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

Forest Pest Methods Laboratory

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Forest Pest Methods Laboratory

2021 Accomplishment Report

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Science and Technology

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2. **Domingue MJ**, Scheff DS, Arthur FH, **Myers SW**. Sublethal exposure of *Trogoderma granarium* everts (Coleoptera: Dermestidae) to insecticide-treated netting alters thigmotactic arrestment and olfactory-mediated anemotaxis. Pestic Biochem Physiol. 2021;171:104742.
3. Elkinton JS, Boettner GH, **Broadley HJ**. Successful biological control of winter moth, *Operophtera brumata*, in the northeastern United States. Ecological Applications. 2021;0(0).
4. Gourgouta M, Baliota GV, Morrison WR, **Domingue MJ**, Athanassiou CG. Comparative Capture of *Trogoderma granarium* (Coleoptera: Dermestidae) and T*. variabile* in Floor Traps in Single Species Releases With Previously Captured Conspecific or Heterospecific Individuals. J Econ Entomol. 2021;114(6):2591-7.
5. **Hickin M**, **Nadel H**, Schal C, Cohen AC. Optimization of a Diet for the Greater Wax Moth (Lepidoptera: Pyralidae) Using Full Factorial and Mixture Design. J Econ Entomol. 2021;114(3):1091-103.
6. Imrei Z, **Domingue MJ**, Lohonyai Z, Moreira JA, Balintne Csonka E, Fail J, et al. Identification of Pheromone Components of *Plagionotus detritus* (Coleoptera: Cerambycidae), and Attraction of Conspecifics, Competitors, and Natural Enemies to the Pheromone Blend. Insects. 2021;12(10).
7. Liebhold AM, Leonard D, Marra JL, **Pfister SE**. Area-wide management of invading gypsy moth (*Lymantria dispar*) populations in the USA. In: Hendrichs J, Pereira R, Vreysen MJB, editors. Area-Wide Integrated Pest Management: Development and Field Application. Boca Raton, FL, USA: CRC Press; 2021. p. 551-60.
8. Liu FL, Rugman-Jones P, Liao YC, Fernandez V, Chien I, **Dodge C**, **Cooperband MF**, Tuan SJ, Stouthamer R. The Attractiveness of alpha-Copaene to Members of the *Euwallacea fornicatus* (Coleoptera: Curculionidae) Species Complex in California and Taiwan. J Econ Entomol. 2021.
9. Mastro VC, Munson AS, **Wang B**, Freyman T, Humble LM. History of the Asian *Lymantria* species Program: A Unique Pathway Risk Mitigation Strategy. Journal of Integrated Pest Management. 2021;12(31):1-10.
10. **Myers SW**, Ghimire MN, Arthur FH, Phillips TW. A Combination Sulfuryl Fluoride and Propylene Oxide Treatment for *Trogoderma granarium* (Coleoptera: Dermestidae). J Econ Entomol. 2021;114(4):1489-95.
11. Salzman S, **Crook D**, Calonje M, Stevenson DW, Pierce NE, Hopkins R. Cycad-Weevil Pollination Symbiosis Is Characterized by Rapidly Evolving and Highly Specific Plant-Insect Chemical Communication. Front Plant Sci. 2021;12:639368.
12. Scheff DS, Baliota GV, **Domingue MJ**, Bingham GV, Morrison III WR, Athanassiou CG. Evaluations of the new deltamethrin-treated all-in-one hermetic bag for the control of the Khapra beetle, *Trogoderma granarium* (Everts). Journal of Stored Products Research. 2021;93:101839.
13. **Simmons GS**, Varela L, Daugherty M, Cooper M, Lance D, Mastro V, Carde RT, Lucchi A, Ioriatti C, Bagnoli B, Steinhauer R, Broadway R, Stone Smith B, Hoffman K, Clark G, Whitmer D, Johnson R. Area-wide eradication of the invasive European grapevine moth, *Lobesia botrana* in California, USA. In: Hendrichs J, Pereira R, Vreysen MJB, editors. *Area*-Wide Integrated Pest Management. Development and Field Application. Boca Raton, FL, USA: CRC Press: 2021; p. 81–596.

14. **Simmons GS**, Salazar Sepulveda MC, Fuentes Barrios EA, Idalsoaga Villegas M, Medina Jimenez RE, Garrido Jerez AR, **Henderson R**, Donoso Riffo H. (2021). Development of Sterile Insect Technique for Control of the European Grapevine Moth, *Lobesia botrana*, in Urban Areas of Chile. Insects. 2021;12(5), 378.

15. **Simmons GS**, Bloem KA, Bloem S, Carpenter JE, Suckling DM. Impact of Moth Suppression/Eradication Programmes Using the Sterile Insect Technique or Inherited Sterility. In: Dyck VA, Hendrichs J, Robinson AS, editors. Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management. 2 ed. Boca Raton, FL, USA: CRC Press; 2021; p. 1007-1050.

16. Tobin PC, Strom BL, **Francese JA**, Herms (Second ed., pp. 1007-1050). DA, McCullough DG, Poland TM, Ryall KL, Scarr T, Silk PJ, Thistle HW. Evaluation of Trapping Schemes to Detect Emerald Ash Borer (Coleoptera: Buprestidae). J Econ Entomol. 2021;114(3):1201-10.

17. Xin B, Zhang YL, Wang XY, Cao LM, Hoelmer KA, **Broadley HJ**, **Gould JR**. Exploratory Survey of Spotted Lanternfly (Hemiptera: Fulgoridae) and Its Natural Enemies in China. Environ Entomol. 2021;50(1):36-45.

Vibroacoustic signals used by spotted lanternfly

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Despite a surge of research on spotted lanternfly (SLF) in the last 10 years, large knowledge gaps regarding its basic biology still exist. Details concerning SLF mate-finding— how mates are located, who finds whom, where courtship and mating occur, timing, and what cues and mechanisms are involved— remain largely unknown. Lanternflies are members of the same suborder that includes the cicadas and tree hoppers. During mate-finding, cicadas are known to broadcast airborne sound and tree hoppers are known to utilize substrate-borne vibrations. Recently, anecdotes describing SLF appearing to respond to acoustic stimuli have been accruing. Therefore, we conducted investigations to determine what role sound and substrate-borne vibrations play in SLF behavior. An improved understanding of SLF behavioral ecology is required to develop and improve survey, detection, control, and mitigation tools for the SLF Program.

In 2020, preliminary experiments tested SLF responses to a tone played from a visually concealed speaker 30 cm from the center of a circular arena where either a 4th instar nymph or adult SLF was released. We found that both 4th instar SLF and adults demonstrated positive phonotaxis, as evidenced by significant orientation toward the source of the tone (Figure 1A).

In 2021, this experiment was expanded upon using a larger, vibration-isolated arena, so that airborne sound waves, ultrasound, and substrate-borne vibrations could each be generated and tested exclusive of each other. Results showed that SLF nymphs could detect and respond to substrate-borne vibrations. The focus then shifted to recording substrate-borne vibrations produced by field-caught adult SLF in the laboratory. During the period where SLF adult emergence is at its peak, between August 17 and November 2, a laser vibrometer was used to record vibrations from cages set up with a potted tree-of-heaven and either 3-5 males, females, or a mix of both sexes (Figure 1B). We found that differences in substrate vibration recordings varied with the developmental progression of the SLF adult life stage. For instance, prior to the SLF mating period observed in the field, only vibrations from honeydew falling on leaves were recorded in the laboratory. Soon after mating was observed in the field, the first SLF substrate vibrations were recorded in the laboratory (Figure 1C). SLF substrate vibrations continued to be recorded throughout the time when courting pairs could be found in the field and became less frequent towards the end of the season. Video recordings taken at the same time revealed that the substrate vibrations intensified when a male-female pair was engaged in courtship. SLF substrate vibrations were recorded only from the cages containing males or both sexes, but not from cages containing only females, suggesting that it is the male who initiates communication with females. Understanding how SLF locate and select mates can inform the development of improved control tools targeting those mechanisms.

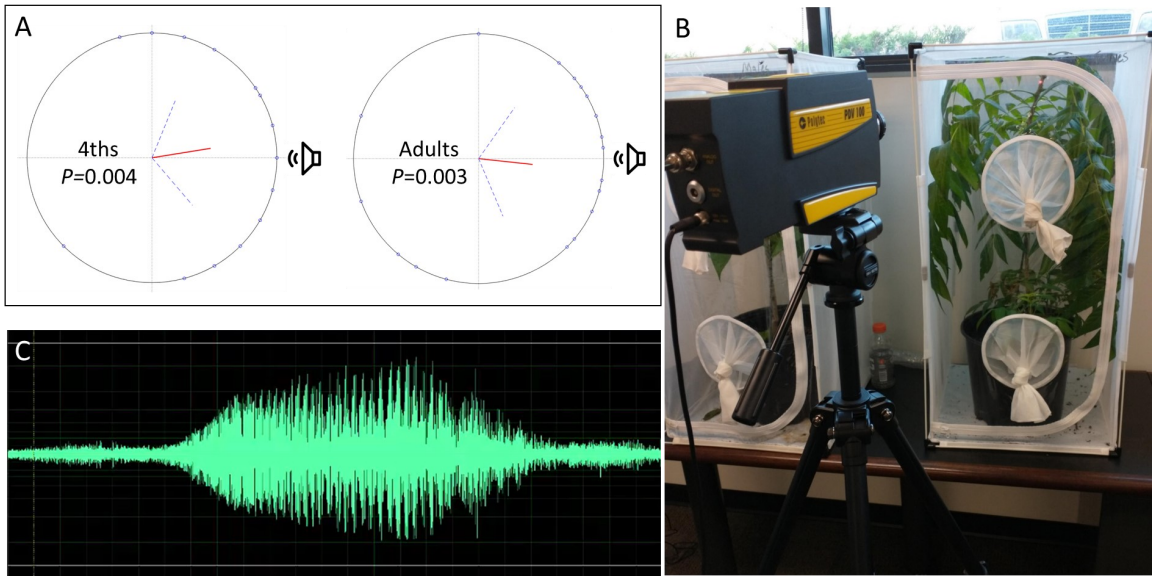

Figure 1. A) Average direction (±SD) SLF exited the arena with respect to the source of the sound. B) Laser vibrometer recording setup. C) Substrate vibration produced by a male SLF.

Using artificial spotted lanternfly aggregations to create "hot" trees

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In stands of similarly sized tree-of-heaven, some trees may seem ignored by spotted lanternfly (SLF) while adjacent trees can be covered with SLF, a phenomenon referred to as “hot” trees. It is currently unknown why one tree is selected over another. Although spotted lanternfly is the most studied fulgorid, the mechanism, cues or factors involved (semiochemicals, vibro-acoustic signals, visual cues, nutritional factors), and biological reasoning (safety in numbers, feeding, mating) for aggregation all remain unknown. It is also unclear if aggregation is driven by insect-produced signals or host factors. SLF aggregations can be observed during the nymphal stages but become more intense in the weeks leading up to and during mating. It stands to reason that aggregation may play some role in locating or attracting mates, however it has been noted that aggregations on some trees will be predominantly female, while aggregations on other trees will be predominantly male [1]. To develop control tools for the SLF Program that exploit aggregation behavior, it is necessary to understand what causes aggregations to form. To determine if aggregations could be experimentally manipulated, we created artificial SLF aggregations on trees in low-density sites and trapped for wild SLF on those trees.

In 2020 and 2021, trees were populated with aggregations of either males or females contained in sleeve cages on the trunks of tree-of-heaven and insect traps were placed above the sleeves. Trap catch was compared between similarly sized, paired trees in 2020 and in 2021 an empty sleeved control tree was added to the experimental design. Results showed that wild sex ratios were significantly more male-biased on male sleeves and female-biased on female sleeves. Male sleeves attracted more males and female sleeves attracted more females than control sleeves in 2021. In addition, the control sleeves attracted significantly fewer SLF in total than either male or female sleeves (Figure 1). Thus, we effectively were able to create “hot” trees using artificial aggregations over time. In addition, we were able to manipulate the sex ratio of the wild SLF to be more male- or female-biased based on the sex of the SLF in the sleeves.

The results suggest that SLF aggregations are formed in response to signals derived from SLF, not necessarily differences between individual trees. However, which signals are responsible for the attraction and aggregation remains to be determined. The fact that sexes were attracted to their own helps form an initial understanding of the underlying mechanism of the skewed sex ratios found in natural aggregations, yet we still do not fully understand how or why this happens, or how males and females ultimately come together for mating. Based on this and our other studies, we hypothesize that SLF may use a combination of long- and short-range cues and signals that change over time. An improved understanding of each step driving SLF to form aggregations and identify mates can inform mechanisms that may be exploited in the development of control tools.

Figure 1. A wild aggregation of SLF (arrow) formed on a tree with an artificial aggregation (sleeved) and not on adjacent trees (circled).

Reference

1. Domingue MJ, Cooperband MF, Baker TC. Skewed adult sex ratios observed early in the North American invasion of *Lycorma delicatula* (Hemiptera: Fulgoridae). Journal of Asia-Pacific Entomology. 2020 Jun 1;23(2):425-9.

Semiochemicals derived from spotted lanternflies evoked antennal responses and attraction in laboratory assays

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Spotted lanternfly (SLF) is capable of hitchhiking to new areas via inadvertent human-assisted movement, making it difficult to predict where new populations will emerge. Early detection is essential for the SLF Program to delineate and mitigate new introductions before establishment. Detection and mitigation tools such as effective lures, mass trapping, and mating disruption rely on powerful attractants called pheromones. The discovery of an attractive pheromone for SLF would advance the development of such early detection and mitigation tools.

Evidence of pheromone use in SLF was demonstrated in behavioral assays using male and female extracts and in field studies using live SLF as lures. To identify attractive volatiles, we developed techniques using SLF antennae as detectors to screen samples in a process known as gas chromatography coupled with electroantennographic detection (GC-EAD) (Figure 1). This technique allowed us to determine potential chemicals involved in conspecific attraction of SLF (Figure 2).

We found more than 50 SLF-derived compounds that evoked antennal responses by both sexes. These were tentatively identified and obtained in pure form for further GC-EAD screening. Although both sexes could detect most SLF-derived compounds, the intensity of antennal responses differed between males and females. Male antennae produced larger responses than female antennae for most of the compounds at fixed concentrations.

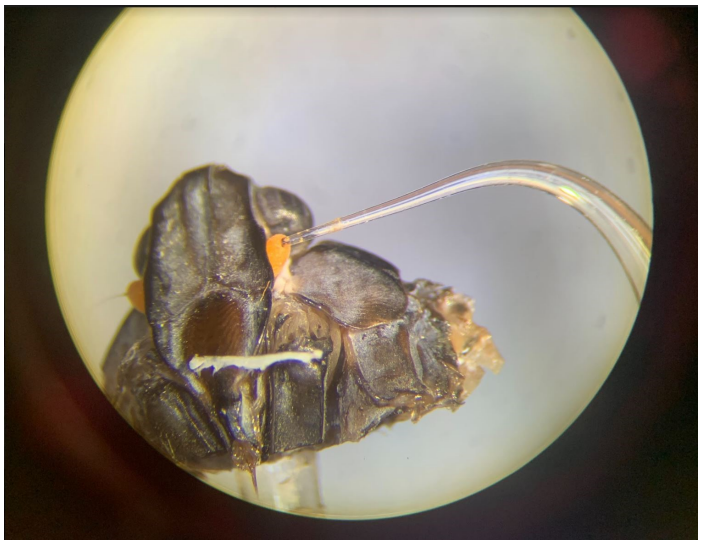
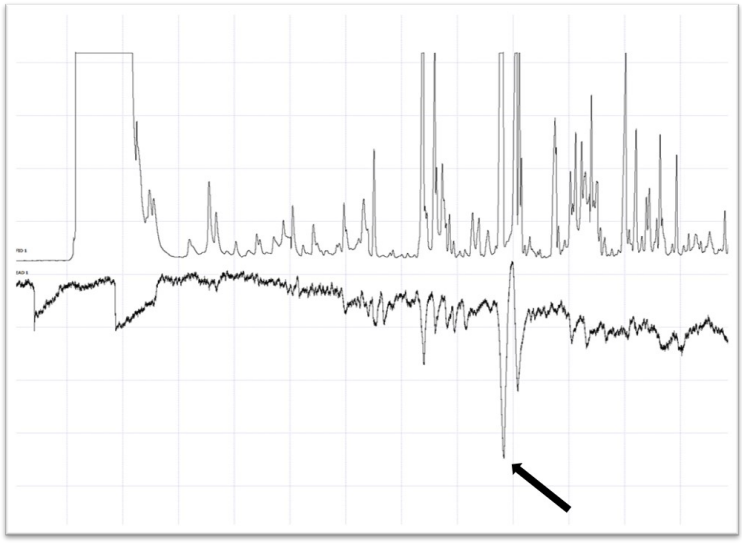


Figure 1. A glass saline electrode connecting to an SLF antenna.



Retention time (minutes)

Figure 2. Representative traces from gas chromatography-electroantennographic detection (GC-EAD).

Behavioral bioassays also revealed that the SLF volatiles or individual compounds evoke different behavioral responses between males and females. For instance, males were attracted to odors from both males and females, while females were attracted to neither sex. In addition, some individual compounds were attractive to only males or to only females, and one compound repelled only females in bioassays.

GC-EAD detection and comparison of the size of antennal responses helped prioritize which compounds to test in the laboratory and field. With further investigation into these compounds, these findings may lead to improved lures for mass trapping or detection.

Survey for native egg parasitoids attacking spotted lanternfly egg masses

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Over the last few years, signs of egg parasitoids have been noted on spotted lanternfly (SLF) egg masses in its invasive range in Pennsylvania and the surrounding area. Signs of native parasitoids include prior reports of attack by the egg parasitoid, *Ooencyrtus kuvanae*, whose primary host is *Lymantria dispar dispar* [1]. Additionally, observations of an *Anastatus*-like wasp interacting with SLF egg masses and emergence holes from prior year SLF egg masses that are consistent with those of *Anastatus* sp. have been noted. While these observations of attack have been infrequent, further investigation is warranted to identify native parasitoids and validate if they are providing sufficient control of SLF populations.

Wasp parasitism was detected at 18 locations spanning Pennsylvania, Delaware, Maryland, and West Virginia in SLF egg mass collections made over the 2020 to 2021 winter (Figure 1). From these locations, 720 current-year egg masses were collected and reared out to identify live parasitoids. Four adult wasps emerged, and two additional wasps that never completed development were subsequently dissected out of the remaining eggs. None of the reared specimens were identified as *O. kuvanae*. Instead, the reared wasps were tentatively determined to be a male *Anastatus* sp., an encyrtid, and a male and female megaspilid (likely *Dendrocerus* sp.). Morphological and molecular approaches confirmed the identification of the *Anastatus* wasp but the species remains unknown. Identification work on the remaining three species is ongoing. The two parasitoid wasps that were dissected out of the SLF eggs were also molecularly determined to be *Anastatus* sp., however comparison of the DNA sequences suggests that they are a different species than the *Anastatus* that was reared out. Interestingly, this second *Anastatus* species matched the sequences of an *Anastatus* wasp that was reared out of a stink bug collected in Newark, Delaware. Molecular tools were also utilized to analyze SLF eggs with wasp emergence holes collected in prior years to identify emerged parasitoids (see Wu, pg. 32).

Analysis of field collections revealed that attack rates by native parasitoids are very low (0.7% of egg masses and 0.04% of individual eggs are parasitized), which indicates that native or resident wasps are not providing population level control of SLF at this time. Therefore, other methods such as classical biological control are needed to manage SLF populations.

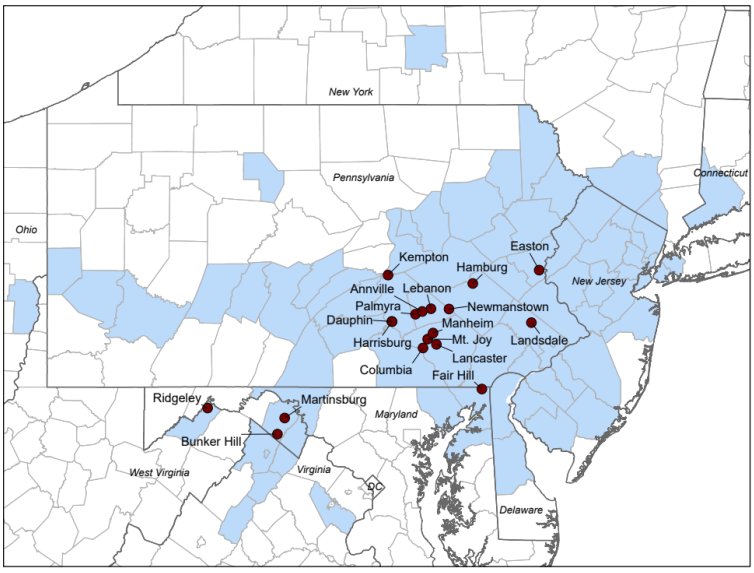

Figure 1. Sites with observed wasp emergence holes between 2020 and 2021 denoted by red dots. Counties with SLF infestation as of summer 2021 are shaded in blue. Map made by Melissa Warden.

Reference

1. Liu H. Occurrence, seasonal abundance, and superparasitism of *Ooencyrtus kuvanae* (Hymenoptera: Encyrtidae) as an egg parasitoid of the spotted lanternfly (*Lycorma delicatula*) in North America. Forests. 2019 Feb;10(2):79.

Host specificity testing of the egg parasitoid *Anastatus orientalis* (haplotype C) reveals non-specificity towards spotted lanternfly

Hannah Broadley[1], Steven Sipolski[1], Danielle Pitt[1,2], Tyler Hagerty[3], Lisa Tewksbury[4], Kim Hoelmer[5], Joe Kaser[5], Charles Bartlett[3], Joseph Elkinton[2], and Juli Gould[1]

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Classical biological methods are being developed to help manage populations of spotted lanternfly (SLF). Of particular interest is *Anastatus orientalis*, an egg parasitoid wasp that has caused significant SLF mortality in its native range of China [1]. By sequencing a portion of the COI barcoding region, we have determined that *A. orientalis* is comprised of distinct haplotypes, which we refer to as haplotype A, B, C, and D. Because haplotypes are genetically distinct, they must be tested as separate candidate biological control agents. Haplotype C was the first haplotype that we discovered and the first haplotype from which we developed a laboratory colony, and so physiological host range testing has been completed first for this haplotype.

No-choice and choice tests were performed on non-target species of eggs, representing a range of species corresponding to taxonomic relatedness as well as having morphologically similar egg masses and occupying the same microhabitat as SLF. We found that *A. orientalis* Haplotype C did not successfully reproduce using the stick bug, mantis, lady beetle, or cockroach egg masses when presented, but they could attack and successfully develop to greater or lesser degrees in 13 more closely related non-target egg masses (Figure 1). *Anastatus orientalis* demonstrated a particularly high attack rate on the silk moth eggs presented and produced both male and female progeny. Choice tests showed that in most cases when *A. orientalis* was presented with SLF eggs along with non-target eggs, SLF was preferred; however, for some silk moth species parasitism was still significant.

Anastatus orientalis Haplotype C does not appear to be sufficiently host specific to use as a biological control agent for SLF. However, we are using the methods developed through this work to accelerate testing of two other, distinct haplotypes of *A. orientalis* (Haplotypes B and D). If either of these latter haplotypes is determined to be sufficiently host specific, they could be suitable candidates for release as classical biological control agents to aid in the management of SLF.

Reference

1. Xin B, Zhang YL, Wang XY, Cao LM, Hoelmer KA, Broadley HJ, Gould JR. Exploratory survey of spotted lanternfly (Hemiptera: Fulgoridae) and its natural enemies in China. Environmental Entomology. 2021 Feb;50(1):36-45.

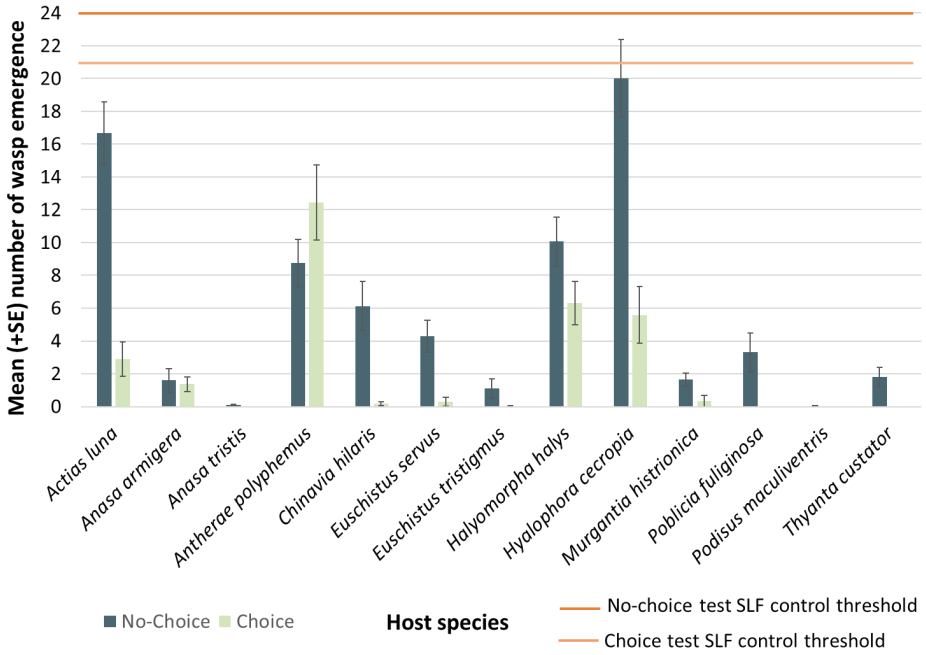

Figure 1. Mean number of wasp emergence from no-choice and choice tests of attacked non-target egg masses (bars) compared to the average attack on controls (threshold lines).

Integrating biocontrol with systemic insecticides to control emerald ash borer in urban forests

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Studies have shown that while emerald ash borer (EAB) parasitoids contribute significantly to control of EAB in regenerating ash trees, most large trees have died by the time parasitoid populations build. Outbreaking populations of EAB produce too many beetles to be controlled by parasitoids in a typical release. Ash trees are often treated with systematic insecticides that offer protection for several years. Therefore, we selected three cities (Boulder, CO; Naperville, IL; Syracuse, NY) that were already treating ash trees as sites in which to conduct parasitoid releases. We assessed parasitism rates in these areas to determine whether insecticide application could cease once parasitoid density increased sufficiently to control EAB.

From 2015 to 2020, two larval parasitoids, *Tetrastichus planipennisi* and *Spathius galinae*, and one egg parasitoids, *Oobius agrili*, established in all three cities. The larval parasitoids additionally showed range expansion past their release sites. In 2019, insecticide treatments ceased in order to determine whether the established parasitoids were sufficient to control EAB. Sentinel logs containing EAB larvae were readily attacked by both *T. planipennisi* and *S. galinae* throughout the summer of 2020. We collected and peeled ash branches each winter and found that by 2020 parasitism of EAB, calculated on a per plot basis ranged from 18-46%, woodpecker predation was between 21-67%, and the density of live EAB larvae had declined. By 2020 many untreated trees were dead or rapidly declining in health, while treated ash trees remained healthy with only a few showing signs of canopy decline.

Parasitoids established and dispersed throughout the cities and EAB density declined to low levels. Now that insecticide treatments have been stopped, we will continue to monitor the health of ash trees and the persistence of the parasitoids at these sites to determine the long-term impact of parasitoids released against EAB in urban forests. As EAB invades more and more urban forests, this study provides evidence that integrating parasitoid releases and treatment of trees with systemic insecticides is a promising tool against EAB. This study has helped provide the EAB Program with tools they can recommend to stakeholders.

Figure 1. Recovery of *T. planipennisi* in yellow pan traps throughout release (top) and control (bottom) plots in Syracuse NY in 2020.

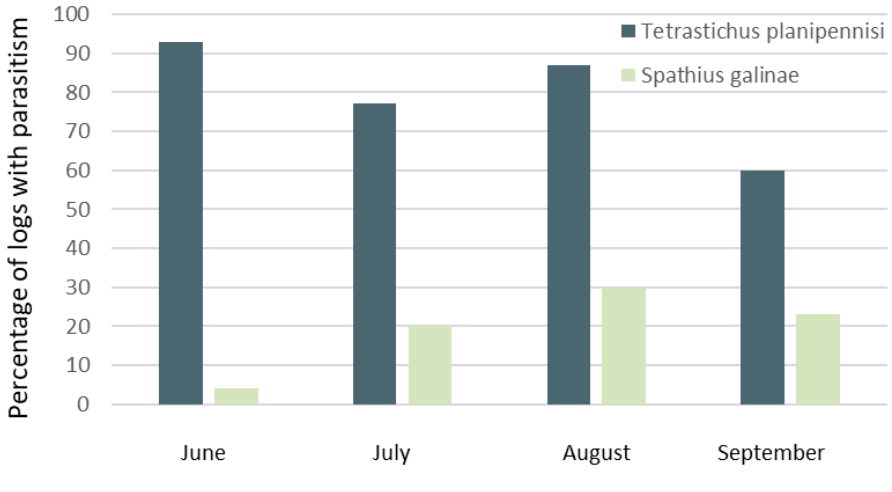

Figure 2. Levels of parasitism by *T. planipennisi* and *S. galinae* deployed in sentinel logs on emerald ash borer throughout the summer of 2021 in Syracuse, New York.

Towards the development of polyphagous shot hole borer biocontrol agents: ambrosia beetle rearing in wood bolts

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Parasitoids of the invasive polyphagous shot hole borer (PSHB) must be studied in the laboratory setting to determine host specificity and efficacy as biocontrol agents before they can be utilized in an integrated pest management strategy. To improve existing protocols, we developed a new rearing method for PSHB and their associated parasitoids that better simulates natural living conditions. The revised protocol uses live wood bolts as an alternative to artificial diet or cuts of wood with waxed ends. An increased production of PSHB and its parasitoids will help to facilitate necessary research in support of an integrated pest management strategy to control its spread.

Figure 1. An 8-week-old shot hole borer rearing setup, with visible branches and leaf growth.

In the revised PSHB rearing protocol, adult beetles are introduced to sealed jars containing a cut of wood which is grown using a simple hydroponic setup (Figure 1). A range of treatments are utilized to reduce mold growth on the wood, bacterial growth in the water, and mite movement between bolts. In trials where 20 mated female PSHB were introduced to bolts of red maple, an average of 4.4 adults successfully bored in and created reproductive galleries. By week 8, newly emerged adults from the next generation had created an average of 28.8 galleries. The revised protocol allows for the continuous reproduction of PSHB on the rearing material for over 16 weeks, representing a major improvement over the previous protocol. Previously, rearing material only remained viable for 12 weeks with limited offspring production seen after 9 weeks. The improved viability of the rearing substrate permits better PSHB larval development, which will allow for subsequent parasitization by wasps and emergence of a new wasp generation that can be studied.

Improved methods for rearing parasitoids of PSHB will allow for a more efficient assessment of host-specificity and evaluation of potential biocontrol agents. Effective rearing methods will also be important for production of PSHB parasitoids, should they be approved for release as biocontrol agents.

Test shipment and quality assessment of white oak veneer logs treated with vacuum and steam

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[2]Virginia Tech University, Department of Sustainable Biomaterials, Blacksburg, VA

The current methyl bromide schedule for oak wilt and oak log export is among the largest used in the USDA Treatment Manual, and methyl bromide alternative development has been identified as a high priority for PPQ. The recent ban by the European Union on imported oak logs treated with methyl bromide has hastened the development of alternatives to support industry. The purpose of this work was to provide quality assurance of combined vacuum and steam treatment to high value, veneer quality white oak logs that are intended for export to Europe. In cooperation with Virginia Tech, vacuum and steam processing has been researched as a methyl bromide alternative by PPQ for logs and other durable commodities [1,2,3,4].

Figure 1. Vacuum steam treatment includes initial processing of white oak logs grouped in a steel chamber

To satisfy industry concerns that vacuum and steam can be a viable treatment option without negatively impacting quality, a treatment experiment was conducted in Elkton, Maryland on veneer quality white oak logs paired with untreated controls. A total of 17 logs were treated to 56°C for 30 minutes at a targeted treatment depth of 5 cm, then placed in a 20 ft shipping container. Untreated control logs were handled similarly and placed in a separate 20 ft container. Both containers of logs were subsequently shipped to Danzer Bohemia in Melnick, Czech Republic for final veneering and grading. The grading staff determined no appreciable difference between treated and untreated veneer samples over a range of metrics that included color, quality, and overall yield.

The results of this study will support a proposed partnership between APHIS-PPQ, industry, and states to place the first commercial vacuum and steam treatment chamber for export logs in the United States. Industry interest in placing additional chambers is expected to grow as methyl bromide use is further restricted worldwide, helping to positively address a major PPQ program goal.

References

1. Bragard, C., K. Dehnen-Schmutz, F. DiSerio, M. Jacques, J. Miret, A. Justesen, A. MacLeod, C. Magnusson, P. Milonas, J. Navas-Cortes, S. Parnell, R. Potting, P. Reignault, H. Thulke, W. van der Wert, A. Civera, J. Yuen, L. Zappala, A. Battisti, J. Douma, D. Rigling, O. Mosbach-Shulz, G. Stanacelli, S. Tramontini, and P. Gonthier. 2020. Commodity risk assessment of oak logs with bark from the US for the oak wilt pathogen *Bretziella fagacearum* under an integrated systems approach. EFSA Journal. 18(12):6352 DOI: 10.2903/j.efsa.2020.6352
2. Chen, Z., M. White and R. Mack. 2017. Evaluating vacuum and steam process on hardwood veneer logs for export. European Journal of Wood and Wood Products. 75:911-918.
3. Juzwik, J., A. Yang, Z. Chen, M. White, S. Shugrue and R. Mack. 2019. Vacuum steam treatment eradicates viable *Bretziella fagacearum* from logs cut from wilted *Quercus rubra*. Plant Disease. 103:276-283.
4. Juzwik, J., A. Yang, S. Heller, M. Moore, Z. Chen, M. White, H. Wantuch, M. Ginzel, and R. Mack. 2020. Vacuum steam treatment effectiveness for eradication of the thousand cankers disease vector and pathogen in logs from diseased walnut trees. Journal of Economic Entomology. 114:100-111.

Sampling and identification of insects on Peru asparagus intended for export to the United States

Scott Myers[1], Amanda Davila-Flores[1], Mandy Furtado[1], Melissa Warden[1], Kendra Vieira[1], and Yunke Wu[1,2]

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A project was developed to generate qualitative and quantitative data on insect pests associated with Peru asparagus exports to the United States. The data generated from this project was used to better inform our understanding of potential pests associated with Peru asparagus imports. This project supports the Agricultural Quarantine Inspection (AQI) program as it informs PPQ on the need for phytosanitary measures to prevent potential pest introduction and establishment.

A cooperative project between USDA and Servicio Nacional de Sanidad Agraria del Perú (SENASA) was established in 2019 to conduct sampling during the asparagus growing season across regions in Peru exporting asparagus to the U.S. market. Asparagus packing houses were selected for sampling based on their volume of production. During the 30-week sampling period 46,800 spears were evaluated by SENASA inspectors at 34 packing houses. Insects were found on 0.71% of spears and 0.37% were infested with regulated pests.

Results suggest that there are no differences in the rate of infestation or the geographic distribution of regulated pest among the asparagus production regions in Peru. Of the 289 specimens molecularly identified, the most significant regulated pest detected was *Helicoverpa armigera*, which represented almost half of the total samples (47.6%) received (Figure 1). Its ability to establish across a broad geographic range makes *H. armigera* a pest of concern for the U.S. Six samples were identified to the genus *Copitarsia* with two of these determined to be *C. corruda* with high confidence. Sequencing data suggest that all six samples are likely *C. corruda* or close relatives. The *Copitarsia* genus, and *C. corruda* specifically, have been associated with Peru asparagus in the past and aspects of their potential risk have been previously covered in USDA risk assessment documents. Several other regulated plant pests were also identified, including one individual each of *Agrotis experta*, *Spodoptera ochrea,* and *Helicoverpa atacamae*. Each belongs to a genus that includes other economically important plant pests.

The results of this project are being used to support risk assessment activities and to evaluate the efficacy of phytosanitary measures for Peru asparagus imports. A full report on the project can be found on SalesForce.

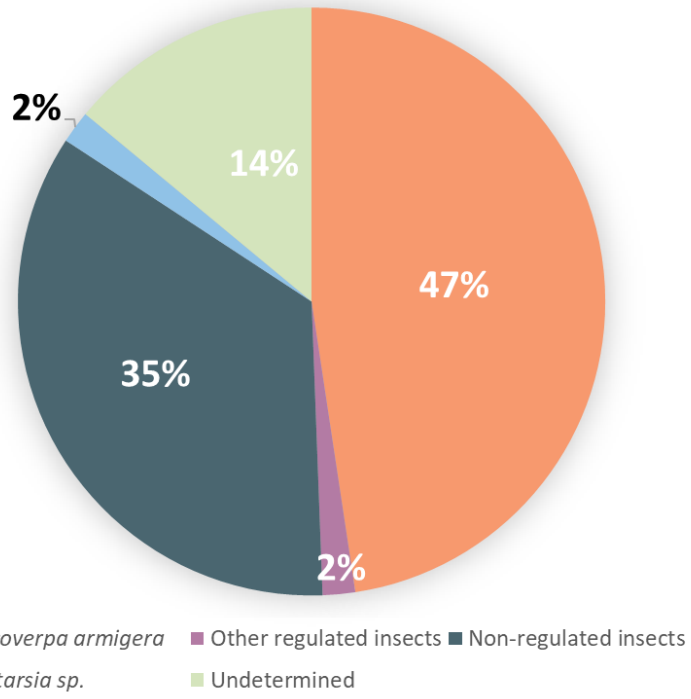

Figure 1. Insects found on Peru asparagus exports.

Potential competitive displacement of native Dermistidae by khapra beetle

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The conditions under which the quarantine pest, khapra beetle, *Trogoderma granarium*, is likely to displace a native grain pest, *Trogoderma inclusum*, were explored by rearing the two species together in different commodities at different temperatures. Understanding the environmental conditions that would allow khapra beetle to displace native dermestids will improve khapra beetle invasion risk assessment. Certain geographic areas may be more prone to khapra beetle invasion depending upon existing pests, climactic factors, and the related management of domestic pests. Enhanced targeted monitoring based upon knowledge of these risks may help prevent khapra beetle establishment.

In a nine-week study, we examined direct competition between the two species, beginning at the adult life stage, on three commodities (corn, wheat, and rice) at either 25 or 32° C. Larval offspring of the species required molecular identification using a restriction fragment length polymorphism method developed at the Forest Pest Methods Laboratory (see Wu, pg. 30). Of 720 larvae sampled, 689 specimens (95.7%) were identified. There were no adult offspring. Commodity type did not appear to influence the proportion of *T. granarium* vs. *T. inclusum*. However, temperature proved to be a strong influence driving the native species to outproduce khapra beetle by 63% at 25°C and 94% at 32°C.

In a subsequent twenty-five-week study, the same two temperatures were compared. In the long-term study, only the wheat commodity was used since competition was not strongly affected by commodity type and wheat resulted in the greatest offspring production at nine weeks. An additional treatment was added by starting the competition events with larvae instead of adults. When starting with larvae at 25°C, both species fared equally well with 44% of all offspring being *T. granarium* (Figure 1). At 32°C the proportion of *T. granarium* rose to 86%. When starting with adults, the proportion of *T. granarium* was very low (<10%) at twenty-five weeks, regardless of temperature.

Cross rearing experiments starting with larvae rather than adults provided critical insight into how *T. granarium* may displace *T. inclusum*. At higher temperatures, *T. granarium* was favored in larval competition studies, while the inverse was shown in adult competition studies. These results emphasize the need for improved detection and mitigation of *T. granarium* larva rather than adults.

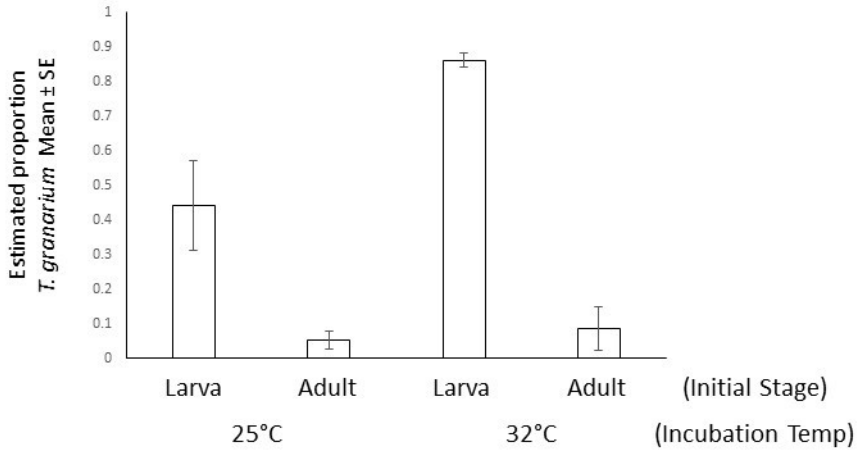

Figure 1. The proportions of *T. granarium* among all offspring in mixed twenty-five-week crosses, separated by life stage used to initiate competition and rearing temperature.

Assessment of khapra beetle movement on substrates to improve trapping approaches

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We examined the climbing ability of khapra beetle, *Trogoderma granarium*, larvae on different substrates as well as the effect of insecticides on their movement (Figure 1). These investigations contribute to a larger effort to develop and improve detection methods for the PPQ Khapra Beetle Program.

Using a plexiglass cube with a concrete floor, exchangeable wall surfaces, and an adjustable platform, we were able to assess larval movement across different surfaces at varying angles. Results showed that sheetrock, plywood, masking tape, and laboratory tape are optimal for khapra beetle climbing [1]. These substrates all share a similar paper-like texture. Cement and other rock-like surfaces also allow climbing but are less ideal, while metal, plastic, or glossy paint-covered surfaces cannot be climbed.

It was determined that khapra beetle larvae can climb on netting impregnated with deltamethrin insecticide (Figure 1D), but short exposures to the insecticide interrupted the ability of larvae to respond to attractive odors [1,2]. In contrast, larvae do not move well on insecticide impregnated plastic bags (Figure 1E) [1,3]. These results indicate that insecticide-treated surface would be challenging to use in an attract-and-kill device. Alternatives such as biopesticides may be considered in the future if they are determined to be more attractive.

Tapes with paper-like surfaces have been shown to increase climbing on metal, plastic and other surfaces that are not conducive to climbing. Insecticide treatment of surfaces negatively impacts semiochemical attraction, making their adoption into attract-and-kill devices challenging. Future work will explore the possibility of using tape corridors that proceed upward toward the wall traps. Understanding the optimal surfaces that facilitate khapra beetle climbing will allow for improved recommendations to the program for the placement of wall traps.

References

1. Domingue MJ, Scheff DS, Leva N, Myers SW. Climbing ability of *Trogoderma granarium* larvae on artificial household and insecticide-treated materials. J Stor Prod Res. 2022; 95:101922.
2. Domingue MJ, Scheff DS, Arthur FH, Myers SW. Sublethal exposure of *Trogoderma granarium* everts (Coleoptera: Dermestidae) to insecticide-treated netting alters thigmotactic arrestment and olfactory-mediated anemotaxis. Pest Biochem Physiol. 2021; 171:104742.
3. Scheff DS, Baliota GV, Domingue MJ, Bingham GV, Morrison WR, Athanassiou CG. Evaluations of the new deltamethrin-treated all-in-one hermetic bag for the control of the Khapra beetle, *Trogoderma granarium* (Everts). J Stor Prod Res. 2022; 93: 101839.

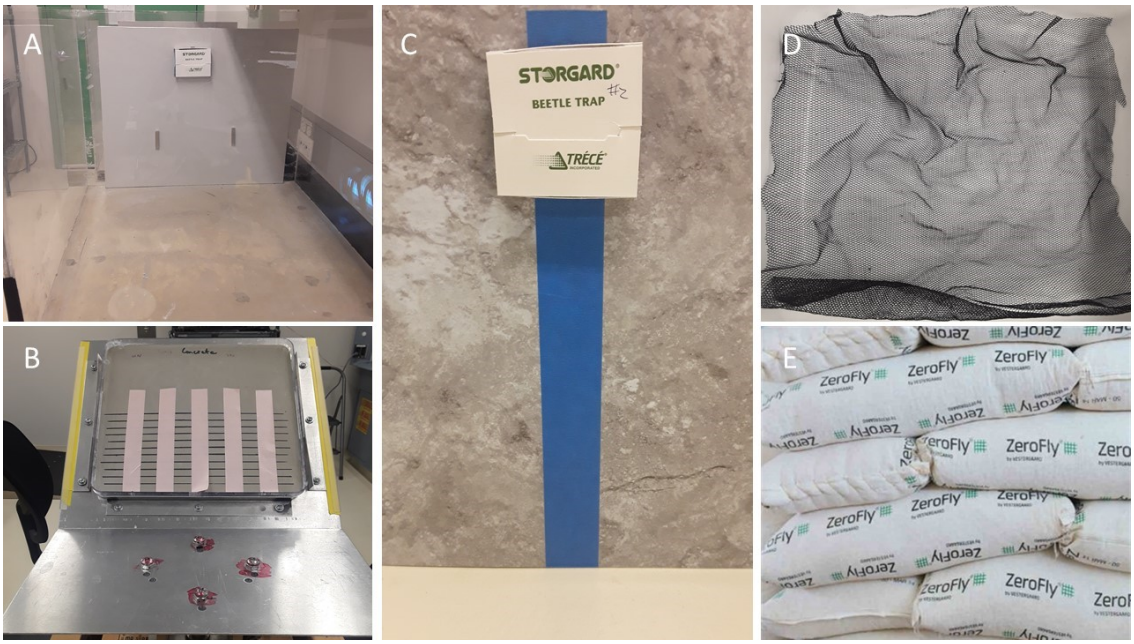

Figure 1. Materials used to assess the ability of *T. granarium* to walk on different substrates on different surfaces toward traps including A) a plexiglass cube hosting an exchangeable wall surface, B) an adjustable platform allowing assessment of different surfaces across varying angles, C) a tape corridor leading toward a wall trap, D) deltamethrin-treated netting, and E) deltamethrin-treated-bags.

Establishment and monitoring of a biocontrol agent of Japanese beetle at key air cargo airports

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Under the right conditions, Japanese beetle (JB), *Popillia japonica*, have been known to congregate near cargo planes and loading areas and can ultimately end up inside of airplanes. Work is being conducted to establish *Ovavesicula popilliae*, a biological control pathogen specific to JB, at major cargo airports in the Eastern and Midwestern U.S. where JB pose the highest risk of being transported. Successful establishment of this pathogen has resulted in significant suppression of JB populations [1], thus greatly reducing JB risk to air cargo operations and protection of agricultural resources.

The JB biological control effort was initiated in 2017 with the participation of PPQ personnel working at six cargo airports in five states. By the 2021 field season, participation in pathogen release and monitoring activities increased to 12 cargo airports in nine states (Figure 1). Seven of the release sites now have epizootic levels of *Ovavesicula popilliae*. The remaining sites are showing positive trends towards biocontrol establishment. Additionally, a molecular PCR tool was developed to screen for pathogen DNA in infected JB, replacing and improving upon a physical dissection method previously utilized [2]. The molecular assay is conducted at the Forest Pest Methods Laboratory to monitor infection levels of JB populations at release sites and to confirm when biological control has been achieved.

Japanese beetle population levels naturally fluctuate, but as the JB pathogen is established beetle numbers rapidly decline and remain low. The pathogen does not spread rapidly on its own, so purposeful introduction and verification of pathogen establishment is important. For sites where the pathogen is established, live infected JB can be collected and moved anywhere within that state to accomplish JB biocontrol where it may be needed. In fact, PPQ officers in Illinois have begun collaborating with university and ARS researchers to establish six regional 'nursery' sites down state to spread the pathogen to impacted businesses and industries including wineries, golf courses, orchards, botanical gardens, etc. The work described has been instrumental in supporting JB control efforts and therefore protecting the agricultural resources of the western states from rapid incursion and damage by Japanese beetle.

References

1. Piombino, M., D. Smitley, and P. Lewis. 2020. Survival of Japanese beetle, *Popillia japonica* Newman, larvae in field plots when infected with a microsporidium pathogen, *Ovavesicula popilliae*. J. Invertebr. Pathol. 174, 107434.
2. Hulbert D., D. Smitley, E. Hotchkiss, P. Lewis, Y. Wu, and J. Smith. 2020. Geographic distribution of *Ovavesicula popilliae* in the United States and sensitivity of visual diagnosis compared with qPCR detection. J. Invertebr. Pathol. 175, 107455.

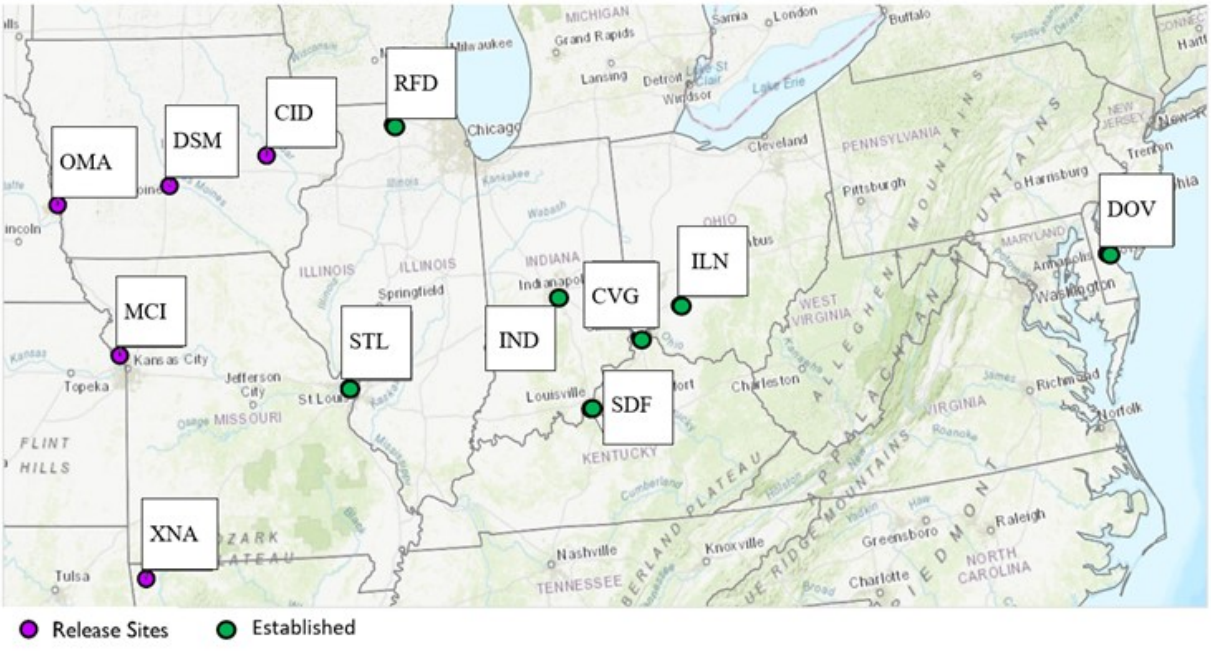

Figure 1. *Ovavesicula popilliae* airport release sites (2017 to 2019) by 3-digit airport code and current establishment status.

Confirmation of an effective control method for spotted lanternfly egg masses

Phil Lewis[1], Amanda Davila-Flores[1], and Emily Wallis[1]

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A simple treatment for spotted lanternfly (SLF) egg masses, using a soybean oil product (Golden Pest Spray Oil, GPSO), was found to be very efficacious over several seasons of research. A pilot study was then planned utilizing field staff working for the SLF Program. Detailed protocols for treatment and treatment monitoring were developed, along with a training video and a geographic information system (GIS) application. The application allowed field crews to establish and monitor efficacy by comparing untreated egg masses to those treated by crews from four states over a 4-month period. Over 1,200 SLF egg masses were assessed post-hatch by direct visual inspection or by assessment of an image uploaded to the GIS application.

The field trial demonstrated that GPSO applied to SLF egg masses in a 1:1 mixture with water is a highly effective treatment (>10-fold reduction in nymphal hatch based on 2,706 nymphs hatching out of an expected 34,452) and can be applied to SLF egg masses at any time of year. Soybean oil treatment completely prevented nymphal hatch in almost 70% of the egg masses. Besides the suppression of egg hatch, it is notable that there was a large reduction in the number of egg masses that yielded ≥ 11 nymphs following treatment. Typically, the majority of eggs hatch in untreated SLF egg masses, however there was a 5-fold decrease in that hatch category when egg masses were treated with GPSO (Figure 1).

This research effort provides the SLF Program with an effective treatment option beyond physical destruction/scraping of egg masses. Field crews can treat egg masses with GPSO when temperatures are 40°F and above. The protocols and training video have been incorporated into the operational manual and are available to field operations crews. The GIS tool allows crews to set up sites, monitor and determine the effectiveness of the treatments over time, and allows for feedback to the SLF Program as new states and field crews begin their own SLF control activities.

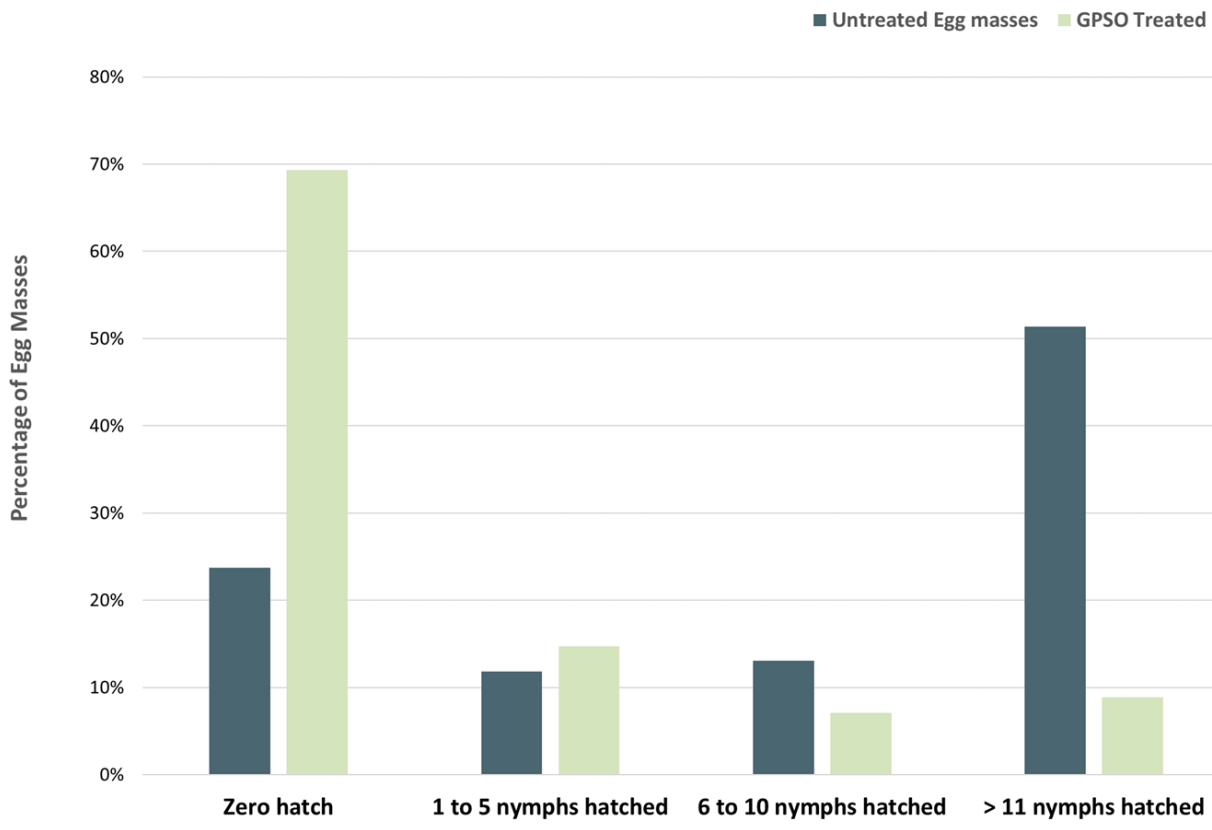

Figure 1. Spotted lanternfly hatch comparison between untreated and soybean oil treated egg masses.

Determining effective range and potential for non-target drift of A1 mist sprayer

Baode Wang[1], David Cowan[1], Scott Myers[1], and Scott Pfister[1]

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Studies were performed to evaluate the A1 "Super Duty" mist sprayer with 8004 and 8006 flat fan nozzles as a possible control tool for the Spotted Lanternfly (SLF) Program. We determined the optimal parameters, the effective range, and non-target drift of the A1 mist sprayer (Figure 1).

Field tests using moisture activated cards arranged at various distances in a mixed woodland indicate that the 0° spray angle produced more coverage on spray cards than both the 45° spray angle and the 0-80° oscillating spray angle for both nozzle types. As expected, spray coverage decreased further from the mist sprayer, with spray cards placed 20-60 ft away from the sprayer showing the most coverage. At distances beyond 100 ft, little to no stains were observed on cards, suggesting that such distances are outside the effective coverage range of the mist sprayer.

To monitor for possible drift, cards were placed behind the sprayer at 20 ft and 50 ft, opposite of the intended spraying direction. Results from these cards showed minimal staining. Additionally, card staining was shown to be as low as 0.01% at a distance of 71 ft and at a 39° angle from the spray direction. Together, these results show that the potential for non-target drift was found to be minimal. This is especially true when applications were made within the recommended wind speed limit of 10 mph.

This project details the performance of the A1 mist sprayer under actual field conditions and provides spray nozzle and cannon angle recommendations that will enable the SLF Program to best utilize the equipment in eradication and suppression efforts. Based on calibration and field testing results, the A1 mist sprayer should be used with the following settings for maximum benefits: 1) vehicle speed of 2 mph, 2) operating pressure at 40 PSI, 3) either 8004 and 8006 nozzles with 4 nozzles installed, and 4) a 0° spray angle. Under these settings, adequate droplet coverage (≥2% stained area) can be achieved from ground level up to 10 ft high.

Figure 1. A1 "Super Duty" mist sprayer set up on a vehicle for field use.

Effect of high temperature treatment on survival of spotted lanternfly eggs

Baode Wang[1], David Cowan[1], and Scott Myers[1]

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Experiments were performed to develop a feasible method to mitigate risk of the spottled lanternfly (SLF) spreading through means of transportation. The two main objectives were to: 1) determine the minimum water temperature and duration required to kill SLF eggs when washing vehicles and equipment, and 2) determine the general heat tolerance of SLF egg masses to hot air and hot water.

Field collected SLF egg masses were exposed to different temperatures and durations of water submersion as well as hot air. Hatch rates were compared to determine the temperature and duration combination that resulted in no nymph hatch, and thus, total mortality.

Water temperature and submersion duration, as well as their combined effects, all contributed significantly to preventing hatch in field collected SLF egg masses. The minimum water bath temperature required to prevent hatch was 50°C at a duration of 210 seconds. Mortality was also reached at higher temperatures with shorter durations. Less than 0.5% hatch was found in the water bath treatment of 80°C and 2 seconds (Table 1).

Additional testing showed that no hatch was observed in the submersion treatment of 80°C and 10 seconds. Exposure to hot air at 40°C for 30 minutes and 50°C for 10 minutes did not affect hatch rate compared with the control. However, hot air exposure at 45°C for 6.5 hours resulted in no hatch.

These results provide useful information regarding temperature and submersion duration that can be applied to wash protocols for vehicles, equipment, and media to mitigate the spread of SLF. Hot water exposures of 80°C for 2 or 3 seconds or at 60°C for 15 seconds provided a high level of control in preventing SLF egg hatch.

Table 1. Treatment temperatures and durations of SLF egg masses immersed in water. Durations shown in **blue** represent treatments with >0.5% hatch rate while those in **orange** represent treatments with <0.5% hatch rate.

Temperature (°C)	Treatment duration (sec)	Treatment duration (sec)	Treatment duration (sec)	Treatment duration (sec)
20	0	1	60	180
50	1	90	180	210
55	1	30	45	70
60	1	15	30	45
70	1	5	10	15
80	0.5	1	2	3

Asian gypsy moth trapping at U.S. military bases in Japan and the Republic of Korea

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Populations of Asian gypsy moth (AGM; *Lymantria dispar asiatica/japonica*, *L. albescens*, *L. postalba, and L. umbrosa),* were monitored at U.S. military bases in Japan and the Republic of Korea to provide support for PPQ's AGM Preclearance and Offshore Program. Adult moth trapping was conducted to investigate the duration and intensity of the moth flight period in support of NAPPO RSPM 33, Guidelines for Regulating the Movement of Vessels from Areas Infested with the Asian Gypsy Moth.

1,284 total AGM were trapped in the Republic of Korea at four military bases—Camp Casey, Camp Humphreys, Camp Carroll, and Camp Walker (Figure 1). Moth flight began in late-June and tapered off by mid-August. In Honshu, Japan trapping was conducted at Camp Zama, Sagami General Depot, and Yokohama North Dock, resulting in capture of 142 total AGM. Based on trapping in Honshu, it appeared that AGM flight began in late June and ended by early August. Additionally, 25 total AGM were caught in Okinawa, Japan at Naha Port and Torii Station.

In 2021, population levels of AGM at trapping sites in the Republic of Korea dropped substantially from the numbers seen in 2020 [1]. In Honshu, Japan population levels also declined, while levels at the sites in Okinawa, Japan remained very low. This trapping survey provided PPQ with valuable information that aids in the determination of relative risk and timing of AGM translocation from these geographic areas.

Reference

1. Cowan, D., Wang, B., Asmundsson, I., Vieira, K., Wu, Y., Palmeri, M., Mackay, J. and Leva, N. Asian gypsy moth trapping at U.S. military bases in Japan and the Republic of Korea. 2020. pp. 37-38. In: Trepanowski N, Viera K, Booth (eds). 2021. Accomplishments Forest Pest Methods Laboratory. United States Department of Agriculture, Buzzards Bay, MA.

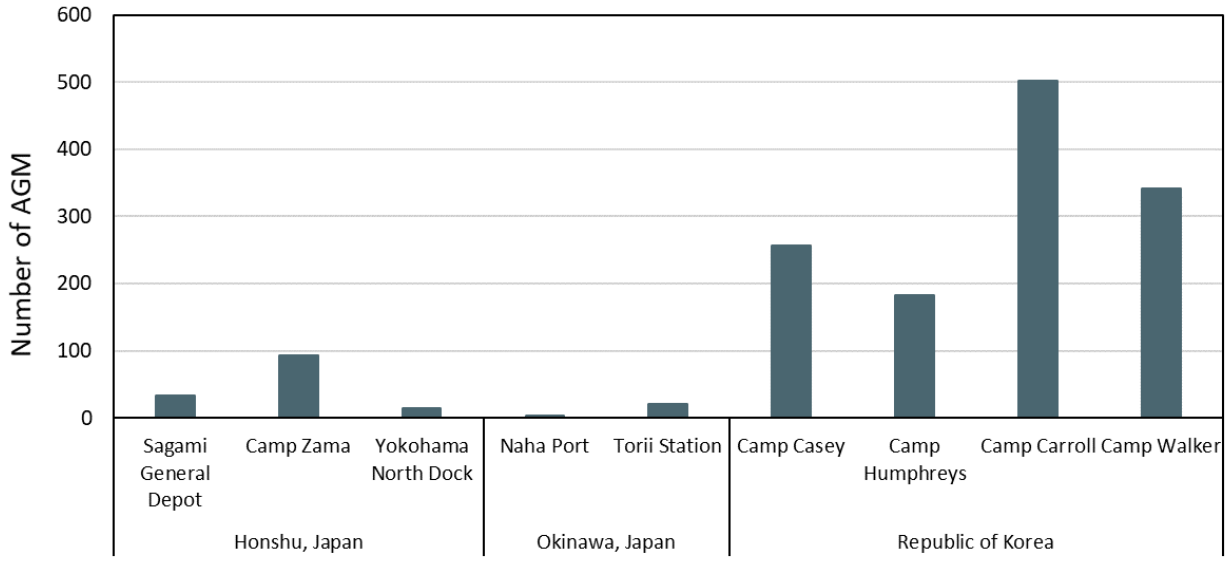

Figure 1. Asian gypsy moth trapping results for Japan and the Republic of Korea in 2021.

2021 Forest Pest Methods Laboratory insect production

Hannah Nadel[1], Carrie Crook[1], Lara Day[1], Mauri Hickin[1], Andrew Landis[1,2], Hannah Landers[1], Sue Lane[1], Chris McCallum[1], Evelyne Barratelli[2,3], and Erica Martin[2,3]

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[2]University of Richmond, Department of Biology, Richmond, VA
[3]Emerald Ash Borer Biocontrol Rearing Facility, USDA APHIS PPQ FO, Brighton, MI

Insects were reared for research and outreach at the Forest Pest Methods Laboratory (FPML) and the Emerald Ash Borer (EAB) Biocontrol Rearing Facility. Rearing efforts supported the Asian Longhorned Beetle (ALB), Gypsy Moth, and EAB Programs, the Cooperative Agricultural Pest Survey (CAPS), and Agricultural Quarantine Inspection (AQI), other federal labs, and domestic and foreign academic customers. Outreach and identification materials in the form of insect specimens, displays, and digital images were provided to the Gypsy Moth Program, CAPS, the APHIS Legislative and Public Affairs Program (LPA), and the National Identification Service (NIS).

Two woodboring beetle species, ALB and EAB, were reared and/or used in support of a variety of research. Eighty-two ALB were utilized by chemical ecologists for research on attractants to support CAPS. Over 1,000 ALB larvae were provided for a study on biological control, which resulted in the development of a novel method that utilizes planting ALB larvae in sentinel logs as a tool for monitoring native parasitoids following their release. Additionally, over 500 specimens were prepared for federal and state outreach programs, and 750 eggs, larvae, and adults were sent to foreign academic researchers. Emerald ash borer and its parasitoids reared at the Brighton EAB Rearing Facility supported our work to develop a rearing system for both groups of insects without reliance on ash wood.

Over 160,000 pupae of European gypsy moth were provided to the Gypsy Moth Slow the Spread Foundation (STS) for research on integrated pest management. STS research focused on development of a system to detect gypsy moth pheromone in air to evaluate mating-disruption treatments, and to study the mechanisms of trapping and mating disruption. We also provided 3,318 egg masses to support research in federal and academic institutions on biological control, pathology, ecology, pesticide efficacy, virology, and molecular biology. Continued production of the gypsy moth virus was supported by provision of 275 egg masses to Andermatt Canada. Twenty-one gypsy moth displays and 100 specimens were prepared and provided to state plant health directors for outreach.

Tens of thousands of European grapevine moth eggs, larvae, and pupae were produced for phytosanitary treatment research conducted for AQI by the Commodity Treatment functional group at FPML. The rearing service supported progress on ethyl formate as an alternative to methyl bromide for killing the pest in imported grapes at U.S. ports of entry.

High-quality USDA stock photos of box tree moth (BTM) life stages were taken of the BTM colony for the LPA Program to increase the public's ability to detect and report sightings of this moth and to broadcast APHIS' efforts to safeguard agriculture (Figure 1) . NIS was supported by provision of BTM reference specimens of all life stages to two APHIS identifiers to confirm domestic BTM detections in infested nursery stock from Ontario, Canada. Hundreds of BTM were provided to a PPA 7721 project to improve production methods for integrated pest management research.

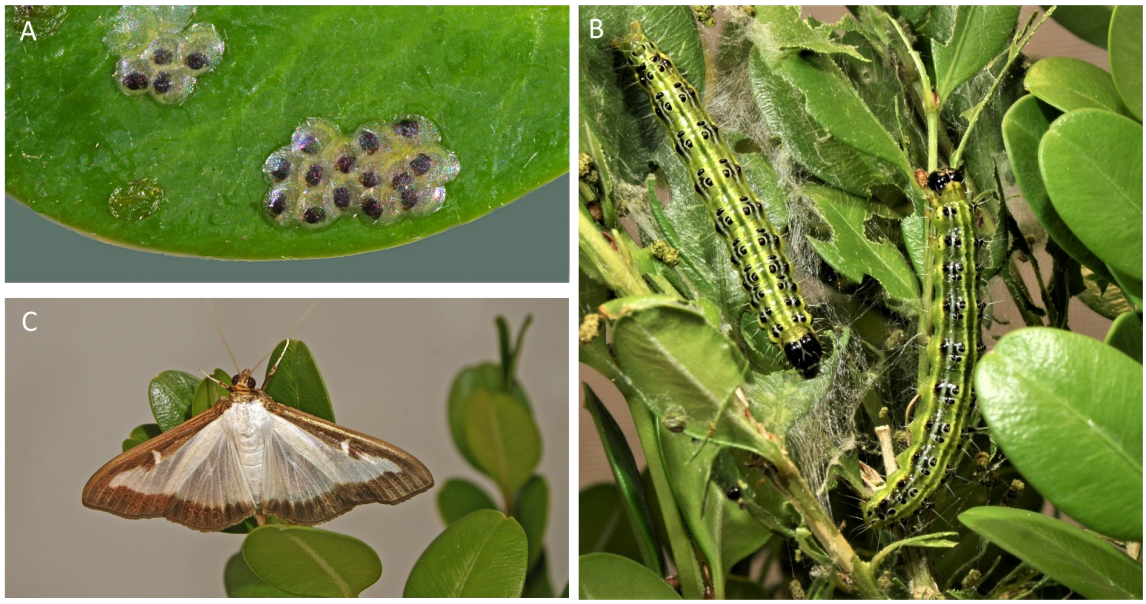

Figure 1. Box tree moth life stages. A) eggs ready to hatch, B) late-stage larvae, and C) female adult.

Improved survey methodology for spotted lanternfly: influence of host, pesticide strips, and trap check frequency on circle trap catch and detection

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Trapping assays were conducted to develop and improve trapping methods for the Spotted Lanternfly (SLF) Program, assisting PPQ, state, and local surveys for SLF in their mission to detect and control new populations. Circle traps have proven to be effective detection tools for SLF when compared with other trapping methods. In 2021, three studies were conducted to 1) compare trap catch and detection on circle traps placed on black walnut and tree-of-heaven in a multistate trapping comparison, 2) determine if pesticide strips could be removed from the circle trap protocol, and 3) determine if circle trap check frequency could be extended from the standard 2-week period to 4- or 8-week intervals.

There was no significant difference in trap catch between tree-of-heaven and black walnut for nymphs, although adult trap catch was significantly higher on tree-of-heaven. However, there was no significant difference in detection rates (the ability to detect at least one insect) between the two host trees at any life stage. Because of the comparable detection rates, black walnut can be used as an alternative host tree for survey sites when tree-of-heaven may not be available or accessible. Additionally, our studies found that traps containing dichlorvos pesticide strips did not catch significantly more SLF than traps without strips across all life stages. As a result, surveyors will be able to forego placing dichlorvos pesticide strips in circle traps, which will ease restrictions on personnel who can place and service circle traps as well as expand the areas where traps can be placed.

No significant difference was detected in season-long catch between 2-, 4-, or 8-week collection intervals, meaning trap check frequency can be extended to 4 weeks, if necessary. Less frequent checks could reduce labor and transportation as less service periods over the field season would be necessary. However, eight-week trap check intervals are not recommended due to the potential for data loss because of trap weathering or sample decomposition. The above findings will offer more flexibility to surveyors and lead to improved detection methods for the SLF program.

Table 1. Spotted lanternfly detection rates in circle traps in a multi-state host comparison between tree-of-heaven and black walnut.

Host	Early Instar Nymphs (n=26)	4th Instar Nymphs (n=21)	Adults (n=16)
Tree-of-heaven	96%	100%	100%
Black walnut	96%	86%	94%

Forest Pest Methods Laboratory CAPS lure support for the detection and survey of pest insects in 2021

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

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Every year the Forest Pest Methods Laboratory (FPML) supports the Cooperative Agriculture Pest Survey (CAPS) community with insect lures that are not always commercially available. These lures support the different insect survey efforts conducted by state and academic partners and helps PPQ safeguard U.S. agriculture and natural resources against the entry, establishment, and spread of economically and environmentally significant pests, and facilitates the safe trade of agricultural products.

Lure composition is based on the latest scientific literature, in-house research, and PPA 7721 funded research with collaborators in the U.S. and other countries. In 2020 the FPML produced 86,195 individual lures. Production totaled 96,572 in 2021, an increase of 12% over the previous year's output (Figure 1).

In addition to CAPS support, FPML performs the Quality Control (QC) of commercially purchased CAPS lures, supports the trap paperboard testing and field evaluations of commercial *Lymantria dispar dispar* string lures, as well as supporting the Forest Service with QC analysis of *Lymantria dispar dispar* mating disruption formulations. Furthermore, FPML supports various local and international research with experimental insect lure formulations.

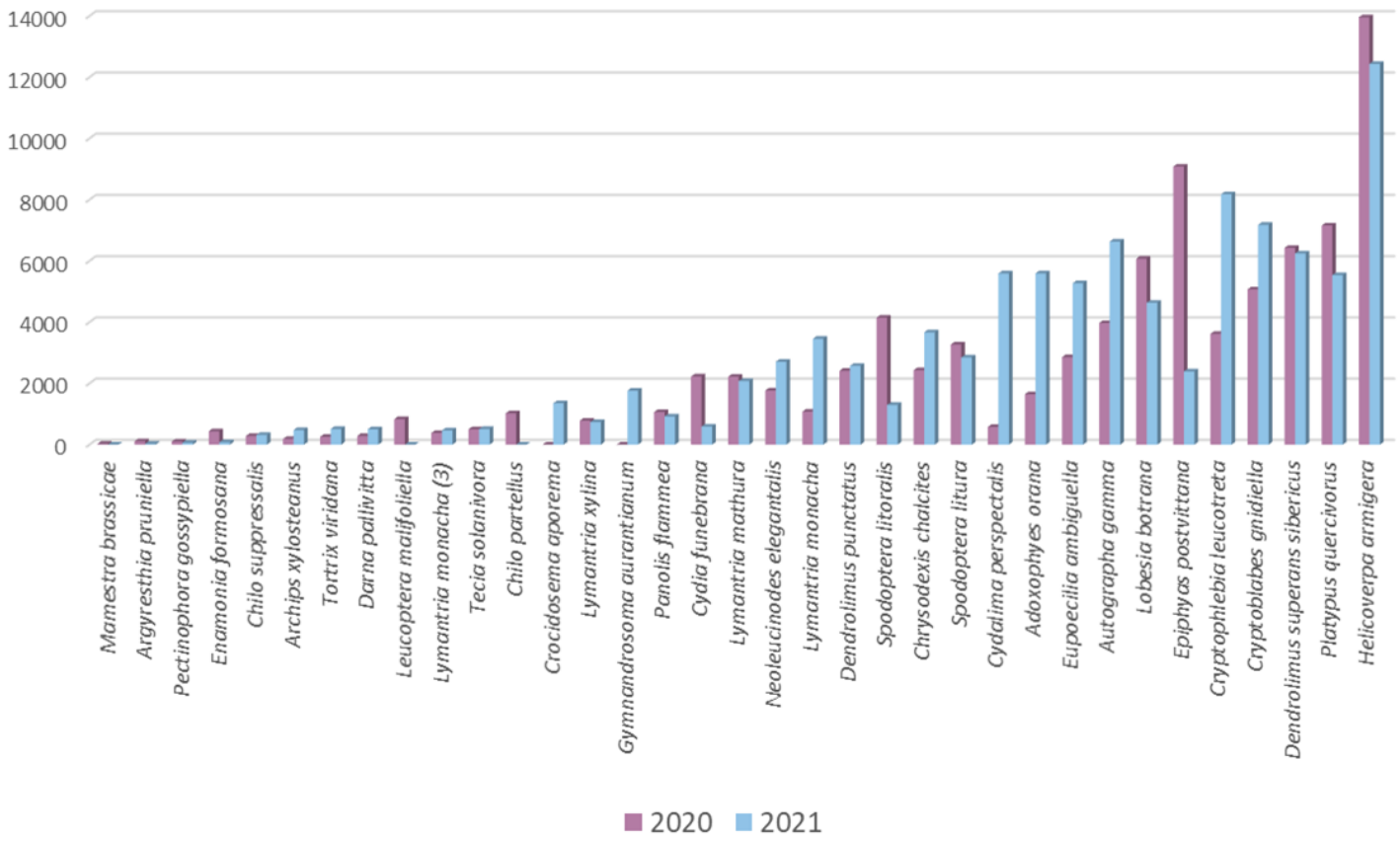

Figure 1. Forest Pest Methods Laboratory CAPS lure production in 2020 and 2021 for 35 different insect species.

2021 Port and Domestic Gypsy Moth Molecular Diagnostics Survey

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In support of the Asian gypsy moth (AGM) and European gypsy moth (EGM) detection programs, molecular diagnostic tools are utilized to identify suspect *Lymantria* specimens trapped domestically and intercepted at ports of entry.

Interceptions were made at nine U.S. ports of entry and one international airport, comprising a total of 105 specimens. Of these, 83 were identified as *Lymantria dispar asiatica/japonica* and two specimens were determined to be *L. d. dispar.* Domestically, a total of 311 trapped specimens were analyzed from areas outside of the established Federal EGM Quarantine. One *L. d. asiatica/japonica* was detected from Stevens County, WA. A total of 277 *L. d. dispar* were identified, with at least one detection from each of the 21 states that submitted from areas outside of the established Quarantine. For counties within the Quarantine, 5,842 specimens were analyzed. No *L.d. asiatica/japonica* were detected and 5,332 specimens were determined to be *L. d. dispar*.

In 2021, a recently validated real-time PCR (qPCR) diagnostic assay for the identification of *Lymantria* sp. was implemented. The qPCR assay has several advantages over the standard assay, including the capacity to distinguish between the *L.d. asiatica* and *L.d. japonica* subspecies as well as identify *L. umbrosa*. qPCR data is generated in real-time as a measure of fluorescence and therefore does not require additional and time-consuming visualization steps. Additionally, the qPCR assay is more sensitive — fewer DNA copies are required to generate positive signals— and therefore can reduce assay failure caused by poor specimen condition. The qPCR assay was used to supplement the standard diagnostic assay data obtained in 2021 and reduced the failure rate substantially (Figure 1). For the 2022 survey season, the gypsy moth qPCR assay will replace the standard assay as the main diagnostic method utilized for analyzing specimens.

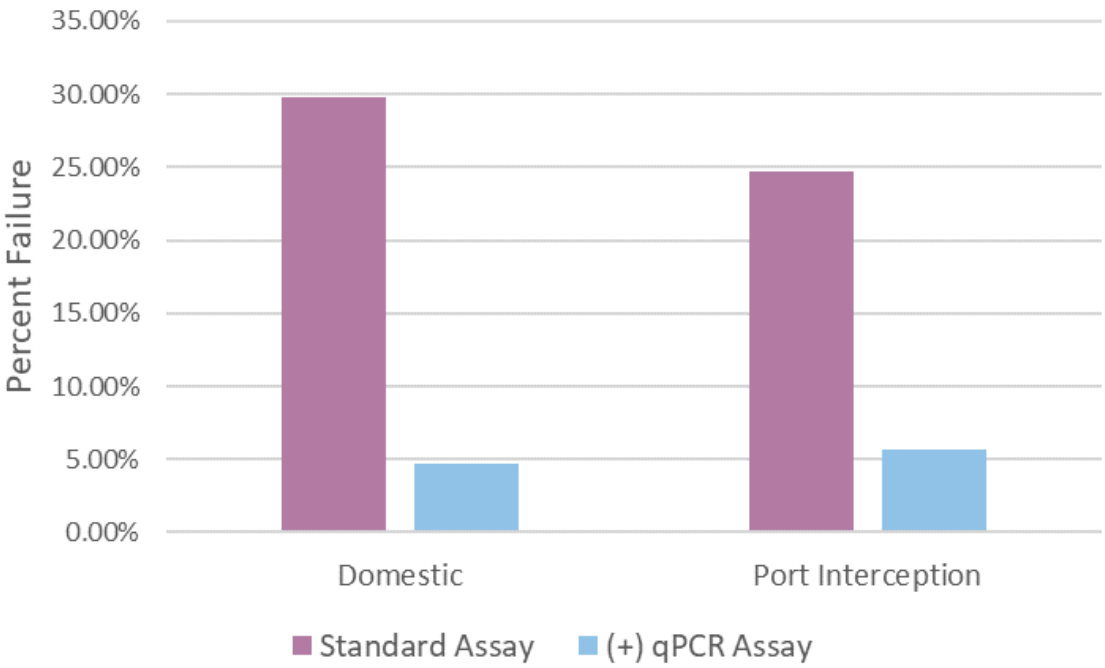

Figure 1. Comparison of the molecular assay percent failure for the domestic and port interception surveys when the standard assay was used alone versus when it was supplemented by processing failures with the qPCR assay.

Reduction of processing time for gypsy moth molecular diagnostics assay

Alana McGraw[1,2], Kendra Vieira[1], Marjorie Palmeri[1,3], and Yunke Wu[1,4]

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Experiments were conducted with the aim of reducing the processing time for molecular diagnostics of suspected Asian gypsy moth (AGM) specimens intercepted at U.S. ports of entry. The current DNA extraction protocol requires overnight incubation of sampled eggs (~18 hours) at 56°C. A range of shorter incubation times were tested to determine whether molecular diagnostic assay results were comparable to the standard incubation period. Reducing the overall processing time will accelerate the molecular identification of AGM, resulting in an expediated time frame from when a sample is received, and results are communicated to the National Identification Services.

DNA was extracted from a random selection of 12 egg masses intercepted from U.S. ports in 2021 with 2-, 3-, and 18-hour incubation periods. The DNA quality and concentration resulting from each treatment was compared through the analysis of cycle threshold (Ct) values, an indirect indicator of DNA concentration, determined by the real-time PCR (qPCR) diagnostic assay for AGM. In general, lower Ct values indicate a higher initial concentration of a specific DNA sequence.

We found that Ct values for DNA extracts incubated for 2 and 3 hours with frequent vortexing at 15 min intervals were comparable to the Ct values of the DNA extracts incubated overnight (Figure 1). The interpretation of the Ct values from each subassay of the AGM qPCR assay indicated that specimens extracted with shorter incubation periods were all correctly identified as either *Lymantria dispar japonica* or *Lymantria umbrosa*. This demonstrates that a shorter incubation period with frequent vortexing is at least as effective for extracting DNA from suspect *Lymantria* egg masses as the more time-consuming protocol. Implementation of the DNA extraction protocol with a 2-hour incubation period provides the capability for same-day molecular identification of urgent specimens and facilitates faster communication of results to stakeholders.

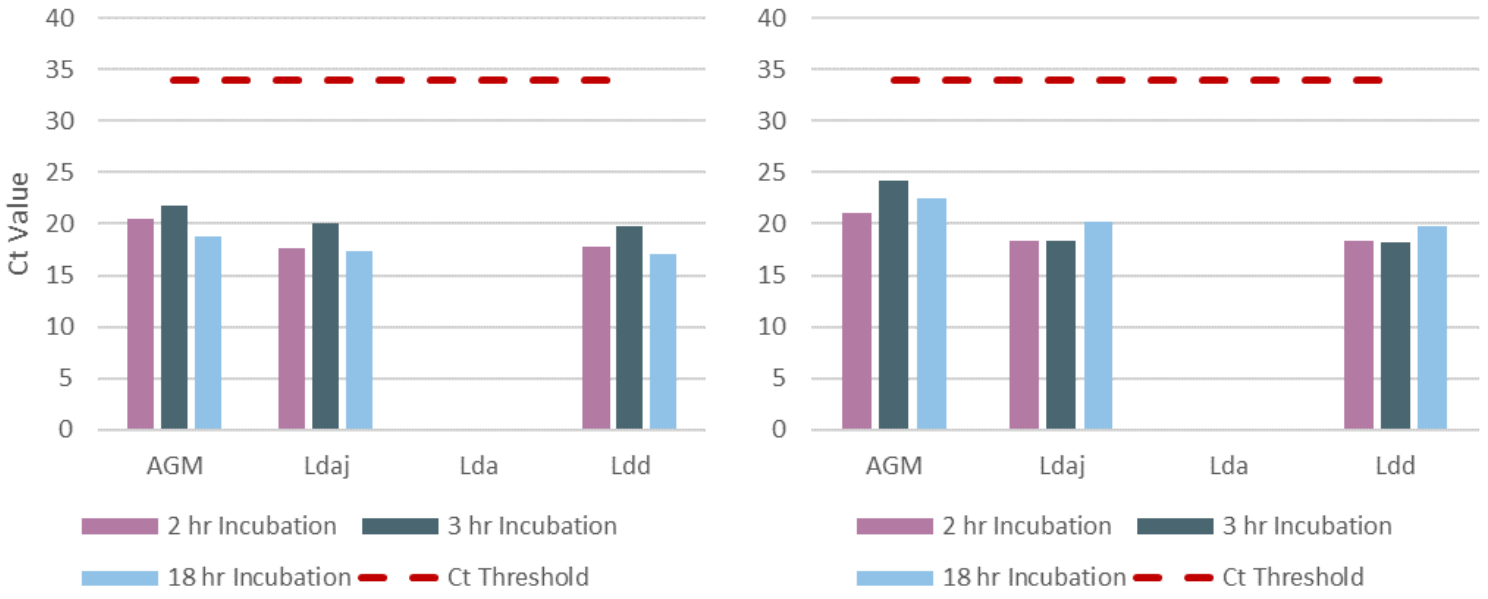

Figure 1. Comparison of the cycle threshold (Ct) values from two representative egg masses collected in the 2021 AGM Port Survey from Oregon (left) and Washington (right) extracted with 2-,3- and 18-hour incubation periods. The four qPCR subassays, AGM (Asia gypsy moth complex), Ldaj (*Lymantria d. asiatica/japonica*), Lda (*Lymantria d. asiatica*), and Ldd (*Lymantria d. dispar*) are used to molecularly determine the identity of suspect specimens. The threshold for a positive reaction is Ct<34 for each subassay. The Ct values for each subassay indicate that both egg masses are *Lymantria dispar japonica*.

Development of a cost-effective and rapid diagnostic assay for *Trogoderma* spp. in USDA colonies

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Colonies of three species of *Trogoderma* are currently maintained at ARS and APHIS laboratories, including khapra beetle (*T. granarium*), warehouse beetle (*T. variabile*), and larger cabinet beetle (*T. inclusum*). Research projects that support the PPQ Khapra Beetle Program, such as direct between-species competition experiments, require accurate species level sample identification. Because morphological identification is difficult for adults and impossible for larvae, a cost-effective diagnostic assay was developed to rapidly distinguish the three species.

Commercial kits for genomic DNA extraction are often expensive (Qiagen DNeasy Blood & Tissue Kit >$3 per sample), so we sought to develop a cheaper approach with comparable effectiveness. Specimens were cut in half and incubated in a solution of 0.05 mg/ml of Proteinase K in 0.1% NP40/TE buffer overnight at 37°C. This ProtK extraction method, costs $0.04 per sample and requires less bench work. Agarose gel electrophoresis showed that PCR products resulting from DNA extractions with the ProtK method were only slightly weaker than those obtained using the Qiagen kit. Average DNA yield was 0.94 ng/ul compared to 2.51 ng/ul with Qiagen, however, the DNA yield for the ProtK method could be even lower than the measured value due to the presence of potential protein contaminant. Nevertheless, the ProtK method resulted in sufficient DNA quality for the successful sequencing of PCR product.

By comparing mitochondrial DNA sequences of khapra beetle, warehouse beetle, and larger cabinet beetle, we discovered characteristic mutation sites that distinguish the three species. Utilizing these characteristic sites, we developed a restriction fragment length polymorphism (RFLP) assay, which requires the use of restriction enzymes to cut specific DNA sequences. After enzyme digestion, each species displayed a unique cut pattern when visualized with gel electrophoresis (Figure 1), allowing for the rapid visual identification of all three species. Notably, two cut patterns were identified for warehouse beetle due to the presence of mutation observed in certain populations which removed one of the two cut sites; however, both cut patterns were still discernable from the other two species.

Using the ProtK DNA extraction method and RFLP assay, we were able to identify 97% of larvae from a direct competition experiment between khapra beetle and larger cabinet beetle (see Domingue, page 18). Compared to other diagnostic methods, such as DNA barcoding ($10 per sample), the newly developed RFLP assay is significantly more cost-effective ($0.30 per sample). The RFPL assay allows for the rapid identification of three species of *Trogoderma* maintained at ARS and APHIS laboratories and has the capacity to screen for these species among field-collected specimens.

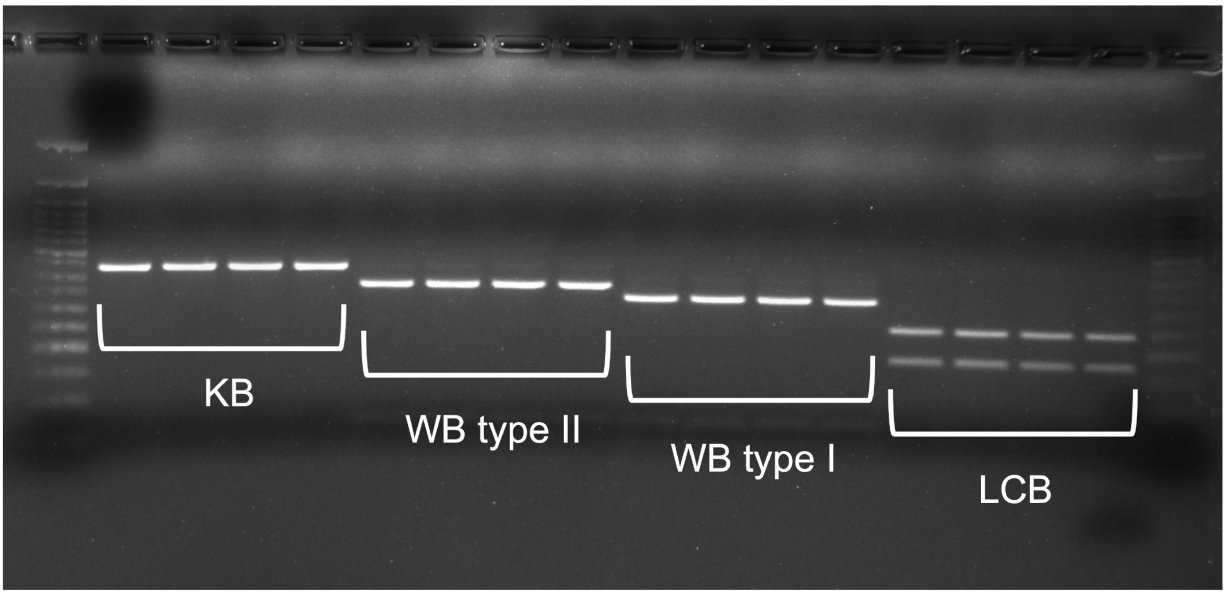

Figure 1. Gel electrophoresis results show that the restriction enzyme cut patterns are different between khapra beetle (KB), warehouse beetle (WB), and larger cabinet beetle (LCB).

Development of a multiplex real-time PCR assay for khapra beetle detection

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In support of the PPQ Khapra Beetle Program, a real-time PCR (qPCR) assay was developed to improve the capacity and efficiency of molecularly identifying khapra beetle (*Trogoderma granarium*) specimens, particularly in trap collections with a large volume of *Trogoderma* spp. samples. Unlike the restriction fragment length polymorphism diagnostic assay (see Wu, pg. 30), the qPCR assay allows for the detection of small traces of khapra beetle DNA within a pooled sample containing non-target, closely related *Trogoderma* species.

The multiplex qPCR assay is composed of two sub-assays using fluorescent probes, one for general detection of the genus *Trogoderma* and one for specific detection of khapra beetle. The general sub-assay was developed using mitochondrial 16S data from Olson et al. [1]. The specific sub-assay was developed using mitochondrial COI sequence data newly generated by this study. When a sample contains khapra beetle DNA, both sub-assays are positive. Alternatively, when a sample contains DNA indicative of *Trogoderma* spp. other than khapra beetle, the general sub-assay is positive, but the specific sub-assay is negative (Figure 1A).

We validated this assay by establishing the standard curve for individual sub-assays as well as the multiplex reaction (Figure 1B). Validation metrics indicate that the qPCR assay passed conventional criteria for qPCR assays [2]. Assay sensitivity was assessed through limit of detection (LOD), which is the smallest DNA quantity that can be confidently detected by the assay. We adopted a more stringent LOD than defined in Bustin et al. [2] by requiring detection at 100% probability with standard deviation between results < 0.5. Under these criteria, assay LOD was determined at the concentration of 50 DNA copies/µl.

The multiplex qPCR assay allows for the potential to detect a low number of khapra beetle in a large bulk sample of non-target, closely related *Trogoderma* species, without the time-consuming process involved in using morphological identification methods. Assay efficacy, efficiency, and sensitivity have been validated.

References

1. Olson RL, Farris RE, Barr NB, Cognato AI. Molecular identification of *Trogoderma granarium* (Coleoptera: Dermestidae) using the 16s gene. J Pest Sci. 2014; 87(4): 701–710.
2. Bustin SA, Benes V, Garson JA, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009; 55: 1–12.

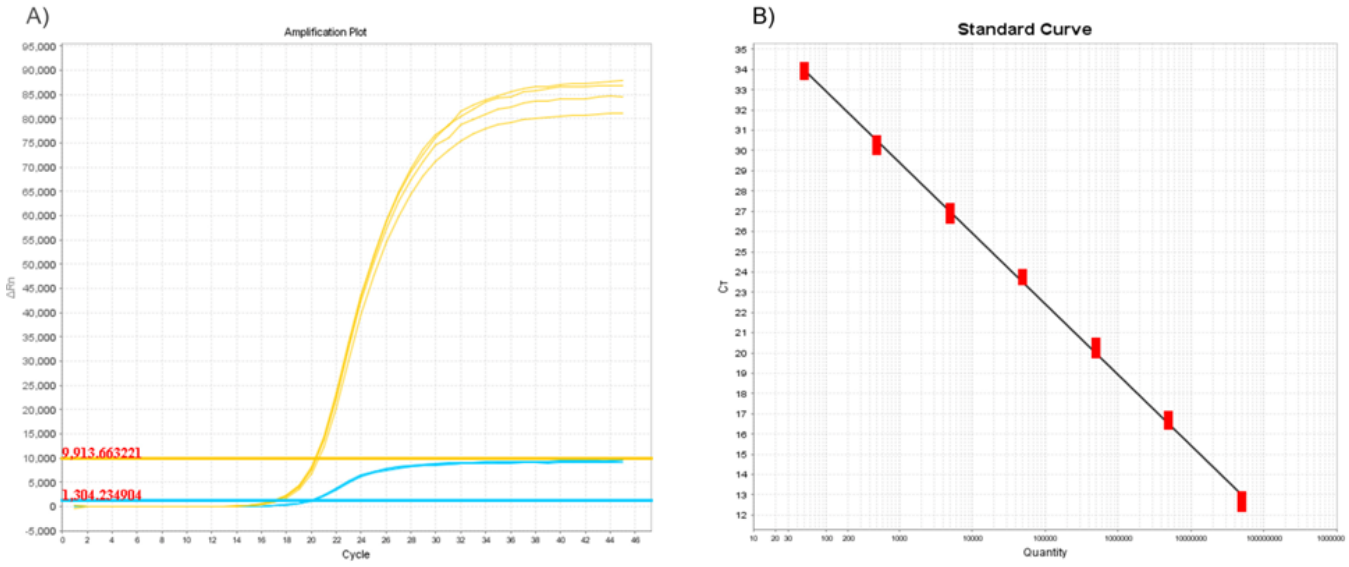

Figure 1. A) Amplification plots for positive detections of khapra beetle DNA with four replicates. Yellow curves passing the horizontal threshold line means positive amplification for the khapra beetle specific sub-assay; blue curves passing the horizontal threshold line means positive amplification for the *Trogoderma* spp. general sub-assay. B) Standard curve of the multiplex assay with high linearity. Serial dilutions are shown as red squares.

Molecular identification of native egg parasitoids from field-collected, parasitized spotted lanternfly egg masses

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Molecular tools were utilized to identify native parasitoid wasps attacking eggs of spotted lanternfly (SLF). This work is an important step in implementing classical biological control methods to support the PPQ SLF Program. While efforts have been made to recover live wasps from field collected SLF eggs (see Broadley, pg. 12), molecular identification provides additional insight into the diversity of native parasitoids. Here, we tested a process that uses traces of DNA from the inside or the surface of empty SLF egg casings to identify emerged parasitoids.

DNA was extracted from 164 individual empty SLF egg casings with wasp emergence holes from 39 egg masses. DNA sequence data were successfully generated for 73 eggs, however 16 of these represented fungal or algal species that likely grew on the eggs. An additional 30 sequences matched SLF, representing DNA from the SLF egg rather than an emerged parasitoid. There were also six sequences representing five mites and one gall midge.

The remaining 21 sequences were successfully assigned to wasps comprising of at least four different species (Figure 1).

One species detected from five SLF eggs was unidentified while another two wasp species were identified as members of the parasitoid superfamily Chalcidoidea. The fourth and most prevalent species, which was detected in 13 parasitized eggs, matched with our reference sequences for *Anastatus reduvii* at 96.6%. This indicates that the parasitoid is certainly a species of *Anastatus* and likely *A. reduvii* or a close relative. *Anastatus orientalis* was ruled out because sequence analysis determined it is only distantly related to the *Anastatus* sp. recovered from SLF eggs (matched at 91.4–92.1%). Despite reports of *Ooencyrtus kuvanae* voluntarily attacking SLF eggs [1], no DNA was detected from this wasp species in our analysis.

Results from this experiment demonstrate a successful method for obtaining DNA data from old, empty SLF egg casings. The use of molecular methods complements the identification efforts made through dissection and rearing to provide a broader picture of native egg parasitoid wasps attacking SLF egg masses (including *Anastatus* sp.), albeit currently at a low parasitism rate.

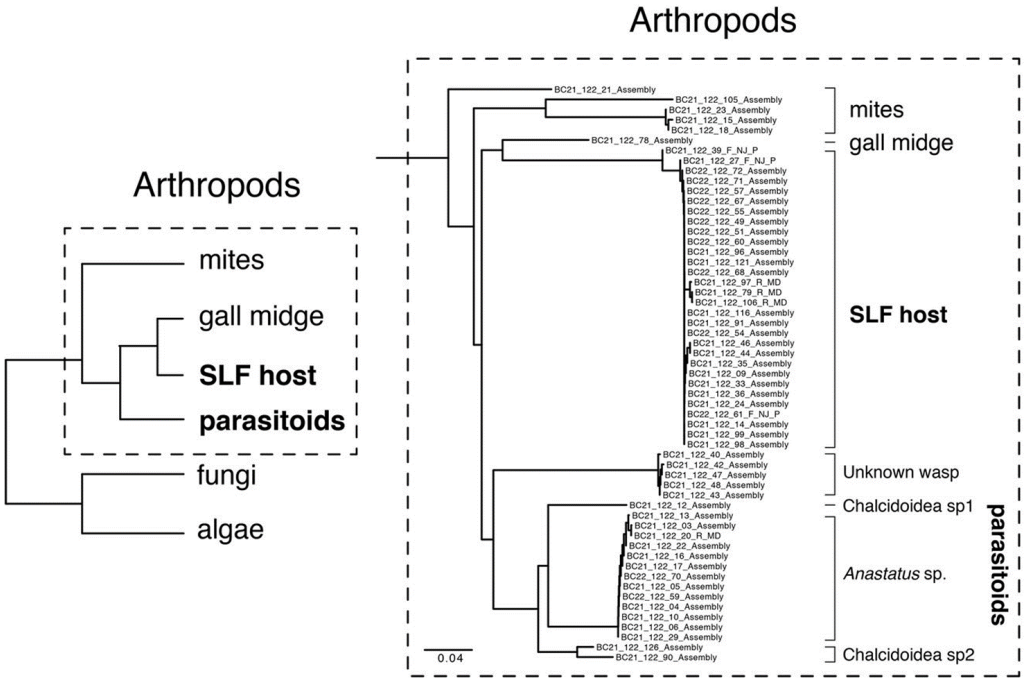

Figure 1. Mitochondrial gene tree showing organisms identified from parasitized SLF eggs. The arthropod portion of the tree is enlarged on the right.

2021 *Lymantria dispar dispar* national risk assessment

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[2]USDA APHIS VS Strategy and Policy, Fort Collins, CO

The *Lymantria dispar dispar* risk assessment supports the Gypsy Moth Program in detecting isolated infestations and limiting artificial spread beyond the known infested area. The risk assessment evaluates spread mechanisms and predicts the likelihood of *L. dispar dispar* detection nationwide, providing a decision tool for allocating PPQ resources.

The risk model is a species distribution model that identifies areas with similar site characteristics to locations where the pest has been found and estimates high likelihood of detection in those areas. The site characteristics are mostly anthropogenic factors such as USPS address forwards from within the federal quarantine zone, population density, and distance from facilities with high volumes of human movement or wood product processing. Short- to intermediate-range natural spread is predicted via distance from prior detections. The model does not consider environmental variables and relies on a post-hoc process of masking out areas that lack suitable host or climate for establishment. A sampling design based on the estimated risk facilitates an easy system of ranking risk by regions such as states or counties.

The model for 2021 was improved by the addition of 2020 trapping data and USPS address forwarding data. Although fewer traps (11,000) were deployed in areas of short-range spread, about 3,500 more traps had at least one positive detection in 2020 compared to 2019. Some notable changes to the model included high-risk spread predictions increasing slightly farther to the west or southwest in Minnesota, Iowa, Illinois, and Indiana. The risk model will be updated again in 2022 to reflect trapping data from the 2021 survey season.

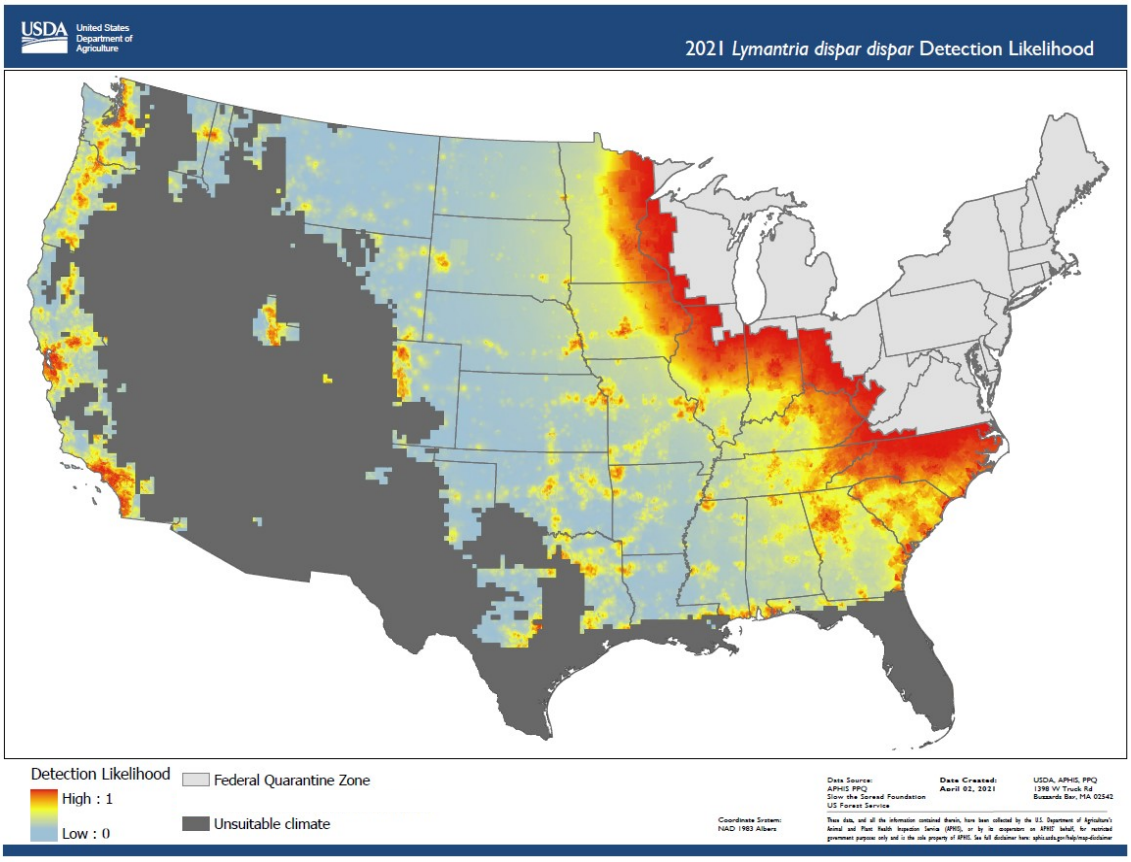

Figure 1. The 2021 *Lymantria dispar dispar* detection likelihood, with areas of unsuitable climate for establishment masked out.

Production and use of the biological control agent *Tamarixia radiata*

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In 2013, a multi-agency team was established to support the biological control of Asian citrus psyllid (ACP), *Diaphorina citri*, in California. The principal biological control agent *Tamarixia radiata* has been reared by the California Department of Food and Agriculture (CDFA), the Citrus Research Board (CRB), the University of California Riverside (UCR), and Foothills Agricultural Research (FAR). The majority of *T. radiata* reared by the program are released throughout regions of California where ACP has become established, with others provided to the ACP biological control program in Arizona and an area-wide IPM demonstration project in Hemet, CA.

A total of 3,342,178 *T. radiata* were produced in 2021 (Figure 1). The majority of *T. radiata* produced (3,024,811) were released in California. However, specimens were also provided to the ACP biological control project in Arizona (112,020) and to the Area-Wide IMP demonstration project in Hemet, CA (184,680). The remaining *T. radiata* were used as starter material in production.

The CRB biological control team, under a cooperative agreement funded by the Citrus Health Response Program, provides methods development support and mass-rearing of *T. radiata* in field cages. The CRB team provided 21% of the total *T. radiata* produced by the California program in 2021. CRB contributed 542,972 *T. radiata* directly to CDFA for release in California and provided 9,500 *T. radiata* to biological control efforts in Arizona and 97,180 *T. radiata* to the area-wide IPM demonstration project.

UCR in collaboration with the Citrus Health Response Program has reared *T. radiata* in separate iso-lines to ensure adequate genetic variability for field adaptation. In 2021, UCR provided 53,640 *T. radiata* to CDFA and shipped 25,320 to Arizona for biological control releases. CDFA also provided 77,200 *T. radiata* to the Arizona biological control project and 87,500 to the Area-Wide IPM demonstration project. Since the project's inception over 23 million *T. radiata* have been released for biological control of ACP. Monitoring of ACP in residential citrus trees by CDFA staff has shown a more than 75% reduction in psyllid populations since 2015, with numbers remaining low even when new flush growth is present.

In Fall 2021, the iso-line colonies used to maintain genetic diversity were transferred from UCR to CRB/CDFA facilities. New iso-lines are being established with *T. radiata* captured in the field in costal, inland, and desert regions of California. Integration of these iso-lines during *T. radiata* production will ensure the continued production of high-quality biological control agents.

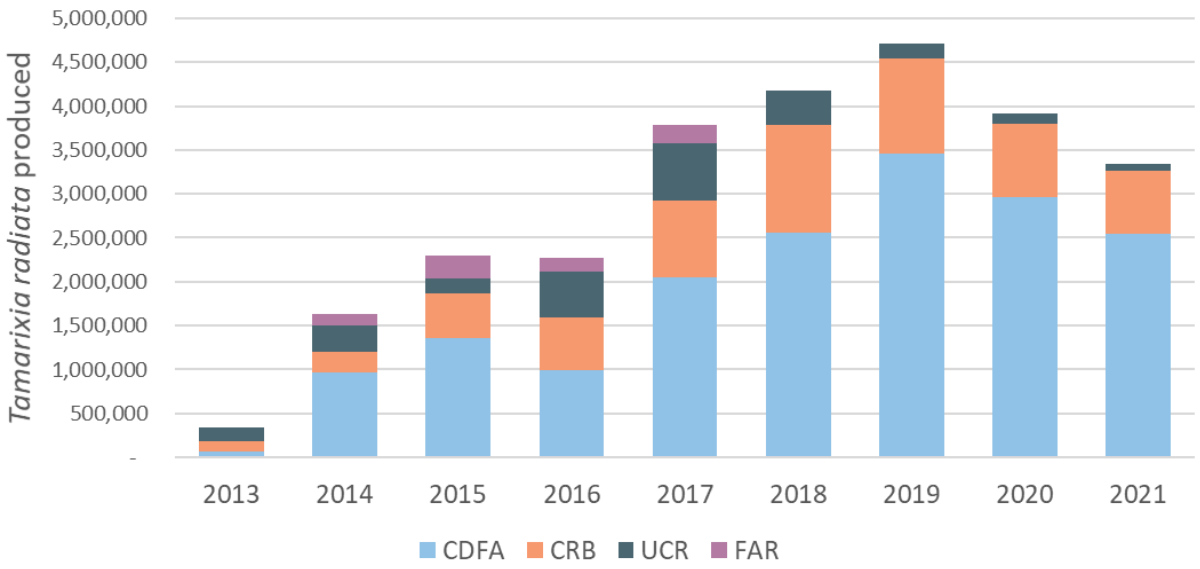

Figure 1. The number of *T. radiata* produced annually by the California Department of Food and Agriculture (CDFA), Citrus Research Board (CRB), University of California Riverside (UCR), and Foothills Agricultural Research (FAR).

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