall approach will increase the likelihood that a small, latent beetle infestation will be discovered by subsequent surveys since not all trees are treated. It also provides less risk when there is a site of interest to the ALB Program, such that areas of concern are not set aside as untreated controls.

Considering the likely limited dispersal of incipient ALB populations along the quarantine boundaries, it is anticipated that this project will demonstrate that a small treatment radius is not only an effective tool for the ALB program, but can also provide reduced costs and improve public perception as compared to host tree removal operations.

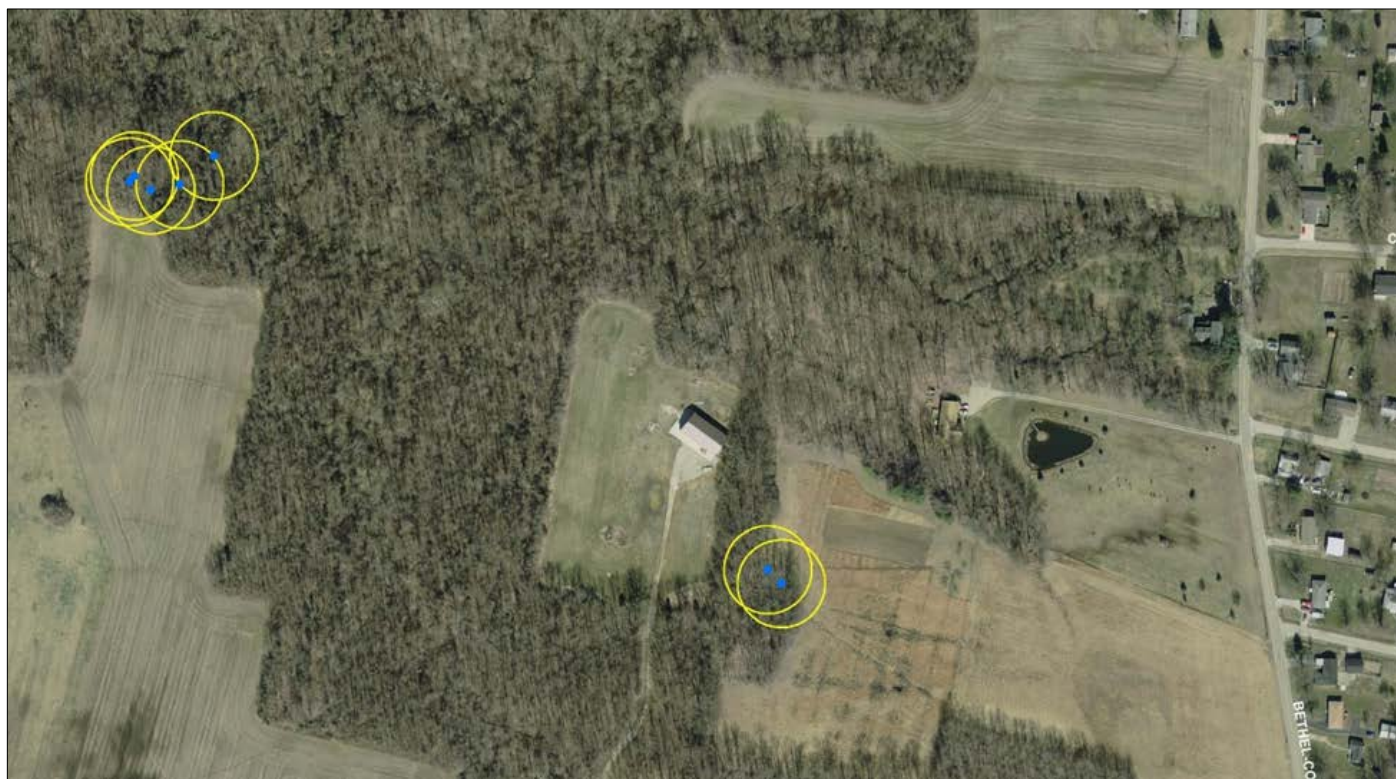


Figure 2. Depiction of 20 meter treatment boundaries centered on infested trees (blue dots).

Evaluating the effectiveness of regulatory controls for the pine shoot beetle

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The discovery of populations of pine shoot beetle (PSB), *Tomicus piniperda*, in Ohio and five surrounding states in 1992 prompted USDA involvement and the establishment of a new pest program for APHIS (Figure 1). PSB was a common intercept on wood articles in ports between 1985 and 1996, before bark-free regulations were implemented, but it's thought to have established in the United States prior to 1985. This invasive bark beetle has slowly expanded its range and is now present in 20 states (Figure 2). The initial economic impact of PSB on Christmas tree growers, timber production and urban areas in the United States was projected to be significant – \$742 million over 30 years (USDA-FS, 1992). In reality, the damage to native pines, plantations and the nursery trade has been minimal and/or not reported.



Figure 1. Pine shoot beetle, *Tomicus piniperda*.

While PSB will likely continue to naturally spread and disperse southward based on the widespread availability of *Pinus* species in southern states, PSB is now thought to be a minor pest. Outbreaks on living trees are typically not self-perpetuating and are readily controlled as a result of intra-species competition. Also, due to its secondary pest status, the agency has been examining the effectiveness and necessity of the PSB regulatory program.

Before APHIS can consider ending the PSB program, or any other pest program that is publicly funded, it must demonstrate to administrators and stakeholders that such a change is economically and scientifically viable and is in the best interests of all involved. State entities, trade partners, and organizations like the National Plant Board are consulted and brought into the process to obtain feedback before announcing any proposed changes to the public through the Federal Register.

Biologically, this species of bark beetle is considered to be a secondary pest of pine and is not able to successfully attack healthy trees. It colonizes fresh timber and dying or damaged pine trees in the early spring. Larvae feed within galleries under the bark, after which they emerge as adults and move to feed on distal shoots in the crown. Overwintering beetles emerge from shoots after a hard frost and move to the base of the trees.

The discovery of a new invasive insect outside of its native range often leads to speculation that the pest will be able to overwhelm previously unencountered host-plant defenses and/or reproduce at increased rates due to a lack of natural enemies. There are many case studies of this phenomenon, but there is also an abundance of literature and historical data on PSB that indicate it is neither capable nor likely to become more problematic, such as:

- 1) **Lack of pest status over a large geographic range**
PSB is classified as a secondary pest species, with occasional outbreaks that have been linked to either poor timber harvesting practices or extreme weather events that result in a large amount of suitable host material.
- 2) **Native competition and predators**
A study determined that native bark beetles were colonizing cut logs alongside PSB in the early spring and that a native clerid along with four other scolytid predators were actively preying on PSB (Kennedy and McCullough, 2002).
- 3) **Native bark beetle complexes**
An analysis conducted on the existing bark beetle community concluded that PSB had limited impact in natural and planted pine stands in the United States and Canada (Haack and Poland, 2001).
- 4) **Management of pine plantations**
Southern and western pine plantations in the United States are typically highly managed and trees are cultivated under intensive forestry practices. Good pest management practices have been shown to be quite effective in limiting PSB populations within managed stands.

The PSB program became ineffective in its operations and the benefits provided by the program were outweighed by the costs. While the possibility exists that PSB may spread at a faster rate and enter southern states sooner without the PSB program, it is anticipated that PSB will be controlled within managed timber stands in the south and will remain a secondary pest. As of May 2017, a proposed amendment to the PSB authority is moving through the regulatory process before being published in the Federal Register. Once that is completed and public comments have been addressed, a final rule will be published outlining any changes.

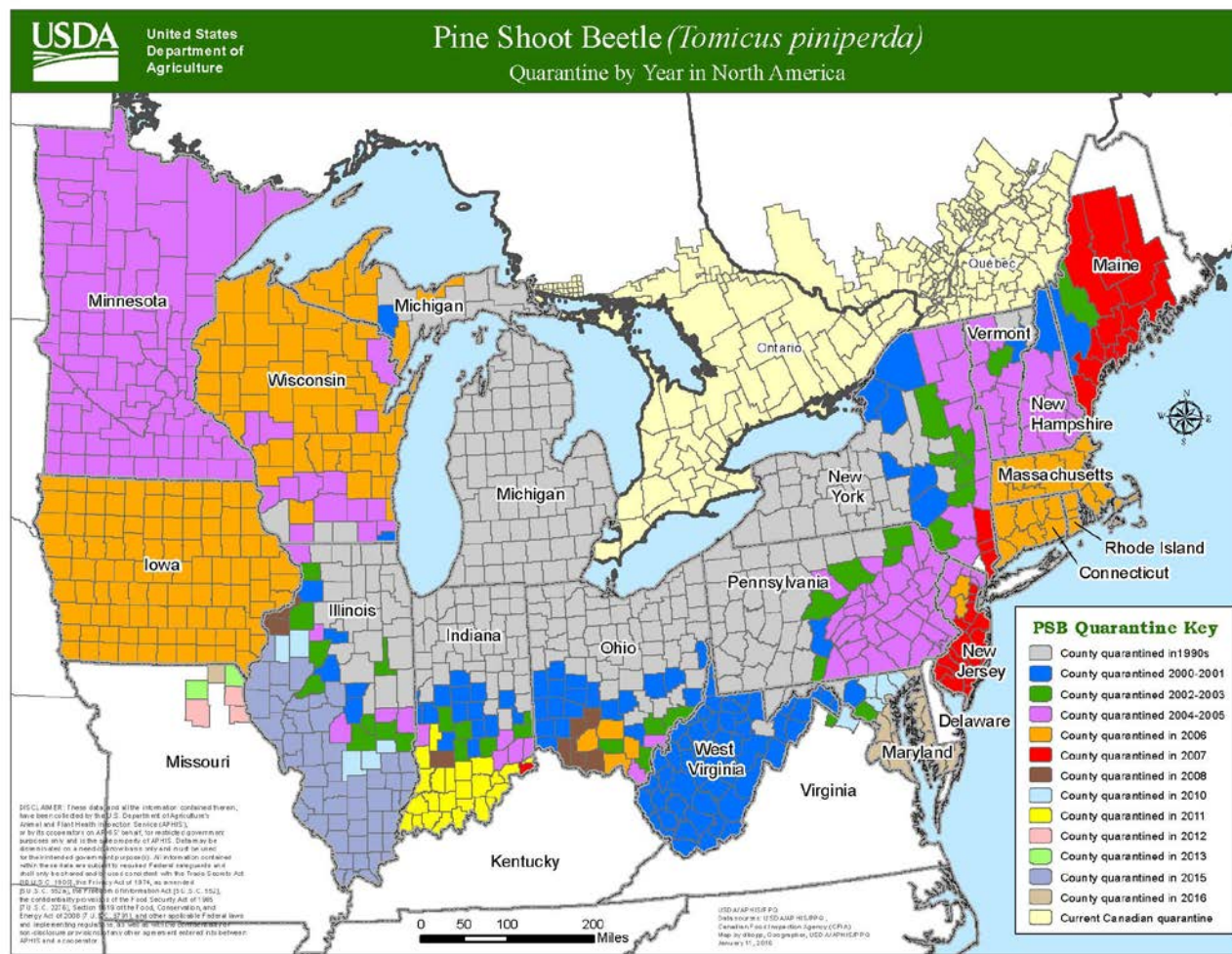


Figure 2. Pine shoot beetle quarantine as of January 11th, 2016.

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Species of insects attacking North American tree species planted in China

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The purpose of this project was to identify insect pests in their native countries that may potentially become devastating to North American trees if introduced and established in the U.S. The susceptibility of North American trees was predicted by observing the degree of attack and the outcome of infestations on tree species which were planted in arboreta, as shade trees, or in commercial plantings in China. More than 30 sites were surveyed from 2014-2016 in the provinces of Shandong, Jiangsu, Anhui, Zhejiang and the municipality of Shanghai. Most of the sites were nurseries importing and planting North American trees such as red maple and blackgum. Other sites were arboreta or parks or streets with ornamental and shade trees. Surveys in eastern China indicated that the majority of North American trees planted in China belong to the following genera: *Acer*, *Betula*, *Carya*, *Cercis*, *Fraxinus*, *Gleditsia*, *Ilex*, *Juglans*, *Lagerstroemia*, *Liquidambar*, *Liriodendron*, *Malus*, *Nyssa*, *Picea*, *Pinus*, *Populus*, *Prunus*, *Quercus*, and *Salix*.

The majority of the insect attacks on North American trees in China were caused by insects in the following families: Buprestidae (e.g., *Agilus planipennis*), Cerambycidae (e.g., *Anoplophora glabripennis*, *A. chinensis*, *Aromia bungii*, *Apriona rugicollis*), Chrysomelidae, Lucanidae, Scarabaeidae, Aphididae, Cixiidae, Diaspididae, Fulgoridae, Lygaeidae, Pentatomidae, Pseudococcidae, Psyllidae, Ricaniidae, Tingidae, Cossidae, Eribidae (e.g., *Lymantria dispar asiatica*), Geometridae, Limacoidae, Psychidae, and Zygaenidae. Figures 1-4 show several examples of woodborers that infest North American trees planted in China.



Figure 1. Red neck (peach) longhorned beetle (RNLB), *Aromia bungii*, was found in most sites surveyed and caused serious damage to *Prunus* trees such as purple cherry plum and midget.



Figure 2. Citrus longhorned beetle (CLB), *Anoplophora chinensis*, can complete its life cycle on red maple trees as small as 1 cm in diameter as well as in a wide range of other host trees species.



Figure 3. Asian longhorned beetle (ALB), *Anoplophora glabripennis*, co-occurred with CLB on some trees in a few sites and infested quite a few tree species such as red maple, Norway maple (*Acer platanoides*), and box-elder (*A. negundo*).



Figure 4. A mulberry longhorned beetle (MLB), *Apriona rugicollis* (referred as *A. germari* in most Chinese literature), on a midget crabapple (*Malus x micromalus* cv. "American").

In addition, Lepidoptera caterpillars, especially species in the families of Eribidae (e.g., Asian gypsy moth, *Lymantria dispar asiatica*), Geometridae, and Limacoidae, primarily feed on leaves of trees, causing serious defoliation. Hemiptera and other insects were also found attacking North American trees in China. For example, *Euphalerus robiniae* psyllid on the eastern redbud, and a Tingidae species feed on the midget crabapple. *Tropidothorax elegans* feeds on leaves of a tulip tree. Spotted lanternfly (*Lycorma delicatula*) adults were found on several species including an ash tree (*Fraxinus* sp.). Eggs and adults of *Dolycoris baccarum* (Pentatomidae) were found on a sweetgum tree. This study is a long term project and will be continued with a focus on woodborers at selected sites. Woodborers are harder to detect and are more likely to become hitchhikers, therefore they are more likely to be introduced.

Characterizing and mitigating risk posed by exotic *Lymantria* species

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Introduction

The Asian gypsy moth complex (AGM) includes the following *Lymantria* species or subspecies whose females possess flight ability: *Lymantria dispar asiatica*, *L. dispar japonica*, *L. albescens*, *L. umbrosa*, and *L. postalba*. In the U.S., these moths, as well as *L. mathura*, *L. monacha*, and *L. xyliina*, are targets of survey and detection efforts as well as offshore vessel certification programs. The goal of this project is to identify and improve risk management measures for these exotic *Lymantria* species.

Cooperative Studies in Russia

In one study, four AGM populations from different geographical areas of Russia were compared for their female wing-fanning periods, which is one of the risk factors contributing to the possibility of AGM females laying egg masses on a vessel. Results showed that unlike females from Novosibirik, South Siberia, and Russia Far East, whose wing-fanning happens after sunset, females from the Trans-Ural region exhibit wing-fanning earlier in the evening and stop before sunset (from 5 PM to 7 PM). This suggests that Trans-Ural females will not fly at night and therefore, pose as less of a risk since it is unlikely that they will be attracted to lights on a vessel called at a port during evening hours.

In another study, trapping experiments using five disparlure concentrations (1-5,000 ug per lure) and three different population densities, found that trap capture of males is density dependent (Figure 1). In addition, trap catch of males declined when lure concentrations were high. In all cases, traps baited with disparlure at the concentration of 10 ug per lure caught more male moths than other concentrations.

Studies were performed to compare the impact of food availability on the longevity of gypsy moths from three different regions: AGM from Altay (Russia) and European gypsy moth (EGM), *Lymantria dispar dispar*, from Volgograd (Russia) and West Virginia (United States). The study simulates the starvation conditions that occur when AGM larvae hatch from egg masses on a vessel in order to assess risk of survival. For each population, 10 egg clusters were randomly placed in 5 Petri dishes, each with 2 egg clusters and under 23°C. The time from when eggs were placed in a Petri dish to when a larva hatched was recorded. Newly hatched larvae were transferred to another Petri dish void of any food and only containing moist paper on the bottom. The number of dead larvae was examined once every 12 hours. The results indicate that for all three populations, the earlier a larva hatches, the longer it survives without food. Furthermore, AGM larvae always survived longer than EGM larvae, and thus present higher risk of infestation.

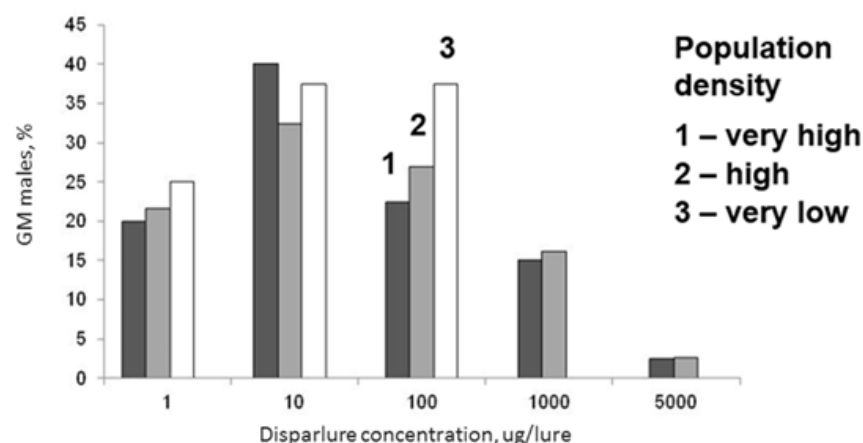


Figure 1. The percent of gypsy moth (GM) males captured per trap relates to population density and lure concentration (population density: very high = 25-50% defoliation and 5 pupae per crown of 1 willow stem; high = no apparent defoliation and 0.1-3 pupae per crown of 1 willow stem; sparse = no defoliation and <0.1 pupa per crown of 1 willow stem).

Cooperative Studies in China

Dispersal studies are important in improving risk management measures for AGM since they can provide insight into AGM's ability to potentially infest ships at ports or to spread from an infested vessel when called at ports or passing through waterways. The dispersal distance of first instar AGM larvae was studied using a release-recapture method in an open field site in China. The study was performed in an area where AGM do not naturally occur at that time of year. Results showed most larvae were recovered within 20 m of the release point, although some larvae were found as far as 30 m, 5 minutes after release with a wind speed of 5.1 meters per second.

Field trapping studies were conducted in China to determine the male gypsy moth population levels in different areas. Traps baited with disparlure, mathuralure, and xylinalure were placed in a few provinces and suburbs of major cities. In areas north of the Yangtze River, more than 3,000 *L. dispar asiatica* males were captured, but no *L. muthura* were found in traps. In the south, a total of 333 individuals (AGM, *L. xyliana* and *L. marginata*) were captured. Disparlure baited traps also captured some males of *L. dissluta* and *L. marginata*, confirming that males of both species react to disparlure.

To understand the infestation biology of the casuarina moth, *L. xyliana*, studies were conducted under laboratory and field conditions to determine the preference of tree species and locations where female moths deposit egg masses. Results from choice tests in cages found that females deposited more egg masses on twigs of *Litchi chinensis* and *Casuarina glauca* than on twigs of *Dimocarpus longan* and *Eriobotrya japonica* (Figure 2). However, the results of our field survey revealed that *L. xyliana* also lay egg masses on many other tree species, although none as preferred as *L. chinensis* and *C. glauca*.

Conclusion

The studies described here help provide a better understanding of exotic *Lymantria* species to improve mitigation responses. This is increasingly important, as these species are intercepted on U.S. vessels and thus are at risk for introduction. Investigation is still underway to understand the effect of extreme environmental conditions on hatching and larval survival of *L. xyliana*.

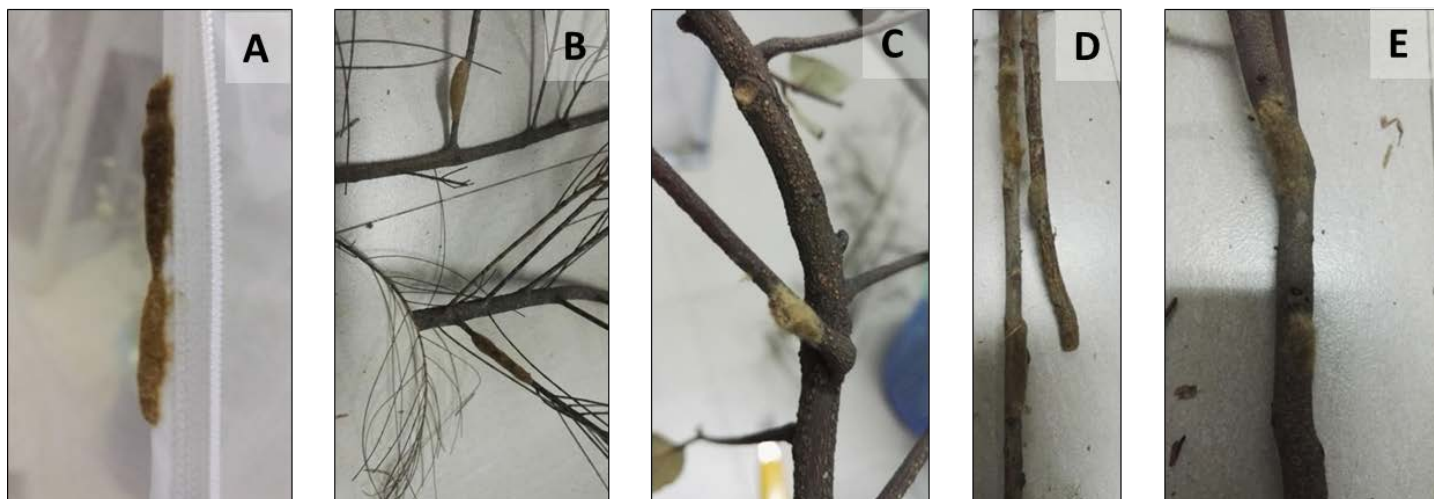


Figure 2. A) Casuarina moth, *Lymantria xyliana*, laid egg masses on the side of the cage B) as well as the twigs of *Litchi chinensis* C) *Casuarina glauca* D) *Dimocarpus longan* and E) *Eriobotrya japonica*.

Evaluating different species of trees for Asian longhorned beetle host suitability

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Uncertainty about the possible hosts for Asian longhorned beetle (ALB), *Anoplophora glabripennis*, in North America has resulted in significant expenditures for survey, control, and program management. A better understanding of the ALB host range has been gained by analyzing ALB eradication program survey data and conducting additional studies. However, there is still a need to refine the ALB host list at the species level. A species level host list would allow for greater targeting of eradication program resources and improve management of ALB in the U.S. The goal of this project was to evaluate selected species of broad leaf trees to determine if they could be ALB hosts.

Cage studies were conducted to determine whether the following tree species can be ALB hosts: *Catalpa bungei* (Figure 1A), *Cercidiphyllum japonicum* (Figure 1B), and *Hibiscus syriacus*. The cage studies were planned to last 3 years for each tree species. The longevity of the beetles, egg sites made, active egg sites, and development of individual beetles were checked and recorded. Female adults caged on *H. syriacus* were found to survive as long as 45 days while males survived up to 30 days. Beetles made a few egg sites on the *H. syriacus*, but active egg sites were only found on 5 of the 25 trees. No exit holes were found on *H. syriacus*, even after three years post caging. Quite a few egg sites were made on *Catalpa bungei* in July, 2016 and active egg sites were found when these trees were checked from July to October, 2016. When ALB were caged with *C. japonicum*, female adults survived up to 56 days, while males only survived up to 48 days. Our data therefore suggest that *C. japonicum* is a suitable host, as many more egg sites and active egg sites were found on trees of this species, whereas *H. syriacus* may not be a suitable host. Whether *C. bungei* is a suitable host or not will depend on whether any ALB can complete development on it, as the active egg sites on *C. bungei* continue to be monitored.



Figure 1. Paired ALB adults were caged with a section of A) *Catalpa bungei* tree and B) *Cercidiphyllum japonicum* tree.

ALB population dynamics in natural conditions with mixed tree species was studied in a “common garden” setting in Beijing. Trees, including *Koelreuteria paniculata*, *Liriodendron tulipifera*, *Alnus incana*, *Acer platanoides*, *Acer mono*, *Aesculus chinensis*, *Fraxinus americana*, and *Populus* spp., were monitored for ALB infestation. This “common garden” was set up in 2003, with 20 species of trees from the U.S. and has not been treated with any pesticide or herbicide. All trees in this garden have been checked periodically each year (at least twice a month from June to October and 1 to 2 times a month from November to May) for ALB active egg sites, exit holes, and number of adult beetles. Natural ALB population began to decline in 2010 at this site. Although quite a few ALB exit holes were seen before 2010 on trees such as *A. mono*, *A. chinensis*, *K. paniculata* as well as other species, exit holes were only found on one of the *A. platanoides* trees in 2016. We speculate that several factors may contribute to this decline.

One factor might be the lessened availability of suitable host trees in the garden, as most of the preferred host tree species, such as *Acer mono*, were heavily infested and/or dead. Additionally, the mixture of tree species and the fact that the site does not undergo pesticide treatment should also benefit ALB natural enemies, such as *Dastarcus helophoroides*, and wood peckers, potentially aiding in ALB population decline.

Surveys for woodborers, including ALB that attack North American trees, were performed at selected sites in Hebei, Yunnan, and Sichuan provinces, and in Beijing, China. Trees in the following genus were surveyed for ALB infestation: *Acer*, *Betula*, *Ulmus*, *Fraxnus*, *Populus*, *Salix*, *Sorbus*, *Cercidiphyllum*, *Platanus*, *Aesculus*, *Albizia*, *Celtis*, *Koelreuteria*, *Castanea*, *Liquidambar*, *Liriodendron*, *Nyssa*, *Prunus*, and *Pyrus*. Some of the survey findings are detailed here. In Hebei, active ALB egg sites were found on *Ulmus pumila*, *Salix babyloica*, and on *Acer truncatum*. In Yunnan, exit holes of the citrus longhorned beetle (CLB), *Anoplophora chinensis*, were found on *Acer rubrum*, and active egg sites of *Apriona rugicollis* were found on *Salix cavaleriei*. In Sichuan, *Populus szechuanica* trees were infested by *A. rugicollis*, while *Batocera horsfieldi* were found infesting *Salix matsudana* 'f. tortuosa' (Figure 2). *Platanus x acerifolia* was infested by CLB. In Beijing, active egg sites and exit holes of ALB were found on *S. babylonica*. In summary, in Hebei and Beijing, high levels of ALB infestation can be found on *Acer*, *Salix*, and *Ulmus* trees, while in Henan, Yunnan, and Sichuan provinces, *B. horsfieldi* infests species in *Salix* and *Olea*, heavily.



Figure 2. A mating pair of ALB on a trunk of *Salix matsudana* 'f. tortuosa' tree.

CLB infests species in *Acer* and *Ulmus*. *Apriona rugicollis* exists in all areas examined in this survey and infests trees in *Salix*, *Malus*, *Ulmus*, and *Lagerstroemia*. Infestations by the above mentioned wood borers might have been erroneously reported as ALB infestations in some areas of China, therefore incorrectly broadening the suspected host range of ALB.

One concern of the ALB eradication program is that removing preferred hosts in the quarantine area, such as striped maple, *Acer penslyvanicum*, may cause remaining beetles to move to less preferred host trees or even feed on unsuitable trees. Therefore, a switch feeding study was carried out on adult ALB in the laboratory. Food source of ALB adults was changed from *Acer penslyvanicum* to either *Betula alleghaniensis*, *B. lenta*, *Fraxinus americana*, *F. pennsylvanica*, *Populus tremuloides* or *Quercus alba*. Adult feeding and longevity, female fecundity, the number of eggs laid, and hatching rate were analyzed as important indicators of tree suitability as a host of ALB. The results suggest that *B. alleghaniensis* and *B. lenta* could serve as hosts, while the others listed above may be less likely to serve as hosts.

In summary, in 2016 the evaluation of *Hibiscus syriacus* was completed, demonstrating that *H. syriacus* is likely not a suitable ALB host. Cage studies were also set up to evaluate a few other tree species, which will continue in 2017 and expand to include more tree species. Field surveying will also be continued, covering more sites and more tree species that could serve as ALB hosts in natural conditions. The adult feeding study will also be continued to include more tree species whose status as ALB hosts are unknown or unconfirmed.

European gypsy moth colony and production

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European gypsy moth (EGM), *Lymantria dispar dispar*, is a destructive forest pest, introduced to the United States in 1869. The Otis Laboratory has maintained the New Jersey Standard Strain (NJSS) colony of EGM for over four decades. The NJSS colony, also referred to as the North American strain, is produced in weekly sub-families for year-round availability to researchers in the United States and abroad. Each sub-family, or cohort, completes development in about 78 days before exposure to 5 months of simulated winter.

Over 38,000 diet cups were prepared for infestation with either eggs (for research) or larvae (for colony) (Figure 1).



Figure 1. Gypsy moth artificial medium being dispensed into rearing containers via an in-house designed manifold.

Approximately 200,000 EGM were reared in 2016 for maintenance of the Otis colony (Figure 2). Additional diet was produced for in-house colony maintenance of Asian gypsy moth strains reared in the Otis Insect Containment Facility as well as for other insects. The colony is monitored daily to assess survival, growth, and development rates of immature life stages. The early detection of anomalies during development is critical, as it allows for the identification of potential causes and for the assessment of their future impact on the colony.



Figure 2. Rearing containers housing 12,000 gypsy moth colony insects in an environmental chamber.

An example of a tracked parameter is the developmental time to reach 50% pupation (DT_{50}) by males and females (Figure 3). Average percent survival to pupation of colony insects is generally 97%, which indicates adherence to the established rearing protocol.

In addition to maintaining the Otis colony, EGM are reared to provide individuals and facilities with material for scientific studies. In 2016, over 25,000 egg masses were provided to establish year-round EGM mass-production at Sylvar Technologies Inc., a Canadian company developing a multiple nucleopolyhedrovirus product for management of GM populations. This host specific Baculovirus is lethal to gypsy moth and will not harm other species, making it advantageous to the environment. Egg masses were also provided to researchers in Germany, Canada, Switzerland, and Austria. Domestically, egg masses were supplied to federal, private and university researchers in nine states. Over 420,000 live larvae were supplied to U.S. Forest Service and universities. An additional 120,000 were reared to support research by the Gypsy Moth Slow the Spread Foundation (STS) on optimizing the mating disruption control strategy.

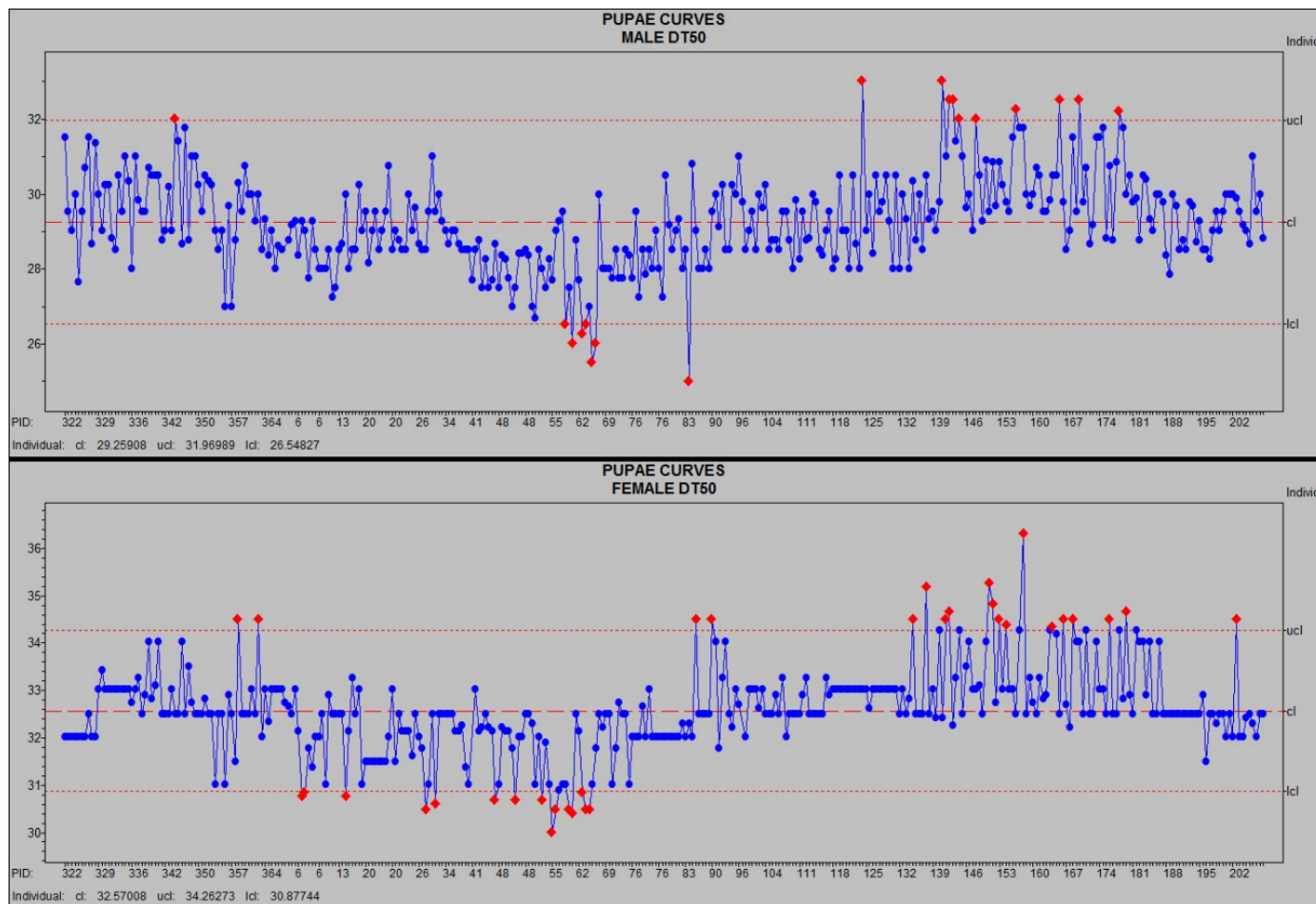


Figure 3. Time to 50% pupation (DT₅₀) in weekly sub-families of the NJSS colony during 2016. Pupation data from sample cups were entered into Northwest Analytics Quality Analyst software (NWA 2012) to track development rates for each sex and to highlight abnormalities. The red dashed lines indicate the average (in days), and the red dotted lines indicate upper and lower acceptable boundaries. Rates exceeding acceptable boundaries are flagged with red.

The STS project, which was established in the year 2000, aims to minimize the national rate of GM spread into uninfested areas. The Otis Laboratory partnered with the foundation to provide 5,650 female pupae and over 111,000 male pupae for the 2016 summer. These internally color-marked pupae were reared for field release to evaluate components of STS's mating disruption program.

The European gypsy moth colony is maintained by a team in the Rearing functional group consisting of a supervisory entomologist and three technical staff members. Specimens from the colony are mass-produced for seasonal research, eggs are provided to other production programs and requests are fulfilled for live insects and display specimens for training and outreach.

REFERENCE

- 1) NWA. 2012. NWA Quality Analyst, v 6.3. Northwest Analytical, Inc., Portland, OR.

Rearing methods for Asian gypsy moth

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Asian gypsy moth (AGM), *Lymantria dispar asiatica*, is a serious forest pest in its native range of Asia. Unlike European gypsy moth (EGM), *Lymantria dispar dispar*, AGM is not currently established in the U.S., furthermore, AGM has a broader host range and the females are capable of flight. If AGM is introduced to the U.S., it has the potential to be an even greater pest than EGM and to spread more rapidly. Currently, five colonies of AGM (Chinese, Korean, Mongolian, Russian Central, and the subspecies *Lymantria dispar japonica* from Japan) are reared at the Otis Laboratory for research aimed at reducing risk of entry into the country, developing trap technology, molecular diagnostics for enhanced detection and identification, and gathering data for risk analysis (Table 1). Additionally, the related rosy moth, *Lymantria mathura* is reared in the insect containment facility. The larvae of the Japanese subspecies and rosy moth are distinguishable from *L. dispar asiatica* (Figure 1).

Rearing is carried out on artificial diet at the Otis Insect Containment Facility at 25°C, 65% RH, and 16L:8D, and takes approximately 35 days from egg hatch to adult. Egg masses are placed in 5°C chill for 120-150 days. Larvae hatch within a week after eggs are removed from chill. About 10 vigorous larvae from each of 30 egg masses are infested onto fresh diet cups. Pupae are collected about 30 days after hatch and adults are bulk-mated (10 pairs per container) with the exception of *L. mathura* (one pair per container). About 30-35 egg masses per colony are placed into chill for the next cohort, with the rest saved as a backup. In 2016, annual cohorts of some colonies were increased to prepare for future studies. Colonies were used in a genetic study and to supply pinned specimens and displays for outreach and training.

Table 1. AGM colony production in 2016.

Strain	Cohorts per Year	# Pupae Produced	% Survival to Pupation	# Matings	# Egg Masses Produced
Chinese	4	847	80	238	228
Japanese	4	1,038	77	320	319
Korean	2	470	73	110	57
Mongolian	4	1,098	89	280	275
Russian Central	2	709	~95	159	159
<i>L. mathura</i>	4	1,039	78	244	218

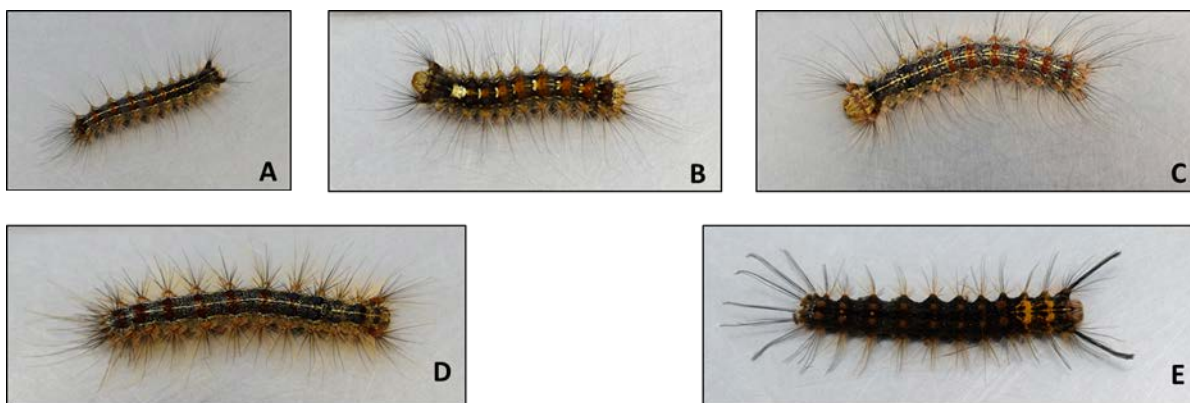


Figure 1. Fourth to fifth instar larvae of Asian gypsy moth (*Lymantria dispar asiatica* and *L. dispar japonica*): A) Chinese, B) Japanese, C) Korean, D) Mongolian and rosy moth E) *L. mathura*.

Support for navel orangeworm mass production

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Navel orangeworm (NOW), *Amyelois transitella*, is native to western U.S. and is a primary pest of tree nuts in California. In 2015, the Phoenix Pink Bollworm Rearing Facility (PBWRF) began developing a mass production system for NOW to supply sterile insects for a planned SIT program in California, where sub-sterile males will be released to mate with females resulting in offspring that are completely sterile. Small colonies from two sources were established at the Otis Insect Containment Facility in February 2016 to answer rearing-related questions that could not be addressed in Phoenix due to limited staffing. The small-scale studies aimed to: 1) test two lighting regimens for best mating success and fertility; 2) determine optimal infestation density per unit diet; and 3) address diet-depth and other diet-related questions (Figure 1 and Figure 2).

The first of the lighting regimens tested had an hour of low light intensity (3 lumens per ft²) at “dusk” and at “dawn”, and the second had low light only at “dawn”. Daytime intensity was 45-48 lumens per ft² at cage level.

Dissections of female reproductive tracts and counts of fertile and infertile eggs indicated no difference in mating success (~91%) or egg fertility (90-97%) between the two lighting regimens.

The optimal unit of diet per larva, and suitable volumetric configurations of the diet (depth and surface area), were defined to improve cost effectiveness and provide options for space efficiency. At densities between 0.4 eggs per gram of diet and 2.5-4 eggs per gram of diet, the fitness parameters of pupa weight and survival rate to adult were unaffected, although pupa weight decreased at higher densities (Figure 3). Survival was only slightly reduced at densities above 2 eggs per gram of diet, but was significantly reduced at the highest density; sex ratio was generally unaffected across densities, averaging 48% males (Figure 4). The optimal infestation density appears to be 4 eggs per gram of diet. At a fixed diet weight and infestation density, a thin layer of diet (2 cm deep) was comparable to 4 cm in terms of survival and pupa weight. Pooled data from various studies showed, however, that 1 cm depth may be acceptable.



Figure 1. Openings of tubes leading from cocoons to the diet surface and freshly emerged adults.



Figure 2. Emerged adults from infestation density studies. The study was combined with a comparison of glass and plastic jars.

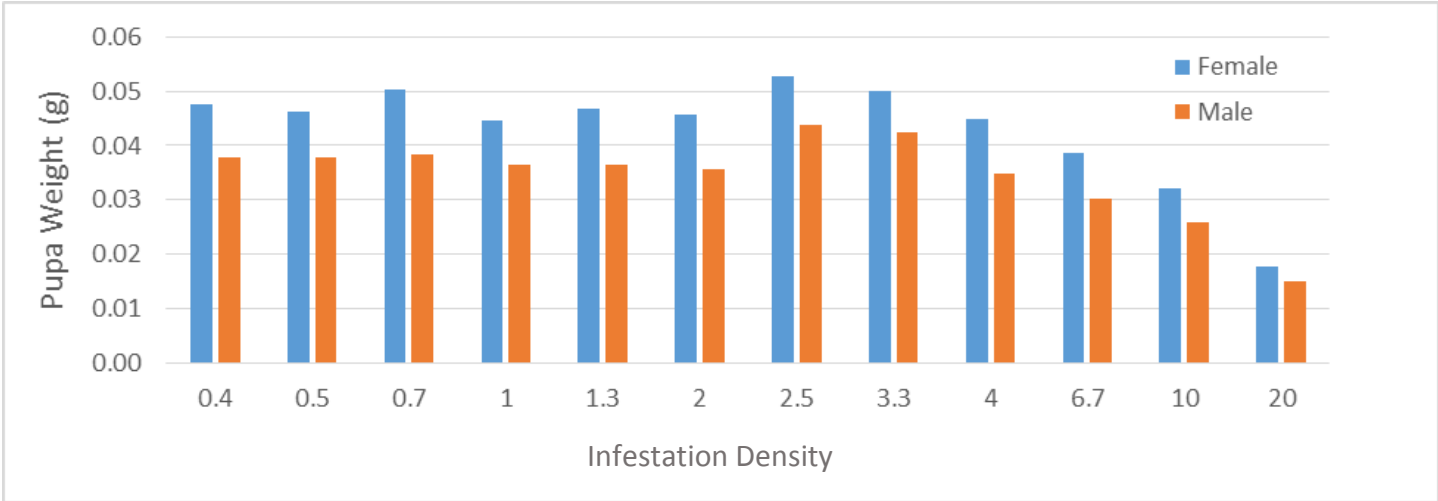


Figure 3. Effect of infestation density (eggs per gram of diet) on navel orangeworm pupa weight.

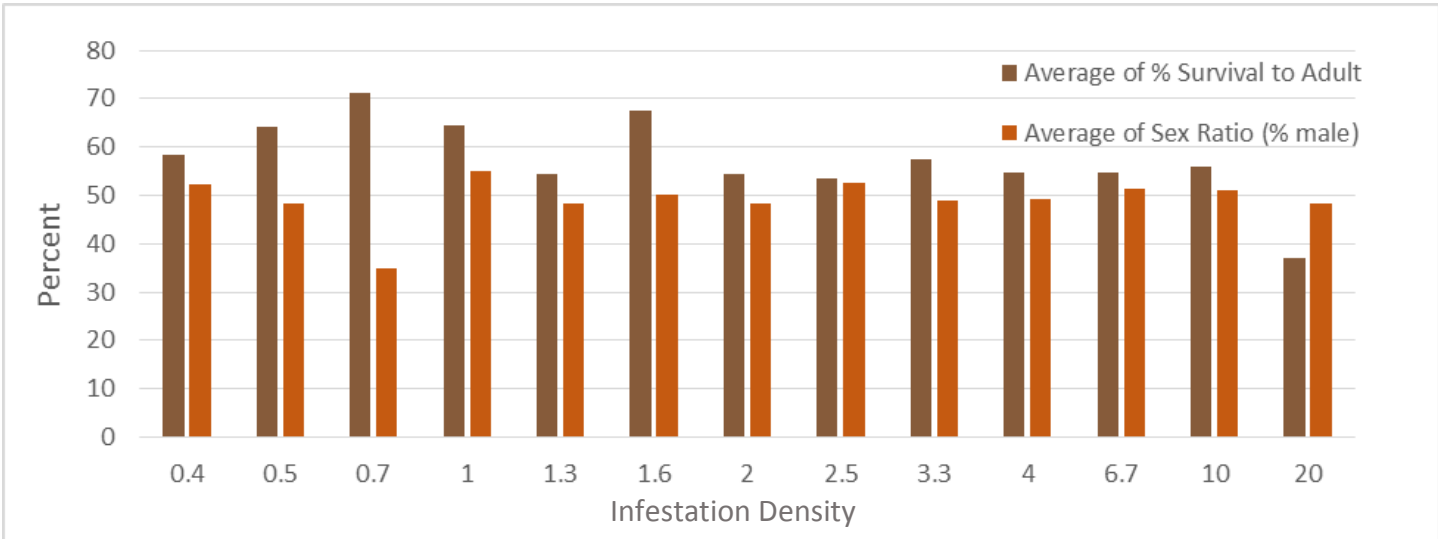


Figure 4. Effect of infestation density (eggs per gram of diet) on survival to adult and sex ratio.

Because the larva spins its cocoon at various depths in the diet and prepares a silk emergence tube to the surface, there was concern that high pupa densities may cause the tubes to constrict and interfere with adult passage to the surface. However, no trapped adults were found in tubes after eclosion, even under extreme crowding. This was found at both Phoenix and Otis Laboratories.

During the colony scale-up phase at Phoenix, NOW diet was prepared by hand; trials began in January, 2016 aiming to automate and expand diet production with a twin screw extruder. Once extruder trials succeeded in producing diet physically similar to hand-mixed diet, both diets were sent to Otis Laboratory for comparison. NOW survival rate and pupa weight were somewhat lower in the extruded diet, but the study confirmed extruded diet development was on track and nearly ready for use.

Asian longhorned beetle production

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Asian longhorned beetle (ALB), *Anoplophora glabripennis*, is one of the most destructive wood-boring pests of maple and other hardwoods in North America. Four strains of ALB are reared at the Otis Insect Containment Facility from the following locations: China, Ohio, Worcester (Massachusetts), and New York. A mixed colony is also maintained with individuals from each of the four strains. These colonies are reared to support research and methods development on control and eradication of ALB populations in isolated locations.

The average weekly production schedules (and the length of each developmental stage) are as follows:

- Eggs: 70-100 (7-14 days)
- Neonates and early instars: 100-150 (2-4 weeks)
- Large instar larvae: 65-85 (5-6 months, including 11 weeks in chill [10°C])
- Pupae 65-75 (~17 days)
- Adults 60-75

In 2016, the colonies entered the following generations: China, 14th; Ohio, 5th; Worcester, 7th; and New York, 4th. Figure 1 illustrates key aspects of the rearing process.

Beetles are individually reared and tracked throughout their development using the in-house designed Barcode-Enabled Tracking System. Each insect is given a unique barcode label (Figure 2) that is scanned at each life event. The database allows us to monitor and track colony-wide life-history characteristics and trends. Additionally, the system can generate specimen-specific life-history reports, providing scientists with a detailed record of insects used in their studies (Figure 2). In 2016, approximately 1,000 adult beetles were supplied for studies on monitoring and trap development, behavior and host specificity, pesticide treatment, and public outreach programs at five U.S. institutions, including the Otis Laboratory (Table 1).

Improvements in ALB rearing techniques and artificial diet are ongoing. Diet development studies support rearing of other longhorned beetle species for biological control studies and provides morphologically identified adults to enhance identification of larvae by molecular methods. Other work plans include assisting chemical ecologists by rearing eggs and early instars in host wood instead of in artificial egg substrates and diet. During the 2016 fiscal year, ALB rearing practices were improved and greatly reduced the annual cost of supplies and diet ingredients. These new methods were shared with other rearing facilities, including the Canadian Forest Service. We continue to develop and test alternative rearing containers to reduce cost and provide options if the current stock becomes unavailable.



Figure 1. A) ALB larvae on diet. B) ALB adult rearing container with maple twigs for feeding. C) ALB mating pair with artificial egg substrate.

Insects Tracker

Point cursor into this search box to scan in or key in Insect_ID >

Search Insect ID

Insect ID:
 Population: OH
 Sex: F
 Feral Date:
 Collection Date -Egg:
 Egg Hatch/On Diet:
 Diet Change:

Chill In - Due:
 Chill In - Actual:
 Chill Out - Due:
 Chill Out - Actual:
 Pupaion_Date:
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 Generation: F1

Parents: Mating Code
 Date of Death:
 Used By: Program Outreach
 Date Removed:
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Year 20XX:
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Standard Automated Routine

Now Active

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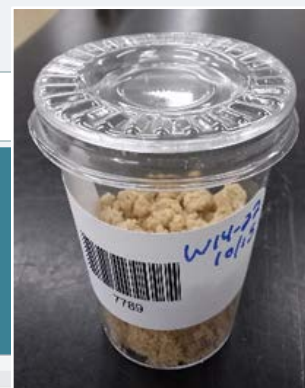


Figure 2. ALB Barcode-Enabled Tracking System specimen-specific life-history page that also details the study/use of the insect. Inset image depicts the barcode label that is used to pull up specimen-specific life-history page.

Table 1. Numbers of ALB life stages and preserved specimens reared and provided in FY16 to U.S. institutions.

Purpose	Eggs	Neonates	Older Larvae	Pupae	Male Adult	Female Adult
Training/Outreach	320	200	140	80	230	275
Chemical Ecology	-	-	-	-	47	62
Biocontrol	-	-	80	-	-	-
Genetic Research	-	-	-	-	25	25
Physiology, Nutrients	-	-	500	-	-	-
Mating pair study	-	-	-	-	10	10
Rearing/diet cup	-	-	500	-	-	-
Host-specificity	-	-	-	-	80	80
Teaching - Academic	-	-	50	30	30	30
Rearing	3,738	2,928	2,648	2,802	992	1,026
Total	4,058	3,128	3,918	2,912	1,414	1,508

Rearing methods for two exotic longhorned beetles

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Introduction

Rearing protocols are being developed at the Otis Insect Containment Facility for two species of exotic cerambycid beetles. These protocols are necessary to produce live adults for pheromone studies and lure development. Velvet longhorned beetle (VLB), *Trichoferus campestris* (Figure 1), was found in several locations in North America and is gaining pest status due to its recent impact on commercial fruit trees in Utah. The other species, mulberry longhorned beetle (MLB), *Apriona rugicollis* (Figure 2), has not been detected in the U.S. but is considered at risk for entry. Both wood-borers feed on a broad range of hardwoods and, in the case of VLB, also on conifers. When live specimens arrived from China in 2014, little information was known about their development or adaptability for rearing. We report on rearing protocols developed for both species and provide some biological information for beetles reared on artificial diet.

Velvet longhorned beetle

The rearing protocol for VLB is still under development and recent successes are encouraging. Egg survival increased from less than 10% to 80% by preventing contact with fluids or moist diet. Larval survival improved to 83% when partially dried ALB diet was used for neonate infestation. However, five months of data show that larval survival increases (92%) when host material, desiccated apple twigs, is used from the neonate stage onward. A chill period is required near the end of the larval stage to induce pupation; larvae are chilled at 10°C in cups with diet for 3 months. The duration of developmental stages on artificial diet and female fecundity are presented in Table 1. The colony is in its third generation and is comprised of 50 larvae on artificial diet, 23 larvae on twigs, and six adults.



Figure 1. Velvet longhorned beetle, *Trichoferus campestris*.

Mulberry longhorned beetle

Eggs of MLB are incubated at 25°C for a week in a covered Petri dish with a thin layer of 1:1 phosphate buffered saline and 1% bleach solution. Neonates are transferred to an artificial diet developed for Asian longhorned beetle (ALB), *Anoplophora glabripennis*, prepared with 10% less water. Larvae are transferred to fresh diet eight times, on average, before pupation occurs. After adult emergence, beetles experience a week-long starvation period, to allow a sclerotization period as it occurs in nature. Male and female pairs are placed in clear 4 liter containers with a 20 cm long mulberry twig (4-6 cm diameter) for oviposition. Each pair is held for about a month to mate and lay eggs, and eggs are harvested weekly. The duration of life stages and female fecundity are presented in Table 1. The third generation of MLB is currently being reared, and is comprised of 400 insects.



Figure 2. Mulberry longhorned beetle, *Apriona rugicollis*.

Table 1. Duration of MLB and VLB life stages reared in artificial diet, and average fecundity at 25°C and ~80-90% RH.

Species	Egg	Larva	Larval Chill	Pupa	Adult	Eggs per Female
MLB	7 days	9-14 months	None	3 weeks	≤ 3 months	~126
VLB	3-8 days	9-14 months	3 months	2 weeks	≤ 9 months	~100

Insect lure formulations

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Increase in global trade has led to more frequent introductions of insect pest species that are not native to the United States. These invasive insects can potentially cause major harm to U.S. agriculture and to the environment. A major part of the research conducted at the Otis Laboratory is focused on developing tools that can detect and prevent the establishment of these invasive insects.

Every year the Otis Chemical Ecology and CAPS Lure Support Group supplies cooperators with specialized insect lure formulations (Table 1). These lures contain insect attractants that attract pest insects to baited traps. The lures are in support of the Cooperative Agricultural Pest Survey (CAPS), Farm Bill research projects, Molecular Diagnostics, and individual research projects of Otis research collaborators.

The semiochemical (pheromones/kairomones) components that are used to attract insect pest are obtained from different manufacturers (both industry and academia) and are subjected to a quality control program. Our quality control program uses both Gas Chromatography (GC-FID) and GC-coupled-mass spectrometry (GC-MS) to verify the composition of the semiochemical lures. Additionally, before formulation of the semiochemical into the different dispenser types occurs, the dosing solutions are subjected to a quantitative analysis (internal standard method) to confirm the correct dosages.

Currently, the Otis Chemical Ecology and CAPS Lure Support Group produces a wide range of lure dispensers including: the standard grey rubber septa, beam caps, plastic polymer strings, and laminates (or plastic laminates). When needed, we also conduct quality control on pre-manufactured industry lures through the analysis of active ingredients by extraction and/or release rate quantification.

The Otis Chemical Ecology and CAPS Lure Support Group plays an active part in the monitoring of different species of gypsy moths, both domestically and abroad. We supply specialized lures, active ingredient analysis of mating disruption formulations, and conduct an annual trapping study at the Joint Base Cape Cod.

In 2016, the Otis laboratory analyzed, formulated, and supplied over 230,000 lures for 60 different insect species (Figure 1).



Figure 1. From left to right: Tanya Dockray, Natalie Leva and Mandy Furtado packaging, sealing, and weighing rubber septa lures.

Table 1. Otis laboratory lure formulations.

Insect Species	Common Name	Lure Dispenser	Insect Species (Con't.)	Common Name (Con't.)	Lure Dispenser (Con't.)
<i>Acrolepia assectella</i>	Leek moth	grey septa	<i>Leucoptera malifoliella</i>	Pear leaf blister moth	grey septa
<i>Adoxophyes orana</i>	Summer fruit tortrix moth	grey septa	<i>Lobesia botrana</i>	Grape vine moth	grey septa
<i>Archips fuscocupreanus</i>	Apple tortrix	grey septa	<i>Lymantria mathura</i>	Rosy moth	string
<i>Archips xylosteanus</i>	Variiegated golden tortrix	grey septa	<i>Lymantria moncha</i>	Nun moth	laminare
<i>Argyresthia conjugella</i>	Apple pith moth	grey septa	<i>Lymantria xyliana</i>		string
<i>Argyresthia pruniella</i>	Cherry blossom moth	grey septa	<i>Melittia cucurbitae</i>		string
<i>Argyrotaenia ijungiana</i>	Grape tortrix	grey septa	<i>Mamestra brassicae</i>	Cabbage moth	polycaps
<i>Autographa gamma</i>	Silver Y moth	grey septa	<i>Neoleucinodes elegantalis</i>	Tomato fruit borer	grey septa
<i>Carposina niponensis</i>	Peach fruit moth	beem capsules	<i>Ostrinia furnacalis</i>	Asian corn borer	grey septa
<i>Chilo partellus</i>	Maize borer	grey septa	<i>Ostrinia nubilalis</i> E strain		grey septa
<i>Chilo suppressalis</i>	Asiatic rice borer	grey septa	<i>Ostrinia nubilalis</i>	European corn borer	grey septa
<i>Chrysodexis chalcites</i>	Tomato looper	grey septa	<i>Pandemis cerasana</i>		grey septa
<i>Clepsis spectrana</i>		grey septa	<i>Pandemis heperana</i>		grey septa
<i>Cnephasia stephensiana</i>	Grey tortrix	red septa	<i>Panolis flammea</i>		grey septa
<i>Cryptoblabes gnidiella</i>	Honeydew moth	grey septa	<i>Paralobesia viteana</i>	Grape berry moth	grey septa
<i>Cryptophlebia leucotreta</i>	False codling moth	grey septa	<i>Paraswammerdamia lutarea</i>		grey septa
<i>Cryptophlebia ombrodeta</i>		grey septa	<i>Platynota ideausalis</i>		grey septa
<i>Cryptophlebia peltastica</i>		grey septa	<i>Platypus quercivorus</i>		grey septa
<i>Cydia coniferana</i>		grey septa	<i>Proeulia</i> spp.		grey septa
<i>Cydia funebrana</i>	Plum fruit moth	grey septa	<i>Sannina uroceriformis</i>	Persimmon borer	grey septa
<i>Cydia illotana</i>		grey septa	<i>Sesia rhynchioides</i>		grey septa
<i>Darna pallivitta</i>		grey septa	<i>Sparganothis pilleriana</i>	Grapevine leafroller	grey septa
<i>Dendrolimus punctatus</i>		grey septa	<i>Spodoptera littoralis</i>	Egyptian cottonworm	laminare
<i>Dendrolimus superans sibericus</i>	Siberian moth	grey septa	<i>Spodoptera litura</i>	Rice cutworm	laminare
<i>Dichelia histrionana</i>		grey septa	<i>Synanthedon</i> spp.		string
<i>Enamonia formosana</i>	Cherry bark tortrix	grey septa	<i>Synanthedon quercus</i>		grey septa
<i>Epiphyas postvittana</i>	Light brown apple moth	grey septa	<i>Synanthedon myopaeformis</i>	Apple clearwing	grey septa
<i>Eupoecilia ambiguella</i>	European grape berry moth	grey septa	<i>Tortrix viridana</i>		grey septa
<i>Gypsanoma aceriana</i>	Poplar pest	grey septa	<i>Yponomeuta malinellus</i>	Apple ermine moth	red septa
<i>Helicoverpa armigera</i>	Old world bollworm	grey septa	<i>Yponomeuta Padellus</i>	Cherry ermine moth	grey septa

Identification of the geographic origin of invasive Asian gypsy moth using genomic data

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Gypsy moth, *Lymantria dispar*, is a polyphagous pest of deciduous forests and trees in urban areas; it is one of the most destructive introduced insects in North America. Indigenous populations of *L. dispar* are found throughout the Japanese archipelago, across much of Asia, throughout Europe, and in parts of North Africa. In 1868 or 1869, a handful of adult moths accidentally escaped from a house in Medford, Massachusetts, where an amateur entomologist, Leopold Trouvelot, was rearing gypsy moths (Forbush and Fernald 1896). Despite early attempts at eradication, gypsy moth populations became established in eastern Massachusetts and gradually expanded their range. Currently, almost all of the northeastern portion of the U.S. is under quarantine for this species (APHIS 2016).

The population structure of gypsy moth is of particular interest because of its wide geographic distribution, potential for invasion, and great economic importance. Patterns of morphological variation in this insect were described in great detail by the evolutionary biologist Richard Goldschmidt, who suggested that *L. dispar* had its origins in Japan and then spread from Japan and/or Korea across Eurasia (Goldschmidt 1934). Currently, three subspecies of *L. dispar* are recognized:

L. d. asiatica from Continental Asia and *L. d. japonica* from the Japanese archipelago (collectively known as AGM) and *L. d. dispar* from Europe and North America (EGM), (Pogue and Schaefer 2007).

This classification broadly corresponds to geographic region of origin, except for the grouping of European and North American populations, which is in agreement with historical accounts of the introduction to North America. In recent years, AGM egg masses have been intercepted frequently on vessels and cargo entering the U.S. and AGM adults have been trapped during U.S. domestic surveys.

The detection of AGM in port interceptions and domestic trapping is alarming because, AGM has a wider host range and stronger flight propensity and capability compared to the established North American population. These traits could allow them to spread even more rapidly (APHIS 2016). Identifying the geographic origin of invasive AGM provides the data necessary to evaluate management strategies and policies that aim to prevent AGM introduction.

Our objective is to characterize the genome-wide genetic differentiation among global populations of gypsy moth with a particular focus on Asian regions. Previous works relied heavily on microsatellite data to address this question (Keena et al. 2008, Wu et al. 2015). Here, using Double Digest Restriction Associated DNA sequencing (ddRADseq), we developed 55 nuclear and 5 mitochondrial loci to genotype field-collected samples as well as AGM intercepted at ports and trapped domestically. A total of 1,328 individuals were genotyped, and samples missing more than 25% of data were not included in the subsequent analysis.

Principal-component (PC) analysis based on allele frequency showed substantial differentiation between AGM and EGM (European and North American populations), with moths from central Asia being intermediate. Most importantly, the results distinguished different populations within AGM (Figure 1).

The PC-2 separated AGM into three major genetic clusters from the following geographic regions: 1) Japan, 2) most parts of China, and 3) a mixture zone composed of northeastern China, Russian Far East, and the Korean Peninsula (Figure 2). The third genetic cluster is likely a result of hybridization between Chinese and Japanese populations. We further tested the diagnostic power of the new data set with two AGM egg masses intercepted in 2014, one at the port in Juneau, Alaska and the other at the port in Houston, Texas. When those eggs were included in the analysis, the Juneau interception fell into the mixture zone whereas the Houston interception was of Japanese origin (Figure 2).

We also genotyped 17 male gypsy moths caught in survey traps in Washington State in the summer of 2015. Fourteen of those moths were determined to be North American and three were confirmed as AGM, which came from multiple sources including Japan and the mixture zone (Figure 2). These results demonstrated that AGM of very different origins, with considerably different genetic makeups, can be introduced to the U.S. within a single year. Because high genetic diversity in invasive species can catalyze colonization success (Rius and Darling 2014), it is critical to stop AGM at ports of entry and detect/eradicate early establishments.

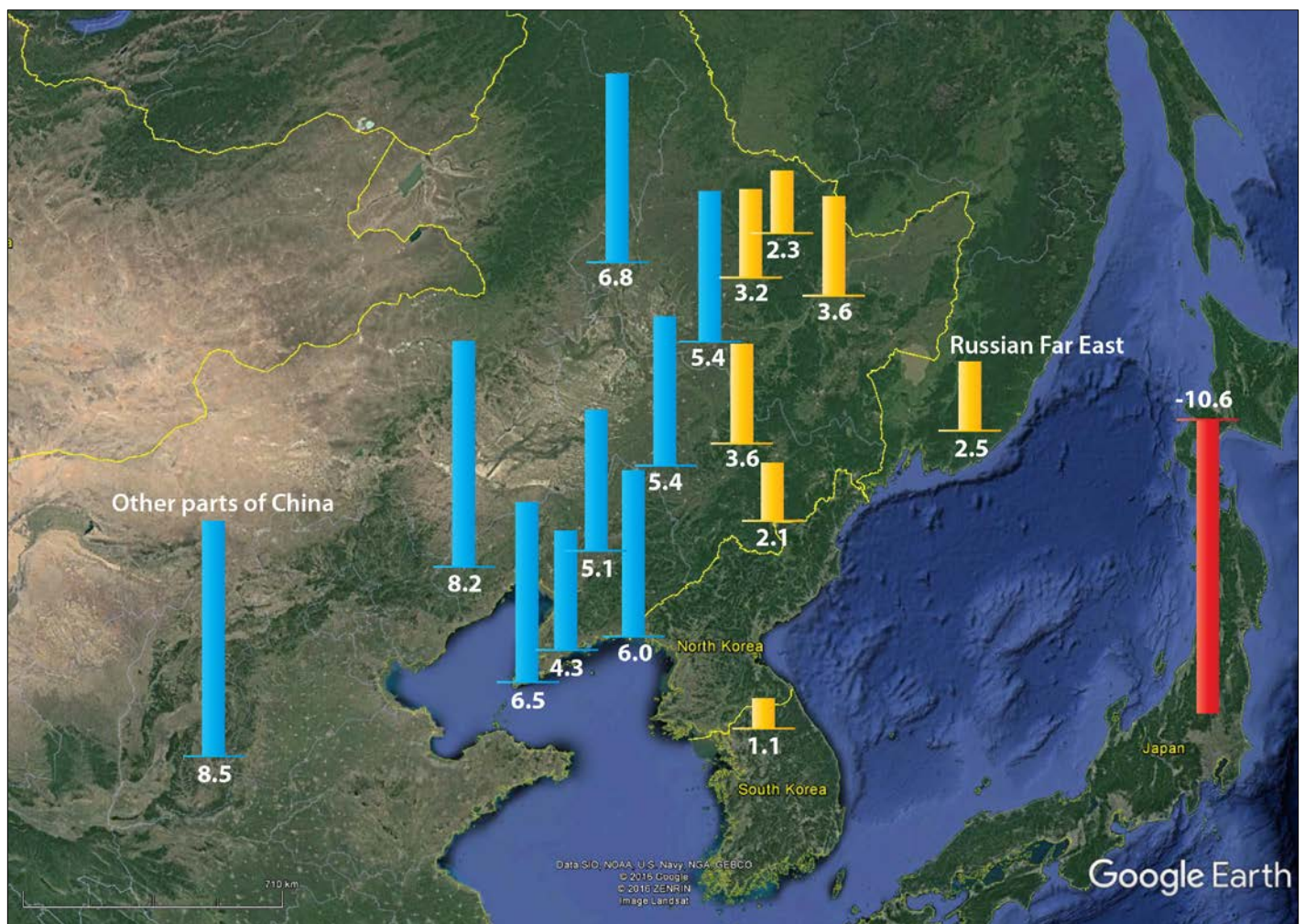


Figure 1. AGM populations have very different PC-2 values and can be separated into three genetic clusters.

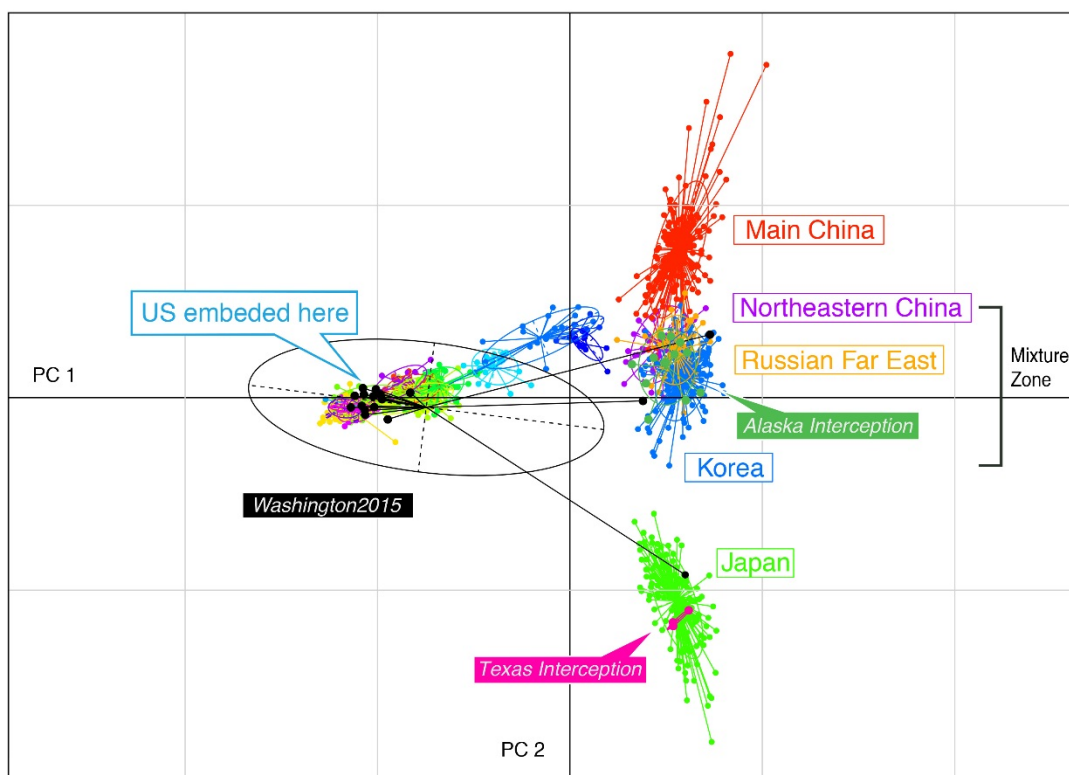


Figure 2. Juneau, Alaska interception fell into the mixture zone whereas the Houston, Texas interception is clearly of Japanese origin. Among the 17 genotyped Washington moths, 14 were determined as North American and 3 were confirmed as AGM. One AGM came from Japan and the other two were from the mixture zone of northeastern China, Russian Far East, and the Korean Peninsula.

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Port and domestic Asian gypsy moth molecular diagnostics survey: 2015-2016

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The Asian Gypsy Moth Molecular Diagnostics Survey originated in 1992, in response to the discovery of the Asian strain of gypsy moth (AGM), *Lymantria dispar asiatica*, in ports in the Pacific Northwest. Since that time, the Otis CPHST Laboratory, in cooperation with state, federal, and academic cooperators, has conducted annual surveys for the presence of AGM in U.S. ports as well as in sensitive domestic locations where there is a high risk of introduction. The survey has proven to be a successful means of detecting the introduction of AGM into the United States. In addition, the survey provides an annual baseline of the domestic gypsy moth population [European gypsy moth (EGM), *Lymantria dispar dispar*] in the United States and has been utilized to monitor and detect the movement of EGM out of the quarantine area into states where this species is currently not established.

AGM and EGM are difficult to distinguish morphologically, therefore samples submitted to the Otis Molecular Biology Group are genetically analyzed to determine if the specimen is of European or Asian lineage.

In the 2015 calendar year gypsy moth life stages were received for molecular analysis from 25 states (Figure 1). A total of 14 adult gypsy moths were confirmed as AGM; 10 from Washington, 2 from Oregon, and 1 each from South Carolina and Georgia. In addition, gypsy moth egg masses were intercepted from 12 U.S. ports on all 3 continental U.S. coasts as well as in San Juan, Puerto Rico (Figure 1) and subsequently confirmed by molecular diagnosis to be AGM (Table 1).

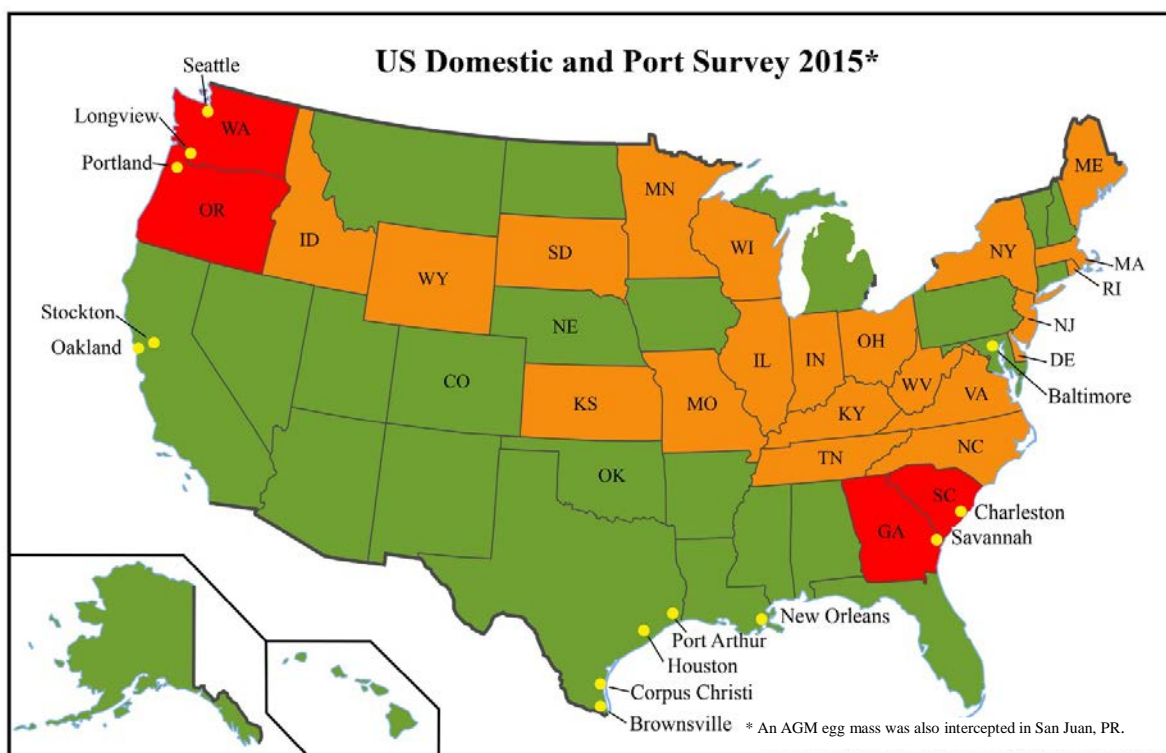


Figure 1. States that participated in 2015 Domestic Survey are labeled. States in red denote detection of AGM, while states in orange denote detection of EGM. U.S. ports where intercepted egg masses were found and sent to the Otis Laboratory for genetic analysis in 2015 are indicated with a yellow dot.

Table 1. Ports from which specimens were received in 2015. Ports that submitted specimens that were confirmed as AGM are denoted with a red asterisk.

Port	Number of egg masses
Baltimore, MD*	5
Brownsville, TX*	4
Charleston, SC*	8
Corpus Christi, TX*	4
Houston, TX*	4
Longview, WA*	1
New Orleans, LA*	18
Oakland, CA*	1
Port Arthur, TX*	2
Portland, OR*	5
San Juan, PR*	1
Savannah, GA	4
Seattle, WA*	3
Stockton, CA	1

During 2016, AGM was not detected in any of the 24 states that submitted samples for analysis — a welcome change from the results of the previous year. In addition, only 1 AGM egg mass was detected in a U.S. port in 2016, a single specimen found in Long Beach, California (Figure 2). However, the year 2016 saw a pronounced increase in the number of gypsy moths trapped in the Northeast (Figure 2). This population increase has likely resulted in the movement of gypsy moth egg masses out of the quarantine area and into the Midwestern and Western states. There was a pronounced increase in the number of confirmed European gypsy moth egg masses collected on transit and inspected at numerous California border crossing stations in 2016 (Figure 2). This trend is expected to continue in 2017.

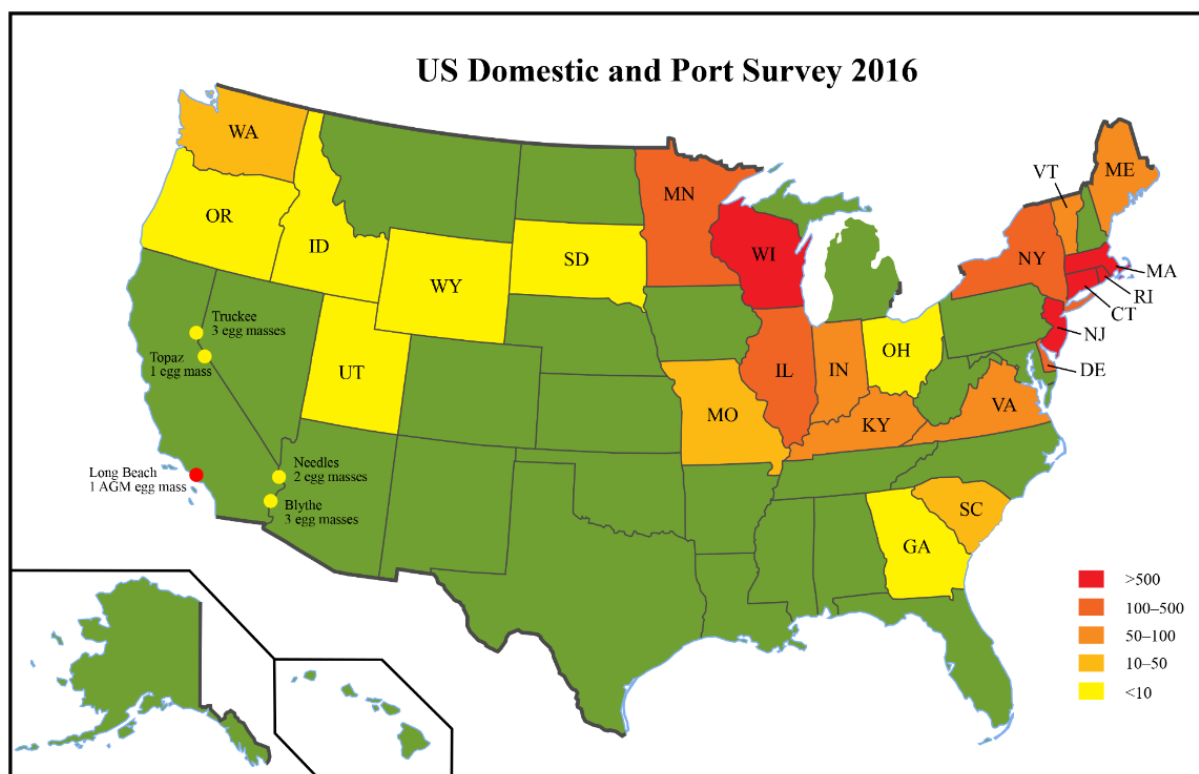


Figure 2. AGM diagnostic survey in 2016. The number of moths received from each submitting state are shown by the color index.

Identification of wood-boring cerambycids, buprestids, and siricids intercepted in trade-associated solid wood packaging material using DNA barcoding and morphology

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Global trade facilitates the inadvertent movement of insect pests and subsequent establishment of populations outside their native ranges. Despite phytosanitary measures, non-native insects arrive at U.S. ports of entry primarily as larvae in solid wood packaging material (SWPM) (Figure 1). Identification of wood-boring larval insects is important for pest risk analysis and management, but is difficult beyond family level due to highly conserved morphology.

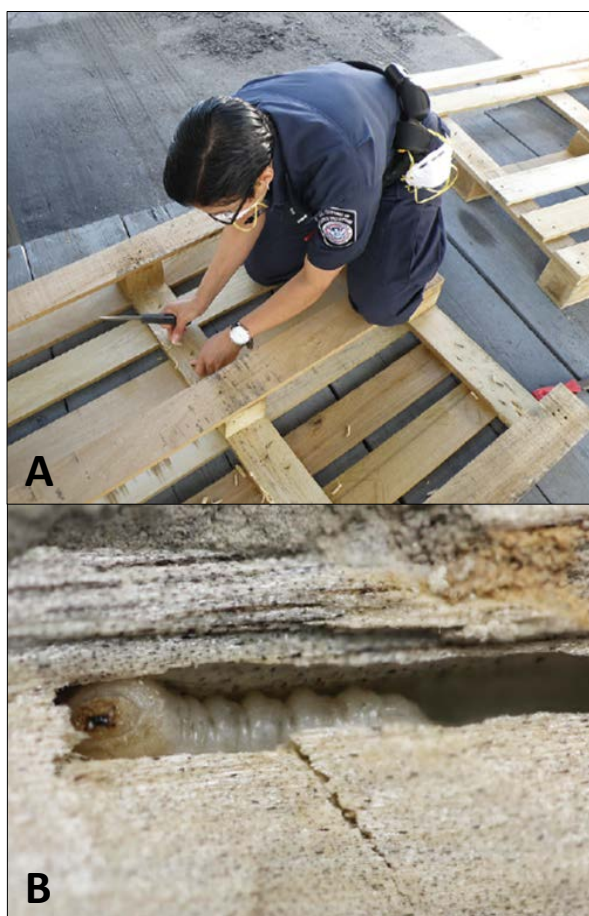


Figure 1. A) CBP agriculture specialist inspecting a pallet. B) Cerambycid larva found inside WPM.

Therefore, we sought to improve the capacity to identify non-native cerambycids, buprestids, and siricids intercepted in SWPM by using a combination of DNA barcoding and rearing larvae to the adult stage (Figure 2). Application of data compiled in this study may facilitate identification of risk factors and at-risk pathways, which will help plant protection agencies focus responses to threats posed by the movement of infested SWPM.

From April, 2012 to March, 2017, we received 1,277 wood borers found by CBP agriculture specialists in SWPM from eleven participating ports: Miami and Port Everglades, Florida; Houston, Hidalgo/Pharr, and Laredo, Texas; Romulus, Michigan; Seattle, Washington; Long Beach, San Diego, and San Francisco, California; Chicago, Illinois. When possible, the infested portion of the SWPM was shipped intact to provide natural host material for rearing larvae to the adult stage. If larvae were removed from or found outside the SWPM, they were placed in individual rearing cups with modified artificial diet developed for Asian longhorned beetle (ALB), *Anoplophora glabripennis* (Dubois et al. 2002). Larvae that arrived dead or died during the rearing process as well as metamorphosed adults were all subject to DNA analysis.

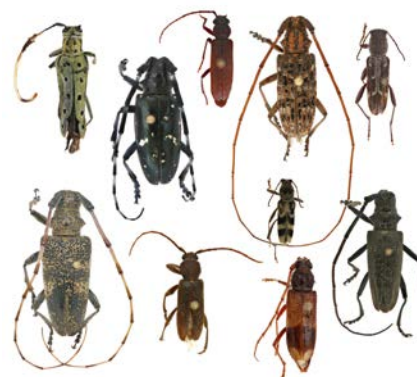
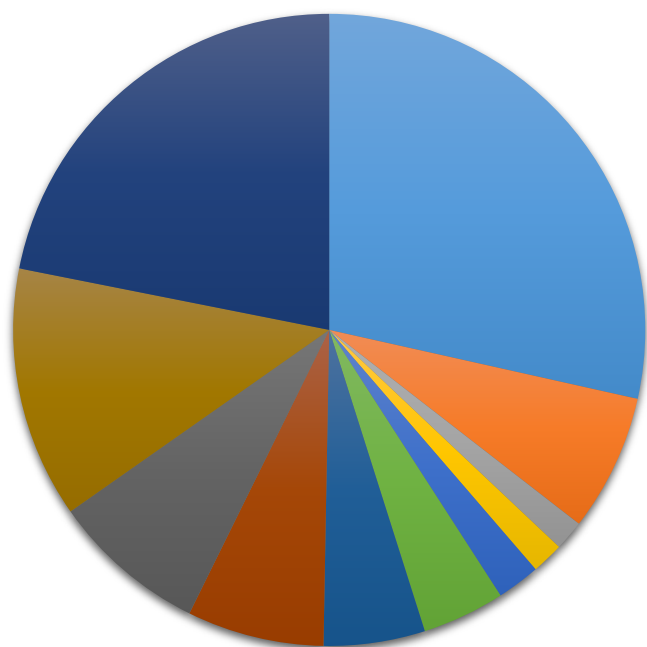
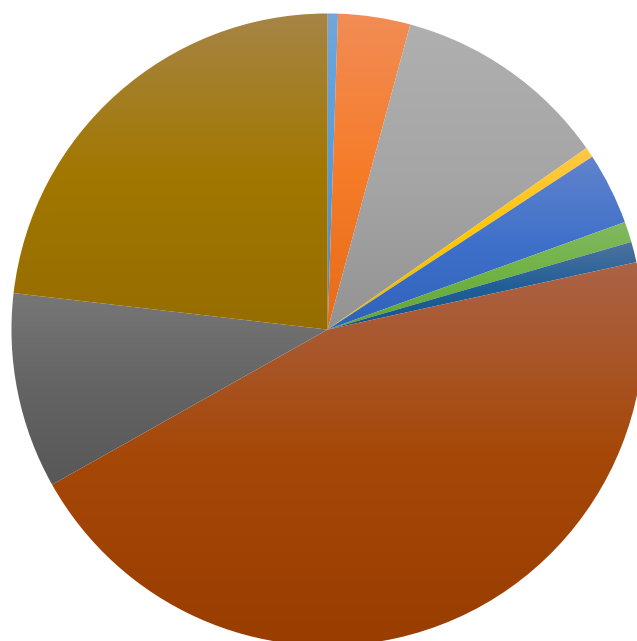


Figure 2. A variety of adult beetles reared from intercepted larvae.

The majority of the intercepted specimens were cerambycids (81%), whereas only 15% were buprestids and 4% were siricids. 180 larvae (14% of intercepted specimens) survived to the adult stage and were identified based on both morphology and molecular methods. We obtained DNA barcode sequences for 749 specimens. Of the 749 specimens, 550 specimens were identified to genus or species by querying sequences in Barcode of Life Database v3 (BOLD) (Ratnasingham and Hebert 2007). Morphological methods identified an additional 51 specimens to genus or species. The 601 specimens identified so far include 32 cerambycid genera, 6 buprestid genera (Figures 3 and 4) and 2 siricid genera. Our approach confirmed ten novel DNA barcodes for seven species of woodborers not in public databases. This study provides important documentation of high-risk pest species (e.g., ALB, intercepted 6 times; *Trichoferus campestris*, 26 times) with potential to cross country borders through the SWPM pathway. Our work and the resulting reference list for cerambycids, buprestids and siricids intercepted in SWPM enhance the capacity of CBP and USDA to identify intercepted wood-boring larvae.



■ Processing, 297
 ■ Miscellaneous genera, 73
 ■ Callidium sp., 16
 ■ Anastrangalia sp., 17
 ■ Tetropium sp., 23
 ■ Xylotrechus sp., 44
 ■ Trichoferus sp., 54
 ■ Monochamus sp., 73
 ■ Inconclusive, 83
 ■ Unknown, 134
 ■ Arhopalus, 228



■ Agrilus sp., 1
 ■ Belionota sp., 7
 ■ Buprestis sp., 21
 ■ Chalcophora sp., 1
 ■ Chrysobothris sp., 7
 ■ Dicerca sp., 2
 ■ Phaenops sp., 2
 ■ Inconclusive, 86
 ■ Unknowns, 19
 ■ Processing, 44

Figure 3. A proportional summary of all cerambycid genera identified.

Figure 4. A proportional summary of all buprestid genera identified.

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Genetic analysis of Asian longhorned beetle in North America

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Asian longhorned beetle (ALB), *Anoplophora glabripennis*, is an important pest insect endemic to China and Korea that was first introduced into the United States in 1996 in Brooklyn, New York. Despite extensive eradication efforts, new beetles are still being found in the U.S. and Canada. Characterizing genetic structure among North American ALB will help us better understand the process of introduction. Carter et al. (2010) analyzed beetles collected from multiple North American locations between 1996 and 2007 but did not include Worcester and Boston (Massachusetts), and Ohio samples, which were only recently detected in 2008, 2010 and 2011, respectively. Therefore, genetic diversity among recent collections are assessed and compared to published data. This will allow for the evaluation of how current populations relate to historical ones and whether there have been new introductions since 2007.

The 1.6 kilo-base pair mitochondrial marker from Carter et al. (2010) was used to sequence 111 beetles from three major persisting populations—Bethel, OH, Farmingdale and Amityville, NY, and Worcester, MA—plus the eradicated Boston infestation. The 13 Bethel ALB all shared the same haplotype, which is closely related to, but differs by two nucleotides from the original population in Toronto, Canada. The difference likely results from base call error during sequencing or editing. In Massachusetts, one common haplotype is shared by most beetles (22 out of 24) sampled from the two sites, indicating that the Boston infestation and the Worcester population are closely related. A single Worcester ALB differs from the common haplotype by three nucleotides. In contrast, one Boston ALB has a notable difference of 17 base pairs, which suggests that this beetle came from a very different genetic lineage. This diverged haplotype has not been seen in any other North American ALB.

Lastly, 74 specimens from Farmingdale and Amityville share an identical haplotype, which is different from the published sequence data from the New York region. Genetic distance shows that MA and NY samples are more closely related to each other than to OH and Toronto populations, and the unique Boston ALB has a large divergence from remaining samples (Figure 1). In summary, mitochondrial haplotypes of recent ALB show a range of differences from beetles collected prior to 2007. Further study is underway to determine whether the observed differences reflect separate introductions or are due to sequencing error.

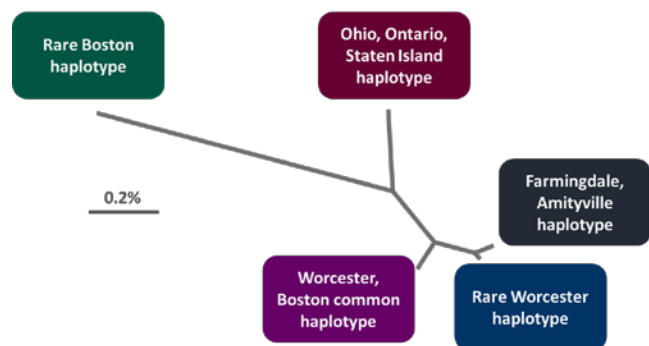


Figure 1. Genetic distances among the five haplotypes recovered from the 111 beetles.

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Asian longhorned beetle host preference

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Asian longhorned beetle (ALB), *Anoplophora glabripennis*, is an invasive pest of hardwoods in the U.S., with current infestations in Massachusetts, Ohio, and New York. Its preferred host list includes maple, *Acer* spp.; horsechestnut, *Aesculus* spp.; birch, *Betula* spp.; willow, *Salix* spp.; elm, *Ulmus* spp.; mimosa, *Albizia* spp.; katsura, *Cercidiphyllum*; ash, *Fraxinus* spp.; London plane tree, *Platanus* spp.; poplar, *Populus* spp.; mountain ash, *Sorbus* spp.; and goldenrain tree, *Koelreuteria paniculata* (Wang 2015).

Eradication programs have visually surveyed millions of trees for signs of ALB, and detection efforts are labor-intensive. Survey data from the eradication programs provide information on both infested and non-infested trees, and we are analyzing those data to estimate the importance of trees on the host list, particularly at the species level. Understanding host preference helps to determine which trees need to be surveyed in which situations, improving cost-effectiveness and efficiency. Starting with the ALB infestation in Worcester, MA, we overlaid the quarantine area with a 300x300 m grid and calculated a preference index for each species *x* for each grid cell, where:

$$\text{index} = \frac{\% \text{ species } x \text{ among infested trees}}{\% \text{ species } x \text{ overall}}$$

Maple is a favored host; red maple in particular has high preference through much of the core of the infestation (Figure 1).

This work is preliminary. Further analysis will investigate individual species with low or no ALB detections, considering factors such as tree size, land cover type, tree density, presence of preferred hosts such as red maple, distance from infested trees, and distance from roads.

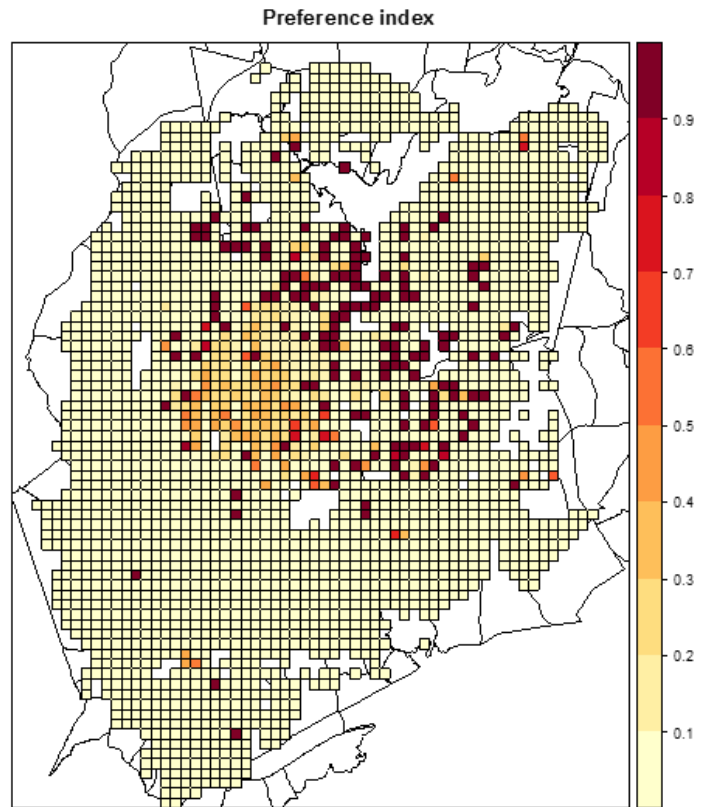


Figure 1. Preference index for red maple in the Worcester, MA, ALB quarantine area.

REFERENCE

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Improving detection tools for emerald ash borer: Effect of trap check frequency and killing agent on trap catch

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Green multi-funnel traps, coated with Fluon, a fluoropolymer, have been found to be comparable to, or better than, purple prism traps in terms of both trap catch and detection of the emerald ash borer (EAB), *Agrilus planipennis*. Since 2015, we have conducted studies to: 1) determine how often multi-funnel traps need to be checked and 2) compare killing agents used in multi-funnel traps.

Survey cost could be substantially reduced if traps could be checked less frequently (Figure 1). We compared trap catch at four trap check intervals over the field season to determine the maximum length of time traps could remain in field while still allowing trap catch to be identified. Intervals were: 1) weekly, 2) every three weeks, 3) every six weeks (to coincide with the changing of the lure), and 4) every twelve weeks (representing a full trapping season). Trap catch was not significantly different among any of the intervals tested. Samples from the 6- and 12-week intervals were still able to be identified and sexed.



Figure 1. Scott Gula lowering a green multi-funnel trap from the canopy to check the contents.

Currently, propylene glycol is the recommended killing agent for EAB multi-funnel traps. Our goal was to compare other killing methods to help reduce costs associated with replacing propylene glycol at every check.

Four treatments were tested:

- 1) standard propylene glycol “wet” cups
- 2) “dry” dichlorvos pesticide strips
- 3) “dry” dichlorvos with an internal funnel (with Fluon) to prevent escape (Figure 2)
- 4) an internal funnel in a dry cup without dichlorvos strips

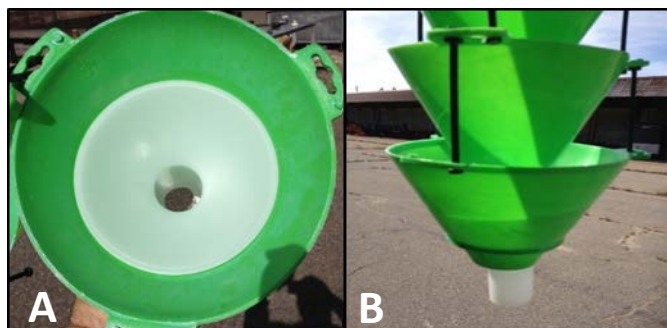


Figure 2. Internal funnel placed in the bottom funnel of a multi-funnel trap. A) Top down view. B) Side view.

There was no significant difference among any of the treatments. Dry cups could be used as an alternative to propylene glycol. The dry cup/internal funnel option without the dichlorvos strip is not recommended for catching EAB because live beetles were found in the traps during the check period. However, this method could be useful for live-trapping other woodborer species.

Improving detection tools for emerald ash borer: Calibrating catch to population density

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The ability to calibrate emerald ash borer (EAB), *Agrilus planipennis*, trap catch to population density would make the green multi-funnel trap a useful tool in the current biological control effort by assisting in targeting sites for future parasitoid releases. In 2015, we began a multi-year, multi-state project to examine the feasibility of calibrating EAB trap catch to population density.

For each plot, 13 traps were set up in a 1 ha grid with an 8-trap outer grid bordering the outside of the plot, 4 traps in an internal 0.25 ha grid around the center point and 1 trap at the center point (Figure 1).

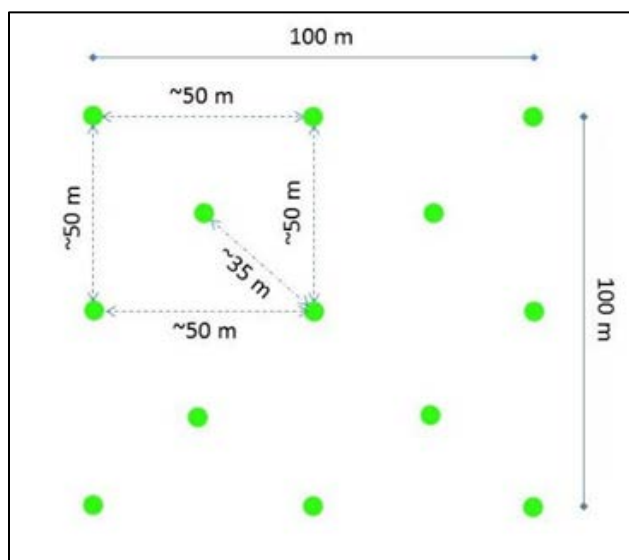


Figure 1. Layout of calibration trapping plot. Green dots represent traps.

All traps were hung approximately 5-8 meters above the ground in an ash tree. In 2015, 4 trapping plots were set up in locations of various stages of EAB infestation in 3 states (Massachusetts, New Hampshire and Ohio).

In order to closely follow “real world” deployment of traps where traps would be placed singly in high risk locations, nine single trap (1 ha, with a trap at the center location only) plots were set up in Massachusetts ($n = 7$) and Ohio ($n = 2$). These long term plots will be monitored for 2-4 years. In

addition to recording trap catch at all plots, yearly assessments are conducted every summer to measure DBH (diameter at breast height) and to record crown damage (on a scale of 1-5) on all ash trees inside each plot. In early 2016, we began felling and peeling 8-20 trees per plot. Trees ranging in size from 10-25 cm diameter were chosen randomly in the plot and cut into 1 m sections (up to 3.5 cm diameter) (Figure 2).

Measurements taken on these trees (Figure 3) include:

number of each EAB life stage found, % gallery cover, number of woodpecker attacks, number of epicormics, and presence/absence of parasitoids.



Figure 2. Everett Booth measuring a felled ash tree to be cut into 1 meter sections.



Figure 3. Everett Booth and Dave Cowan peeling ash logs to look for signs of EAB infestation.

Using green and purple multi-funnel traps as general survey tools for wood-boring beetles

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As part of an ongoing project to improve survey tools for emerald ash borer (EAB), *Agrilus planipennis*, we conducted a multi-state comparison of three currently available traps on a variety of host and non-host tree genera (Figures 1-3). This project also included a general woodborer survey comparing buprestid genera caught in each of the trap designs tested. A similar study was conducted in Poland.

We compared the following four trap treatments:

1) green, fluoned multi-funnel traps baited with z-3-hexenol, 2) unbaited green, fluoned multi-funnel traps, 3) unbaited, fluoned purple multi-funnel traps and 4) unbaited purple prism traps. In 2013, the four treatments were placed on one of four host genera: 1) *Fraxinus*, 2) *Pinus*, 3) *Acer* and 4) *Quercus* (red oak group only). In 2014, traps were placed on five host types: 1) *Fraxinus*, 2) *Populus*, 3) *Betula*, 4) *Quercus* (red oaks), 5) *Quercus* (white oaks). Replicates in both the 2013 and 2014 consisted of a single host/non-host genus.

Identifications of the trap catch have been completed for 56 of 72 replicates placed in 2013 (Table 1) and 75 of 101 replicates placed in 2014 (Table 2). Specimens from the remaining replicates are still being identified. Among the different genera, more EAB were caught on ash than on non-host trees. Overall, in 2013 and 2014, EAB detections were higher on green multi-funnel traps than on purple multi-funnel and prism traps.

A total of 30 and 27 species have been identified from traps placed in 2013 and 2014, respectively. In general, green multi-funnel traps caught more non-EAB *Agrilus* species than purple traps. From the 2013 trapping season 56 replicates per trap type have been identified. Of those traps, 43 green baited multi-funnels, 42 green unbaited multi-funnels, 15 purple prisms and 18 purple unbaited purple multi-funnels caught *Agrilus* species. In 2014, out of 75 trap replicates, 51 green baited multi-funnels, 52 green unbaited multi-funnels, 30 purple prisms and 36 purple unbaited purple multi-funnels caught *Agrilus* species. More *Chrysobothris* and *Dicerca* species were caught on purple traps than on green traps. In 2013 green multi-funnel traps caught more *Anthaxia*.

In 2013, we tested the same trap and lure combinations in Poland that were tested in the U.S. Traps were placed on the following host genera: 1) *Fraxinus*, 2) *Quercus*, and 3) *Pinus*, in a total of 10 replicates. A total of 2,015 *Agrilus* beetles representing 11 species were caught (Table 3). Similar to results found in the U.S., more beetles were caught on green traps than on purple traps. Among other buprestid genera, more *Phaenops (cyanea)* were caught on purple traps than on green traps.

Preliminary results from the general buprestid survey have shown that green multi-funnel traps may serve as a useful tool for the capture and detection of other *Agrilus* species besides EAB.

Table 1. Mean number (\pm SE) of buprestid beetles (and species) caught in each genera on each of four trap types in a multi-state (MA, MD, MI, MO, TN, WV; $n=56$) field comparison conducted in 2013. The number in parentheses below the mean catch represents the total number of traps of a type in which the genus was caught. Results in bold represent significant differences among trap types within a genus. ^a Does not include *A. planipennis* (EAB). ^b When traps were placed on *Fraxinus* spp., EAB were captured on 10 of 14 baited green multi-funnel traps, 12 of 14 unbaited green multi-funnel traps, 9 of 14 purple prism traps and 9 of 14 purple multi-funnel traps.

Genus	Green Multi-funnel Baited	Green Multi-funnel Unbaited	Purple Prism Unbaited	Purple Multi-funnel Unbaited
<i>Acmaeodera</i> (3 species)	0.09 \pm 0.05 (4 traps)	0.09 \pm 0.06 (3)	0.0 \pm 0.0 (0)	0.6 \pm 0.3 (5)
<i>Actenodes</i> (1 species)	0.02 \pm 0.02 (1)	0.02 \pm 0.02 (1)	0.0 \pm 0.0 (0)	0.02 \pm 0.02 (1)
<i>Agrilus</i> (30 species) ^a	16.1 \pm 5.4 (43)	21.6 \pm 7.6 (42)	1.9 \pm 1.0 (15)	2.3 \pm 1.3 (18)
<i>A. planipennis</i>	21.0 \pm 13.2 (25) ^b	4.9 \pm 1.8 (20) ^b	2.0 \pm 0.7 (13) ^b	3.8 \pm 2.6 (12) ^b
<i>Anthaxia</i> (4 species)	3.4 \pm 1.8 (23)	1.4 \pm 0.5 (16)	0.04 \pm 0.03 (2)	0.2 \pm 0.1 (4)
<i>Brachys</i> (2 species)	0.05 \pm 0.04 (2)	0.05 \pm 0.04 (2)	0.0 \pm 0.0 (0)	0.0 \pm 0.0 (0)
<i>Buprestis</i> (3 species)	0.04 \pm 0.03 (2)	0.05 \pm 0.03 (3)	0.00 \pm 0.00 (0)	0.07 \pm 0.04 (3)
<i>Chrysobothris</i> (5 species)	0.3 \pm 0.1 (7)	0.1 \pm 0.1 (3)	1.6 \pm 1.3 (12)	8.7 \pm 3.7 (34)
<i>Dicerca</i> (7 species)	1.0 \pm 0.5 (5)	0.1 \pm 0.03 (5)	0.02 \pm 0.02 (1)	2.3 \pm 1.0 (24)
<i>Phaenops</i> (2 species)	0.02 \pm 0.02 (1)	0.0 \pm 0.0 (0)	0.02 \pm 0.02 (1)	0.0 \pm 0.0 (2)
<i>Poecilonota</i> (1 species)	0.0 \pm 0.0 (0)	0.02 \pm 0.02 (1)	0.0 \pm 0.0 (0)	0.0 \pm 0.0 (0)
<i>Ptosima</i> (2 species)	0.11 \pm 0.06 (3)	0.1 \pm 0.1 (2)	0.0 \pm 0.0 (0)	0.0 \pm 0.0 (0)
<i>Spectralia</i> (1 species)	0.02 \pm 0.02 (1)	0.0 \pm 0.0 (0)	0.0 \pm 0.0 (0)	0.04 \pm 0.03 (2)
<i>Texania</i> (1 species)	0.0 \pm 0.0 (0)	0.0 \pm 0.0 (0)	0.0 \pm 0.0 (0)	0.02 \pm 0.02 (1)

Table 2. Mean number (\pm SE) of buprestid beetles (and species) caught in each genera on each of four trap types in a multi-state (CO, MA, MD, MI, MO, NY, OH, PA, WV; $n=75$) field comparison conducted in 2014. Results in bold represent significant differences among trap types within a genus. The number in parentheses below the mean catch represents the total number of traps of a type in which the genus was caught. ^a Does not include *A. planipennis* (EAB). ^b When traps were placed on *Fraxinus* spp. in infested areas, *A. planipennis* were captured on 8 of 12 baited green multi-funnel traps, 8 of 12 unbaited green multi-funnel traps, 10 of 12 purple prism traps and 7 of 12 purple multi-funnel traps.

Genus	Green Multi-funnel Baited	Green Multi-funnel Unbaited	Purple Prism Unbaited	Purple Multi-funnel Unbaited
<i>Acmaeodera</i> (1 species)	0.0 \pm 0.0 (0 traps)	0.01 \pm 0.01 (1)	0.0 \pm 0.0 (0)	0.01 \pm 0.01 (1)
<i>Actenodes</i> (1 species)	0.01 \pm 0.01 (1)	0.0 \pm 0.0 (0)	0.0 \pm 0.0 (0)	0.0 \pm 0.0 (0)
<i>Agrilus</i> (27 species) ^a	8.7 \pm 2.1 (51)	8.8 \pm 2.3 (52)	3.0 \pm 1.3 (30)	1.6 \pm 0.4 (36)
<i>A. planipennis</i>	5.5 \pm 4.1 (25) ^b	7.0 \pm 1.8 (17) ^b	2.0 \pm 0.7 (14) ^b	3.8 \pm 2.6 (10) ^b
<i>Anthaxia</i> (6 species)	0.2 \pm 0.1 (7)	0.3 \pm 0.1 (6)	0.01 \pm 0.01 (1)	0.01 \pm 0.01 (1)
<i>Brachys</i> (3 species)	0.7 \pm 0.3 (17)	2.0 \pm 1.0 (23)	0.0 \pm 0.0 (0)	0.01 \pm 0.01 (1)
<i>Buprestis</i> (2 species)	0.01 \pm 0.01 (1)	0.04 \pm 0.02 (3)	0.03 \pm 0.02 (2)	0.15 \pm 0.05 (10)
<i>Chrysobothris</i> (3 species)	0.2 \pm 0.1 (11)	0.1 \pm 0.04 (6)	1.2 \pm 0.5 (19)	2.4 \pm 0.3 (47)
<i>Dicerca</i> (3 species)	0.3 \pm 0.1 (12)	0.3 \pm 0.1 (15)	0.5 \pm 0.3 (14)	2.3 \pm 1.0 (39)
<i>Mastogenius</i> (1 species)	0.0 \pm 0.0 (0)	0.01 \pm 0.01 (1)	0.0 \pm 0.0 (0)	0.01 \pm 0.01 (1)
<i>Phaenops</i> (1 species)	0.0 \pm 0.0 (0)	0.0 \pm 0.0 (0)	0.0 \pm 0.0 (0)	0.04 \pm 0.03 (2)
<i>Poecilonota</i> (1 species)	0.0 \pm 0.0 (0)	0.02 \pm 0.02 (1)	0.0 \pm 0.0 (0)	0.03 \pm 0.03 (2)
<i>Ptosima</i> (2 species)	0.01 \pm 0.01 (1)	0.0 \pm 0.0 (0)	0.01 \pm 0.01 (1)	0.01 \pm 0.01 (1)
<i>Spectralia</i> (1 species)	0.01 \pm 0.01 (1)	0.03 \pm 0.02 (2)	0.12 \pm 0.08 (4)	0.03 \pm 0.02 (2)
<i>Texania</i> (1 species)	0.0 \pm 0.0 (0)	0.0 \pm 0.0 (0)	0.0 \pm 0.0 (0)	0.03 \pm 0.03 (1)

Table 3. Mean number (\pm SE) of buprestid beetles (and species) caught in each genera on each of four trap types in field comparison conducted in Poland in 2013 ($n=10$). Results in bold represent significant differences among trap types within a genus. The number in parentheses below the mean catch represents the total number of traps of a type in which the genus was caught.

Genus	Green Multi-funnel Baited	Green Multi-funnel Unbaited	Purple Prism Unbaited	Purple Multi-funnel Unbaited
<i>Agrilus</i> (11 species)	64.6 \pm 25.2 (9)	155.4 \pm 78.8 (9)	0.3 \pm 0.2 (2)	3.3 \pm 1.3 (2)
<i>Anthaxia</i> (1 species)	0.0 \pm 0.0 (0)	0.0 \pm 0.0 (0)	0.3 \pm 0.2 (2)	0.1 \pm 0.1 (1)
<i>Chrysobothris</i> (2 species)	0.0 \pm 0.0 (0)	0.0 \pm 0.0 (0)	0.0 \pm 0.0 (0)	0.3 \pm 0.2 (2)
<i>Phaenops</i> (1 species)	0.5 \pm 0.3 (2)	0.4 \pm 0.3 (2)	0.0 \pm 0.0 (0)	10.3 \pm 6.0 (4)
<i>Trachys</i> (1 species)	0.2 \pm 0.1 (2)	0.0 \pm 0.0 (0)	0.0 \pm 0.0 (0)	0.0 \pm 0.0 (0)



Figure 2. A green multi-funnel trap.



Figure 1. A purple multi-funnel trap.



Figure 3. A purple prism trap.

Isolation and identification of a male-produced attractant pheromone for the invasive velvet longhorned beetle, *Trichoferus campestris*

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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 (Figure 1). Larvae of VLB can be transported in wood packing material and are frequently intercepted at ports and warehouse facilities; a total of 29 interceptions were recorded between 2012 and 2015. Populations of VLB have been found outside of the native range of the species, including near Salt Lake City, Utah. A national program for the eradication of VLB has not yet been implemented, partially because the biology of VLB in North America is not well understood, and because trapping protocols have not been fully evaluated or optimized. Prior to 2017, the only CAPS approved method for surveying for VLB was visual survey and black light trapping.



Figure 1. 1) Male and 2) female adult *Trichoferus campestris*.
From Grebennikov et al. 2010.

Following capture of specimens attracted to traps used in a field trapping assay, male and female VLB were sent off for sectioning. Cross-sectional analysis of the prothoraces of collected beetles indicated that pheromone production is present in males, but not in females (Figure 2).

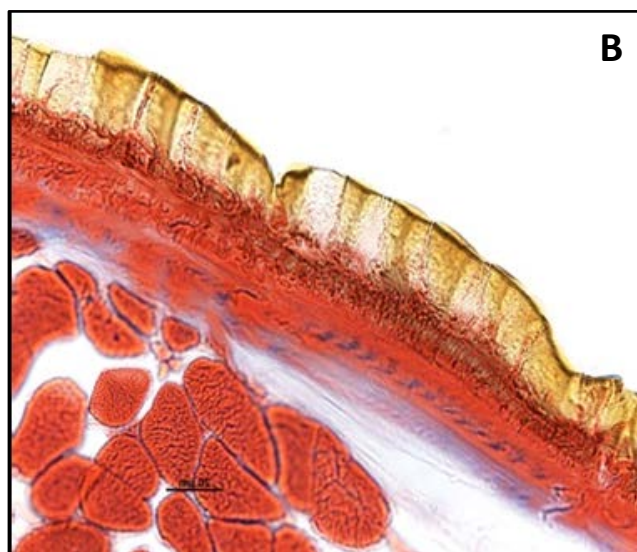
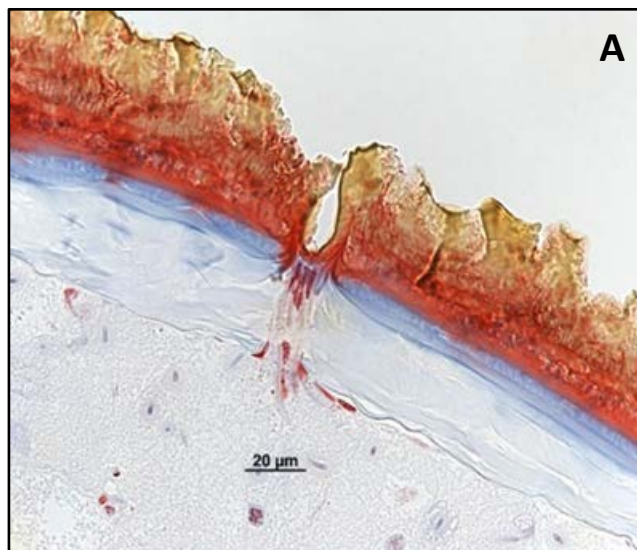


Figure 2. Cross-sections of A) male and B) female prothoraces at 60x showing possible pheromone production glands present in males, but absent in females.

Since the detection of VLB in 2014, trapping assays have been conducted in Salt Lake and Utah counties, UT with the goal of identifying a possible attractant compound for VLB. These trapping assays also evaluated the effects of trap design, color, and placement height on the number of beetles captured. The initial trapping assays (2014-2015) focused on comparing compounds utilized by other cerambycine species along with an ethanol control. Results showed that there were no significant differences among treatments, with the exception of the ethanol control.

In 2015, laboratory aerations were performed to collect head-space volatiles from beetles live-captured from the field in UT (Figure 3). Several chemical structures were identified from the headspace of males that were not present in females. These structures were identified as 4 stereoisomers (in a 10:1 ratio) of a novel 2, 3-alkanone, trichoferone. Following this identification, trapping assays were conducted in 2016 to compare number of adult beetles captured in traps baited with trichoferone versus traps baited with ethanol lures and unbaited traps. In two separate assays, more than 3,000 total beetles were caught, with significantly more caught in traps baited with either a 4- or 2-stereoisomer blend of trichoferone (differences significant at $p < 0.05$).

Separately, in Pennsylvania, a single VLB adult was captured in a trap baited with trichoferone, detecting VLB in a location not previously known to be infested.



Figure 3. Portable volatile collection system used to collect headspace volatiles from adult VLB.

In assays to evaluate the effect of trap height on trap catch, significantly more beetles were captured on traps placed in the canopy than on traps placed near the ground (differences significant at $p < 0.05$). More beetles were caught on standard Fluon coated black intercept panel traps, (Figure 4) therefore preliminary results suggest varying trap color and design does not increase trap catch. Trapping assays were also conducted in a recently detected, low-density population in White Plains, New York. A total of 66 traps baited with ethanol lures caught 2 beetles. In 2017, trapping assays will focus on a dose-response study for trichoferone, and on comparing VLB catch in trichoferone-baited traps at two heights. The results of this work will lead to lure and trapping recommendations to assist state and U.S. federal personnel and land managers in developing monitoring surveys for VLB.



Figure 4. Black intercept panel trap.

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Response of adult Asian longhorned beetles to isothiocyanates in laboratory bioassays

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Asian longhorned beetle (ALB), *Anoplophora glabripennis*, is an invasive cerambycid that can damage a wide range of deciduous trees. It has caused extensive damage in multiple sites in North America. Species in the genera *Acer*, *Betula*, *Salix*, and *Ulmus* are the preferred hosts in North America. A key component in limiting the spread of the infestation is detection. Synthetic pheromones paired with host plant volatiles have been used as attractants in monitoring programs for ALB. However, synthetic pheromones appear to have limited efficacy when ALB populations are small.

A more attractive lure would improve detection and monitoring efforts. We hypothesized that adult ALB would be attracted to isothiocyanates (ITCs) and that ITCs could increase the efficacy of pheromone lures in the field. In other beetle families a particular enzyme, myrosinase, allows beetles to sequester glucosinates, a defense chemical produced by plants, and release ITCs as a sex-attractant pheromone.

Since ALB has a gene coding for the production of myrosinase, we investigated the potential attractiveness of four ITCs (allyl, isobutyl, phenyl and methyl) in a Y-tube bioassay (Figure 1).

Unmated mature beetles that were 7-60 day(s) old were given a choice between a control and 1.0, 0.1, or 0.01 mg concentration of each of the four ITCs. In each assay, a single beetle was given 15 minutes to make a choice (Figure 1). Only one ITC, allyl ITC, showed significant attractiveness to ALB over the control (Table 1). We will continue this investigation in 2017 with this ITC along with others to determine if this compound could help increase the efficacy of lures in the field. We will also combine this compound with the known farnesene component of the ALB pheromone and incorporate mated males and females into the study.

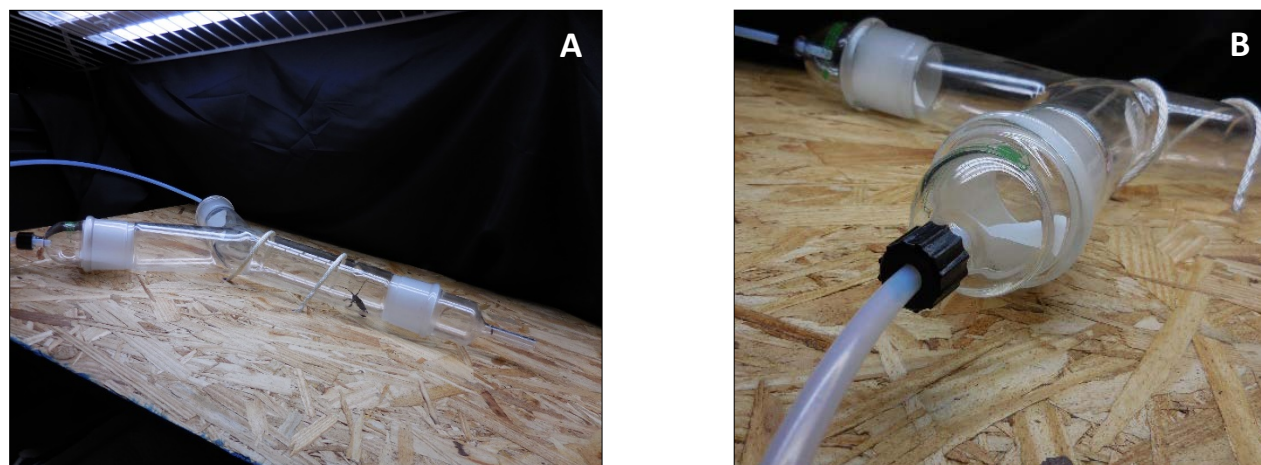


Figure 1. A) ALB inside glass Y-tube with ITC sample on one side and ethanol control on the other. B) Detail of Y-tube apparatus.

Table 1. Results of a Y-tube bioassay. Due to low sample size, concentration of each treatment and beetle sexes were combined for analysis.

Isothiocyanate	No. beetles tested	No. choosing ITC	No. choosing control	χ^2 statistic (P)
Allyl ITC	50	21	6	8.33 (P=0.004)*
Isobutyl ITC	50	6	14	3.20 (P=0.074)
Phenyl ITC	50	12	12	0.00 (P=1.000)
Methyl ITC	50	15	16	0.032 (P=0.857)

Comparing trap colors for Asian longhorned beetle

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Currently, survey protocol for Asian longhorned beetle (ALB), *Anoplophora glabripennis*, consists of visual inspection of trees, which involves searching for ovipositional scars, exit holes and other signs of infestation. This survey work, conducted by climbers and ground crews, is expensive and labor intensive. Adding trapping to the current ALB survey method could serve as a cost reducing measure. While several promising beetle- and host-produced compounds have been identified, to date there is no effective trapping protocol for ALB.

The goal of this work is to develop a more effective trap for ALB. Studies were conducted to determine if trap color influences trap catch. In 2015, we conducted electroretinogram assays to determine ALB retina sensitivity at different wavelengths of light in the UV spectrum. Based on these results, we selected several colors in the blue/UV (450-495), green/yellow/orange (550-600) and deep red/purple (660+) ranges for use in a laboratory color choice bioassay. A single beetle was placed in the center of an arena (12 inch cube). One color was tested at a time against standard black corrugated plastic.

Based on this work, three paint colors were chosen for trapping assays: dark blue (450 nm, 28% reflectance), purple (412 nm, 21% reflectance; 670 nm, 43% reflectance) and yellow (570 nm, 63% reflectance). These paints were applied to intercept panel traps, and the three colors were compared to black intercept panel traps, the current standard traps for ALB (and most cerambycids) (Figure 1). All traps were coated with Fluon, a fluoropolymer shown to increase trap catch of many beetles by making the trap surface more slippery. All traps were placed on the trunk of a host tree: a) maple in Ohio in 2015, b) willow in Beijing (Tongzhou Canal Park) in 2016 and c) poplar in Ningxia (near Yanchi) in 2016. The top of the trap was removed and the trap was secured to the trunk using 4 foot long cable ties in an attempt to attract and capture beetles walking up and down the trunk.

In 2015, traps were placed on maple trees in Ohio, but no beetles were caught presumably due to low population density. Therefore, in 2016, traps were placed in two more heavily infested locations in ALB's native range in China: on willow trees in Beijing, and on poplar trees in Ningxia A.R. A total of 86 beetles were caught, but there were no significant differences among the colors tested.

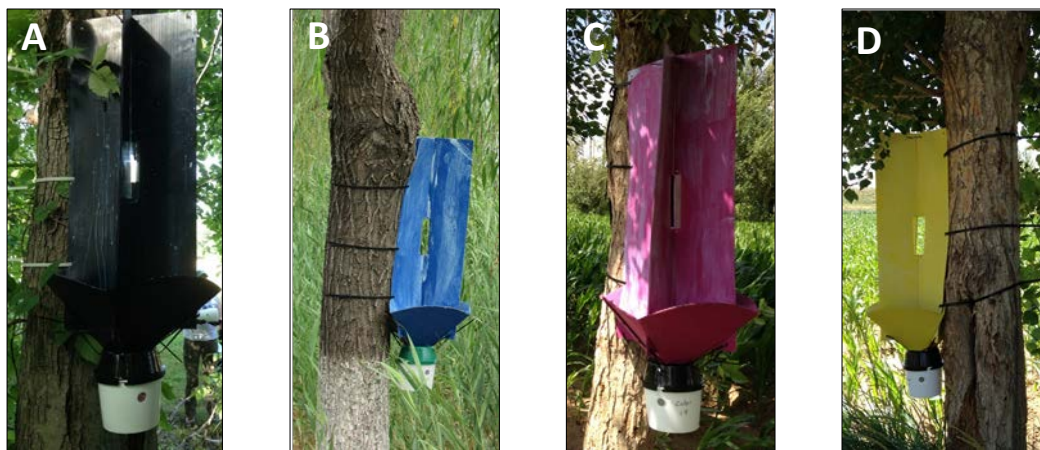


Figure 1. Modified intercept panel traps attached to the trunk of host trees in a trap color comparison. A) Black (standard, control). B) Dark blue. C) Purple. D) Yellow.

Recent development and advances in survey and detection tools for Asian longhorned beetle

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Asian longhorned beetle (ALB), *Anoplophora glabripennis*, is considered to be one of the most serious invasive pests of deciduous trees in North America.

An efficient monitoring trap is needed to detect new introductions and assess population densities of established infestations. Previous studies on ALB have shown that males produce a two-component pheromone, which consists of a 1:1 blend of 4-(n-heptyloxy) butan-1-ol and 4-(n-heptyloxy) butanal (Zhang et al. 2003). Our laboratory recently identified a third male produced component ((3E-6E)- α -farnesene) which enhanced female attraction over a short range (Crook et al. 2014). Volatiles from female ALB are currently being examined to identify potential pheromone components that work better at a longer range. Wickham et al. (2012) demonstrated that ALB female contact pheromones were also a precursor that underwent abiotic oxidation to yield more volatile components.

Antennally active components (heptanal, nonanal and hexadecanal) caught more beetles than controls in trapping studies (Wickham et al. 2012) but the lure was believed to be missing some further components. Our main goal has been to identify any missing female produced oxidation components using Gas Chromatography coupled electroantennography detection (GC-EAD) analysis.

Adult female hexane body washes were loaded into quartz cuvettes and exposed to UV light for several hours (Figure 1). GC-EAD recordings revealed the presence of at least two new antennally active aldehydes (Figure 2, Peaks 2 & 4). Olfactometer behavioral assays (Figure 3) will help determine if these new aldehyde components will enhance the current 3-component aldehyde blend developed by Wickham et al. (2012). We are also developing new color traps and trap designs for ALB and are testing to see if they can be visually enhanced by the addition of 3D printed ALB models.

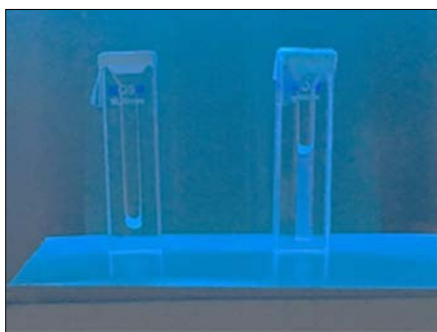


Figure 1. Female body wash extracts in quartz cuvettes under UV lighting.



Figure 3. ALB adult in olfactometer assay.

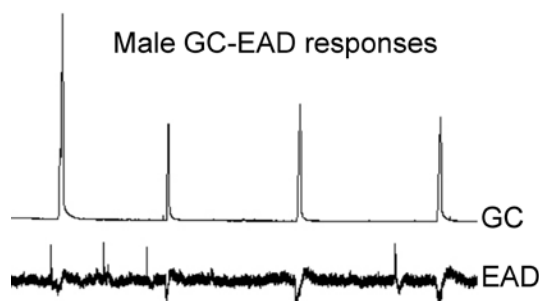


Figure 2. Male antennal responses to female produced aldehydes.

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Salinas Field Station annual support to cooperative projects

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The Salinas Field Station's scientific assistance to USDA's cooperative researchers is an integral constituent of the Plant Protection and Quarantine (PPQ) mission to safeguard U.S. agriculture and natural resources against the entry, establishment, and spread of economically and environmentally significant pests and to facilitate the safe trade of agricultural products. Responsibilities of the Salinas Station include rearing and maintaining a colony of Light Brown Apple Moth (LBAM), *Epiphyas postvittana*, to support multiple research projects, as well as coordinating and providing support to collaborative research.

LBAM production is comprised of the setup of four larval trays per week from January through September, and then three per week from October to December. Each tray yields an average of 1,580 pupae per tray (271,000 pupae per year). Environmental rearing parameters include a constant temperature of 22°C (± 2°C) and target 60% humidity conditions with a 16:8 hour light/dark cycle.

Moths are fed 7.5% sucrose solution while in egg cages, and pink bollworm diet as pupae. As part of continuing efforts to improve LBAM production a study compared the use of coffee filters as a pupal substrate versus diet alone. Because LBAM are tortricids, they "roll" leaf like material around their pupae. Deprived of leaf like material in a standard diet tray they pupate in silk along the edge of the tray or burrow into the diet to pupate. When provided a light flexible paper medium like coffee filter approximately 40% of the moths pupate in the silk on the side of the tray, or on the coffee filters, compared to 30% of moths pupating in silk in the control trays (Figure 1). Although the sample size for this study was too small to be definitive, it is consistent with observations from rearing. The advantage of this method is that it is much easier to harvest pupae from the substrate than to pick them from the insect diet.

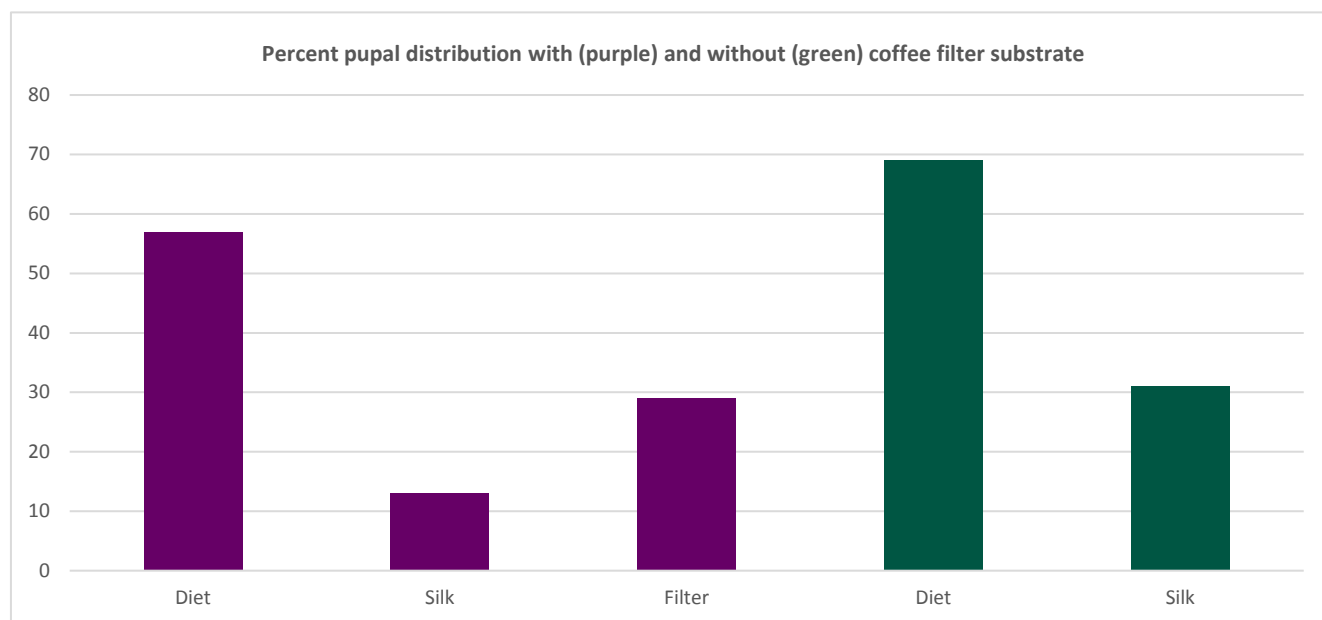


Figure 1. Results of coffee filter experiment showing a 10% increase in moths pupating in desired location with addition of coffee filters.

The emerald ash borer (EAB), *Agrilus planipennis*, is an invasive pest that is damaging ash trees throughout the North Eastern United States. In an effort to control this pest, APHIS supports a biological control laboratory in Michigan, which maintains a year round emerald ash borer colony for research. However, since ash trees are fully deciduous in the Northeast, the Michigan laboratory is unable to collect host plant material for the EAB colony during the winter months. Several years ago, a row of tropical ash, *Fraxinus uhdei*, trees were planted at the Salinas Station to fill this gap (Figure 2).



Figure 2. Tropical ash trees at CPHST California Station.

Salinas Station provided 14 shipments of 3-4 lbs of ash leaves between January and April, 2016 to support the maintenance of APHIS's EAB colony in Michigan. Additionally, a shipment of two species of olive wood was sent in January to cooperators at Wright State University in Ohio to investigate the possibility that EAB may be able to use olive wood as an alternate host. A full description of all materials shipped to cooperators in 2016 can be seen in Table 1.

CPHST Salinas Field Station will remain an adjunct to the cooperative research efforts that defend our nation's agricultural resources.

Table 1. Support materials and specimens provided to cooperators by CPHST California Station in 2016.

Material Type	Quantity Provided	Destination	Purpose
LBAM eggs	9,800	U.C. Berkley	Mills Lab project
LBAM pupae	18,720	U.C. Davis	Nursery regulatory treatment project
LBAM pupae	1,500	University of Arizona	Stable isotope project
LBAM adults	4,373	University of Arizona	Stable isotope project
Ash tree leaves	14 cooler units	USDA Brighton, Michigan Lab	EAB biological control project
Colander cages and navel orangeworm eggs	2	USDA Phoenix, Arizona Lab	Navel orangeworm project
Olive wood	4 cooler units	Wright State University, Ohio	Emerald ash borer project

Development of regulatory nursery treatment protocols for light brown apple moth

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California Department of Food and Agriculture (CDFA) regulates wholesale ornamental plant nurseries operating within a light brown apple moth (LBAM), *Epiphyas postvittana*, state interior quarantine (SIQ). These nurseries must have an integrated pest management program in place that includes regular monitoring and insecticide treatments based on LBAM detections. The nursery must conduct trapping and monthly inspections of plants intended for shipment. If any LBAM are found, nursery operators must apply a foliar insecticide treatment to plants intended for shipment, targeting the detected LBAM life stage. After treatment, the nursery must be re-inspected by program officials and cleared of any holds before shipping can resume.

At the inception of the LBAM regulatory program, nursery production and shipping were identified as significant risk pathways. An analysis of trapping records from the first years of the LBAM program suggested that human assisted movement was an important factor in the spread of LBAM (Suckling et al. 2011). LBAM infestation of nursery production along the central California coast has resulted in significant costs to the nursery industry and to the cooperative LBAM regulatory program. Recent outbreaks in new California coastal counties associated with movement of ornamental nursery plants highlight the challenges of controlling LBAM spread and the ongoing risk to nursery production. Research completed by the University of California documented the degree and duration of control expected from existing treatments and identified new treatments that may serve as part of a regulatory strategy to reduce the risk of spreading LBAM through transport or shipping (Tjosvold and Murray 2012).

A population survey of LBAM was conducted in 2016 from January to April on publically accessible sites around San Diego County, CA; 42,069 plants were scouted and 21 leafroller larvae were collected. The samples were then moved by permit to cages in the San Diego County Department of Agriculture Weights and Measures Entomology Laboratory, where 16 of 21 leafrollers were reared to the pupal stage. A single LBAM was positively identified by the USDA from the leafroller collection on a Brazilian pepper tree, *Schinus terebinthifolius*, in a parking lot near a residential neighborhood in Rancho Santa Fe. The remaining 15 leafrollers were reared in an effort to detect any natural enemies that might be present. Repeated searches in the same area yielded several more leafroller larvae, but no LBAM to date.

In addition to LBAM population surveys, four experimental trials were conducted at the USDA-ARS Station in Salinas, CA. Nine chemicals from the CDFA approved pesticide list were tested: Acelepryn, Conserve, Mainspring, Malathion, NeemOil, Pedestal, Pyrellin, Provaunt, and Scimitar. Scimitar and Malathion proved to be the only products to cause significantly reduced LBAM egg viability compared to the control (Figure 1). However, Mainspring, Acelepryn and Conserve were found to be sufficient for LBAM neonate control or eradication (Figure 2). CPHST California Station reared a total of 18,720 LBAM in support of this project.

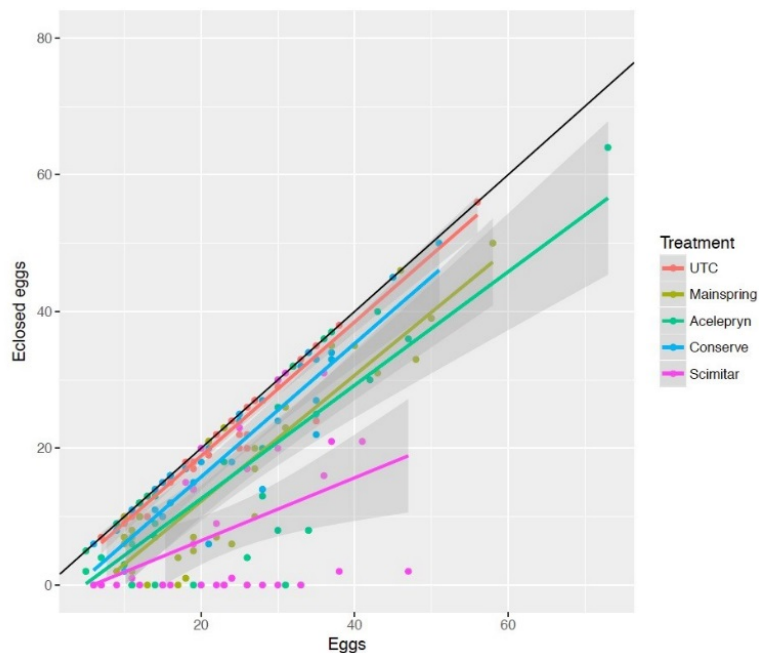


Figure 1. The relationship between the pretreatment number of eggs on the x-axis and the number of enclosed eggs on the y-axis in response to selected treatment applications. Shaded areas around a line indicate the 95% confidence interval.

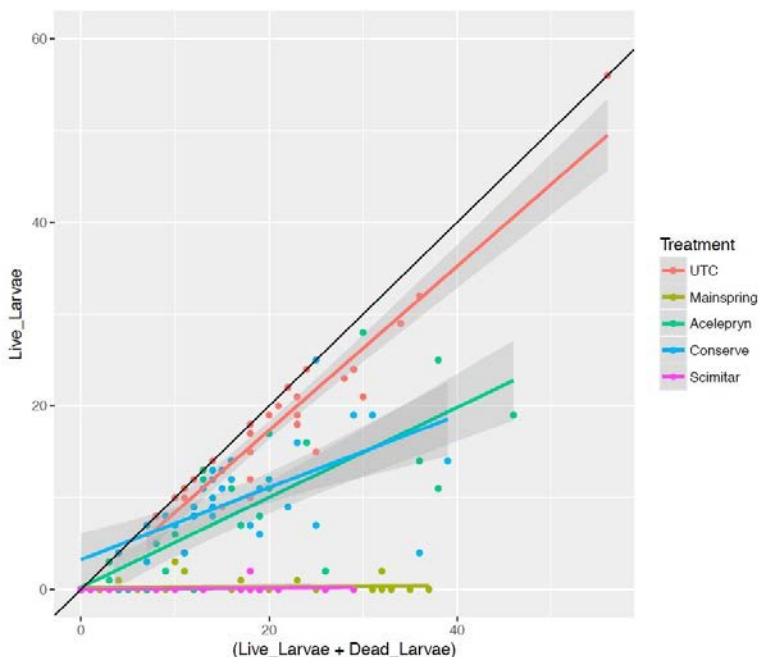


Figure 2. The relationship between the total number of larvae on the x-axis to the number of live larvae on the y-axis in response to feeding on treated leaves. Shaded areas around a line indicate the 95% confidence interval.

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Methods development in support of area wide program to eradicate European grapevine moth in California

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In the fall of 2009, the first confirmed North American detection of European grapevine moth (EGVM), *Lobesia botrana*, was made in Napa County, California. Based on its status as a significant grape pest in other parts of the world, the establishment of EGVM in California presented significant production and export issues for grapes, as well as for other fresh market agricultural commodities. Over the past seven years, an intensive California statewide survey and area-wide eradication campaign was undertaken in partnership with agricultural officials at local, state and federal levels, university scientists, and the wine, table grape and raisin industries. These efforts resulted in a dramatic decline in moth captures from over 100,000 moths in 2010, to one in 2014, and none in 2015 (Figure 1). In August of 2016, all previously infested areas in California were declared eradicated of EVGM.

The decision to pursue the eradication effort was based on the limited geographic area and host range of the EGVM infestation, the availability of several effective tools for monitoring and control, and the strong support of the affected grape production industry. The eradication campaign employed a coordinated logistical, regulatory, and technical effort that included:

- 1) A statewide monitoring effort using a network of moth pheromone traps and in field monitoring that allowed for regular communication of survey results (placed into a geographic information mapping system) to program officials
- 2) An area-wide hand-application of mating disruption dispensers to all infested grapes, including use in urban environments within infested zones
- 3) Area-wide applications of insecticides (implemented by treatment coordinators) where application timing was determined by degree day modeling for each infested region
- 4) A robust regulatory program that initiated and maintained a quarantine of infested areas that regulated movement of fruit, farming equipment and winery processing wastes
- 5) An extensive outreach program to grape growers, wineries, pest control specialists, and the public
- 6) Formation of a technical advisory group along with a robust methods development and research effort to provide guidance to the operational program and to develop and test tools needed to support the program

The methods development effort supported the eradication program by: developing enhanced detection methods for vineyards under mating disruption, testing efficacy and residual control of insecticides, testing mating disruption formulations, evaluating the impacts of winery processing methods on EGVM mortality, developing methods to determine EGVM biofix (biological event) to improve degree day models in California, developing EGVM rearing methods, testing the quality of pheromone lures and trap monitoring, and a spatial analysis of trapping data to determine program effectiveness and to analyze invasion pathways.

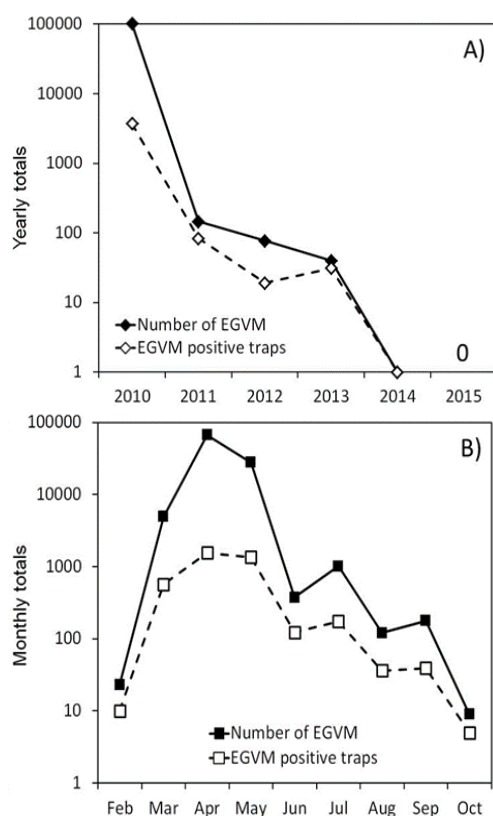


Figure 1. Overall A) yearly and B) seasonal trends (2010) in *Lobesia botrana* (EGVM) trap catch in California.

Development of stable isotopes and new biochemical tools for identification of sterile insects and determination of pest origin

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The goals of this project were to: 1) develop new identification tools for differentiating sterile insect technique (SIT) insects from wild-type for the following eradication programs: pink bollworm (PBW), *Pectinophora gossypiella*, Mediterranean fruit fly (Medfly), *Ceratitidis capitata*, Mexican fruit fly (Mexfly), *Anastrepha ludens*, and light brown apple moth (LBAM), *Epiphyas postvittana*, and 2) to develop and apply new isotopic and fatty acid analysis tools for identification and trace-back to probable origin and host identity of invasive plant pests found in Cooperative Agricultural Pest Survey (CAPS) programs. More robust and reliable identification tools for SIT programs are needed because mistaking sterile released insects for wild-target pests can trigger costly response measures or delay eradication declarations. New isotopic and fatty acid analysis methods can be used to determine location of origin for new pest detections, and to determine host plant use for species using multiple hosts.

Isotopic differences in samples as small as 150 micrograms, i.e. one fly, moth or beetle leg, can easily be detected using new laser-based technology (CM-CRDS Picarro-USA). We used this technology to rapidly determine isotopic (carbon isotope, ¹³C) and fatty acid differences between wild- and lab-reared insects of PBW, Medfly, Mexfly, and LBAM. Results from Medfly and Mexfly analyses are described below.

Samples collected from various types of glue traps were analyzed to determine if the glue would affect the sample signal, and if there are suitable methods for cleaning samples that would preserve the original isotopic ratios. We also determined how much sample is needed for analysis, and how insect samples should be prepared and preserved for further analysis.

Both Medflies and Mexflies reared on artificial diets were found to be significantly different ($F=1836$, $n=136$, $p<0.0001$) and ($F=1479$, $n=60$, $p<0.0001$), respectively, from those reared on mango or guava, with no overlap in carbon isotope signature. This suggests that ¹³C can be used to distinguish between mass reared flies from wild flies reared on fruit (Figures 1 and 2). Fatty acid fingerprint analysis of wild Mexflies, subsequently reared from guava or mango, and mass-reared Mexflies (MRI) reared on sugar cane bagasse diet, showed clear differences between fly types ($n=5$) (Figure 3). This suggests this method could be a useful tool in determining the host origin of the flies caught on the traps.

LBAM reared on artificial diet were significantly different ($F=144$, $n=130$, $P<0.0001$) from wild moths reared on plant diets with no overlap in carbon isotope signature (letters denote statistically different groups with $\alpha=0.0001$) (Figure 4). ¹³C can be used to distinguish between mass reared LBAM from wild LBAM reared on host plants.

There was no significant difference between PBW reared on artificial diet versus wild PBW reared on plants with a great deal of overlap in isotope values (letters denote statistically different groups with $\alpha=0.0001$) (Figure 5). As there is no separation between mass reared PBW from wild PBW reared on host plants, ¹³C cannot be used to distinguish between mass reared PBW from wild PBW reared on host plants, though it may be possible when other isotopes are included in the analysis.

We found that trap glue has a strong ¹³C signal and glue should be cleaned from samples (up to 5% glue acceptable) before analysis. If possible, samples free of glue should be selected, or traps without thick soft glue (such as the new hard glue traps) should be used. It was also demonstrated that with careful and simple washing, traces of C associated with alcohol storage can be removed.



Figure 1. ¹³C signatures of Mediterranean fruit fly (Medfly), *Ceratitidis capitata*, reared on different diets. Significance was determined using the Tukey-Kramer test, illustrated on the right side of the graph ($F=1836$, $p<0.0001$).

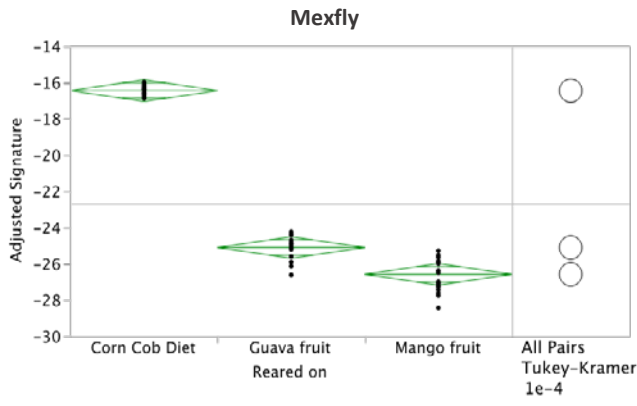


Figure 2. 13C signatures of Mexican Fruit fly (Mexfly), *Anastrepha ludens*, reared on different diets. Significance was determined using the Tukey-Kramer test, illustrated on the right side of the graph ($F=1479, p<0.0001$).

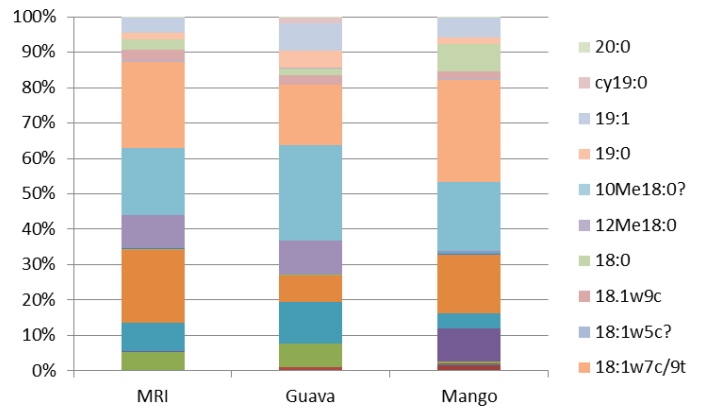


Figure 3. Fatty Acid Analysis of Mexican Fruit fly (Mexfly), *Anastrepha ludens*, reared on different diets.

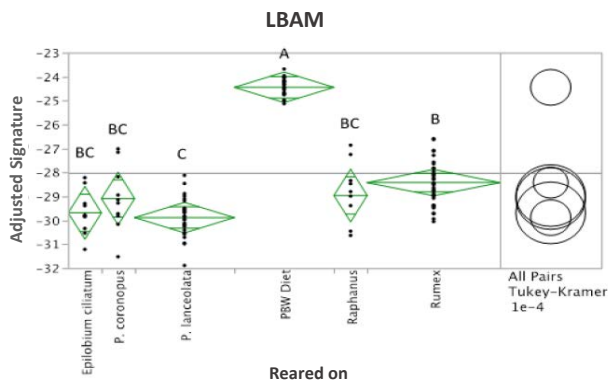


Figure 3. 13C signatures of LBAM, *Epiphyas postvittana*, reared on different host plants. Significance was determined using the Tukey-Kramer test, illustrated on the right side of the graph ($F=144, n=130, P<0.0001$). Letters denote statistically different groups with $\alpha=0.0001$.

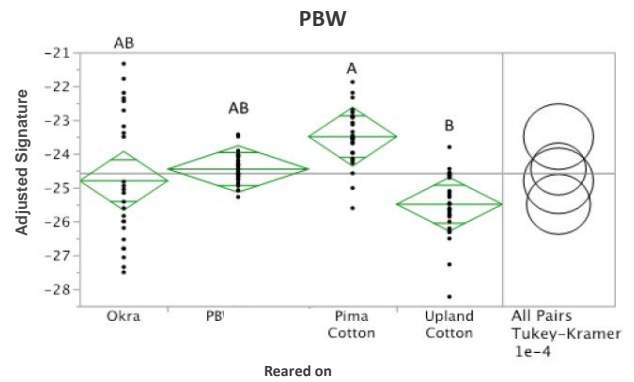


Figure 4. 13C signatures of PBW, *Pectinophora gossypiella*, showing no significant difference between PBW reared on artificial diet versus wild PBW reared on plants with a great deal of overlap in isotope values (letters denote statistically different groups with $\alpha=0.0001$).

Identification of host plant for Asian citrus psyllid and *Tamarixia radiata* rearing

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Asian citrus psyllid (ACP), *Diaphorina citri*, the vector of Huanglongbing (HLB) disease caused by the bacteria *Candidatus Liberibacter asiaticus*, was first detected in Florida in 1998 and in California in 2008. There is no cure for HLB, therefore it has severely impacted the citrus industry in Florida. Two significant parasitoids of ACP, *Tamarixia radiata* and *Diaphorencyrtus aligarhensis*, have been imported from Pakistan and released in 2011 and 2013, respectively. Mass produced *T. radiata* wasps are released mostly in urban backyards of Southern California to help suppress the ACP population and slow the spread of ACP and HLB. Due to the specificity of these parasitoids, their mass production relies on our ability to successfully mass produce ACP. ACP can be reared on many types of *Citrus* as well as related species such as orange jasmine, *Murraya paniculata*, and Curry leaf plant, *Bergera koenigii*. Curry leaf is a preferred host plant for greenhouse rearing of ACP and its parasitoid *T. radiata* in California. Curry leaf plants are grown from seeds collected from several sources and show a wide range of variations. The three most common phenotypes (Figure 1) based on leaf characteristics were described as:

- 1) Large-leaf: ~17.7 cm long, 19.6 average leaflets and light green color
- 2) Small-leaf: 10.9 cm long, 13.1 leaflet, light green color
- 3) Dark-leaf: 15.2 cm long, 16.8 leaflets and dark green color



Figure 1. Curry leaf phenotypes: Large-leaf (left); Small-leaf (middle) and Dark-leaf (right).

A study was conducted at California Department of Food and Agriculture greenhouses (near Mt Rubidoux, Riverside, CA) to compare these three types of curry leaf plants for *T. radiata* production. Eight curry leaf plants and one *Citrus volkameriana* (used as an oviposition stimulator) were used within each BugDorm cage (BD2400) (Megaview, TW). Each cage was inoculated with 500 ACP adults. When the nymphs reached the 3rd to 4th instar, 100 to 150 adult *T. radiata* were introduced into each cage. The study was repeated five times. The number of cages evaluated at each setup date is presented in Table 1.

The large leaf variety of curry leaf plant produced an average of 1,694 wasps per cage, which was significantly higher compared to the numbers of *T. radiata* produced per cage with small leaf (922 wasps per cage) and dark leaf (482 wasps per cage) type plants (Table 2). A separate study showed that dark leaf type plants produce fewer of the young flush leaves necessary for ACP oviposition than the large leaf variety. New leaves on the large leaf variety also remained suitable for ACP oviposition longer than other phenotypes, which also might have helped to produce more ACP and thereby more *T. radiata* wasps.

Table 1. Number of cages setup with different type of curry leaf plant on each date.

Setup date	Plant Type		
	Large-leaf	Small-leaf	Dark-leaf
3/2/2016	3	18	7
3/16/2016	8	7	15
3/23/2016	-	1	9
3/29/2016	2	3	8
4/6/2016	5	5	2
Total	18	34	41

Table 2. Mean number of *T. radiata* wasps produced per cage by different types of curry leaf plant on each date.

Setup date	Plant Type		
	Large-leaf	Small-leaf	Dark-leaf
3/2/2016	903	553	598
3/16/2016	907	617	320
3/23/2016	-	985	454
3/29/2016	560	1,093	594
4/6/2016	3,881	2,564	971
Mean of all observations	1,694	922	482

Asian citrus psyllid inoculation rate and plant pruning for *Tamarixia radiata* production

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Biological control of Asian citrus psyllid (ACP), *Diaphorina citri*, using parasitoids *Tamarixia radiata* and *Diaphorencyrtus aligarhensis* is being implemented in Southern California. *Tamarixia radiata* wasps are mass produced and released mostly in urban backyards of Southern California to help suppress the ACP population and slow down the spread of both ACP and the huánglóngbìng (HLB) disease it vectors. Curry leaf plant, *Bergera koenigii*, has been the preferred host plant to rear ACP and *T. radiata*.

Studies were conducted at the California Department of Food and Agriculture (CDFA) greenhouses near Mt. Rubidoux (Riverside, CA) to identify appropriate plant management practices (leaf pruning) and determine the appropriate ACP density necessary for inoculation of *T. radiata* production cages. Treatments included removal or retention of mature leaves on curry leaf plants and three ACP densities in a factorial randomized complete block (RCB) design. In the first study, five plants (low plant density) were used, where the plant to ACP ratio was either 1:20, 1:40 or 1:60. In the second study eight plants per cage (high plant density) were used, where the plant to ACP ratio was either 1:12.5, 1:25 or 1:37.5. Total number of *T. radiata* produced in each cage was counted during collection and compared among treatments.

Under low plant density, higher numbers of *T. radiata* were produced when mature leaves were retained on the plants. The 1:40 ratio of ACP adults per plant produced the highest number of *T. radiata* (1,522 per cage) when leaves were retained (Table 1). Removal of mature leaves led to higher numbers of ACP adults feeding and ovipositing on young flush, sometimes causing death of the flush tips and leading to production of fewer *T. radiata*. Under higher plant density, greater numbers of *T. radiata* were produced in cages where mature foliage was removed (Table 2). Cages inoculated with 25 adult ACP per plant and removed of mature leaves produced 1,405 wasps per cage. Retention of mature leaves acted as distraction sites for adult ACP leading to fewer adults actually feeding and ovipositing on young flush.

Table 1. Mean number of *T. radiata* produced per cage as affected by foliage removal and ACP density (low plant density).

Mature leaves	No. of ACP	Plant: ACP ratio	<i>T. radiata</i> per cage
Retained	100	1:20	1,123
	200	1:40	1,522
	300	1:60	1,211
Removed	100	1:20	1,055
	200	1:40	809
	300	1:60	1,011

Table 2. Mean number of *T. radiata* produced per cage as affected by foliage removal and ACP density (high plant density).

Mature leaves	No. of ACP	Plant: ACP ratio	<i>T. radiata</i> per cage
Retained	100	1:12.5	470
	200	1:25.0	517
	300	1:37.5	839
Removed	100	1:12.5	991
	200	1:25.0	1,405
	300	1:37.5	1,316

During this time period, it was common practice to retain mature leaves on the host plants. Based on the results of these studies, a third study was conducted to compare the then practice of using 700 ACP adults per cage (77.8 ACP per plant) with 300 ACP adults per cage (33.3 ACP adults per plant) while retaining the mature foliage on plants. The number of *T. radiata* produced in each cage was counted as well as the number of plant tips that were flush or dead due to ACP feeding.

The use of 300 ACP adults per cage resulted in production of 1,544 *T. radiata* adults, on average, while the cages inoculated with 700 ACP adults produced an average of 1,230 wasps per cage. In a second round of experimentation, cages inoculated with 300 ACP adults produced an average of 978 *T. radiata* wasps, while the cages inoculated with 700 ACP adults produced 1,109 *T. radiata* wasps (Figure 1).

Cages inoculated with 300 adult ACP averaged 3.9 dead tips per cage, compared to 8.5 dead tips per cage in cages inoculated with 700 adult ACP. There was a significantly higher number of live flushing tips in the cages with 300 ACP adults (9.9 tips per cage) compared to the cages with 700 ACP adults (3.5 tips per cage) (Figure 2).

These results showed that 33 ACP adults per plant is adequate to inoculate cages. Using fewer ACP can save resources (number of ACP required, number of plants to be used for their production, collection time) and also may result in quicker recovery of the plants. The CDFA production program has adopted the use of introducing 300 adult ACP into the production process.

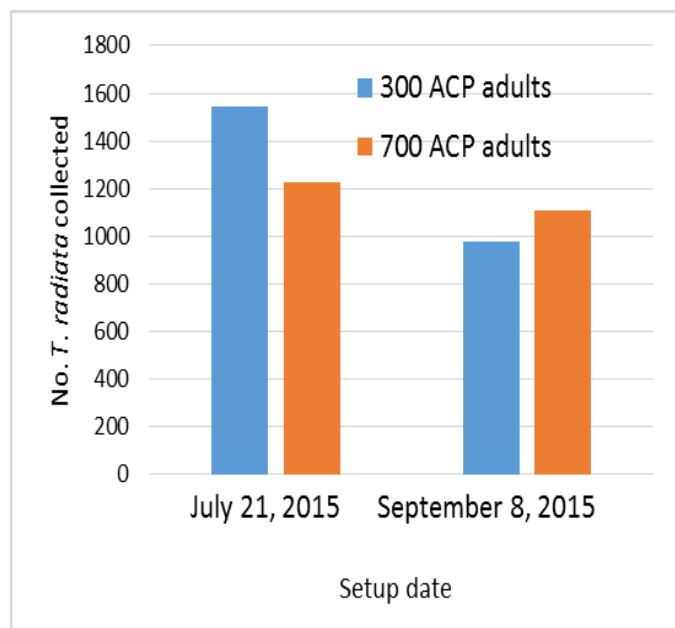


Figure 1. Effect of ACP inoculation rate on number of *T. radiata* produced.

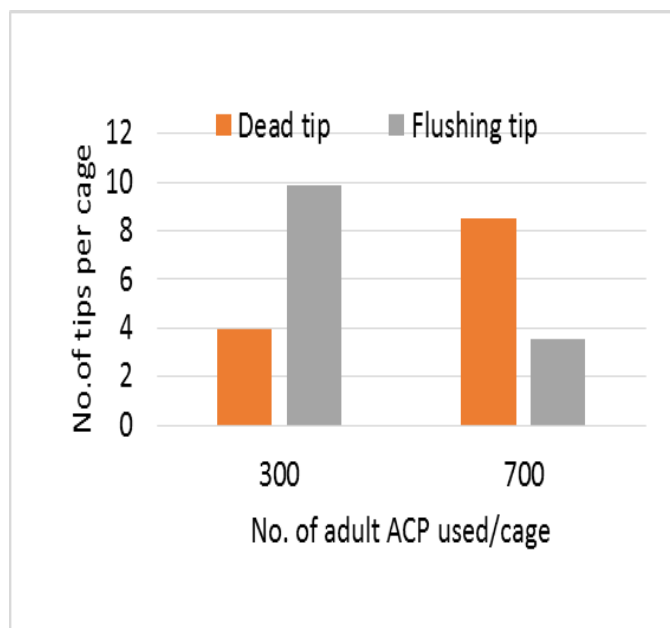


Figure 2. Effect of ACP inoculation rate on host plant tip mortality.

Appropriate inoculation timing for *Tamarixia radiata* production

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Tamarixia radiata is an ectoparasitoid of Asian citrus psyllid (ACP), *Diaphorina citri*. In a mass production system, it is important to inoculate *T. radiata* adults to ACP colonies at an appropriate time, as introducing them too early may result in high host mortality due to host feeding. Introducing *T. radiata* too late may lead to hosts escaping parasitism and emerging as ACP adults.

A study was conducted at the Foothill Agriculture Research Facility (Corona, California) in a temperature-controlled (27.8°C) room. Adult ACP were allowed to lay eggs on curry leaf plants for a seven day period; the plants were then covered with a sleeve cage. Following the initial ACP introduction, 10 *T. radiata* were added to each sleeve cage on either day 10, 11, 12 or 13. *Tamarixia radiata* and ACP adults produced in each sleeve cage were collected. The degree days (DD) accumulated by the ACP nymphs were calculated from temperature data collected by a HOBO device and using the lower temperature threshold of 10.5°C.

The ACP nymphs were mostly 3rd and 4th instars on day 10, and were found feeding on leaf midribs with a few migrating to the tip of the stem. On day 11, they were mainly 4th instars (with some earlier instar nymphs) and were found migrating further down on the stem. On day 12 and 13, most of the nymphs had developed into 4th and 5th instars and were migrating down on the main stem.

Cages inoculated with *T. radiata* 10 days after ACP inoculation produced only 92 wasps and 2 ACP adults, while those inoculated with *T. radiata* on day 11 produced 215 wasps — the highest number produced in this experiment (Table 1). Inoculations that occurred on day 12 or 13 produced fewer *T. radiata* wasps, more ACP adults, and had higher proportions of ACP adults that escaped parasitization. In conclusion, at 27.8°C (82°F), ACP cages should be inoculated with *T. radiata* on the 11th day, or after 186 DD of heat accumulation.

Table 1. Production of *T. radiata* wasps as affected by ACP nymph age (mean of 5 replications, $F_{3,11}=3.16$, and $p=0.005$).

Inoculation timing	Mean DD accumulation	<i>T. radiata</i> produced	ACP adults produced
10 days	169.4	92.4	2.0
11 days	186.1	215.0	33.4
12 days	202.8	68.5	50.2
13 days	219.4	47.0	49.0

Inoculation densities for *Tamarixia radiata* production

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A single *Tamarixia radiata* adult female can kill about 500 Asian citrus psyllid (ACP), *Diaphorina citri*, nymphs during its life span, approximately 80% by parasitization and the rest by host feeding. In the parasitoid rearing process, the ratio of host to parasite can affect the productivity and efficiency of the system. Too many wasps can result in high host feeding mortality leaving few nymphs for parasitization, whereas too few wasps may not be able to keep up with high ACP density. California Department of Food and Agriculture mass rearing program was initially using 200-300 wasps per cage, however we have sought to identify a standard inoculation density to optimize productivity and efficiency.

Three densities of *T. radiata* (100, 150 and 200 wasps per cage) were evaluated under greenhouse conditions. A total of 78 cages (26 cages per treatment) were tested on three different dates in 2015. There were 10 cages assigned to each treatment in September and 8 cages each at the October and November setups. Seven hundred ACP adults per cage (with nine plants) were used in the first study but only 300 ACP adults per cage (with nine plants) were used in the subsequent two studies. *Tamarixia radiata* wasps were introduced in each cage when ACP nymphs reached 3-4th instar. *Tamarixia radiata* adults were counted and collected daily once they began to emerge to determine the total number of *T. radiata* produced from each cage. The increase factor for *T. radiata* was calculated by dividing the total wasps produced by the number of wasps initially used.

The introduction of 100, 150, or 200 *T. radiata* per cage produced an average of 859, 853 and 626 *T. radiata* adults, respectively. The mean number of *T. radiata* produced was not significantly different among the three densities of *T. radiata* introduced. The increase factor of *T. radiata* was highest (8.68) when only 100 *T. radiata* wasps per cage were used and was significantly higher than the increase factor (3.26) when 200 wasps per cage were used. Using 150 wasps per cage resulted in an increase factor of 5.94, which was not significantly different from the increase factor for 100 or 200 wasps per cage. Results of the studies are summarized in Table 1. Following the findings of this study, 100 *T. radiata* wasp per cage has been adopted as the standard inoculation rate.

Table 1. Number of *Tamarixia radiata* produced with variable introduction rates.

<i>T. radiata</i> used	<i>T. radiata</i> produced	Increase factor
100	858.7	8.68a
150	853.1	5.94ab
200	626.0	3.26b
F-values	0.95	8.75
p-values	0.39	<0.001

Evaluation of six *Citrus* rootstock varieties as host plants in field cage insectary production of *Tamarixia radiata*

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One of the most important factors in mass-production of the Asian citrus psyllid (ACP), *Diaphorina citri*, parasitoid *Tamarixia radiata* is the selection of host plants. Host plants must be able to support large populations of ACP and also be durable enough to withstand pruning during the harvest of *T. radiata* pupae. Data from previous seasons have demonstrated that among *Citrus* scion varieties, grapefruit, lemon, and Persian lime trees are superior hosts for *T. radiata* field cage production, while sweet orange and Mexican lime trees tend to be poorer hosts. All scion varieties tested so far are able to withstand two consecutive harvests at most before they weaken. After this, the ACP population on the tree must be eliminated and the field cage removed and moved to a fresh host.

Unlike scion varieties, rootstocks are bred for rapid growth and resilience, giving them potential to be excellent hosts for *T. radiata* field cage insectaries. Evaluation of six varieties, planted in an experimental plot at Cal Poly Pomona in 2013, was started in 2016. The varieties tested are Carrizo citrange (*C. sinensis* x *Poncirus trifoliata*), trifoliolate (*Poncirus trifoliata*), rough lemon (*C. jambhiri*), volkameriana (*C. volkameriana*), sour orange (*C. x aurantium*), and macrophylla (*C. macrophylla*). Plots of six plants each, caged and pruned two at a time, were prepared for *T. radiata* production between March and October, 2016.

Plants were hedged down to approximately 6 feet in height and were hedged on the sides to make space between individual plants. After hedging, the height of each plant was measured, as well as the diameter at the widest points along the North-South and East-West axes. These measurements were used to estimate the volume of each plant's canopy, assuming an approximately cylindrical shape.

Cages were inoculated with 2,000 to 3,000 adult ACP approximately one week after pruning. When nymphs were present and the majority were 2nd and 3rd instars, 250 adult *T. radiata* were introduced to the cage. When the majority of nymphs reached 3rd and 4th instars, additional *T. radiata* were released to bring the total wasp to nymph inoculation ratio to 1:50. The number of nymphs present in a cage was estimated by calculating the average number of nymphs per leaf flush, then multiplying this average by the total number of ACP-infested flush points counted in the cage. The number of times plants in a cage were harvested, the dry mass of foliage collected at each harvest, the total number of *T. radiata* produced per cage, and the total increase factor per cage (*T. radiata* produced divided by *T. radiata* inoculated) are summarized in Table 1.

Table 1. Rootstock cage data from the 2016 field season. Mass of harvested flush is taken as dry mass after *T. radiata* emergence. Increase factor = TR produced/TR inoculated (* indicates only ACP-infested material harvested).

Variety	No. Harvests	TR used	Dry mass (g) of foliage collected at				Total TR Produced	TR Increase Factor
			Harvest 1	Harvest 2	Harvest 3	Harvest 4		
Carrizo citrange	2	1,030	1,334	2,136	--	--	18,286	17.75X
Carrizo citrange	1	250	412	--	--	--	342	1.37X
Trifoliolate	3	500	1,818	1,058	542	--	19,433	38.87X
Trifoliolate	0	0	--	--	--	--	0	NA
Sour orange	3	1,500	2,758	2,863	586	--	23,601	15.73X
Sour orange	1	500	2,758	--	--	--	14,169	28.34X
Rough lemon	1	500	1,207*	--	--	--	341	0.68X
Rough lemon	2	1,100	4,392	1,940	--	--	82,730	75.21X
Volkameriana	2	1,200	2,952	2,950	--	--	24,939	20.78X
Volkameriana	2	1,100	5,457	298*	--	--	30,652	61.30X
Volkameriana	2	1,100	2,311	1,302	--	--	16,038	14.58X
Macrophylla	4	1,000	1,612	2,323	2,020	1,752	61,820	61.82X
Macrophylla	1	500	2,596	--	--	--	32,690	65.38X

Trees in the sour orange, trifoliolate, and macrophylla cages were harvested three consecutive times. Harvest mass decreased drastically in the third harvests on sour orange and trifoliolate due to decreased flush production. Harvest mass did not decrease in the macrophylla cage after the first harvest. The first volkameriana cage produced sufficient flush for a third harvest, but the cage had become infested with green lacewing, which eliminated the ACP population. After initial pruning, Carrizo citrange plants produced significantly fewer flush points per cubic foot than sour orange, rough lemon, and volkameriana plants (Figure 1). No statistically significant difference in flush production was found in flush re-growth after the first harvest (Figure 2).

The ability to harvest host plants multiple times is an important step toward increasing field cage efficiency. Multiple harvests reduce the time spent on building an additional cage and pruning new trees, as the previous harvest will induce new flush production. Unparasitized ACP adults emerging from nymphs before a harvest are also sufficient to re-establish infestation without need of further inoculations. In many cages there will also be sufficient *T. radiata*, either adults that emerged before harvests or mummies that were missed, to make further *T. radiata* inoculations unnecessary. Thus far, macrophylla and rough lemon stand out for each having had one or more extraordinarily productive cages. Trifoliolate and Carrizo citrange, on the other hand, appear to produce less flush than the other varieties. Evaluation of rootstock varieties will continue in 2017.

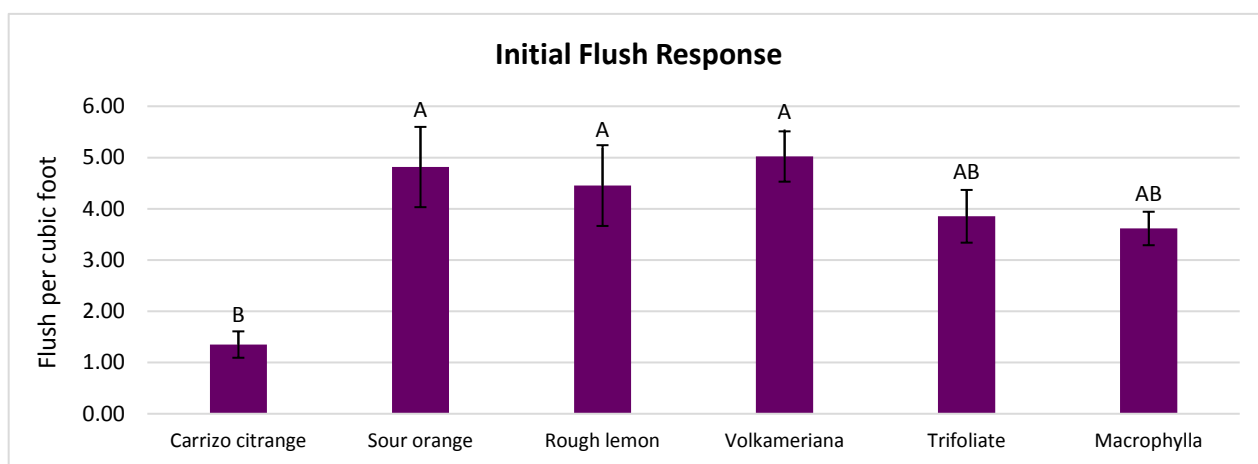


Figure 1. Average (\pm SE) new flush per cubic foot after initial pruning. Columns not sharing a letter are significantly different (Tukey test; $p < 0.05$).

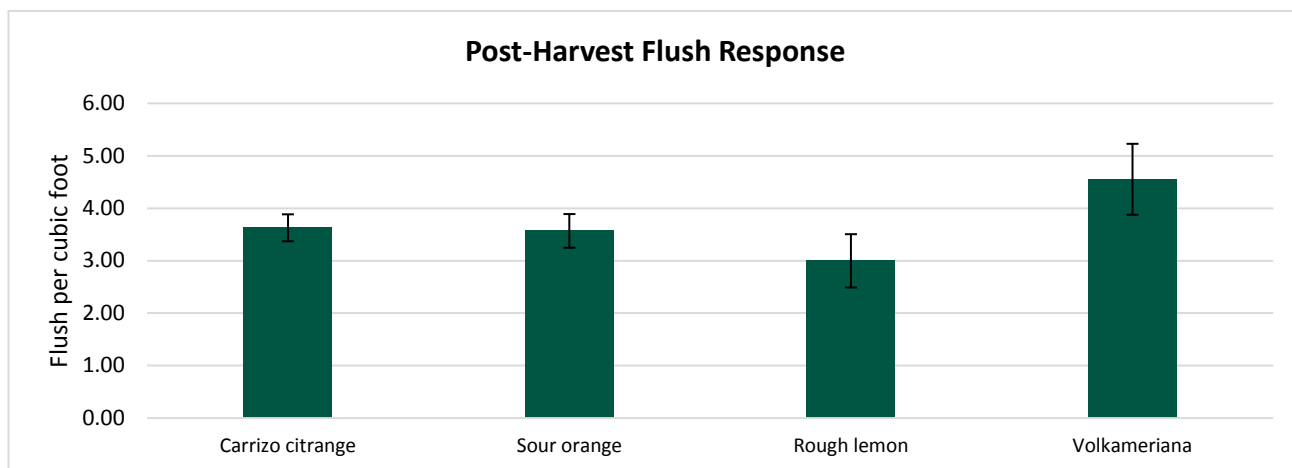


Figure 2. Average (\pm SE) flush per cubic foot after first *T. radiata* harvest. No significant differences were found (ANOVA, $p = 0.43$, $F = 0.94$).

Improving mass-production of *Tamarixia radiata* in field cage insectaries to contribute to biological control of Asian citrus psyllid in Southern California

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Efforts to mass-produce the Asian citrus psyllid (ACP), *Diaphorina citri*, parasitoid, *Tamarixia radiata*, in field cage insectaries began at the Citrus Research Board Laboratory (CRB) in Southern California in 2013. The goals of this project were twofold: to contribute *T. radiata* to Southern California biological control efforts, and to make field cage insectaries a viable and efficient means of mass-production for *T. radiata*. In 2016, record production of *T. radiata* was achieved, with a total of 605,493 wasps produced. Our group provided 592,921 of these *T. radiata* to the California Department of Food and Agriculture for release in Southern California and 14,166 to the biological control efforts in Yuma, Arizona. Smaller numbers of *T. radiata* were provided to University of California, Riverside researchers and used in inoculation of new field cage insectaries. Field cage-produced wasps accounted for more than 26% of total *T. radiata* production by the cooperative effort in 2016 (Table 1).

A total of 31 cages were harvested for *T. radiata* in the 2016 season. Eleven cages used single grapefruit trees in a commercial orchard near Mentone, CA as host plants for ACP and *T. radiata* rearing. The other 20 cages were located in an experimental *Citrus* plot on the Cal Poly Pomona campus. Of these, one used curry leaf plants as hosts, two used Mexican lime shrubs, one used a plot of three Eureka lemon trees, and 16 used six-plant plots of *Citrus* rootstock varieties. Total *T. radiata* production and cage efficiency, as measured by *T. radiata* increase factor (number of *T. radiata* produced divided by number of *T. radiata* inoculated), were higher in 2016 than all other years (Figure 1). These production figures, as well as other measures of production, are compared in Table 2. Although fewer cages were set up in 2016 than in 2015, greater *T. radiata* production was achieved.

With hardier rootstock varieties being used as host plants this season, more cages were harvested multiple times for *T. radiata* than in previous years, with some being harvested as many as four times. Field cages were also more efficient in 2016 than in previous years, in part due to improved *T. radiata* inoculation strategy. Cages not being used in inoculation experiments had *T. radiata* inoculations split into an early release on 2nd and 3rd instar nymphs and a second release on 3rd and 4th instar nymphs. This allows for parasitism of a greater proportion of ACP nymphs as they develop at varying rates in the field. The total number of *T. radiata* introduced to a cage was calculated for an overall wasp to nymph inoculation ratio of approximately 1:50, which analysis of past data suggests is an appropriate ratio. This season also had the largest number of exceptional *T. radiata* harvests seen so far, including the largest harvest to date of 64,542 *T. radiata* (Table 3).

The first burst of *T. radiata* production in April, 2016 came from cages set up in 2015 that were left up over winter with established populations of ACP inside. Leaf flush and ACP nymphs began to appear in these cages in March, and *T. radiata* were introduced at the appropriate stages. The first new cages of 2016 were set up in mid- to late March, but were not harvested until late May. Production did not exceed 10,000 *T. radiata* in any of these cages. The majority of *T. radiata* production took place between June and October, with the highest peak in production occurring in September. This roughly corresponds to the seasonal variation in temperature, with production being higher during periods of high temperature (Figure 2).

Table 1. Annual production of *T. radiata* in Southern California.

Produced by:	2013	2014	2015	2016	TOTAL
CRB lab	--	32,515	30,195	1,526	64,236
CRB field	119,142	207,941	480,702	605,493	1,294,136
FAR	--	137,524	265,961	147,850	551,335
UCR	161,057	296,881	165,445	523,015	985,341
CDFA	60,626	963,373	1,355,240	990,290	3,308,903
Total produced	340,825	1,638,234	2,297,543	2,268,174	6,203,951

Table 2. Comparison of *T. radiata* production by year.

Year	Number of cages	Number of harvests	Average TR per cage (\pm SE)	Maximum TR per cage	Average increase factor (\pm SE)	Maximum 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

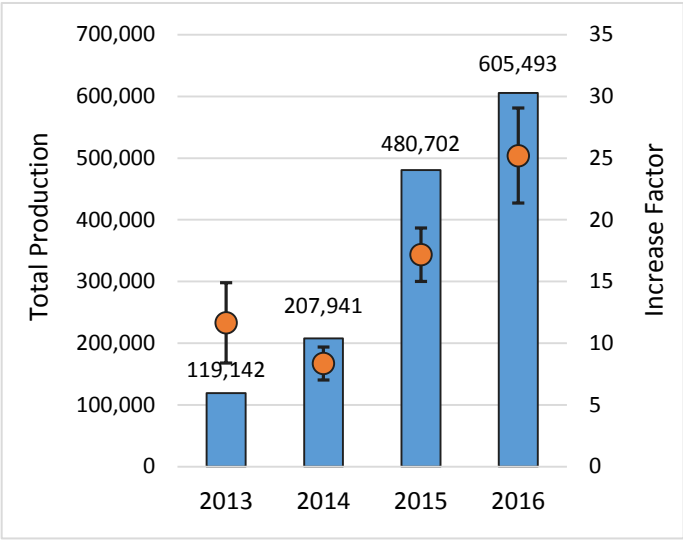


Table 3. The five largest harvests of 2016.

Harvest date	Host variety	Harvest number	TR produced
8/29/2016	Volkameriana	1	30,652
10/28/2016	Macrophylla	1	32,690
7/27/2016	Macrophylla	2	41,438
9/16/2016	Grapefruit	1	53,440
10/18/2016	Rough lemon	2	64,542

Figure 1. Total production (blue columns) and average (\pm SE) increase factor (orange circles) of *T. radiata* for each year.

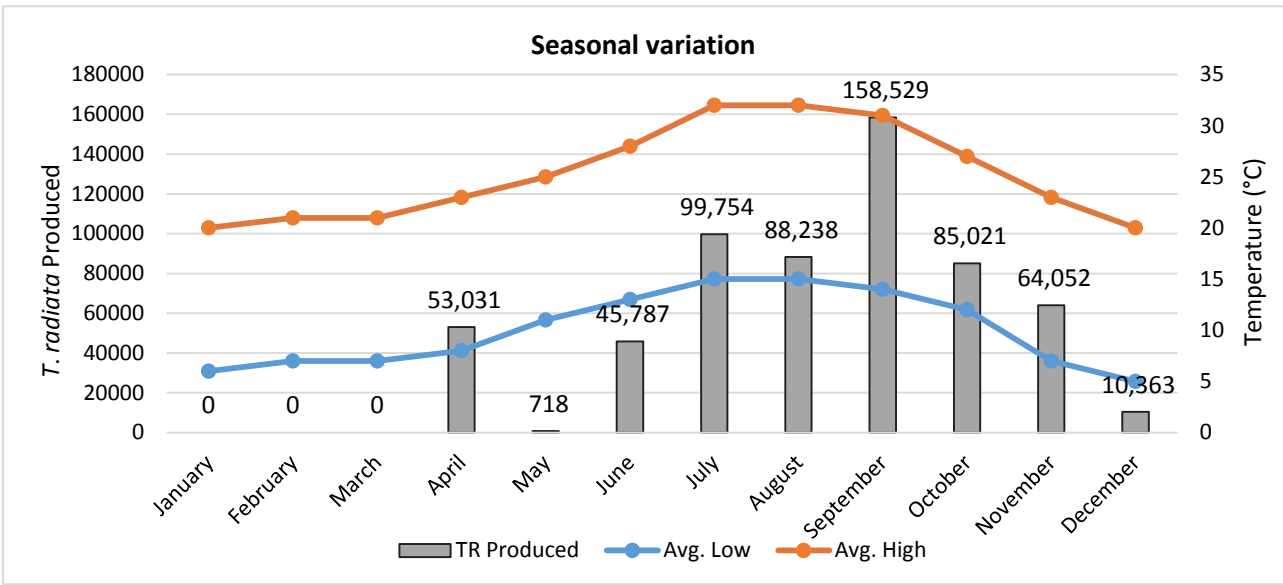


Figure 2. Columns: monthly production of *T. radiata* from field cages. Lines: average monthly high and low temperature in Pomona, CA. Temperature data from www.intellicast.com.

Investigation of three relative inoculation rates for establishing *Tamarixia radiata* in field cage insectariesRuth E. Henderson¹, Raju R. Pandey¹ and Gregory S. Simmons²¹Citrus Research Board, ACP Biocontrol Laboratory, Riverside, CA²Otis Laboratory Salinas Field Station, USDA-APHIS-PPQ-S&T-CPHST, Salinas, CA

The Citrus Research Board biological control group has been producing *Tamarixia radiata*, a parasitoid of Asian citrus psyllid (ACP), *Diaphorina citri*. These parasitoids are produced in field cage insectaries and have contributed to biological control releases since 2013. Continuous efforts are being made to maximize the efficiency of the field cage insectary rearing system. Increasing the level of parasitism by *T. radiata* could improve rearing efficiency. A key step to improving parasitism levels is finding the best *T. radiata* to ACP nymph ratio to ensure that there are enough parasitoids to attack the available nymphs, but not so many that competition between wasps leads to a loss in productivity. An analysis of production data from past seasons shows a relationship between the ratio of *T. radiata* to ACP nymphs at inoculation and the number of *T. radiata* produced from the subsequent harvest. While data from 2013 and 2014 suggested maximum *T. radiata* production from inoculations of approximately 1 wasp to 60 ACP nymphs, the addition of data from 2015 and 2016 shifted this maximum closer to 1 wasp to 50 nymphs (Figure 1).

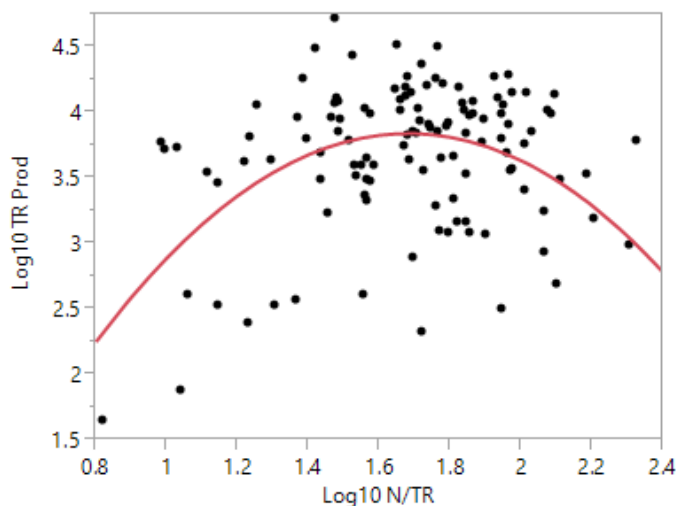


Figure 1. The relationship between *T. radiata* production and ACP nymphs per wasp at inoculation (both log base 10 transformed). The maximum of the curve lies at approximately 48 nymphs per *T. radiata* inoculated (adjusted $R^2=0.20$).

A relative *T. radiata* inoculation rate trial was started in 2015 and continued in the 2016 field season. In this trial, sets of cages on three grapefruit trees were set up simultaneously in a commercial orchard near Mentone, California. When the majority of ACP nymphs in each cage reached 3rd and 4th instars, the total number of ACP infested and un-infested leaf flush points on each tree were counted. Three flush points were randomly selected on each side (North, South, East, and West) of the tree and the number of nymphs on each of these flush points were counted. For each cage, the average number of nymphs per flush point was multiplied by the number of infested flush points on the tree to estimate the total number of nymphs present in the cage. Based on this number, each tree was assigned to one of three *T. radiata* inoculation rates:

- 1) Low rate: 1 *T. radiata* per 90 ACP nymphs
- 2) Medium rate: 1 *T. radiata* per 60 ACP nymphs
- 3) High rate: 1 *T. radiata* per 30 ACP nymphs

The total number of *T. radiata* produced and the *T. radiata* increase factor are shown in Figure 2. Increase factor is derived by dividing the *T. radiata* produced by the *T. radiata* inoculated. Additionally, estimated parasitism (*T. radiata* produced divided by estimated ACP nymphs) was also compared between treatments.

Three replications of this experiment were completed in 2015. Another three were completed in 2016. Statistical analysis did not uncover any significant difference between treatments in either the number of *T. radiata* produced or increase factor. However, general trends indicated that the average *T. radiata* increase factor is highest in the low inoculation rate treatment, but estimated parasitism and number of *T. radiata* produced are highest in the high inoculation rate. The medium inoculation rate is intermediate in both number of *T. radiata* produced and increase factor. This suggests that although the most number of *T. radiata* is produced when a high inoculation rate is used, a low inoculation rate strategy is more efficient in terms of the number of wasps that will be produced for each that is used.

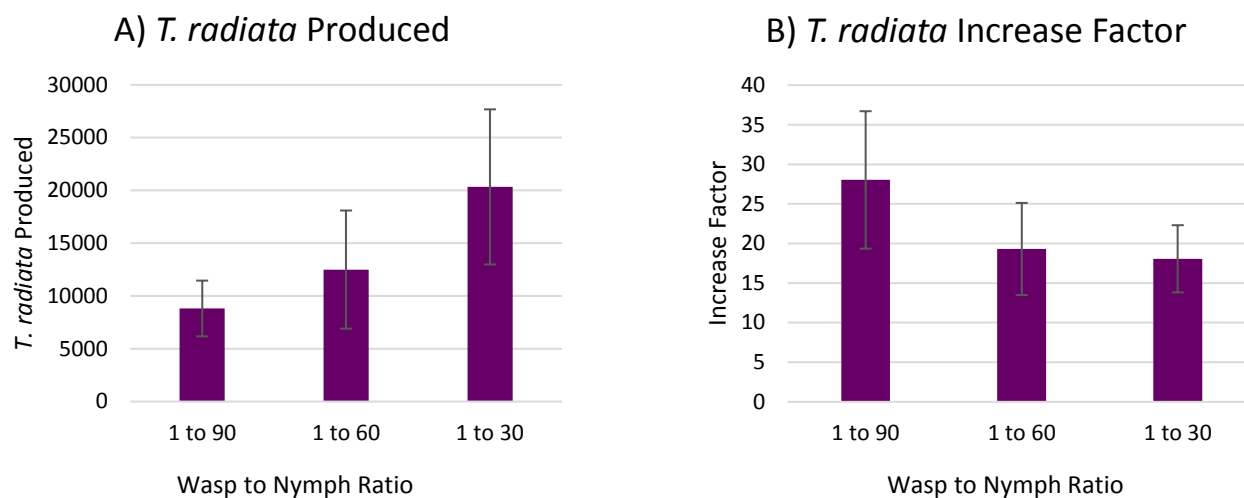


Figure 2. A) The average *T. radiata* produced. B) *T. radiata* increase factor. Error bars represent SE.

Another factor examined was relative parasitism, or the proportion of adult *T. radiata* emerging from harvested materials. Although this measure does not inform us of the actual percent parasitism in a cage, as many unparasitized ACP have already eclosed as adults before harvest, it can be used as a way to compare the level of parasitism between cages. In this study, relative parasitism was significantly higher in the high inoculation rate treatment than in the medium inoculation rate treatment. Relative parasitism in the low inoculation rate treatment was not statistically different from either the high or medium inoculation rate treatments (Figure 3). This experiment will be continued in 2017, focusing on only the medium and high inoculation rates.

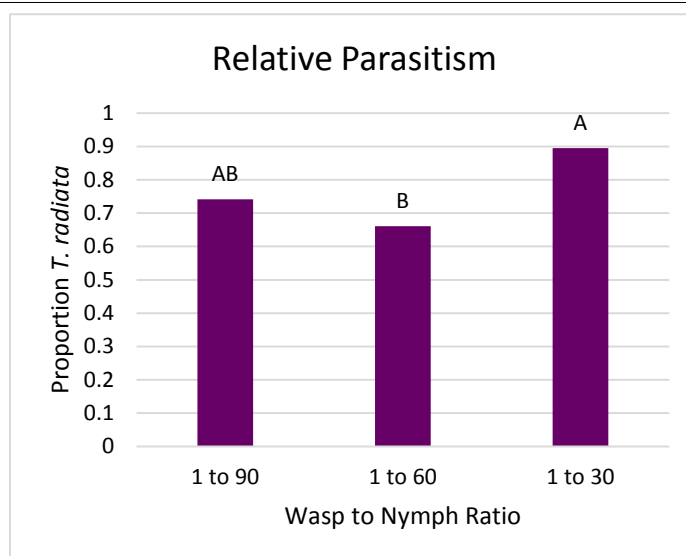


Figure 3. Relative parasitism, the proportion of *T. radiata* among adult insects emerging from harvested materials. Columns not sharing a letter are significantly different (Tukey test; $p < 0.05$).

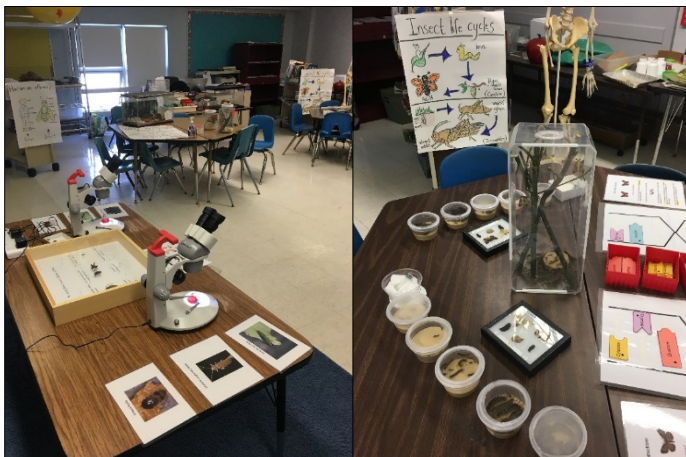
Outreach conducted by Otis Laboratory in 2016

Introduction

During the 2016 fiscal year Otis Laboratory staff organized and volunteered in numerous outreach activities including: giving presentations at local elementary schools, judging at science fairs, organizing Moth Night, and participating in a local PBS news show. In addition, Otis provided specimens and Riker displays for both outreach and training to USDA staff and state agricultural departments. The staff's passion for science and insects is demonstrated through their commitment to engage with the community.

Outreach to elementary schools

Otis Laboratory staff gave five presentations to local elementary schools and one presentation to a local homeschool group. Presentations centered on teaching the basics of entomology and informing how research at Otis helps safeguard U.S. agriculture and natural resources against invasive pests. Students examined pinned invasive beetles and live gypsy moth life stages reared at Otis, completed a DNA puzzle that raises awareness of the importance of molecular diagnostics, and learned about biological control and its role in the fight against emerald ash borer. With approximately 300 students participating in these outreach events, it served as an excellent opportunity to interact with the community and inspire the next generation of young scientists.



Invasive beetle display, gypsy moth life cycle display, and DNA puzzle at elementary school outreach event.

Outreach to Yale University

A scientist from the Otis Laboratory conducted a lecture and discussion session in a Forest Health and Sustainability class at the Yale School of Forestry and Environmental Studies. The session focused on the role of regulatory agencies in protecting U.S. forests from exotic, invasive pests and drew largely on the history and diversity of gypsy moth programs to provide examples.

Science fair judging

Otis Laboratory staff volunteered at three schools to judge science fair projects at both the local and regional level. Staff participation provided constructive feedback for the students and introduced them to the importance of scientific discourse.

PBS local affiliate WGBH visits Otis Laboratory

Christina Quinn, host of a local news magazine show, interviewed members of Otis Laboratory on current biological control efforts to manage the spread of emerald ash borer and Asian longhorned beetle (WGBH News). This interview allowed Otis staff to educate a wide audience on APHIS's mission to protect American agricultural and natural ecosystems from invasive plant pests.



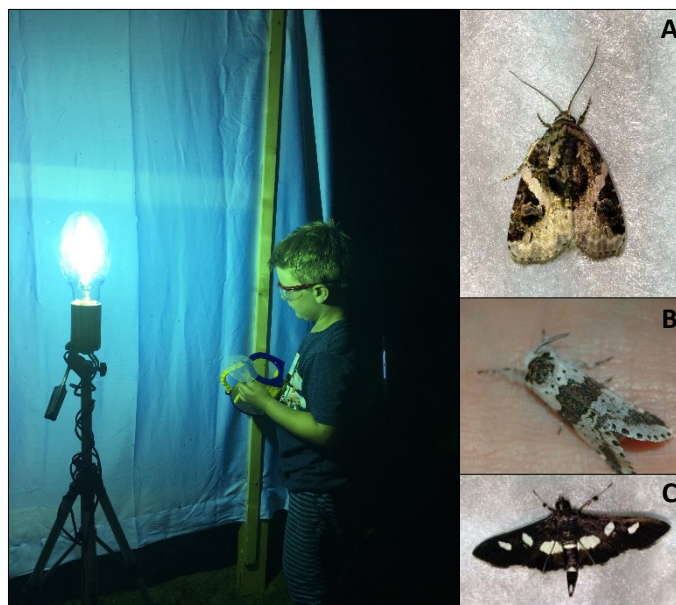
WGBH interviewing staff in Otis containment facility.

Moth Night

In honor of National Moth Week, a group of Otis Laboratory employees organized an evening of moth-gazing at Mass Audubon’s Long Pasture Wildlife Sanctuary in Barnstable, MA. Both professional and hobbyist entomologists and naturalists identified the local moth population as they appeared on an illuminated backdrop. The event was attended by about 20 people, including Otis staff, friends, family members, and sanctuary staff.

Invasive insect specimens for training and outreach

The Otis Laboratory Rearing functional group prepares pinned and unpinned adults, pupae, larvae preserved in alcohol, irradiated (killed) eggs, Riker displays, and autoclaved branches or slices of wood bearing insect galleries. The specimens are primarily provided to federal and state identification, detection, and interception programs. A summary of the specimens prepared and distributed in 2016 is presented in Table 1.



Moth Night illuminated backdrop with observed native moths:
 A) pink-barred pseudostrota, *Pseudeustrotia carneola*
 B) white furcula, *Furcula borealis* C) grape leaf roller, *Desmia maculalis*.


Table 1. Specimens provided for training and outreach in 2016.

Recipient	Purpose	Species	Life stage				
			Eggs/ masses	Larvae	Pupae	Dead adults	Riker display
SPHD, Georgia	Training & outreach	European gypsy moth	-	-	-	-	10
Washington State Department of Agriculture	Training	European gypsy moth	-	-	-	213	-
US Customs Agency Germany	Training & outreach	European gypsy moth	-	-	-	-	3
Oregon Department Of Agriculture	Training	European gypsy moth	13	-	-	263	-
USDA APHIS PPQ Portland, OR	Training	European gypsy moth	20	-	-	-	-
USDA APHIS PPQ Champaign, IL	Training & outreach	European gypsy moth	10	-	-	-	-
CA Dept. of Food & Agriculture	Training	European grapevine moth	-	-	-	200	-
Multiple Organizations	Training & outreach	Asian longhorned beetle	320	340	80	505	-

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







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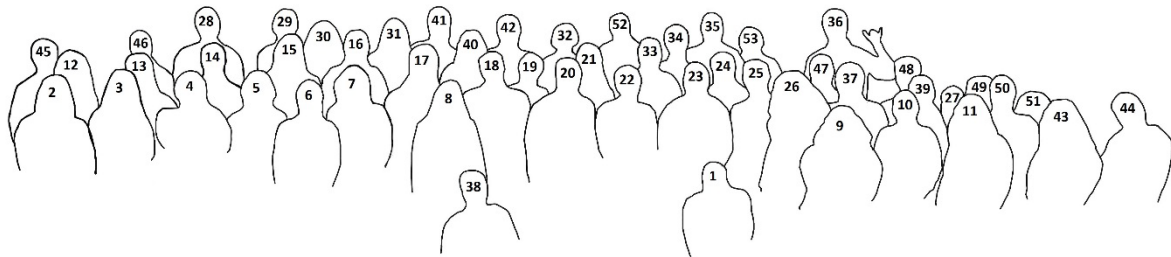
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2. Mauri Barrett
3. Nevada Trepanowski
4. Isaiah Canlas
5. Natalie Leva
6. Kendra Vieira
7. Sindhu Krishnankutty
8. Julia Mackay
9. Charyl Calfina
10. Alyssa Pierce
11. Lenora Macnevin
12. Carrie Crook
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14. Everett Booth
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16. Tanya Dockray
17. Ty Cummins
18. Phil Lewis
19. Hannah Landers

20. Bill Panagakos
21. Chris McCallum
22. John Molonogoski
23. Dave Cowan
24. Ron Mack
25. Scott Myers
26. Miriam Cooperband
27. Carl Wilcox
28. Mike Salhany
29. Melissa Warden
30. Emily Franzen
31. Juli Gould
32. Hannah Nadel
33. Scott Pfister
34. Yunke Wu
35. Joe Francese
36. Damon Crook
37. Greg Simmons

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38. Andrew Luxon
39. Mike Garrity
40. Breanne Aflague
41. Shane McCallister
42. Dave Lance

Visitors at Forest Pest Meeting

43. Robyn Rose
44. Josie Ryan
45. Ben Slager
46. Joe Beckwith
47. John Crowe
48. Russ Bullock
49. Greg Parra
50. Dustin Grant
51. Deb McPartland
52. Ron Weeks
53. Paul Chaloux

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- Sue Lane
- Dave Mills
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