USDA Animal and Plant Health Inspection Service
U.S. DEPARTMENT OF AGRICULTURE

Forest Pest Methods Laboratory

2023 Accomplishment Report

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Buzzards Bay MA

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Plant Protection and Quarantine

Science and Technology

This report was edited by: Amanda Davila-Flores, Everett Booth, and Angela Hoover
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Publications

*FPML employees and cooperators are indicated in bold

1. **Broadley HJ**, **Sipolski SJ**, Pitt DB, Hoelmer KA, Wang X-y, Cao L-m, et al. Assessing the host range of *Anastatus orientalis*, an egg parasitoid of spotted lanternfly (*Lycorma delicatula*) using Eastern U.S. non-target species. Front Insect Sci. 2023;3.

2. **Cooperband MF**, Wickham JD, **Warden ML**. Factors guiding the orientation of nymphal spotted lanternfly, *Lycorma delicatula*. Insects. 2023;14(3).

3. Dias VS, Hallman GJ, Araújo AS, Lima IVG, Galvão-Silva FL, Caravantes LA, et al. High cold tolerance and differential population response of third instars from the *Zeugodacus tau* complex to phytosanitary cold treatment in navel oranges. Postharvest Biol Technol. 2023;203.

4. **Domingue MJ**, **Wu Y**, **Vieira KA**, **McGraw AR**, **Furtado M**, Athanassiou CG, et al. Direct competition and potential displacement involving managed *Trogoderma* stored product pests. Sci Rep. 2023;13(1):3656.

5. **Faal H, Canlas IJ, Cosse A**, Jones TH, Carrillo D, **Cooperband MF**. Investigating photo-degradation as a potential pheromone production pathway in spotted lanternfly, *Lycorma delicatula*. Insects. 2023;14(6).

6. **Hickin M**, **Nadel H**, Carlson J, Conway H. Effects of adult-diet modifications on sexual maturation rate, body weight, and quantity of sperm transfer by male Mexican fruit fly (Diptera: Tephritidae). J Econ Entomol. 2023;116(4):1110-8.

7. Liu H, Wang X, **Cooperband MF**. Editorial: Focus on spotted lanternfly. Front Insect Sci. 2023;3:1292590.

8. Morris TD, **Gould JR**, Fierke MK. Establishment and impacts of emerald ash borer (Coleoptera: Buprestidae) parasitoids released at early- and post-invasion sites. J Econ Entomol. 2023;116(4):1155-64.

9. Noriyuki S, Matsumoto I, Yoshikawa T, Kaneko S, **Broadley HJ**. Bird predation on Roseau cane scale as revealed by a web image search and querying a citizen monitoring database. Ecosphere. 2023;14(6).

10. Roques A, Ren L, Rassati D, Shi J, Akulov E, Audsley N, et al. Worldwide tests of generic attractants, a promising tool for early detection of non-native cerambycid species. NeoBiota. 2023;84:169-209.

11. Siderhurst MS, **Murman KM**, Kaye KT, Wallace MS, **Cooperband MF**. Radio telemetry and harmonic radar tracking of the spotted lanternfly, *Lycorma delicatula* (White) (Hemiptera: Fulgoridae). Insects. 2023;15(1).

12. **Wu Y**, **Broadley HJ**, **Vieira KA**, **McCormack JJ**, **Losch CA**, Namgung H, et al. Cryptic genetic diversity and associated ecological differences of *Anastatus orientalis*, an egg parasitoid of the spotted lanternfly. Front Insect Sci. 2023;3.

13. **Wu Y**, **Domingue MJ**, **McGraw AR**, **Vieira KA**, **Palmeri MZ**, **Myers SW**. Development of an array of molecular tools for the identification of khapra beetle (*Trogoderma granarium*), a destructive beetle of stored food products. Sci Rep. 2023;13(1):3327.

Rearing and life history evaluations of *Dryinus sinicus*, a promising biological control agent of spotted lanternfly

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The USDA APHIS Spotted Lanternfly 5-Year Strategy highlights the necessity for the development of effective and innovative biological control techniques to limit the advancement of spotted lanternfly (SLF). To this end, the Forest Pest Methods Laboratory (FPML) is developing and optimizing methods for rearing and has evaluated the life history and host range of a promising biological control agent, *Dryinus sinicus* (Hymenoptera: Dryinidae). *Dryinus sinicus* is a univoltine nymphal parasitoid of spotted lanternfly that is native to China [1]. To establish a colony of *D. sinicus*, a multi-faceted rearing strategy has been developed in which host plants (*Ailanthus altissima*) are propagated from seeds, SLF nymphs are reared from field collected egg masses to 2nd or 3rd instar nymphs to serve as hosts, and parasitoids (*Dryinus sinicus)* are mated and reared through their entire life cycle concurrently.

Combined work at FPML optimizing the storage of SLF egg masses and improving *D. sinicus* cocoon storage and hatch, has resulted in the extension of the *D. sinicus* rearing period from one generation a year (adults emerging only in early summer) to three generations reared from February to November. Over the last three years, the production of the FPML colony increased from 95 male cocoons in 2021, to 775 cocoons including the first female progeny in 2022, to 2,125 cocoons including 666 females in 2023. Mating of *D. sinicus* was achieved by confining males and females together in small rearing cups in natural lighting prior to oviposition. We found that females can live for up to six weeks and the shorter-lived males generally live for less than two weeks. The *D. sinicus* colony is approximately 30% female when using the current rearing method exposing wasps to many nymphs at once in cages; however, it is possible to increase this to roughly 70% when wasps are introduced to a SLF nymph individually in an arena. Additionally, we observed that cocoon length can be used as an effective metric for predicting the sex of *D. sinicus*, with females generally having a cocoon length of over 7mm and males measuring under 7mm.

The improved colony has allowed for the following advancements: the investigation of optimized rearing strategies for *D. sinicus*, the ability to run studies with *D. sinicus* from February to November rather than just in June, the establishment of a secondary colony with USDA ARS, and the ability to start host range specificity testing.

References:

1. Xin, B, Zhang, Y, Wang, X, Cao, L, Hoelmer, KA, Broadley, HJ and Gould, JR. Exploratory survey of spotted lanternfly (Hemiptera: Fulgoridae) and its natural enemies in China. Environ Entomol. 2021; 50(1) 36–45.

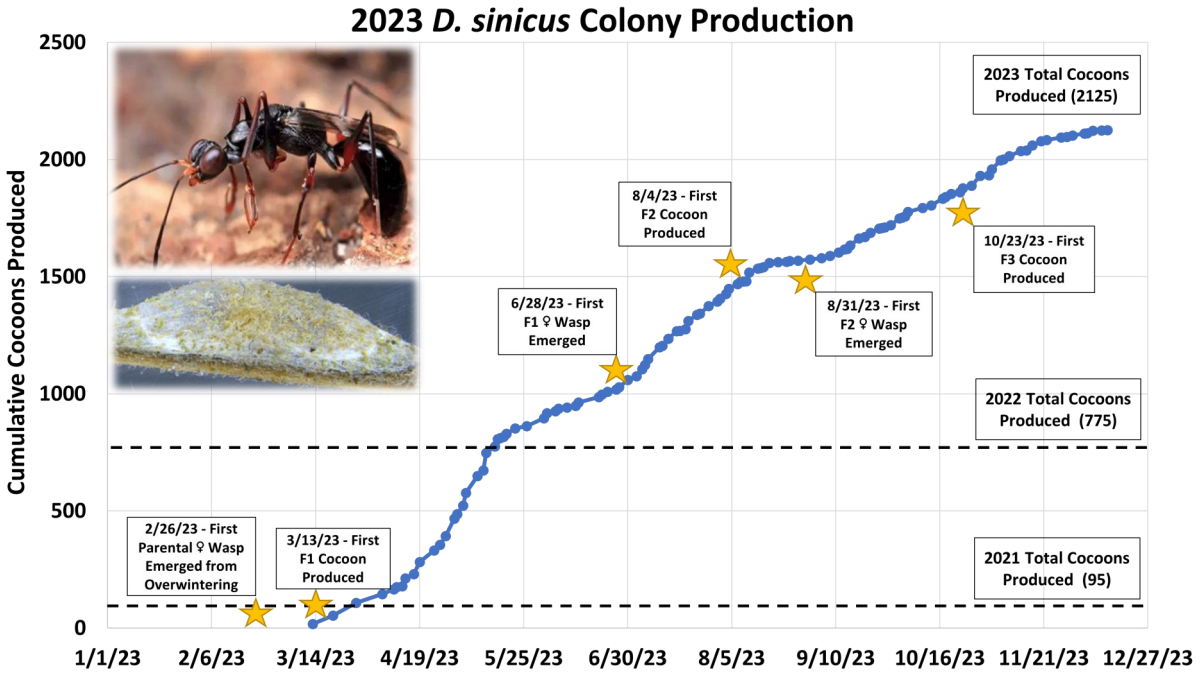

Figure 1. The cumulative number of cocoons produced in our *D. sinicus* colony throughout the 2023 rearing season with markers indicating 2021 and 2022 cocoon totals for comparison. Dates when subsequent generations of *D. sinicus* (cocoons and adults) were reared are also displayed on this figure.

Host range testing of *Anastatus orientalis* Haplotypes B, C, and D shows a lack of specificity

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Anastatus orientalis is an egg parasitoid of the spotted lanternfly, SLF, *Lycorma delicatula* from the native range of SLF. It was used as a biological control agent for invasive SLF in South Korea and we have been testing it as a possible classical biological control agent for managing invasive SLF in the United States. Molecular analysis has separated *A. orientalis* into six haplotype groups (A-F) [1] and we have been conducting host range testing with the three dominant haplotypes—B, C, and D. Previous work showed that *A. orientalis* Haplotype C attacked eggs of non-target insects, including species from the families Coreidae, Fulgoridae, Pentatomidae, and Saturniidae [2,3]. Haplotypes B and D are both genetically and biologically distinct from Haplotype C, exhibiting significantly different diapause behaviors when reared under different conditions [1]. Host range testing was conducted on Haplotypes B and D to evaluate their specificity for SLF and viability as classical biocontrol agents.

Haplotypes B and D were tested on eleven non-target species previously shown to be attacked by Haplotype C, spanning two orders and four families. Haplotypes B and D attacked all non-target species tested in no-choice testing. Haplotype B, C, and D wasps all attacked silk moths, *Actias luna* and *Antheraea polyphemus*, the brown marmorated stink bug, *Halyomorpha halys*, and the native planthopper, *Poblicia fuliginosa* at a relatively high rate (Figure 1). In choice testing, Haplotype D attacked seven of eight non-target species tested, and Haplotype B attacked seven of nine species tested. In addition, Haplotype D produced the highest proportion of female progeny in SLF and silk moth eggs, the largest eggs included in the study, a trend that had previously been identified in Haplotype C testing [2]. Significantly fewer progeny were produced from the non-target egg masses than from SLF egg masses run simultaneously in both the no-choice and choice tests for all three haplotypes, suggesting *A. orientalis* prefers to parasitize SLF over non-target species but will use non-targets when SLF is not available.

These results show that the genetic and biological differences between Haplotypes B, C, and D do not translate into significant differences in host specificity because all three haplotypes attacked non-target species at a similar rate. Work on *A. orientalis* has concluded at the Forest Pest Methods Lab. Field studies in China and behavioral studies at the USDA ARS laboratory in Newark DE will be completed in 2024.

References:

1. Wu Y, Broadley HJ, Vieira KA, McCormack JJ, Losch CA, Namgung H, Kim Y, Kim H, McGraw AR, Palmeri MZ, Lee S. Cryptic genetic diversity and associated ecological differences of *Anastatus orientalis*, an egg parasitoid of the spotted lanternfly. Front. Insect Sci. 2023; Jun 2;3:1154651.

2. Broadley HJ, Sipolski SJ, Pitt DB, Hoelmer KA, Wang XY, Cao LM, Tewksbury LA, Hagerty TJ, Bartlett CR, Russell AD, Wu Y. Assessing the host range of *Anastatus orientalis*, an egg parasitoid of spotted lanternfly (*Lycorma delicatula*) using Eastern US non-target species. Front. Insect Sci. 2023; Apr 18;3:1154697.

3. Gómez Marco F, Yanega D, Ruiz M, Hoddle MS. Proactive classical biological control of *Lycorma delicatula* (Hemiptera: Fulgoridae) in California (US): Host range testing of *Anastatus orientalis* (Hymenoptera: Eupelmidae). Front. Insect Sci. 2023; Feb 20;3:1134889.

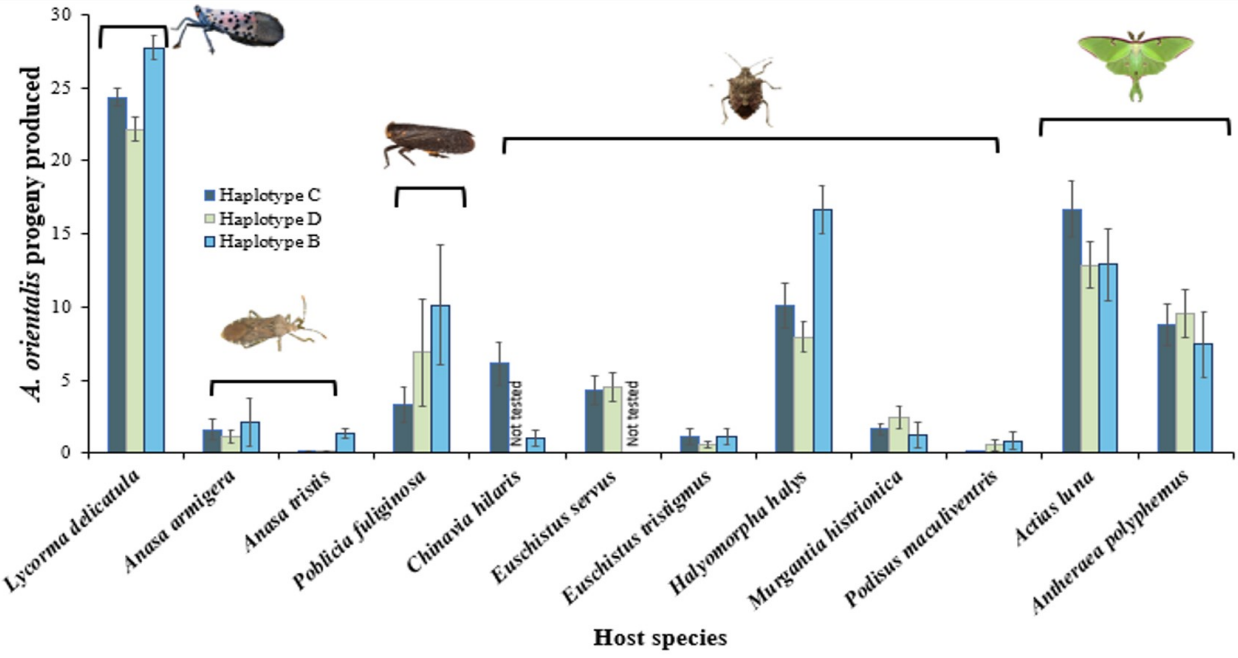

Figure 1. The mean (±SE) number of progeny produced by *A. orientalis* Haplotypes B, C, and D in SLF controls and non-target species during no-choice testing. In this figure, the haplotypes are listed in the order they were tested—C, D, and then B.

Optimization of polyphagous shot hole borer, *Euwallacea fornicatus,* rearing in wood bolts

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A laboratory colony of polyphagous shot hole borer, PSHB, *Euwallacea fornicatus* is maintained at the Forest Pest Methods Laboratory (FPML) to support classical biological control efforts in collaboration with the University of California, Riverside. This colony is easily reared and maintained on sawdust-based artificial media, yet this unnatural rearing setup is not ideal in facilitating interactions with parasitoids in classical biocontrol experimentation. A rearing system needed to be developed that mimics natural conditions while also supporting a robust PSHB population. Prior work utilizing live bolts was successful in filling these needs but was only previously tested on red maple, *Acer rubrum*. In an effort to further maximize PSHB production, we explored host suitability using a variety of hardwood species.

Using our wood bolt rearing method developed on red maple, we performed an experiment to compare PSHB colonization success and reproductive capacity in five different hosts: red maple, striped maple, *Acer pensylvanicum*, American beech, *Fagus grandifolia*, black birch, *Betula lenta*, and red oak, *Quercus rubra*. Ten replicates were performed for each tree species, and each bolt received 20 adult female PSHB.

The number of active galleries was counted after 24 and 48 hrs, and thereafter once per week for eight weeks (covering approximately two PSHB generations). We found that the average number of active galleries significantly differed between the five hosts after eight weeks (Figure 1). Average active gallery counts between the five hosts after four weeks was only marginally significant, suggesting that host tree species does not have a strong effect on initial colonization success, but strongly affects PSHB survival and reproductive ability over time. Of the five hosts we tested, American beech produced the highest number of PSHB and active galleries after two generations, followed by red maple and striped maple (Figure 1).

Based on these results we are incorporating American beech into wood bolt production of PSHB and its natural enemies. These results allow us to maximize rearing of natural enemies of PSHB on wood bolts. Optimizing PSHB and parasitoid production on wood bolts not only provides us with sufficient colony numbers for experiments, but also establishes an arena and methodology for performing host specificity testing of PSHB natural enemies.

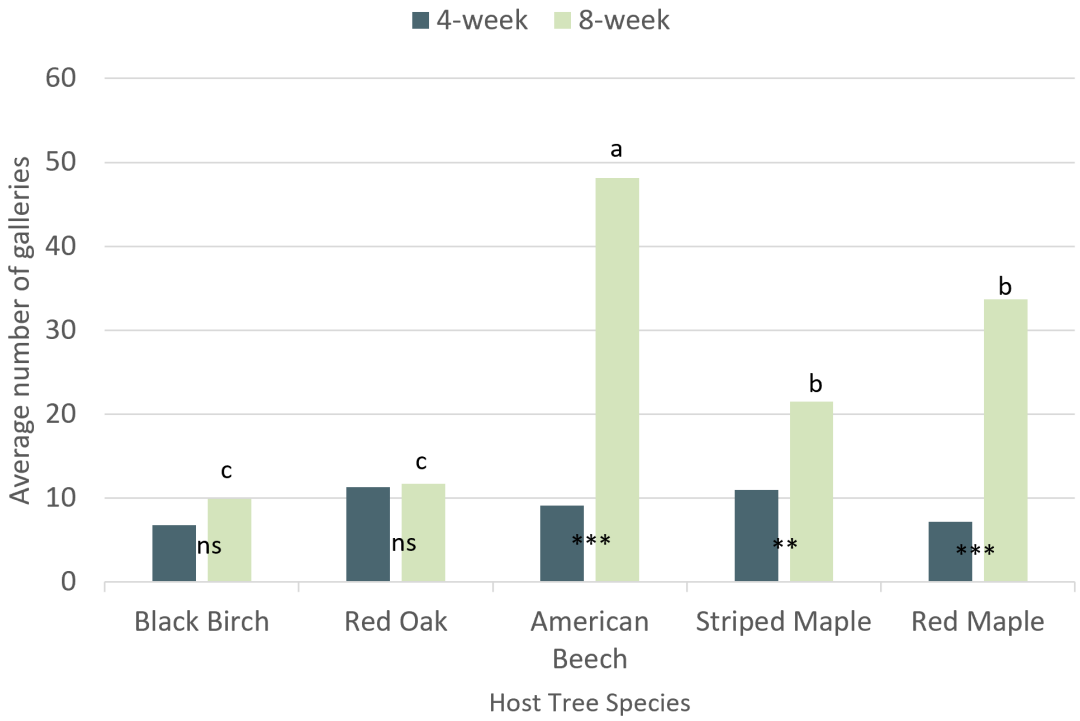

Figure 1. Average active gallery counts at four- and eight-weeks post-colonization for each host species tested. Asterisks denote significance between timepoints within host treatments; letters denote significance between final active gallery counts between host treatments.

Development of rearing methods for *Phymastichus* sp., a parasitoid of polyphagous shot hole borer, *Euwallacea fornicatus*

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Establishing a classical biological control program for polyphagous shot hole borer, PSHB, *Euwallacea fornicatus* has proven challenging due to the difficulty of collecting and rearing its parasitoids. An undescribed species in the genus *Phymastichus* (hereafter referred to as *Phymastichus* sp.) (Hymenoptera: Eulophidae) is the priority parasitoid species associated with PSHB due to its prevalence in its native range, its short and simple life cycle and reproduction, and its presumed high host specificity based on other species in the genus. *Phymastichus* sp. is an adult endoparasitoid that attacks PSHB as they are walking on or boring galleries into a host tree. PSHB that are parasitized by *Phymastichus* sp. do not get the chance to reproduce, as they become immobilized by the parasitoid before they can construct a gallery and lay eggs. By developing and further advancing a rearing method for *Phymastichus* we would be working towards creating the foundations needed for a successful PSHB biological control program.

In February 2023, we received a shipment of PSHB-infested wood from Taiwan that contained *Phymastichus* sp. Wood bolts infested with PSHB from our laboratory colony were used to rear subsequent generations of *Phymastichus* sp. When introducing parasitoids to wood bolts, 20 additional PSHB were also added to the bolts to provide fresh host material. While this method continued to produce new generations of *Phymastichus* sp., it was not sufficient to establish a stable laboratory colony. Around the time that F7 *Phymastichus* sp. were emerging, we modified our method to incorporate multiple additions of 10-20 PSHB to the bolt over the course of several hours. While labor- and time-intensive, this method increased the number of hosts that the *Phymastichus* sp. were able to utilize and resulted in positive colony growth for the first time since F2. Since then, we have been steadily growing our *Phymastichus* sp. colony, which has now achieved record numbers (Figure 1) and saw F12 emerging at the time of writing this report. For the first time, this colony is self-sufficient and no longer requires supplementation of parasitoids from the field. Our focus is now shifting toward space-saving efforts to miniaturize the rearing set-up and maximize *Phymastichus* sp. production.

This development is vital to the advancement of the biological control program for PSHB. It not only provides high numbers of *Phymastichus* sp. for basic biology studies, but also allows us to begin host specificity testing for this species, a crucial component of any biological control program and one that until now has been hindered by parasitoid availability. It also represents a significant milestone in ambrosia beetle biological control research, since this is the first report of a hymenopteran parasitoid of an ambrosia beetle being established in a laboratory colony.

Figure 1. *Phymastichus* sp. colony emergence numbers over the course of 11 generations in the laboratory. Rearing methods were modified during F7 emergence.

Integrating insecticides and biological control to manage Emerald Ash Borer in urban forests

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Ash trees (*Fraxinus* spp.) are valuable street trees commonly planted in urban environments in the 1990s prior to arrival of emerald ash borer, EAB, *Agrilus planipennis*. Insecticide treatments can provide consistent control of EAB and are used to protect high-value trees; however, treatments are expensive, and control only lasts a few years between reapplications. Biological control of EAB seeks to provide long-term control, thus, several parasitoids have been released, including the larval parasitoids *Tetrastichus planipennisi* and *Spathius galinae*. In 2015, we released parasitoids in three cities (Syracuse, New York, Naperville, Illinois, and Boulder, Colorado) while the cities were simultaneously treating high-value street trees with insecticides. We hypothesized parasitoids would be able to establish on EAB in untreated trees and spread throughout the cities while treated trees remained healthy. We also hypothesized that as available ash was depleted and parasitism increased, EAB densities would fall and that biocontrol agent populations would prevent EAB population rebound as insecticide treatments were halted.

Three-hundred trees were selected in each city for monitoring: 100 untreated (UT), 100 treated (T), and 100 trees from which treatment was removed in 2018 (RT) once EAB densities declined to $\leq$ 10 larvae/m^2 (Figure 1). Untreated trees rapidly declined as expected. *Tetrastichus planipennisi* successfully established in all three cities, while *S. galinae* only established in Syracuse and Boulder. Parasitoids spread throughout each city, and the numbers recovered in yellow pan traps and sentinel logs generally increased over time. After treatment was removed, T trees maintained a healthy crown while minor decline was present in some RT trees. Despite this shift, most RT trees remained un-infested (Figure 1) and retained healthy canopies, while infested trees exhibited high apparent parasitism of EAB and low EAB densities.

Our study shows that the use of insecticides to protect mature trees during the initial outbreak of EAB is compatible with the release of EAB parasitoids. We have evidence that these parasitoids can suppress EAB densities, often below or close to the expected damage threshold of EAB. However, some trees from which insecticide treatments were removed showed a minor but significant reduction in crown class, possibly because the threshold tolerance for EAB density is lower in trees that are subject to other urban stressors such as construction, road salt, or compacted soils. We recommend that as treatments are removed, arborists should closely monitor tree health and re-treat threatened trees. Even with some re-treatment, we anticipate fewer trees needing treatment and the treatment intervals being extended, saving time and resources in battling EAB in urban forests.

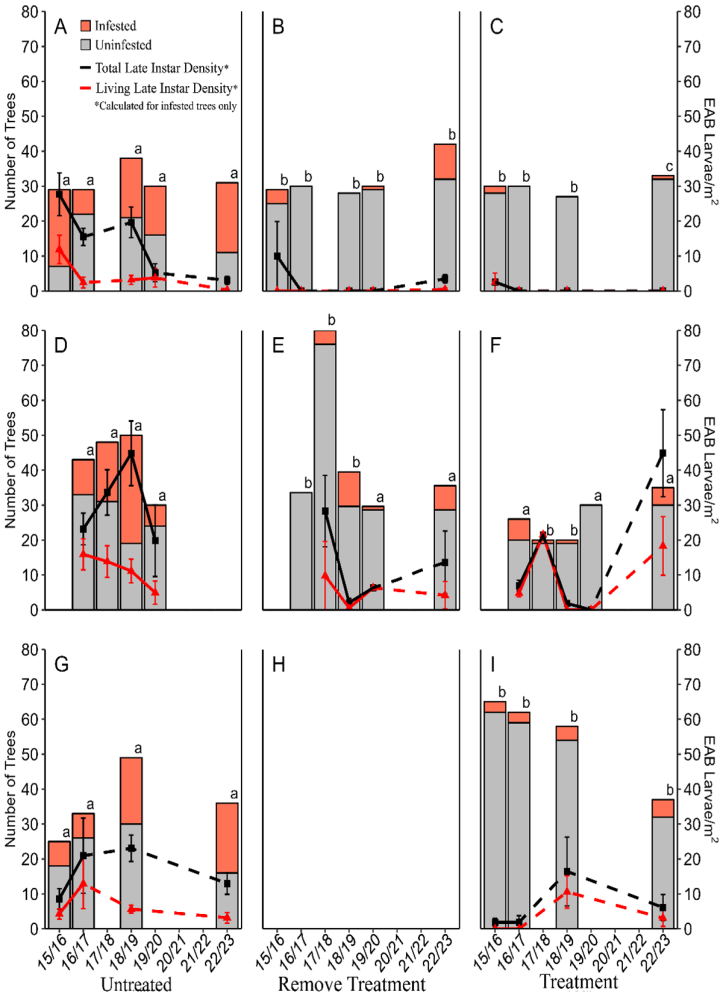

Figure 1. Results of branch sampling in Syracuse (A–C), Naperville (D–F), and Boulder (G–I). Bars indicate number of trees sampled in a given year, grey portions of bars are trees not infested with EAB, orange portions were infested trees. Significant differences among treatments within each sampling year are indicated by letters above bars, e.g., in Syracuse, there were significantly more UT trees (all "a"s) infested with EAB than RT and T trees every year sampled – and only in 22/23 were there differences among all three treatments. Lines are associated with the secondary axis and indicate mean EAB density (per m2) (± SE) within infested trees only, with the black line being total late instar EAB density, and the red line the density of living late instar EAB.

Radiofrequency development for solid wood packaging material (SWPM) treatment

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Radio frequency (RF) heating, is a process in which electromagnetic waves are generated to produce heat. The adoption of the radio frequency treatment standard into ISPM-15 in 2013 required a thorough operational understanding of commercial scale application before the technology could be successfully implemented for SWPM. Researchers from APHIS-PPQ, Penn State, and private industry partner RF Kiln Tech Limited assembled a team under a cooperative research and development agreement that designed and placed a 50 KW RF heat treatment system at Penn State. Testing was conducted on bulk pre-pallet components (stringers, decking boards, blocking) to insure proper treatment to the 60°C/1 min dielectric standard. This work supports both pest exclusion and methyl bromide alternatives as outlined by APHIS-PPQ.

A test matrix was developed that considered varied pallet components, wood species, and moisture content. Heating uniformity at the 60°C for 1 min hold was established throughout the bulk stack through a number of operational enhancements that included solid state power supply upgrade, electrode design modification, and insulation placement. An industry friendly temperature monitoring system was developed that leveraged exterior wood stack temperatures due to volumetric heating.

This RF heating research will lead to a treatment certification in North America through the American Lumber Standards Committee and the Canadian Lumber Standards Accreditation Board for use on pallet components. This technology will ultimately be placed in commercial use in other countries as an ISPM-15 treatment option.

Figure 1. Removal of a bulk test pack of radiofrequency (RF) heated pallet components from the treatment cylinder. Aluminum electrodes are sandwiched between each grouping of wood, and temperature monitoring leads are placed at selected locations within the stack.

Vacuum steam heat treatment for log export

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Methyl bromide treatment on logs for export has been one of the most utilized phytosanitary applications in the USDA Treatment Manual. APHIS PPQ partnered with Virginia Tech and Phytovac LLC to test vacuum and steam in combination as a phytosanitary treatment for export logs. High profile pests and pathogens of oak (oak wilt, *Bretziella fagacearum*) and walnut (thousand cankers disease, *Geosmithia morbida*) were included in the testing, along with quality test shipments of logs, in an effort to provide regulatory authorities and industry with confidence in the treatment. The work supports PPQ methyl bromide alternative development for commercial application.

The treatment science has been completed, but regulatory approval in the European Union (EU) for oak and walnut logs and market access initiatives in China remain a focus of the research group. In 2023, a comprehensive dossier on vacuum steam development for oak and walnut logs was submitted to the European Commission for review. Members of the research group also visited with Chinese Inspection and Quarantine officials and members of the log industry at various locations in China to gauge interest in the new log treatment technology and explore potential partnerships.

Adoption of vacuum steam for treatment of export logs will provide an important alternative for countries that are trading with the United States. Many, like the EU, no longer accept methyl bromide treated logs, thereby creating hardship for the log exporting industry. Vacuum steam as a competitive, economical treatment alternative could help reduce the use of methyl bromide in trade with countries like China that still allow use.

Figure 1. White oak logs loaded on pallet inside a 5’ wide x 5’ high x 8’ long steel vacuum steam chamber prior to treatment. The steel treatment chamber is nested within a conventional enclosed trailer for transport to field site for testing

Evaluation of ethyl formate and CO_2 as a phytosanitary treatment for imported table grapes

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[2]FDF Fundacion para el Desarrollo Fruiticola, Santiago Chile

Imported table grapes are treated with methyl bromide as a condition of entry into the U.S. to prevent the introduction of regulated pests that threaten US agriculture. This is the largest single use of methyl bromide in the US. Alternatives to methyl bromide fumigation are needed because it is an ozone-depleting substance and a hazardous air pollutant. Developing an alternative would allow the continued safe trade import of table grapes, as well as provide an option for reducing methyl bromide use in the US.

One potential alternative is the fumigant ethyl formate combined with CO_2 (16.7% Ethyl Formate, 83.3% balance CO_2) which offers advantages over methyl bromide for worker and consumer safety, and environmental impact. Known commercially as eFUME™, it is in EPA registration review with a proposed label for use on fresh commodities.

Laboratory studies were performed at FPML and Fundacion para el Desarrollo Fruticola in Chile, to evaluate a cold treatment at 0°C followed by ethyl formate/CO_2 fumigation as a phytosanitary measure for imported Chilean table grapes. Two pests classified as medium-risk for introduction in a recent PPQ risk assessment were evaluated; the false Chilean flat mite, *Brevipalpus chilensis;* and the European grapevine moth, *Lobesia botrana*. The goal of this project was to establish treatment parameters to provide quarantine-level efficacy for both pests.

Brevipalpus chilensis was tolerant to cold treatment. Only the egg stage showed significantly higher mortality from the control group after 10 d at 0°C. Cold treatment of *L. botrana* found that the pupa stage was most tolerant followed by eggs and then larvae. When fumigation with ethyl formate and CO_2 followed the cold treatment the egg stage of *B. chilensis* and pupa stage of *L. botrana* were the most tolerant to the combined treatment. The egg stage of both species was used for confirmatory tests because *L. botrana* pupae are not associated with the pathway. Initial doses of 60 and 73 mgL^{-1} resulting in concentration × time products of 149.7±1.3 and 220.5 ±4.3 h- mgL^{-1} were effective in preventing egg hatch in *L. botrana* and *B. chilensis*, respectively (Figure 1).

These results support the use of cold treatment followed by a 4-hour ethyl formate and CO_2 fumigation as an effective phytosanitary treatment for life stages of *B. chilensis* and *L. botrana* associated with the import pathway for table grapes. This treatment provides a viable option for reducing methyl bromide use in the U.S., and has additional potential uses for other important pests.

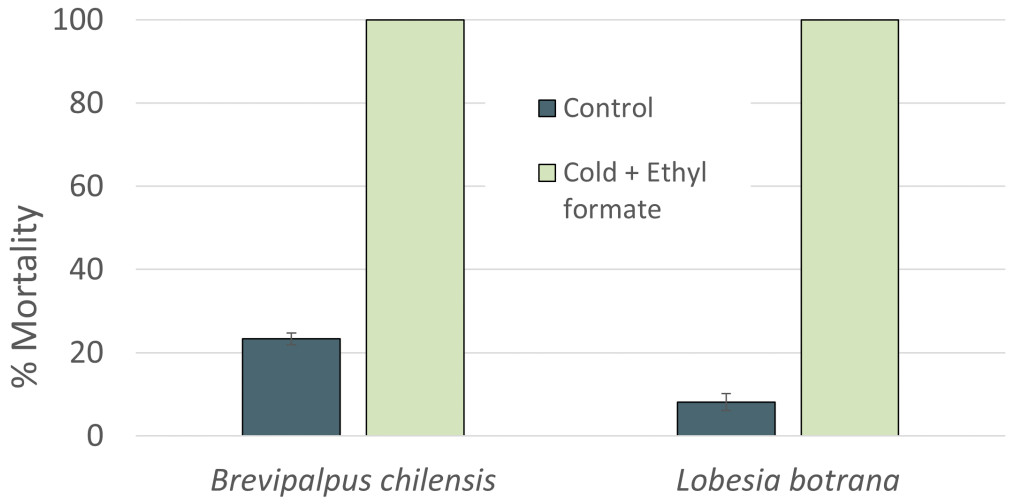

Figure 1. Confirmatory tests of *Brevipalpus chilensis* (N=7,332) and *Lobesia botrana* (N=10,004) eggs using a 12-day cold treatment at 0°C followed by fumigation with ethyl formate and CO_2 at 5°C

Spongy moth ultraviolet light emitting diode light trap comparison test

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A pheromone-based trap is currently used to detect and monitor spongy moth, *Lymantria dispar dispar*, and flighted spongy moth complex, FSMC, *Lymantria dispar asiatica*, *L. d. japonica*, *L. umbrosa*, *L. albescens* and *L. postalba* populations, however, these traps do not attract female moths . Previous studies in China and Russia have demonstrated that ultraviolet A light (UVA) light traps can be used to trap both sexes of flighted spongy moth complex, but the traps tested have been large cumbersome devices that rely on 12-volt batteries. The solar powered UVA (365-370 nm) light-emitting diode (LED) light circuit that we have developed uses small rechargeable 18650 lithium-ion batteries as a power source and solar panels to recharge the batteries. The circuitry was easily fitted to both the large delta traps, and container traps of our own design.

The circuit and traps were field tested using male spongy moth as a surrogate for flighted spongy moth complex. Five trap designs were tested; three variations of a container trap with different hole configurations (two, four, and a taped two-hole), a large Delta trap fitted with new 2023 circuitry, and a large Delta trap fitted with a similar circuit that was developed in 2022.

Delta traps, using the 2023 or 2022 circuit performed equally as well as each other, and caught twice as many moths as the two-hole container trap (Table 1). The four-hole container and taped two-hole container trap caught fewer moths.

Based on these observations, delta traps fitted with UVA LED circuitry could be used for detection purposes during domestic Port Environs Surveys or to aid in delimitation surveys and eradication efforts of introduced populations. The traps could also be utilized abroad by PPQ's Preclearance and Offshore Programs to monitor populations of FSMC at ports of interest and to aid in the determination of the relative risk of FSMC hitchhiking on marine vessels from these locals.

Table 1. Number of male flighted spongy moth collected in five different types fitted with UVA LEDs.

Trap Type:	New Delta	Old Delta	2-Hole	4-Hole	Taped 2-Hole
# Male SM Collected	**202**	**211**	**101**	**85**	**51**

Figure 1. Five variations of FSMC traps fitted with UVA LEDs. From left to right, three versions of container traps (taped 2-hole, 4-hole, and 2-hole), old style Delta with 2022 circuitry, and the new Delta trap modified with 2023 circuitry. Photos taken in daylight (top) and at night (bottom).

2023 Forest Pest Methods Laboratory insect production and outreach

Lara Day[1], Evelyne Baratelli[2,3], Cori Carson[2,3], Carrie Crook[1], Megan Doner[1,4], Jie Fang[1,3], Hannah Landers[1], Sue Lane[1], Erica Martin[2,3], Christine McCallum[1], Hannah Nadel[1], Christine Swank[1,3], Mary Watson[2,3], and Mauri Hickin[1]

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Insect colonies were reared at the Forest Pest Methods Laboratory (FPML) and Emerald Ash Borer Biocontrol Rearing Facility to provide life stages and specimens for various research projects and outreach programs. These efforts supported the Asian longhorned beetle, ALB, *Anoplophora glabripennis*, spongy moth, *Lymantria dispar* species complex, box tree moth, BTM, *Cydalima perspectalis,* and EAB programs, Cooperative Agricultural Pest Survey (CAPS), Agricultural Quarantine Inspection, other federal labs, and research and education at domestic and foreign academic institutions.

Colonies of ALB and citrus longhorned beetles, CLB, *Anoplophora chinensis* were reared at FPML for various uses. Approximately 305 ALB specimens were used for research on attractants in support of the CAPS program. An additional 459 live larvae and adults were provided for a biological control study that planted ALB larvae in sentinel logs to monitor for native parasitoids. Seventy-two larvae as well as 40 adult ALB and 24 adult CLB were provided for training USDA detector dogs. Over 700 preserved ALB specimens were provided to federal and state outreach programs.

Emerald ash borer reared at the Brighton, MI EAB facility supported the development of a wood-free rearing system for EAB and its parasitoids. Thirteen artificial diets were prepared at FPML using a twin-screw extruder and sent to Brighton for testing and rearing.

The USDA National Spongy Moth Slow the Spread project was provided 78,625 pupae for research on integrated pest management (IPM), focusing on developing a system to detect pheromones, evaluate mating-disruption techniques, and evaluate trapping methods. Additionally, 4,640 egg masses were provided to federal and academic institutions to support research on biological control, pathology, ecology, pesticide efficacy, virology, and molecular biology. Commercial production of *Lymantria* virus was supported by providing 1,655 egg masses to Andermatt, Canada. Eleven spongy moth displays were prepared and provided to state plant health directors for outreach use.

A colony of Old World bollworm, OWB, *Helicoverpa armigera* was reared at FPML and used to support several research projects. With PPQ collaborators, OWB and corn earworm, CEW, *Helicoverpa zea* were used to complete a study on hybrid genetics, using 230 OWB and 450 CEW. Also, 60 OWB pupal specimens were provided for an identification project. A new IPM project with external collaborators was started using 28 moths and collaborations will continue in FY24.

The FPML BTM colony provided over 3000 insects for studies aimed at developing an artificial diet for mass rearing. Pesticide research was conducted with PPQ and academic collaborators and used over 13,000 eggs and 1,700 larvae for testing. About 300 eggs and 75 larvae were provided for training a BTM detector dog (Figure 1A & 1B). Preserved BTM larvae, pupae and adults were used to provide 78 Riker mounts to states in support of outreach and identification.

Figure 1. Lucky, a detector dog, and Shane Phillips, a detector dog trainer from Pennsylvania Department of Agriculture, visited FPML to train Lucky to detect BTM in the FPML containment facility (A). She tested her training by searching the boxwood plants outside (B) for scent tubes charged with BTM odors.

Increasing egg hatch of long-term stored *Lycorma delicatula* egg masses

Megan Doner[1,2] and Mauri Hickin[1]

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Host range testing and colony maintenance of the spotted lanternfly SLF nymphal parasitoid *Dryinus sinicus* relies on a supply of early instar SLF nymphs hatched from field collected egg masses. SLF egg masses are only available for collection within the Northeast from late October to early May, so optimal cold storage and emergence conditions are needed to maintain the viability of stored egg masses. Groups of egg masses were collected in Pennsylvania in November 2022 and either immediately cold stored or treated with a step-down temperature regimen emulating Pennsylvania fall conditions before being cold stored. Egg masses were also collected in February 2023 and cold stored up to 8-months. A post-cold storage emergence temperature treatment emulating Pennsylvania spring conditions was tested, and nymph emergence was compared to the 25℃ (16:8 L:D) emergence regime. The goal of this study was to find the optimal storage and emergence regimens to obtain nymph hatch throughout the year from November- and February-collected egg masses cold stored at 5℃ in 62±5% relative humidity for up to 8-months.

November egg masses from all treatment groups had lower emergence than the February egg masses. The November egg masses placed directly into 25℃ post cold storage showed the lowest percent emergence while egg masses treated with the step-down PA fall temperatures before cold storage were shown to have a marginally higher percent emergence. February egg masses treated with the PA spring temperature regime after cold storage had the highest percent emergence from all the egg masses processed this year. The egg masses placed directly into 25℃ post cold storage had higher percent emergence than the November collection, but significantly less than the February collected, PA spring treated egg masses (Figure 1). Post-emergence dissections revealed the fate of unhatched eggs was a combination of desiccation and increased cold storage depending on timing of egg mass collection.

Cold-stored egg masses treated with a PA spring warm up temperature regimen increased nymph hatch in moderate humidity conditions for both egg mass collection groups. The February collected egg masses had significantly higher emergence rates than the November collected egg masses even when treated with a PA fall pre-storage temperature regime. Therefore, using February collected egg masses along with the PA spring warm-up regime to increase nymphal hatch would be beneficial to produce the necessary nymphs required to maintain an active *Dryinus sinicus* colony. This would also reduce field collection efforts and space needed for egg mass storage that may limit nymph production.

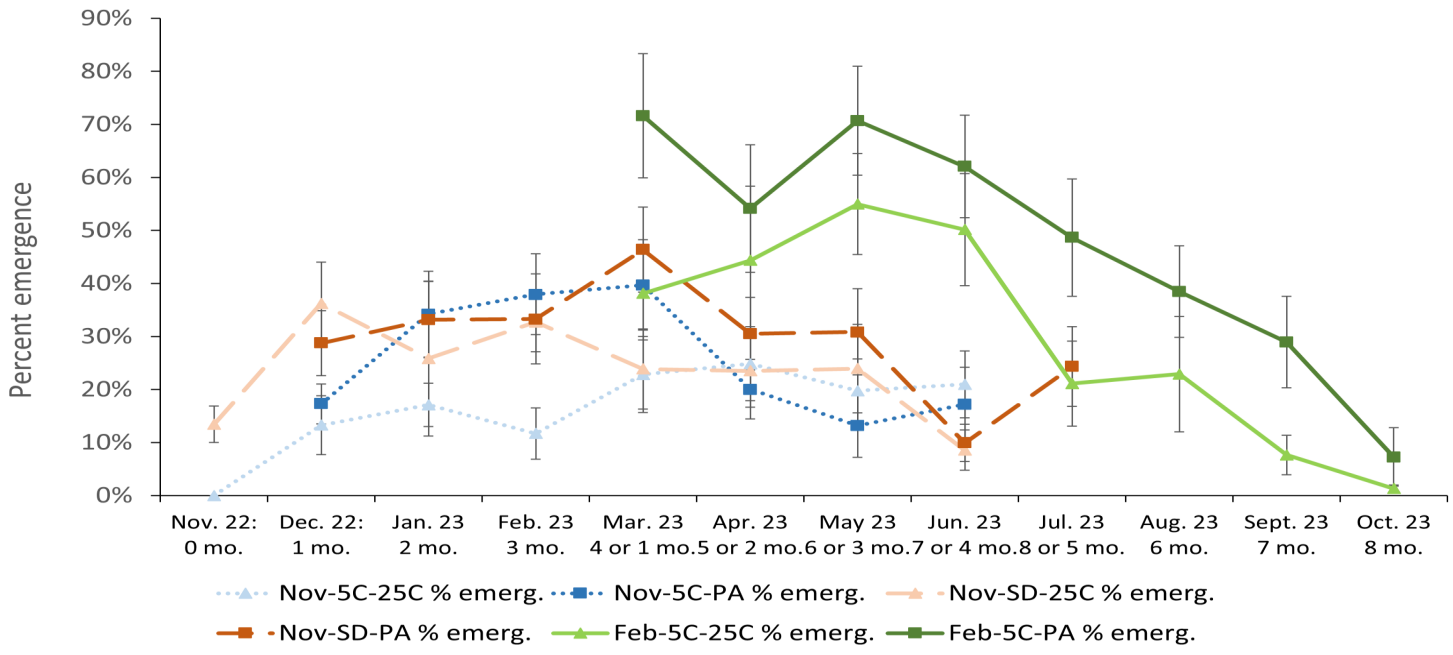

Figure 1. Mean percent monthly emergence of SLF nymphs from egg masses collected in November 2022 and February 2023 and cold-stored for 7 or 8 months prior to emergence. Eggs collected in November experienced either PA fall then 5℃ (step down 'Nov-SD') or were placed directly into 5℃ (Nov-5C) after collection, and February collected egg masses were placed directly into 5℃ (Feb-5C). Post chill conditions for both collection periods were either PA spring then 25℃ (PA) or placed directly into 25℃ (25C) to warm-up before emergence. Displayed values are mean ±SE.

Development of a twin screw extruded diet for emerald ash borer, *Agrilus planipennis* larval rearing

Mauri Hickin[1], Hannah Nadel[1] Evelyne Baratelli[3,4], Cori Carson[3,4], Megan Doner[1,2], Kristine Grayson[3], Sue Lane[1], Erica Martin[3,4], and Mary Watson[3,4]

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Multiple species of emerald ash borer, EAB, *Agrilus planipennis* parasitoids are reared at a biocontrol facility and released at heavily invaded sites across the United States to combat EAB. Currently, EAB host rearing is dependent on ash wood, but a viable artificial diet would substantially reduce cost and time spent sourcing ash host material. A handmade diet lacking host material was developed by Keena et al. (2015), however, rearing results have been inconsistent. Furthermore, this handmade diet is labor intensive to produce in large quantities using the recommended preparation methods. Twin-screw extrusion technology can produce a diet that has the optimal 50% moisture, and consistency similar to phloem, while also extruding thin flat sheets necessary for EAB larval feeding. The goal of this project was to develop a twin screw extruded diet that supports EAB larval development to late instar within six weeks and to test whether wheat germ oil (WGO) was needed in the diet. WGO is costly and has a short shelf-life and removing it from the diet would reduce the cost of diet production.

Prior work testing neonates on handmade diet sought to obtain the optimal container and infestation methods necessary for increasing the number of neonates that reached late instar within six weeks. Results from this work (Figure 1B) were then applied to several twin-screw extruded diets developed and produced at the Forest Pest Methods Laboratory (FPML) and then sent to the biocontrol mass-rearing facility in Brighton, MI for further testing. The performance of the optimized extruded diet was compared to the handmade diet. The optimized extruded diet with WGO increased late instar survival per individual rearing dish by 56.2% compared to the handmade diet with WGO (Figure 1A). Additionally, we observed both the extruded and handmade diets that contained WGO demonstrated a slight increase in late instar survival and therefore can be considered as part of optimal extruded diet formulation.

Twin screw extrusion technology has enhanced the rearing operation for EAB larvae. The extruded diet produced late instar larvae at six weeks of development more consistently and at a higher rate than handmade diet. Wheat germ oil was shown to be marginally better for producing late instar larvae, and future diets will contain the oil to ensure the optimal diet is used. The extruded diet has optimal consistency and does not require labor to roll out into thin layers, or time to dry in a hood. Moreover, the larvae produced using the diet were exposed to *Spathius galinae* and successful parasitism was achieved. Future work includes optimizing parasitism enclosures for all larval parasitoids and determining a mass-rearing method for EAB larval rearing using the extruded diet.

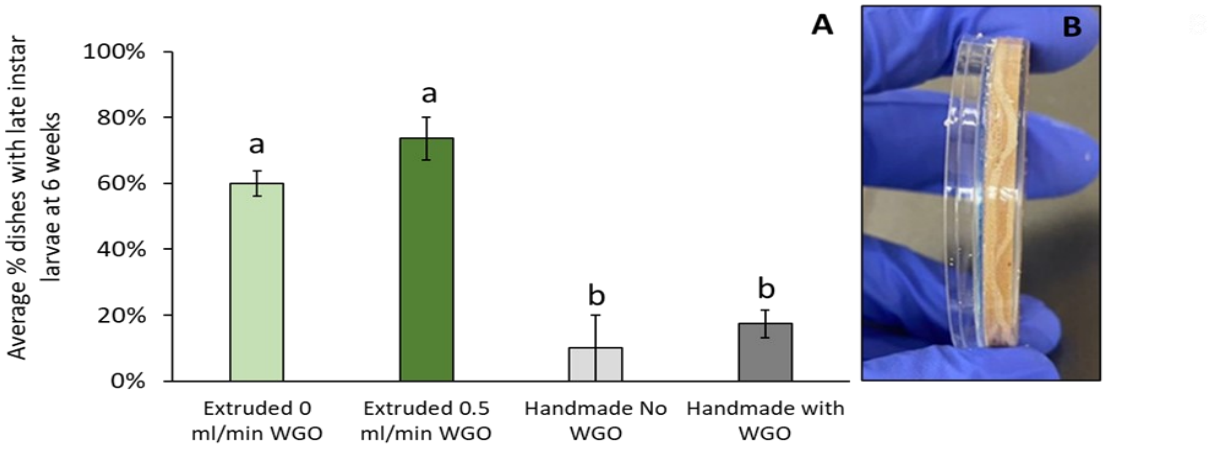

Figure 1. (A) Average percent of dishes with late instar EAB larvae at six weeks of development. Production of late instar larvae at six weeks was significantly higher for extruded diet compared to handmade diet (One-way ANOVA $F = 15.2675$; df = 3,32; $P < 0.0001$), but no significant difference was observed between treatment for the addition or absence of wheat germ oil (WGO). Groups that do not share letters are significantly different from each other (Tukey's honestly significant difference test, $p < 0.05$). (B) Image of a fourth instar EAB feeding on extruded diet in the optimized container method using two petri dish lids, with the top lid inverted to ensure no air gap is present between the diet and the larval gallery.

Development of an artificial diet that supports the full life cycle of box tree moth, *Cydalima perspectalis*

Mauri Hickin[1], Hannah Nadel[1], Megan Doner[1,2], Jie Fang[1,3], and Christine Swank[1,3]

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A colony of box tree moth, BTM, *Cydalima perspectalis*, has been reared in containment at the Forest Pest Methods Laboratory since 2020 and is currently used for biological research, IPM studies, and biocontrol testing. Colony rearing methods rely heavily on host plant cuttings from *Buxus microphylla*, (cv. Winter gem), which are costly and difficult to source, especially in winter months. Store bought plants can also be treated with chemicals, resulting in poor colony health. Using plant material for rearing is time-consuming and variability in plant quality can cause unstable colony production output. While an artificial diet can be difficult to develop due to the host-specific feeding of larval BTM, incorporating one into the rearing system, has the potential to reduce time spent caring for plants and changing plant material in rearing cages. The goal of this work was to develop an artificial diet that supports the full lifecycle of BTM.

A total of 51 different diets were developed and tested from 2021-2022, and in 2023, a proprietary commercial diet was purchased (Insecta F2, Nihon Nosan Co. Ltd, Yokohama, Japan) that induced pupation. While this commercial diet showed that an artificial diet with added plant material could be developed, this diet was difficult to obtain, expensive, the proportion of ingredients was unknown, and larvae fed this diet had several pupal deformities. Several in-house diet formulations were then tested that had a mixture of ingredients and ratios resembling the commercial diet, spongy moth diet and silkworm diet. From these trials, a new in-house artificial diet was developed at FPML that contained 30% boxwood leaf powder and supported the full life cycle of BTM. The larvae fed on the diet, developed at similar rates as larvae that fed on plant cuttings, and adults laid viable eggs. On average, the FPML in-house diet supported 66% pupation, which was marginally higher than the commercial-diet and plant cutting pupation rates (Figure 1A). In addition, the FPML diet-reared pupae were significantly heavier than pupae that developed on the commercial diet or plant cuttings during the same time period as the diet testing (Figure 1B).

Once the FPML in-house diet is fully optimized to reduce the amount of dried plant material, it can be incorporated into the rearing system. Insects reared on diet will be easier to maintain, and more synchronous in their development. As a result, more life stages will be available for biology research, IPM studies, and biocontrol testing. Future work will focus on optimizing the diet to produce consistently high-quality insects, and the development of methods to incorporate the optimized diet into the rearing system.

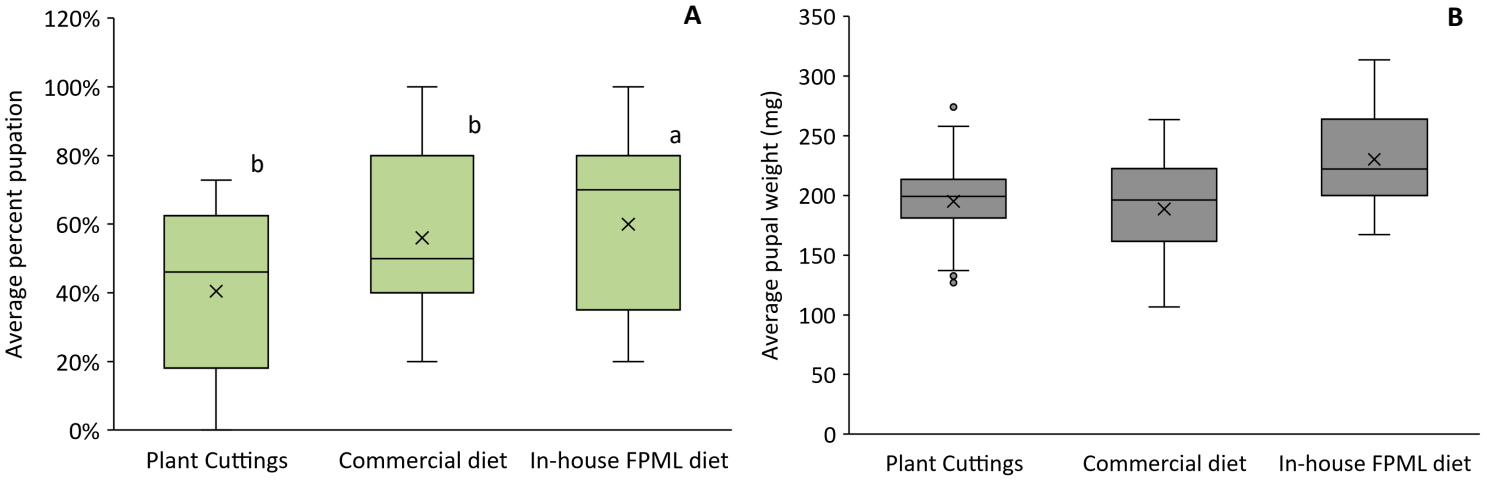

Figure 1. Average percent pupation (A) and average pupal weight (B) of BTM that fed on the commercial diet, FPML diet or plant cuttings. No significant differences in pupation rates were shown among larvae fed either diet or plant cuttings (One-way ANOVA F = 2.1497; df = 2,36; P = 0.1312). Average pupal weight was higher for larvae fed the FPML diet compared to the commercial diet and plant cuttings (One-way ANOVA F = 13.7370; df = 2,125; P < 0.0001). Groups that do not share letters are significantly different from each other (Tukey's honestly significant difference test, $p < 0.05$).

Update of trap and lure testing in support of CAPS and Field Operations

Joseph Francese[1], Everett Booth[1], Sarah Devine[1,2] and Emily Franzen[2,3]

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[2]Xavier University, Department of Biology, Cincinnati, OH
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As part of FPML's Cooperative Agricultural Pest Survey (CAPS) and Survey Supply and Procurement Program (SSPP) support, we conducted field trapping and lure assays to test new methods, lures, and traps for potential use in CAPS surveys. Pests that were targeted in these efforts were cerambycids, buprestids, and spongy moth, *Lymantria dispar*. Additionally, since FPML was in closer proximity to trapping sites, we provided supplemental trapping support to Field Operations to assist with invasive pest surveys in eastern MA for box tree moth, BTM, *Cydalima perspectalis* and a new cerambycid pest, *Xylotrechus pyrrhoderus*.

A 6-component, generic cerambycid lure was made available to all states as part of the CAPS program in 2023. Originally piloted by a small number of states in 2022, this new lure is a departure from traditional single-target CAPS lures in that it allows for use of a single trap that has the potential to attract a wider range of cerambycid species. More attractant compounds will be tested in 2024 and 2025 to add to the lure.

New delta traps from vendors Web-Cote and AlphaScents were compared against standard Scentry delta traps for efficacy in trapping spongy moth, *Lymantria dispar*. The glues used in the Web-Cote and AlphaScents traps, hot-melt thin layer adhesives which have been shown to be effective in trapping spongy moth, were tested against a standard trap with the tangle-foot type glue. Traps were left in the field and cardboards were tested in the laboratory, and while all the traps met the standards for water saturation, Web-Cote caught more spongy moths than the standard traps. However, there was greater sample loss to scavengers in the hot-melt traps than in the standard traps. Trap-caught spongy moths were provided to the FPML Molecular Diagnostics group for extraction and identification purposes. Results were presented to the SSPP and the Spongy Moth Cross Functional Working Group. All three new versions of the delta traps have been added to the survey supply for future use.

We assisted Field Ops by placing and monitoring a small number of traps for BTM recently found on Cape Cod. FPML's proximity to the newly detected areas made it optimal to trap locally.

Intercept panel traps baited with the 6-component generic lure were also placed at vineyards in Eastern MA to assist Field Ops with surveys for the invasive cerambycid, *Xylotrechus pyrrhoderus*. This species is a known pest of grape vines in its native range in East Asia. This allowed Field Ops staff to concentrate on the areas closer to their home office, while we conducted trapping close to FPML. No new infestations were found outside of the original discovery area in Chicopee, MA.

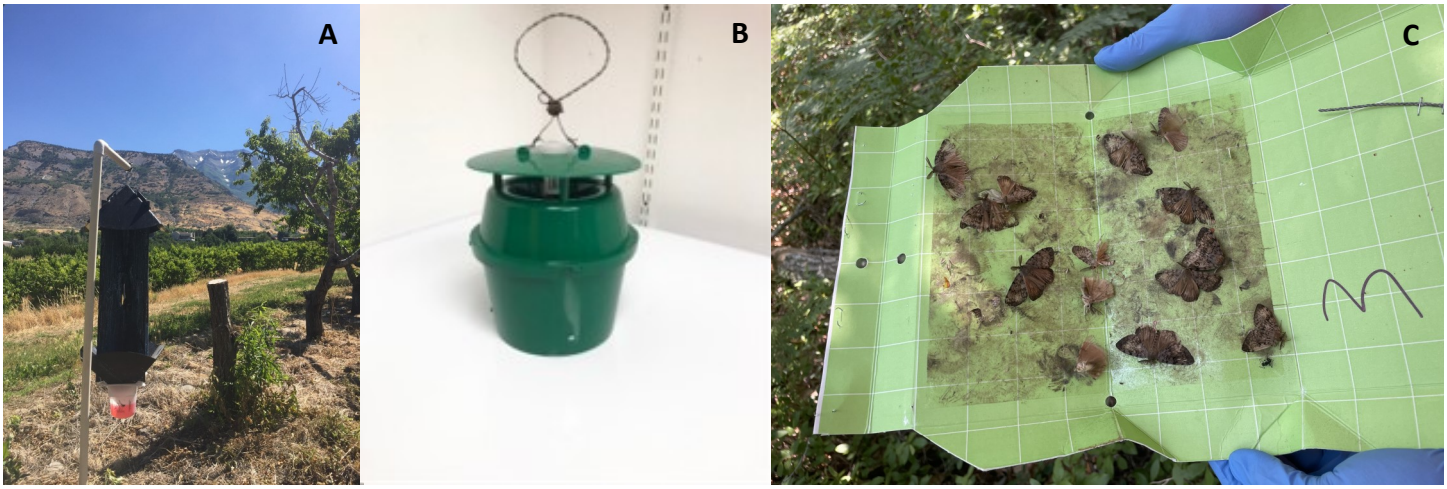

Figure 1. Three traps used in studies reported: A) intercept panel trap for cerambycids, B) unitrap for box tree moth and C) cardboard delta trap for spongy moth.

Assessing the efficacy and impacts of circle trap density on reducing SLF populations

Joseph Francese[1], Everett Booth[1], Sarah Devine[1,2], Miriam Cooperband[1], Kelly Murman[1], and Emily Franzen[2,3]

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The circle trap has been shown to be an effective tool for the detection of spotted lanternfly, SLF, *Lycorma delicatula* with traps catching thousands of lanternfly nymphs and adults in high-density sites. Because of these high catch numbers, several states and the SLF Program wondered whether increasing the number of traps at pesticide-restricted sites (i.e. wetlands, aquifers, etc.) would help reduce SLF populations.

In 2022 and 2023, we conducted trapping assays at sites in Greenwich, Connecticut (which also encompassed a portion of New York) (n = 5), and Vevay, Indiana (n = 6). In treatment plots (10m x 10m), a methyl salicylate lure-baited circle trap was placed on every tree stem $\geq$ 5cm. Control plots, where no traps were set, were placed adjacent to each treatment plot. Traps were checked bi-weekly and a five minute visual survey was also conducted in each quadrant of each plot.

Traps caught the most SLF during 1st instar emergence, while visual surveys of this stage appear to underestimate SLF population density. Visual surveys appear to match trap numbers when 4th instars and adults emerge. In either case, if traps were an efficient tool for controlling populations of SLF, we would hypothesize that both visual survey counts and trap catch would decrease over time. However, in 2023, we caught more SLF in circle traps at all population densities than we did in 2022, with mixed visual survey counts.

The standard visual surveys resulted in a highly variable predictor of SLF density and highlighted some of the challenges associated with estimating population densities for such a mobile pest. There is little evidence that increasing circle trap density can be used as an effective control strategy, especially without an effective lure. However, circle traps have been shown to be a better detection tool than visual surveys.

Figure 1. One quadrant of a 10m x 10m treatment plot with circle traps placed on every stem greater than 5cm.

Forest Pest Methods Laboratory CAPS lure support for the detection and survey of pest insects in 2023

Anna Stevenson[1], Natalie Leva[1], Julia Mackay[1], and Allard Cossé[1]

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In 2023, the FPML produced 102,629 individual insect lures in support of the Cooperative Agriculture Pest Survey (CAPS). This amount was similar to the 2022 lure production which totaled 105,857 lures. For both 2022 and 2023, the three biggest lure productions were for box tree moth, *Cydalima perspectalis*, old world bollworm, *Helicoverpa armigera*, and false codling moth, *Thaumatotibia leucotreta*.

Our biggest state stakeholders in 2023 for CAPS lures were PPQ programs in California, New York, Florida, and Illinois. During 2022 and 2023, we made efforts to automate and modernize the lure production lab with industrial pouch printers, robotic pouch openers, and package modifications. These efforts streamlined production and resulted in a reduction of required staff from around five staff members during peak production to one.

In addition, FPML supported the CAPS program with quality control (QC) lure analysis, the Forest Service with QC analysis of *Lymantria dispar dispar* mating disruption formulations, and supported various local and international researchers with experimental insect lure formulations.

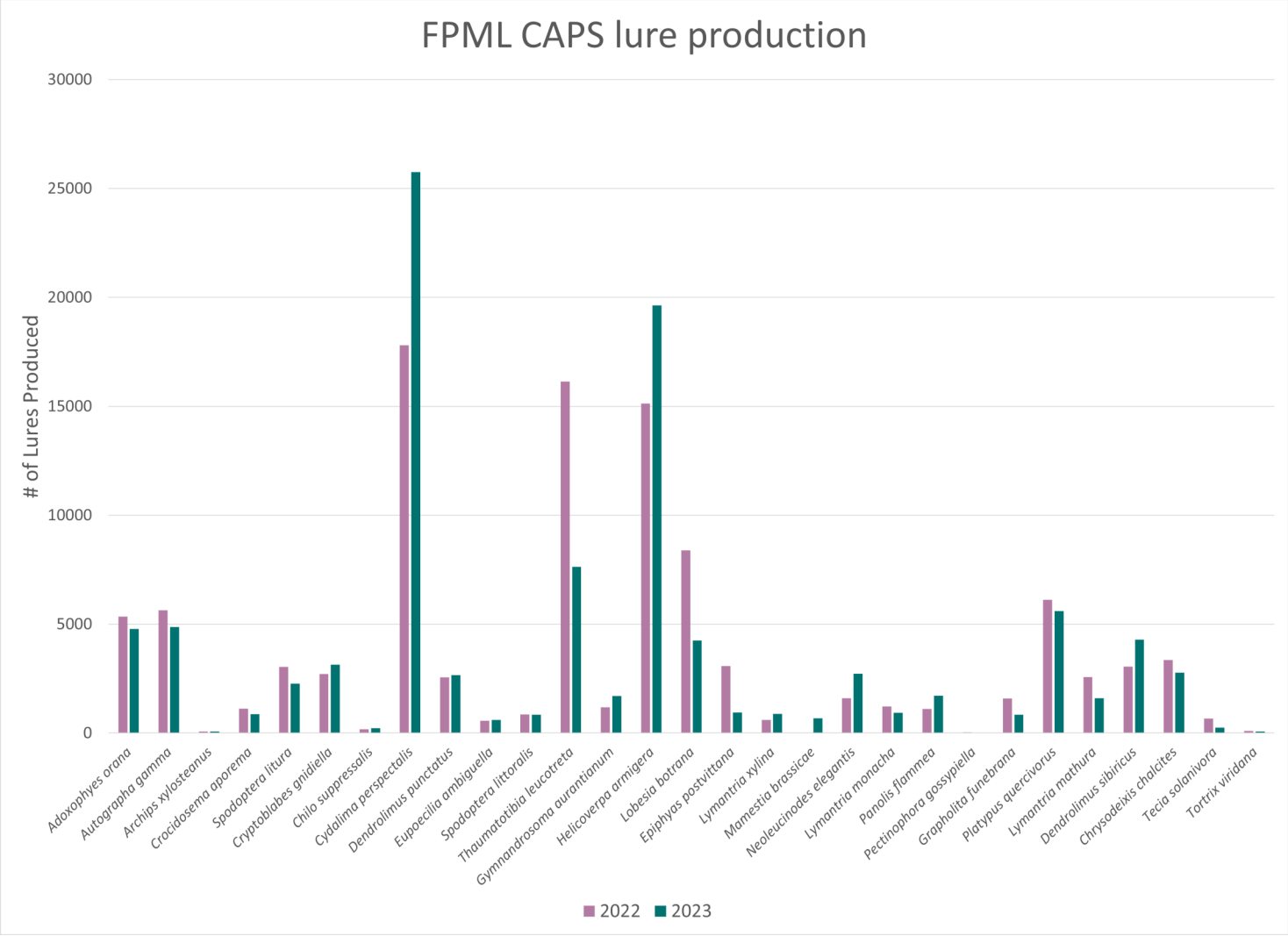

Figure 1. Forest Pest Methods Laboratory CAPS lure production in 2022 and 2023 for 29 different insect species.

2023 Port and Domestic Spongy Moth (*Lymantria dispar* species complex) Molecular Diagnostic Survey

Brian Silva[1,2], Caroline Davock[1,2], Megan Doner[1,3], Daniel Laffoon[1], and Yunke Wu[1]

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The Forest Pest Methods Laboratory supports PPQ's Spongy Moth Programs by utilizing molecular tools to identify possible *Lymantria* specimens trapped domestically and at U.S. ports of entry. The utilization of real-time PCR (qPCR) has led to faster processing of samples and a growth in diagnostic capabilities. All *Lymantria* samples from 2023 were analyzed through real-time qPCR which allowed for quick and accurate detection of suspect *Lymantria dispar dispar*, *L. d. asiatica, L. d. japonica,* and *L. umbrosa.*

In 2021, assay failure rate was about 19.2% when standard PCR was used as the primary diagnostic tool. In 2022, assay failure rate decreased to about 2% with the addition of qPCR sequencing (Figure 1). In 2023, the assay failure rate further decreased to only 1.5% with the optimization of qPCR methods.

For 2023 U.S. ports surveys, 20 specimens were intercepted, 11 *L. d. asiatica,* five *L. d. dispar*, three *L. d. japonica* and one unknown. For the domestic survey, 11,432 specimens were received from 34 states (Figure 1). Current guidelines require that every specimen collected from states outside of quarantine be processed for identification. Of the 11,432 samples, 46% were from outside quarantine. Most of these samples came from two states, Iowa and Minnesota, which are at the spongy moth population frontline, consistent with the large volume of samples.

Even though the number of samples received and processed over the past few years has increased, assay failure rate has decreased with the implementation of qPCR (Figure 2). The utilization of real-time PCR has led to faster and more accurate processing of samples.

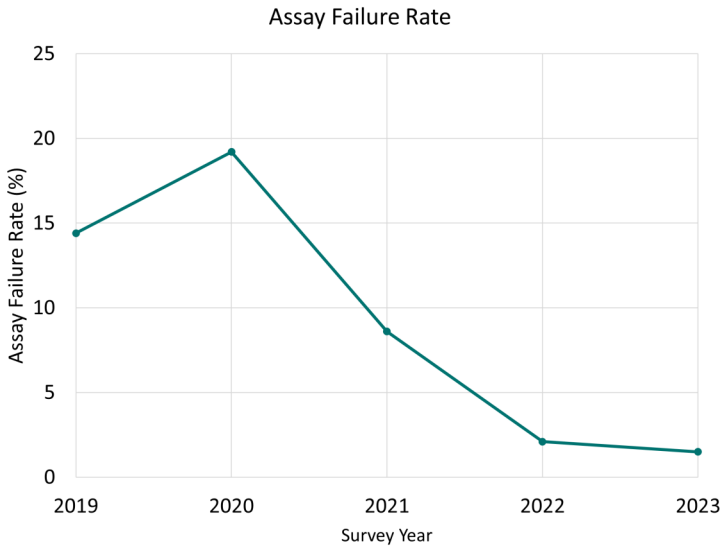

Figure 1. Assay failure rate over time showing decreases over the years along with the adoption of the current qPCR assay.

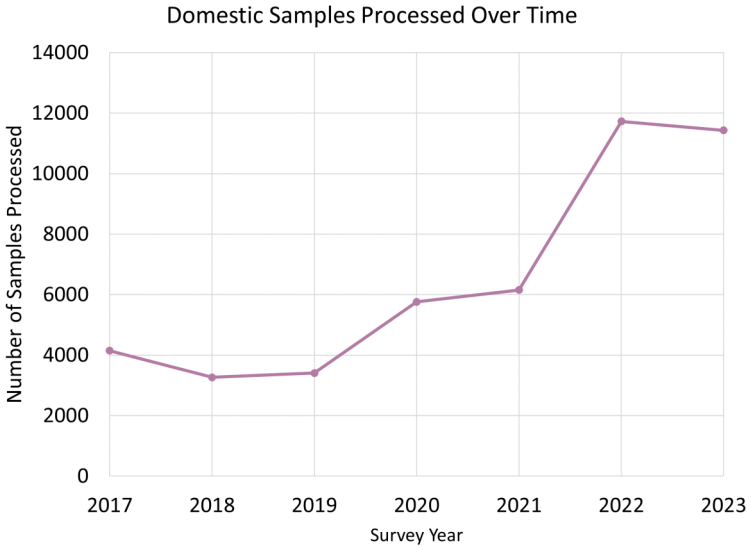

Figure 2. Number of spongy moth samples processed from domestic surveys over the last seven years, with the number being almost doubled in 2022 and 2023.

Development of a ddPCR assay for molecular diagnostics of khapra beetle (*Trogoderma granarium*)

Yunke Wu[1] and Scott Myers[1]

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Droplet Digital Polymerase Chain Reaction (ddPCR) technology is a digital PCR method utilizing massive sample partitioning to achieve absolute quantification of target DNA. This method provides the highest sensitivity compared to existing diagnostic methods. Khapra beetle, *Trogoderma granarium*, is a quarantine pest that infests a wide variety of stored grains and processed dried food and animal products. We aimed to develop a ddPCR assay for detecting khapra beetle in a large background of closely related, non-target beetle samples with further potential usage in environmental DNA (eDNA) surveys.

Development of the ddPCR assay was based on a real-time khapra beetle PCR assay previously published by FPML [1]. Similar to the real-time PCR assay, the ddPCR assay has a multiplex format that includes a genus-wide general assay from the mitochondrial 16S gene (16S assay) and a specific assay from the COI gene targeting khapra beetle (NJ assay). Probes for the two assays were labeled with fluorophore HEX and FAM, respectively. We first determined the optimal annealing temperature of the ddPCR reaction by running a temperature gradient from 56°C to 65°C. The largest and cleanest separation between positive and negative droplets were observed at 59°C, which is lower than the annealing temperature of the real-time PCR. We further tested the direction of the probes (i.e., sense vs. anti-sense) following the manufacturer's guideline that ideal probes have more nucleotide cytosines than guanines. Results indicated better performance by the sense strand-probes. Lastly, the ddPCR assay was evaluated using two closely related congeneric species, the warehouse beetle, *T. variabile,* and the larger cabinet beetle, *T. inclusum*. As expected, the general assay amplified both species along with khapra beetle, whereas the specific assay only produced positive droplets for khapra beetle but not the other two close relatives (all NO CALLS, Figure 1). With the auto-threshold option in the manufacturer's software QuantaSoft Analysis Pro, the limit of detection of the ddPCR assay could be 5 copies of khapra beetle DNA or even less per sampling well.

The development of ddPCR technology allows for the potential to detect a relatively low number of khapra beetles in a larger bulk sample of non-target organisms without the time-consuming process involved in using morphological identification methods. The assay can be delivered to other S&T labs and National Identification Services to improve capacity and efficiency in identification of a large volume of *Trogoderma* spp. samples.

References:

1. Wu Y., Domingue M.J., McGraw A.R. et al. (2023) Development of an array of molecular tools for the identification of khapra beetle (*Trogoderma granarium*), a destructive beetle of stored food products. Scientific Reports, 13, 3327.

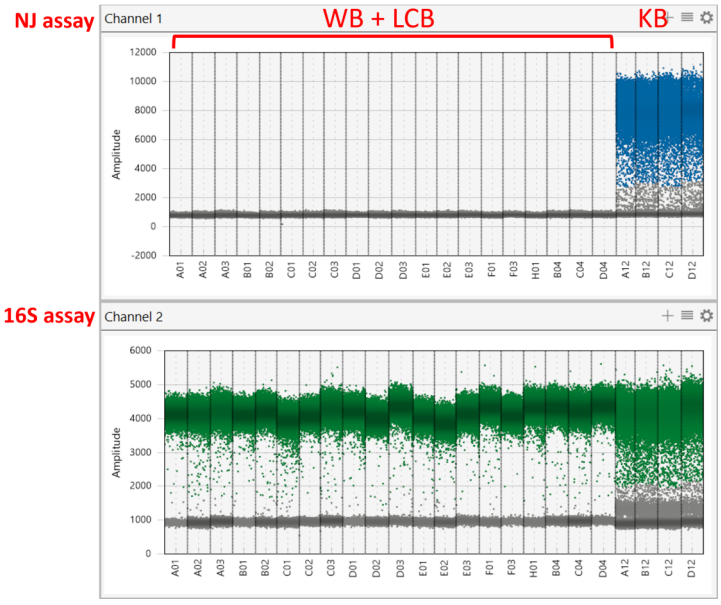

Figure 1. ddPCR assay output showing the 16S assay with positive droplets in all three beetle species, khapra beetle (KB), warehouse beetle (WB), and the larger cabinet beetle (LCB), whereas the NJ assay only amplified positive droplets in khapra beetle.

Development of a multiplex real-time PCR assay for detecting *Anastatus reduvii* among other native parasitoids of spotted lanternfly

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In 2021, a study conducted at FPML demonstrated the variety of native egg parasitoid wasps that attack spotted lanternfly, SLF, *Lycorma delicatula* egg masses in the eastern United States [1]. To better detect these parasitoids, particularly *Anastatus reduvii*, which was the most abundant wasp species recovered from the 2021 study, we aimed to develop a real-time PCR assay to identify DNA of *A. reduvii* accurately and efficiently from SLF egg masses. *Anastatus reduvii* is widespread in eastern US and Canada and has been reared and released as a biological control agent against brown marmorated stink bug, BMSB, *Halyomorpha halys*.

Mitochondrial COI sequences were generated from seven *A. reduvii* specimens reared from SLF egg masses collected in Tullytown, Pennsylvania, as well as six voucher specimens from the colony maintained by the Insect Biology and Management Program, North Carolina State Extension. One additional *A. reduvii* DNA sequence was downloaded from the Barcode of Life Data System (accession no. GMGSS069-13). Based on the alignment of the 14 sequences, primers and a TaqMan probe were designed with < 0.5% genetic divergence. To ensure successful DNA extraction and high DNA quality, a second general assay [2] was developed as a control to be combined with the above assay. The resulting multiplex assay was tested using DNA of *A. reduvii* and two other *Anastatus* species, *A. orientalis* and *A. mirabilis;* the general assay amplified all three species, while the specific assay only amplified *A. reduvii* (Figure 1). Assay metrics were evaluated using six serial dilutions of 1:5 of DNA extracted from a single *A. reduvii* leg. Each serial dilution included four replicates. Standard curves were computed based on the first five serial dilutions, as the sixth dilution (1:15625) was no longer detectable by the assay. For the *A. reduvii*-specific assay, amplification efficiency was estimated at 98.2% with a linearity of 0.993. The general assay had an amplification efficiency of 111.2% and a linearity of 0.989. These metrics fall within or are very close to conventional standards of real-time PCR assay.

Development of a real-time PCR assay for this native parasitoid of SLF helps to provide a better estimate of the impact of natural enemies on the invasive SLF populations. It also provides the SLF program a survey tool to monitor any changes of the parasitism rate by native wasp species, especially in places where SLF population are growing and thus may become a preferred host for the native parasitoids.

References:

1. Wu Y., Broadley H., Palmeri M. et al. (2022) Molecular identification of native egg parasitoids from field-collected, parasitized spotted lanternfly egg masses. FPML 2021 Accomplishment Report, 32.
2. Mittelberger C, Obkircher L, Oberkofler V, Ianeslli A, Kerschbamer C, Gallmetzer A, Reyes-Dominguez Y, Letschka T, Janik K. Development of a universal endogenous qPCR control for eukaryotic DNA samples. Plant Methods. 2002; 16:53.

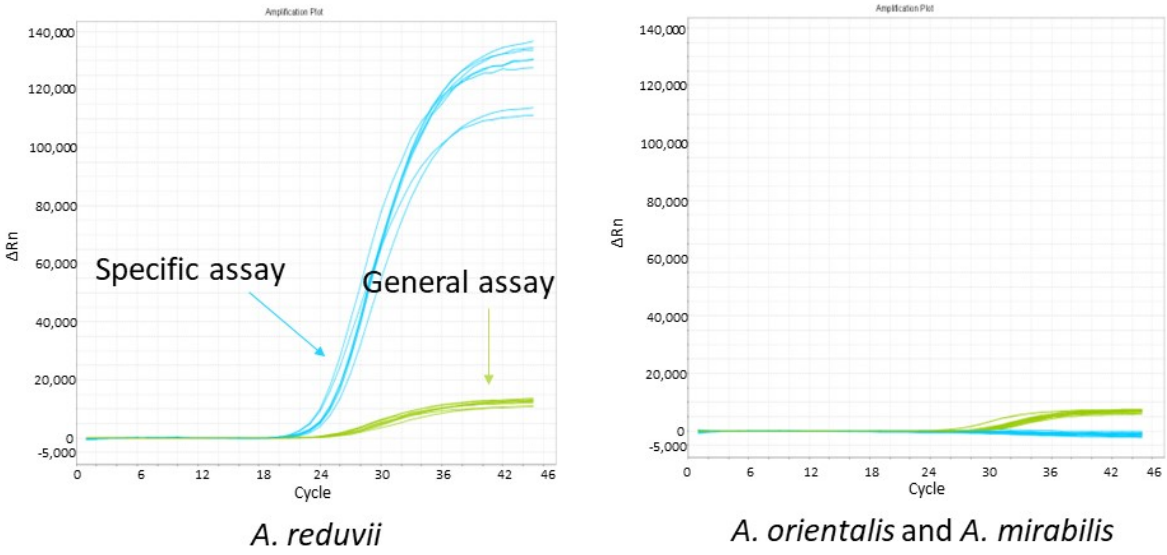

Figure 1. Left: the multiplex real-time PCR result showing positive amplification for both the specific (blue) and general (green) assay when the sample is *Anastatus reduvii*. Right: only the general assay shows positive amplification when the sample is other *Anastatus* species, such as *A. orientalis* and *A. mirabilis*.

Establishing classical biological control of Asian citrus psyllid, ACP, *Diaphorina citri*, in Arizona

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The Asian citrus psyllid, ACP, *Diaphorina citri* is managed in commercial citrus in Arizona with coordinated pesticide treatments; however, ACP can potentially re-invade managed areas from untreated adjacent residential areas and other unmanaged citrus. From 2013 to 2023, the parasitoid *Tamarixia radiata* (Hymenoptera: Eulophidae) was released in residential areas in Arizona to establish classical biological control of ACP in dooryard citrus trees. The *T. radiata* reared in California were sourced from the Punjab region of Pakistan due to close climate match with citrus growing regions in the Western US. Releases were conducted in Yuma and Lake Havasu City from 2013 to 2019 and in San Luis, Wellton, Ajo, and Nogales from 2019 to 2023.

A total of 845,000 *T. radiata* were released over the course of the program. Presence of Pakistani-origin *T. radiata* at non-release sites was confirmed in Yuma in 2015 and 2016 [1]. Adult *T. radiata* were recovered from panel traps at four non-release sites in Ajo in 2022 and one site in San Luis in 2023. Immature *T. radiata* were recovered from parasitized nymphs at a non-release site in Nogales in 2022, and at two sites in San Luis and two sites in Wellton in 2023 (Figure 1). Specimens underwent COI gene sequencing to identify *T. radiata* haplotypes. Two haplotypes that occur in the Pakistani-origin colonies were identified among specimens recovered in San Luis, one from Wellton specimens, seven from Ajo specimens, and four from Nogales specimens. All *T. radiata* recovered from San Luis, Wellton and Ajo belonged to Pakistani-origin haplotypes as did the majority of *T. radiata* recovered from Nogales. A *T. radiata* haplotype common in the field in Mexico, which was found in Nogales and Ajo prior to biological control releases, was also present in the specimens recovered from Nogales. Evidence suggests that the Pakistani-origin strain of *T. radiata* has become established in all four recent release areas.

The *T. radiata* are reared in an iso-line system to preserve genetic diversity, which will allow for adaptation of *T. radiata* to local field conditions. The establishment of this strain of *T. radiata* should have the best potential for biological control of ACP in residential and other unmanaged citrus.

References:

1. Gomez-Marco F, Gebiola M, Baker BG, Stouthamer R, Simmons GS. Impact of the temperature on the phenology of *Diaphorina citri* (Hemiptera: Liviidae) and on the establishment of *Tamarixia radiata* (Hymenoptera: Eulophidae) in urban areas in the lower Colorado Desert in Arizona. Environmental entomology. 2019;48(3):514-23.

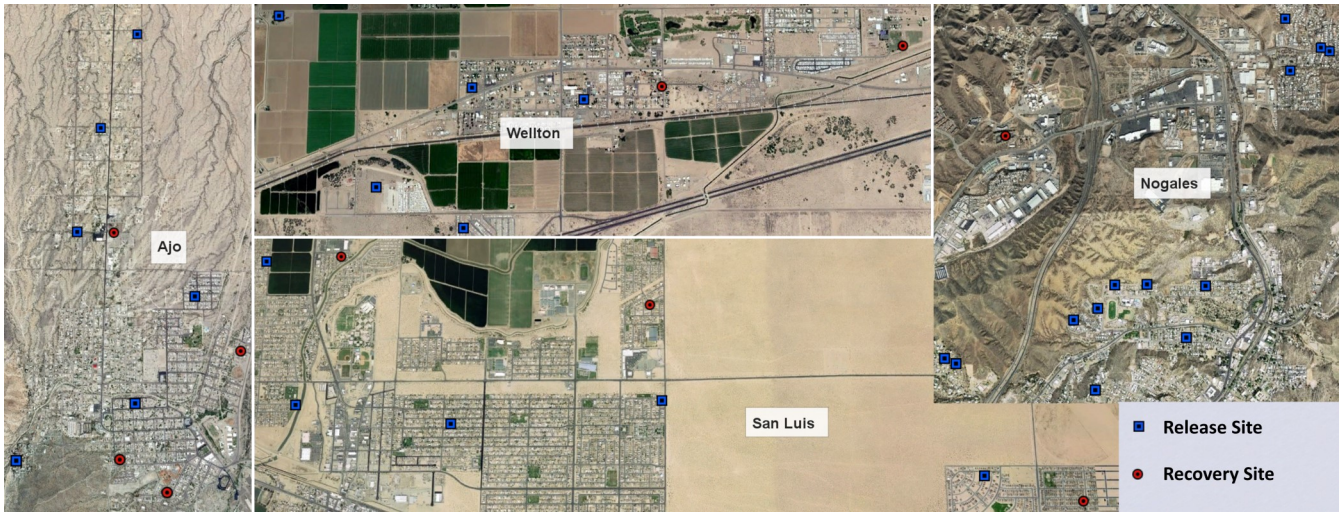

Figure 1. Biological control release sites (blue squares) and sites from which *T. radiata* were recovered (red circles) in Ajo, Wellton, San Luis, and Nogales, AZ.

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