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

Forest Pest Methods Laboratory

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

Forest Pest Methods Laboratory

2022 Accomplishment Report

Buzzards Bay MA

Salinas CA • Bethel OH

United States Department of Agriculture

Animal and Plant Health Inspection Service

Plant Protection and Quarantine

Science and Technology

This report was edited by: Kendra Vieira, Everett Booth, and Amanda Davila-Flores
It was designed and formatted by: Kendra Vieira, Everett Booth, and Amanda Davila-Flores
Cover designed by: Amanda Davila-Flores

Contents

Molecular Biology

Quantitative Risk Analysis

Salinas Field Station

Publications

*FPML employees and cooperators are indicated in bold

1. **Cooperband MF**, **Murman K**. Responses of adult spotted lanternflies to artificial aggregations composed of all males or females. Frontiers in Insect Science. 2022;2.
2. Cortez AO, Chu CL, **Broadley HJ**, Lo YS, Chen YC, Gates MW, et al. Exploratory surveys in Taiwan of the roseau cane scale *Nipponaclerda biwakoensis* Kuwana (Hemiptera: Aclerdidae) and its associated parasitoids. Journal of Applied Entomology. 2022;146(5):596-606.
3. **Domingue MJ**, Scheff DS, **Leva N**, **Myers SW**. Climbing ability of *Trogoderma granarium* larvae on artificial household and insecticide-treated materials. Journal of Stored Products Research. 2022;95.
4. **Faal H**, **Cooperband MF**, **Canlas I**, Carrillo D. Evidence of Pheromone Use in a Fulgorid, Spotted Lanternfly. Forests. 2022;13(10)1639.
5. **Faal H**, Meier LR, **Canlas IJ**, **Murman K**, Wallace M, Carrillo D, et al. Volatiles from male honeydew excretions attract conspecific male spotted lanternflies, *Lycorma delicatula* (Hemiptera: Fulgoridae). Frontiers in Insect Science. 2022;2.
6. Freistetter NC, **Simmons GS**, Wu Y, Finger DC, Hood-Nowotny R. Tracking global invasion pathways of the spongy moth (Lepidoptera: Erebidae) to the United States using stable isotopes as endogenous biomarkers. Ecol Evol. 2022;12(7):e9092.
7. **Gould J**, Fierke MK, **Hickin M**. Mortality of Emerald Ash Borer Larvae in Small Regenerating Ash in New York Forests. J Econ Entomol. 2022;115(5):1442-54.
8. Lampiri E, Baliota GV, Morrison WR, **Domingue MJ**, Athanassiou CG. Comparative Population Growth of the Khapra Beetle (Coleoptera: Dermestidae) and the Warehouse Beetle (Coleoptera: Dermestidae) on Wheat and Rice. J Econ Entomol. 2022;115(1):344-52.
9. Liu FL, Rugman-Jones P, Liao YC, Fernandez V, Chien I, **Dodge C**, et al. The Attractiveness of alpha-Copaene to Members of the *Euwallacea fornicatus* (Coleoptera: Curculionidae) Species Complex in California and Taiwan. J Econ Entomol. 2022;115(1):116-23.
10. Lombardo JA, Sullivan BT, **Myers SW**, Ayres MP. Are southern pine forests becoming too warm for the southern pine beetle? Agricultural and Forest Meteorology. 2022;315.
11. Nixon LJ, Jones S, Dechaine AC, Ludwick D, **Hickin M**, Sullivan L, et al. Development of rearing methodology for the invasive Spotted Lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae). Frontiers in Insect Science. 2022;2.
12. Rohde BB, **Cooperband MF**, **Canlas I**, Mankin RW. Evidence of Receptivity to Vibroacoustic Stimuli in the Spotted Lanternfly *Lycorma delicatula* (Hemiptera: Fulgoridae). J Econ Entomol. 2022;115(6):2116-20.
13. Schneider SA, **Broadley HJ**, Andersen JC, Elkinton JS, Hwang S-Y, Liu C, et al. An invasive population of Roseau Cane Scale in the Mississippi River Delta, USA originated from northeastern China. Biological Invasions. 2022;24 (9):2735-55.

Modified trap design tailored to spotted lanternfly multimodal communication

Miriam Cooperband[1] and Kelly Murman[1]

[1]Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA

Spotted lanternfly, SLF, *Lycorma delicatula*, survey and detection tools play a critical role in identifying infestations in new areas and assessing the spread and intensity of existing SLF populations. Currently, circle traps rely on the host tree to attract SLF. Semiochemical lures alone have produced weak to no attraction in the field and failed to compete with naturally occurring cues and SLF aggregations [1]. Attracted SLF often aggregate below traps but fail to enter them. After determining that SLF uses a combination of olfactory [2-4], visual [1], and vibratory [5] cues to orient and aggregate [1-6], we aimed to improve trap efficacy by recruiting live SLF captured in traps to emit natural signals that could more efficiently lure additional SLF into traps.

SLF circle traps were modified (Figure 1) so that instead of using a plastic capture bag with a kill strip, a mesh capture bag was attached to the trap and to the tree trunk, allowing captured SLF to feed through it and produce natural signals, such as honeydew [3], body volatiles [2], plant damage volatiles from feeding, and substrate vibrations [5], that attract conspecifics at both long and short range. Mesh and plastic bag circle traps targeting adult SLF were deployed with positions rotated every two weeks to control for variation between trees. The newly designed traps with mesh bags captured significantly more adult SLF than circle traps with plastic bags during four weeks in August prior to mating (Early) and the first two of October after oviposition started (Late), with improved detection in early August.

The use of mesh bags pinned to the tree trunk instead of plastic bags can significantly enhance trap efficacy for adults by using the live SLF as the lure. This was true particularly in the first month after emergence and the first two weeks of oviposition. Future work will focus on additional improvements and test for efficacy during mating time.

References:

1. Cooperband MF, Wickham JD, Warden ML. Factors Guiding the Orientation of Nymphal Spotted Lanternfly, *Lycorma delicatula*. Insects. 2023 Mar 11;14(3):279.
2. Faal H, Cooperband MF, Canlas I, Carrillo D. Evidence of pheromone use in a fulgorid, spotted lanternfly. Forests. 2022 Oct 7;13(10):1639.
3. Faal H, Meier LR, Canlas IJ, Murman K, Wallace M, Carrillo D, Cooperband MF. Volatiles from male honeydew excretions attract conspecific male spotted lanternflies, *Lycorma delicatula* (Hemiptera: Fulgoridae). Frontiers in Insect Science. 2022 Sep 27;2.
4. Derstine NT, Meier L, Canlas I, Murman K, Cannon S, Carrillo D, Wallace M, Cooperband MF. Plant volatiles help mediate host plant selection and attraction of the spotted lanternfly (Hemiptera: Fulgoridae): a generalist with a preferred host. Environmental Entomology. 2020 Oct;49(5):1049-62.
5. Rohde BB, Cooperband MF, Canlas I, Mankin RW. Evidence of receptivity to vibroacoustic stimuli in the spotted lanternfly *Lycorma delicatula* (Hemiptera: Fulgoridae). Journal of Economic Entomology. 2022 Dec 1;115(6):2116-20.
6. Cooperband MF, Murman K. Responses of adult spotted lanternflies to artificial aggregations composed of all males or females. Frontiers in Insect Science. 2022 Sep 8;2:981832.

Figure 1. SLF circle trap fitted with a plastic bag and kill strip (left); a newly modified circle trap with a mesh bag pinned to the tree allows SLF to feed and produce aggregation signals after being trapped (center); a closeup shows SLF feeding inside a mesh bag (right).

Field responses by adult spotted lanternflies to natural honeydew and body extracts

Miriam Cooperband[1] and Kelly Murman[1]

[1]Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA

Much effort has focused on identifying long-range attractants for spotted lanternfly, SLF, *Lycorma delicatula,* lures for survey, detection, and mitigation. We discovered that SLF likely communicate through pheromones emitted from their honeydew [1] for aggregation [2] and from their bodies for mating [3]. Honeydew and whole-body extracts were attractive, particularly to males, in laboratory bioassays. Investigations to identify the responsible compounds revealed numerous antennally active components, and several were found to be attractive in laboratory bioassays. However, no experimental pheromone lures have been effective in the field so far. Therefore, identifying the crucial blends and ratios of pheromone components requires additional work.

We conducted a proof-of-concept test in low-density field sites using natural honeydew (collected onto ribbons from heavy infestations elsewhere) and whole-body extracts of SLF in diffuser lures (by collecting ~6000 SLF/week elsewhere and extracting them in hexane) to determine whether pheromones could be the basis for designing effective lures. Natural materials were used to ensure no pheromone components were missing or used in the wrong ratios. Sex ratios of SLF extract lures matched field conditions where SLF were collected. Traps and lures were refreshed three days per week. We tested SLF attraction to (1) the whole-body extracts in combination with honeydew on ribbons, or (2) whole-body extract with no honeydew added (clean ribbons), or (3) controls (clean hexane and clean ribbons) (Figure 1A) (N=10).

Since release rates of diffusers varied with field conditions (rain, wind, sun, temperature), the amount of extract (or control hexane) released was compared to how many male and female SLF were caught over that period. We examined this linear relationship for all three treatments (Extract + Honeydew, Extract only, and Control), excluding blocks with all zeros in a trapping period, since SLF density started off low. There was no significant relationship between capture of either sex and release rate for controls or SLF body extract alone. However, there was a significant positive relationship between the extract release rate in the presence of honeydew for both males and females (Figure 1B). Thus, the presence of both extract and honeydew significantly increased capture rate of both sexes, but particularly males.

This study provides additional evidence that pheromones derived from both SLF honeydew and body volatiles are used in SLF aggregation and mating, and demonstrated proof-of-concept that if the correct chemical blend is identified, the development of a pheromone lure for adult SLF may be possible. Whether to continue pursuit of SLF pheromones is a consequential consideration due to the upfront investment of time and resources it may require. Should efforts to identify SLF pheromones continue, researchers should not ignore the role that honeydew plays in SLF communication.

References:

1. Faal H, Meier LR, Canlas IJ, Murman K, Wallace M, Carrillo D, Cooperband MF. Volatiles from male honeydew excretions attract conspecific male spotted lanternflies, *Lycorma delicatula* (Hemiptera: Fulgoridae). Frontiers in Insect Science. 2022 Sep 27;2.
2. Cooperband MF, Murman K. Responses of adult spotted lanternflies to artificial aggregations composed of all males or females. Frontiers in Insect Science. 2022 Sep 8;2:981832.
3. Faal H, Cooperband MF, Canlas I, Carrillo D. Evidence of pheromone use in a fulgorid, spotted lanternfly. Forests. 2022 Oct 7;13(10):1639.

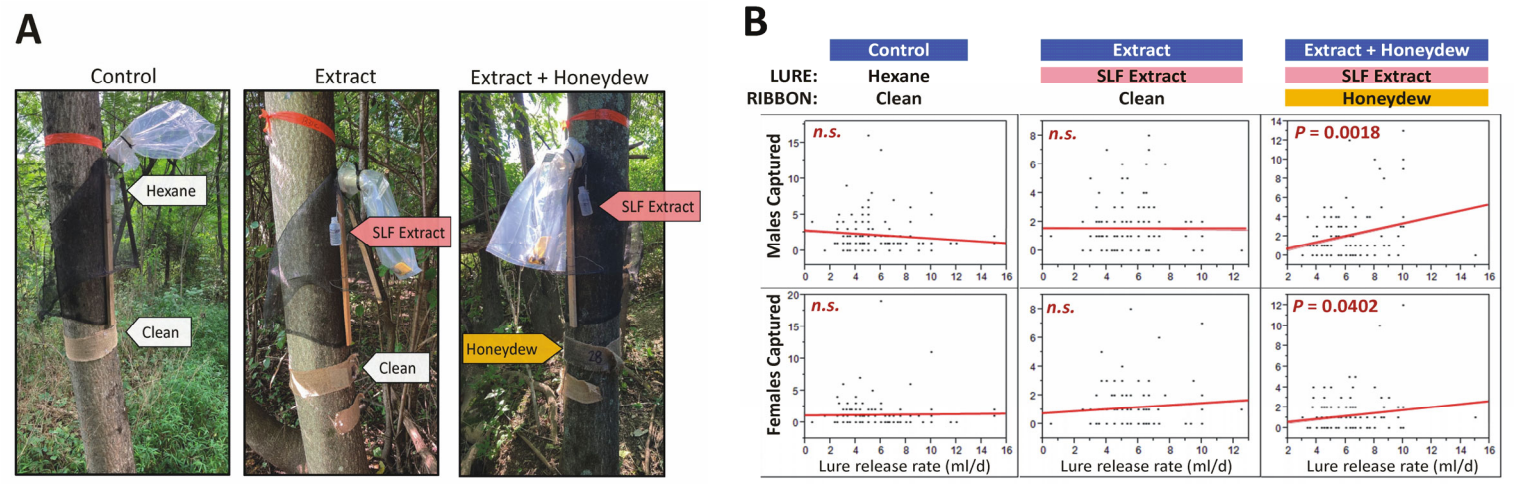

Figure 1. Photographs of a block of trees with the three treatments of lures containing SLF body extracts and honeydew collected on ribbons and their controls (A), and resulting captured males and females (B) by lure release rate for the three treatments. A positive linear regression existed between lure release rate and both males and females captured only in the presence of honeydew.

Seasonal variation in spotted lanternfly male attraction to conspecific volatiles

Hajar Faal[1,2,3], Isaiah Canlas[1], Kelly Murman[1], Sam Stella[1,4], Ann Ray[4], Matthew Wallace[5], Daniel Carillo[2], and Miriam Cooperband[1]

[1] Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA
[2] University of Florida, Department of Entomology and Nematology, Homestead, FL
[3] Texas A&M University, Kingsville Citrus Center, Weslaco, TX
[4] Xavier University, Biology Department, Cincinnati, OH
[5] East Stroudsburg University, Biology Department, East Stroudsburg, PA

Development of pheromone-based traps for spotted lanternfly, SLF, *Lycorma delicatula*, is still an essential element in pest management programs. These traps are species-specific and can detect and monitor the spread of SLF population in new areas. Currently, no pheromone has been identified for this invasive species, although previous laboratory studies using whole body extracts of SLF, adults demonstrated that they use conspecific pheromones to locate each other. Due to the presence of heavy waxy materials in their body extracts we could not chemically process those samples to identify attractive volatiles. In 2022, we used a new method in which we collected SLF body headspace volatiles from different physiological stages and examined them in dual-choice bioassays.

Similar results were achieved using the new method: adult males, were significantly attracted in dual-choice bioassays toward body volatiles of both male and female SLF during feeding stages and prior to mating (Early). In addition, males significantly preferred male body volatiles immediately prior to mating (pre-Mid), whereas their attraction quickly shifted to only female body volatiles during mating (Mid). Neither of the body volatiles from males or females were attractive to males after mating and oviposition (Late).

Despite the lack of attraction of females to body volatiles from either sex in dual-choice bioassays, a trend of female attraction to body volatiles from same-sex conspecifics was observed immediately prior to mating (pre-Mid). The switch in attraction to volatiles from the same or opposite sex between Early, pre-Mid, and Mid phases indicated a behavioral plasticity between physiological phases in males. This finding enhances our understanding of how SLF males behave in the field and provides a possible explanation for the observation of extreme male- and female-biased sex ratios on different trees just before mating time.

In the current study, we report attraction of SLF to body volatiles of their conspecifics. With further investigation, identification of a pheromone attractant for SLF may lead to early detection and monitoring of this pest in newly invaded areas.

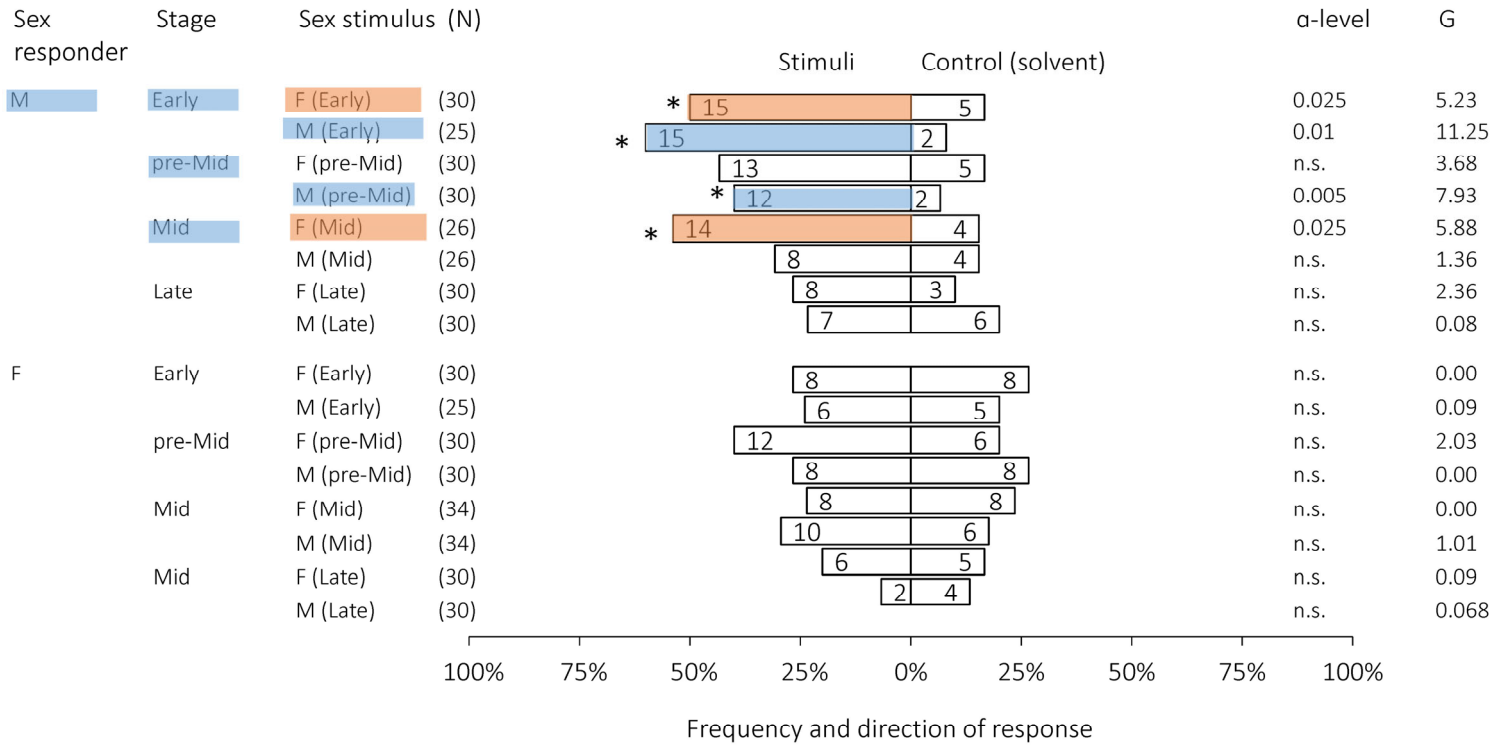

Figure 1. The preference of individual adult male (M) and female (F) spotted lanternflies from different physiological stages, in response to headspace body volatiles of their conspecifics against a solvent control in dual-choice olfactometer assays. Significant preferences are denoted by asterisks (Chi Square test, $G > 3.841$, $P < 0.05$).

Antennal sensitivity differences of male and female spotted lanternflies correlate to their different behavioral preferences

Hajar Faal[1,2,3], Isaiah Canlas[1], Kelly Murman[1], Sam Stella[1,4], Tappey Jones[6], Allard Cossé[1], Ann Ray[4], Matthew Wallace[5], Daniel Carillo[2], and Miriam Cooperband[1]

[1] Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA
[2] University of Florida, Department of Entomology and Nematology, Homestead, FL
[3] Texas A&M University, Kingsville Citrus Center, Weslaco, TX
[4] Xavier University, Biology Department, Cincinnati, OH
[5] East Stroudsburg University, Biology Department, East Stroudsburg, PA
[6] Virginia Military Institute, Department of Chemistry, Lexington, VA

Spotted lanternfly (SLF) antennae respond to numerous chemicals, necessitating their prioritization in a rapid and efficient manner. Using gas chromatography coupled with electroantennograms (GC-EAG), we examined the relative sensitivity of male and female spotted lanternfly antennae to 40 compounds, previously found in SLF host plant, body, and/or honeydew volatile collections. We tested these compounds in a pure form and at a fixed dose. This study is an important step toward understanding the role of olfaction in modulating SLF behavior.

Our results showed that the intensity of antennal responses to these compounds differed between males and females. Considering the four largest EAG responses for each sex, male antennae responded most strongly to honeydew volatiles. Host plant volatiles and one honeydew volatile elicited the four strongest EAG responses from female antennae. These results aligned with previous behavioral observations where we found that SLF males significantly attracted to honeydew volatiles in dual-choice bioassays, with an attractive trend for females. Females, but not males, were attracted to methyl salicylate, and both sexes were attracted to sulcatone, both compounds found in their preferred host plant *Ailanthus altissima*. Females were not attracted to body volatiles, whereas males were. We also found that male antennae produced larger antennal responses than female antennae to most chemicals.

Based on their antennal responses, aggregation behavior, and attraction in bioassays, we hypothesize that females may need higher concentrations of semiochemicals than males to initiate attraction, and that females may be guided more by host plant volatiles, and males by conspecific cues such as honeydew. This study determined the sensitivity and specificity of SLF adults to a set of semiochemicals, helping prioritize compounds for two-choice bioassays testing in the lab.

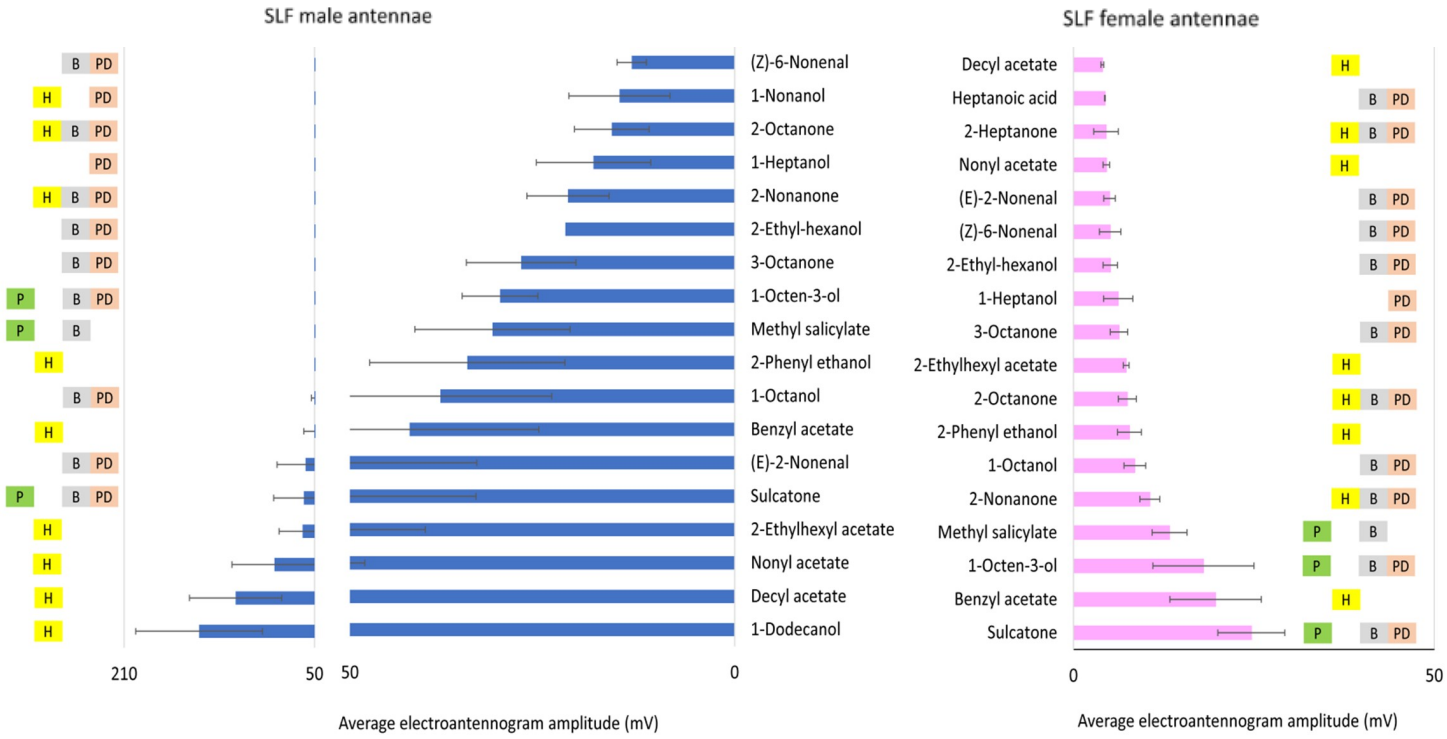

Figure 1. The average amplitudes (mV) for the 18 largest antennal responses of spotted lanternfly (SLF) males and females to a series of SLF-derived semiochemicals (H: honeydew, P:plant, B: body, and PD: photodegraded body volatiles) tested by gas chromatography coupled with electroantennograms. In each test, 50 ng of a given compound was applied to the antenna.

Developing rearing methods for *Aprostocetus* sp., a candidate biological control agent for roseau cane scale

Mike Martinson[1,2], Hannah Broadley[1], Jeremy Andersen[2], Tanner Sparks[3], Rodrigo Diaz[3], Michael Gates[4,5] and Juli Gould[1]

[1]Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA,
[2]University of Massachusetts, Department of Environmental Conservation, Amherst, MA
[3]Louisiana State University, AgCenter, Baton Rouge, LA,
[4]Systematic Entomology Laboratory, USDA ARS, Beltsville, MD
[5]Smithsonian Institute, National Museum of Natural History, Washington, DC

Classical biological control is being studied as a potential management tool for roseau cane scale (RCS), *Nipponaclerda biwakoensis*, as part of Roseau cane die-off research led by the Louisiana State University AgCenter. Roseau cane scale is an invasive grass scale native to Asia that has established in the Mississippi River Delta in Louisiana on naturalized roseau cane (Delta variety *Phragmites australis*) and is associated with stress and dieback of the cane. Roseau cane is the dominant plant species of the Mississippi River Delta and provides critical ecosystem services including erosion control, protection against storm surges, and contributions to estuary habitat. The purpose of this work is to select a priority candidate biological control agent and develop a laboratory colony of the selected wasp species for host range testing.

We identified *Aprostocetus* spp. as potential biological control agent(s) due to their prevalence and high parasitism rate in the native range and because species from this genus have been used previously as successful biological control agents. Additionally, *Aprostocetus* wasps were recently discovered for the first time in Louisiana as an adventive population on RCS. Our collections of *Aprostocetus* across sites in Asia from 2019 to 2021 show a high degree of genetic diversity, suggesting the presence of seven cryptic species ranging from 2.31% to 11% divergence. One of these clades is now present in Louisiana. With the help of collaborators at Chungbuk National University, Kunsan National University, and Seoul National University, we collected parasitized RCS across sites in South Korea and hand carried them back to Forest Pest Methods Laboratory's (FPML) Insect Containment Facility to develop a rearing system and establish a laboratory colony. *Aprostocetus* accounted for 16% of total emergence (184 individuals) with a slightly female skewed sex ratio. *Aprostocetus* were exposed to RCS using three different rearing methods: 1) isolated scale in a petri dish, 2) small plants inoculated with scale, and 3) cut stems with established scale (Figure 1). Neither scale isolated in tubes nor those on cut stems and exposed to gravid females produced wasp progeny. However, by exposing wasps to scale reared on small potted plants, we have recovered progeny and are initiating a laboratory colony.

Aprostocetus has been identified as the priority species for investigation in the search for potential biological control agents for RCS. We are now shifting our focus to prioritize rearing and studying the *Aprostocetus* sp. recently found adventively in Louisiana. We will evaluate its potential role in the invasive population and possible non-target effects. Utilizing the rearing methods developed at the FPML, we have produced an F1 generation of *Aprostocetus* and will continue to maintain a laboratory colony for use in host range testing and other studies.

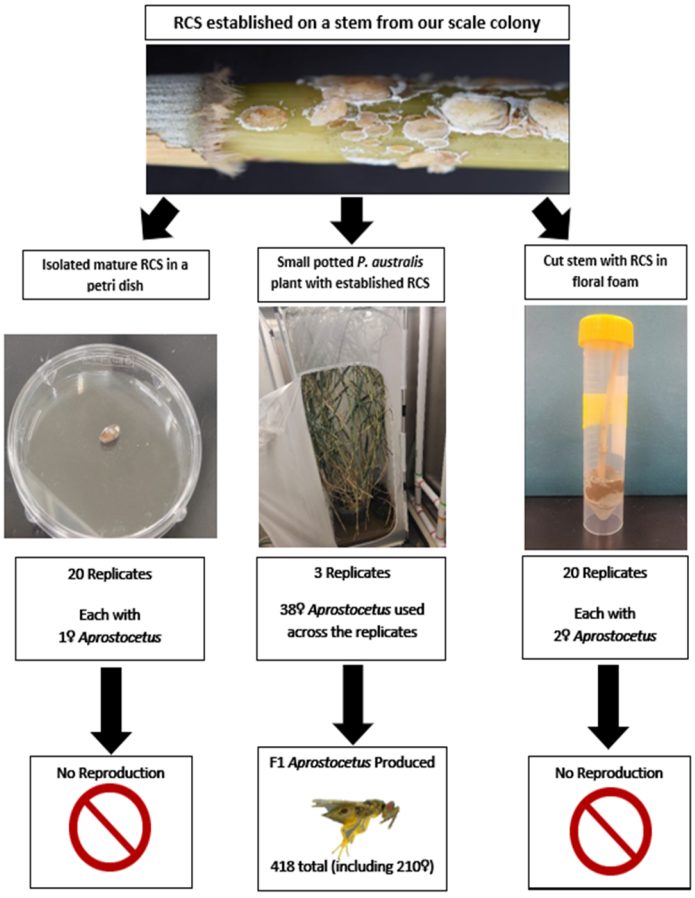

Figure 1. Schematic of our experimental rearing setups for developing a colony of *Aprostocetus* wasps. We tested three rearing protocols and found that rearing with potted plants worked most successfully.

Establishing laboratory colonies of non-target ambrosia beetles for host specificity testing

Christine Dodge[1,2], Zachary Lee[1,3], Aaron Weber[1,3], and Juli Gould[1]

[1]Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA
[2]University of California, Department of Entomology, Riverside, CA
[3]University of Massachusetts, Department of Environmental Conservation, Amherst, MA

Classical biological control methods are being developed to help manage invasive populations of polyphagous shot hole borer, (PSHB), *Euwallacea fornicatus*. To support these efforts, laboratory colonies of non-target ambrosia beetles (tribe *Xyleborini*) were established for use in host specificity testing of PSHB parasitoids. In Summer 2021 we trapped for ambrosia beetles in Massachusetts to determine the species diversity present in three different forest types. In Summer 2022 we applied this knowledge to live trap ambrosia beetles for the purpose of establishing laboratory colonies of a diverse range of species.

Field sites in 2021 were Douglas State Forest (Worcester Co.), Middleborough (Plymouth Co.), and western Cape Cod (Barnstable Co.). A total of 17 ambrosia beetle species were collected and identified based on morphology (Figure 1). The greatest species diversity was found in Douglas State Forest, where 15 of the 17 species were collected. In 2022, nine species were obtained via live collection efforts in Douglas State Forest and Middleborough. We established reproducing colonies of six of those species in the laboratory: *Xylosandrus crassiusculus*, *Xylosandrus germanus*, *Anisandrus sayi*, *Xyleborinus saxesenii*, *Xyleborus ferrugineus*, and *Xyleborus intrusus*. These represent the four most encountered species, as well as two rare species (Figure 1).

Methods were developed at the Forest Pest Methods Laboratory to successfully rear field-collected ambrosia beetles, allowing for their use in host specificity testing of potential classical biological control agents for PSHB. Colonies are maintained in 50 mL centrifuge tubes and reared on sawdust -based artificial media, which saves on space and resources needed to collect and maintain woody host material. Polyphagous shot hole borer is reared on artificial media made from avocado, a preferred host in its invasive range. To rear non-target ambrosia beetles, we experimented with three alternative hosts common in Massachusetts forests: American beech, striped maple, and black birch. Preliminary results suggest that several non-target ambrosia beetle species produce more offspring and have higher colony establishment success on artificial media made from the local tree species rather than avocado. Formal experiments measuring colony establishment success and reproductive output of each of these species on each type of artificial media are on-going and will enable us to further maximize colony production of each species.

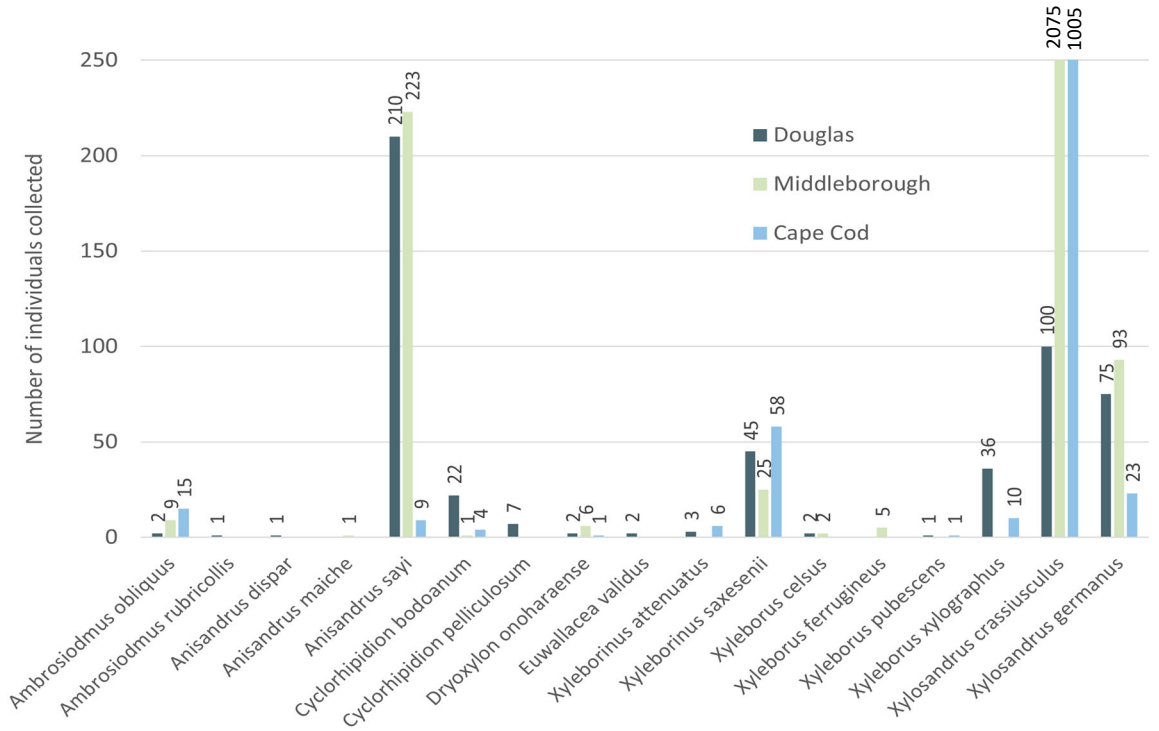

Figure 1. Number and diversity of ambrosia beetles collected in Summer 2021. Exact values are listed since certain records of *Xylosandrus crassiusculus* are too large to display on the chart.

Tolerance of *Zeugodacus tau* populations to phytosanitary cold treatment

Vanessa S. Dias[1], Guy Hallman[2], Alexandre S. Araújo[1], Inajara V. G. Lima[1], Fabio Galvão-Silva[1], Jhonatan S. Aguilar[1], Luis A. Caravantes[1], Carlos E. B. Cáceres[1], Marc J. B. Vreysen[1], and Scott Myers[3]

[1]Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, IAEA, Vienna, Austria
[2]Phytosanitation, 3917 Estancia Drive, Oceanside, CA
[3]Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA

The limited options for phytosanitary treatment of *Zeugodacus tau*, a highly polyphagous fruit pest, poses a quarantine risk for citrus imports into the United States. To assess nonchemical phytosanitary treatment schedules for this pest, in support of PPQ's Fruit Fly and Agricultural Quarantine Inspection Programs, cold tolerance of *Z. tau* populations were compared and efficacy of a single treatment schedule against the most cold-tolerant population in citrus was determined. Additionally, the susceptibility and suitability of *Citrus sinensis* to *Z. tau* populations were assessed in field cages.

Exploratory tests comparing the tolerance of *Z. tau* third instars from wild strains of Palampur (India), Fujian (China), and Baipayl (Bangladesh) and a laboratory strain from Fujian to cold treatments (≤ 1.7 °C for 3, 8, 10, 13, 15,16, 18, and 20 days) showed that Fujian-wild and Palampur were the most cold-tolerant populations (Figure 1). Confirmatory tests exposing 41,331 third instars from Fujian-wild and Palampur to ≤ 1.7 °C for 22 days yielded four survivors; however, none of the four were able to develop to the adult stage (Table 1). Despite the low levels of infestation observed in *C. sinensis*, *Z. tau* could infest intact harvested oranges placed on trees under field cage conditions and sustain its development to the adult stage.

These results confirm that the cold treatment of 22 days at ≤ 1.7 °C prevents *Z. tau* from developing to the adult stage with a high confidence level. Results also support the current PPQ cold treatment schedule T107-o as a phytosanitary measure against *Z. tau*, which helps to safeguard U.S. agriculture from the risk of introduction and establishment of *Z. tau* through citrus imports.

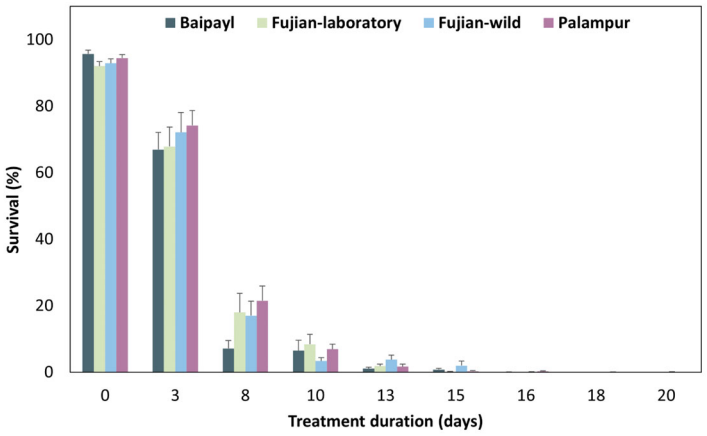

Figure 1. Mean survival (%) of four populations of the *Zeugodacus tau* complex third instars reared in oranges exposed to 1.40°C ± 0.02 (mean ± SEM) for 0 to 20 days.

Table 1. Confirmatory test with *Zeugodacus tau* third instars from Fujian-Wild and Palampur treated at 1.40 ± 0.02°C (mean ± SEM) for 22 days in oranges.

Treatment duration	Population	N	Treated fruit	Treated larvae	Live larvae	Larvae/fruit (mean ± SE)	Mortality (mean ± SE)
0 (control)	Fujian-wild	7	65	9,014	8,037	139 ± 19	10.23 ± 1.54
0 (control)	Palampur	4	13	1,321	1,074	102 ± 29	21.18 ± 8.19
22 days	Fujian-wild	7	242	36,512	4	151 ± 9	99.99 ± 0.00
22 days	Palampur	4	68	4,819	0	71 ± 11	100.00 ± 0.00

Irradiation as an effective phytosanitary treatment against *Drosophila suzukii*

Inajara V. Gomes[1], Jhonatan A. Sanchez[1], Jennifer L. Andrade[1], Carlos Caceres[1], Marc J. B. Vreysen[1], Scott Myers[2], and Vanessa S. Dias[1]

[1]Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, IAEA, Vienna, Austria
[2]Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA

Currently, the only available phytosanitary treatment option for *Drosophila suzukii* is fumigation with methyl bromide. To validate the first nonchemical post-harvest treatment for this species, the efficacy of a phytosanitary irradiation dose for *D. suzukii* in blueberries and cherries was evaluated. This work contributes to the Agricultural Quarantine Inspection programs of PPQ, supporting the development of the first phytosanitary irradiation treatment against *D. suzukii.*

Large-scale confirmatory tests evaluated the efficacy of minimum absorbed doses ranging from 70 to 80 Gy against the late pupal stage of *D. suzukii.* Prevention of egg hatching was considered the treatment endpoint. A total of 35,168 insects were irradiated, and egg laying on blueberries was assessed for two weeks. While non-irradiated *D. suzukii* females laid 23,975 eggs in blueberries and cherries, no egg was laid by irradiated females (Table 1).

These results provide data to support a non-chemical treatment for inclusion in the International Standard on Phytosanitary Measure 28 of the International Plant Protection Convention. An internationally recognized phytosanitary irradiation treatment might benefit U.S. exports of soft and stone fruit to *D. suzukii* free areas.

Table 1. Total number of eggs laid and eggs hatched by irradiation treated *Drosophila suzukii* on both blueberries and cherries.

Dose (Gy)	Host	Total number of insects treated	Total number of eggs laid	Total number of eggs hatched
0	Blueberry	11,915	16,862	13,054
80	Blueberry	26,556	0	0
0	Cherry	3,672	7,113	5,049
80	Cherry	8,612	0	0

Effect of Beauveria spore treatment on khapra beetle trapping bait

Michael Domingue[1,2], Mandy Furtado[1], and Scott Myers[1]

[1]Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA
[2]Kansas State University, Department of Entomology, Manhattan, KS

Work was conducted at the Forest Pest Methods Laboratory (FPML) to explore the possibility of using *Beauveria bassiana* spores in trapping systems for the khapra beetle, *Trogoderma granarium.* Previous work has determined that these spores are effective in killing khapra beetle [1]. However, the effect of adding *B. bassiana* spores to the wheat germ and pheromone lures used in program traps is unknown. In the existing design, khapra beetle and any other insects will enter a collection tray containing the wheat germ and are able to survive, develop, and produce offspring while the traps wait servicing. It is necessary to assess potential effects of spore treatment on the efficacy of current khapra beetle trapping systems before its possible integration into attract-and-kill and early detection options for this pest.

Dome traps were placed in 50 × 50 cm enclosures with 30 insects for five replicated events of two treatments. The experimental treatments consisted of *B. bassiana* (BotaniGard®ES: Strain GHA) spores in liquid added to the wheat germ normally used in program traps, while the control traps did not contain the spores. A pheromone lure (Trece™), like that used in program traps, was also added to the spore-treated and control traps. The dome trapping system used here is different from the wall trapping systems used in program-supported monitoring efforts, but the bait in controls is otherwise similar. The dome traps were selected to safely contain the spores within the FPML insect containment facility and prevent spread to other insect colonies.

The mean ± SE percentage of larvae found in the traps was 93 ± 5% for controls and 98 ± 2% for the spore-treated group (Figure 1), which was not statistically different according to a logistical regression model (F=0.02, d.f.= 1,7, p= 0.90).

Results suggest that at close range, *B. bassiana* spores do not interfere with attraction of insects into khapra beetle traps. Additional validation in the field is needed to determine the utility of *B. bassiana* for use in attract-and kill applications, and thus whether the technology can be adapted for program objectives.

References:

1. Mantzoukas S, Lagogiannis I, Kitsiou F, Eliopoulos PA. Entomopathogenic action of wild fungal strains against stored product beetle pests. Insects. 2023 Jan 14;14(1):91.

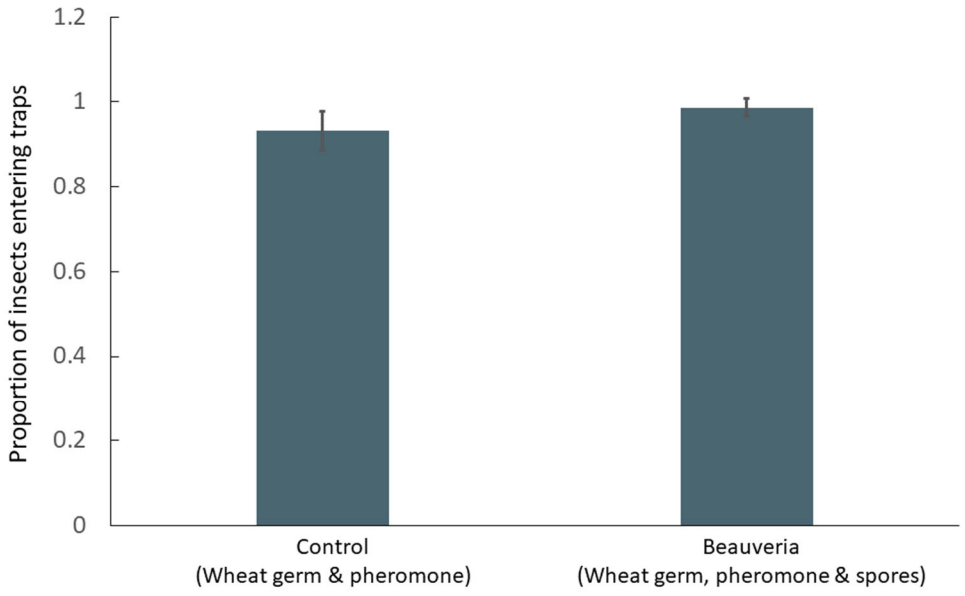

Figure 1. Mean (± SE) proportion of *Trogoderma granarium* larvae entering dome traps baited with wheat germ and pheromone (control) compared to those where *Beauveria* spores were also added.

An effective trap for spotted lanternfly egg masses

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

[1]Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA

Traps effective in targeting spotted lanternfly, SLF, *Lycorma delicatula*, egg masses have the potential to be a valuable tool for PPQ's SLF Program in terms of monitoring, mitigation, and supporting future research efforts. A trap has been developed at the Forest Pest Methods Laboratory that is attractive to ovipositing SLF females; insects enter the trap area and readily lay eggs on the trap substrate. Work was conducted across several field seasons to assess various trapping configurations and materials and determine trapping efficiency.

We have identified an efficient, durable, low-cost trap that attaches to the lower trunk area of an SLF host tree and can be set up and left in the field until collection. The trap is made of roofing material affixed around the trunk of the tree with a second layer of material inverted and held away from the tree such that the appearance is that of a lamp shade (Figure 1A). SLF females readily enter the trap and lay eggs on the thin, flexible trap surface.

A total of 1,943 egg masses were collected from 105 traps, 95.5% of which were laid on the surfaces of 73 vertically oriented traps. The average number of egg masses per trap at a lower density site was 9.6, while two of the six study sites yielded averages of 47.1 and 54.4 egg masses per trap. Three individual traps captured 98, 102 and 111 SLF egg masses. Traps oriented vertically stimulated SLF females to focus oviposition on the trap substrate and very few egg masses were noted on horizontal traps, above or below the traps, or on the trunks of the paired control trees. The traps provide an environment and a material on which SLF females will greatly concentrate their egg masses.

The trapping system outlined here would aid in the collection of SLF egg masses needed for active biological control and other research efforts, and could replace the destructive and labor intensive chipping method currently used. In addition, this trap has the potential to be a useful tool for detection, monitoring, and mitigation of SLF populations.

A short document with links to trap materials and a description on how to construct the egg mass trap can be found here: https://www.stopslf.org/stopslf/assets/File/LST-Construction-for-SLF-Egg-Masses.pdf

References:

Lewis P, Davila-Flores A, Wallis E. An effective trap for spotted lanternfly egg masses. Frontiers in Insect Science. 2023 Apr 17;3:1154510.

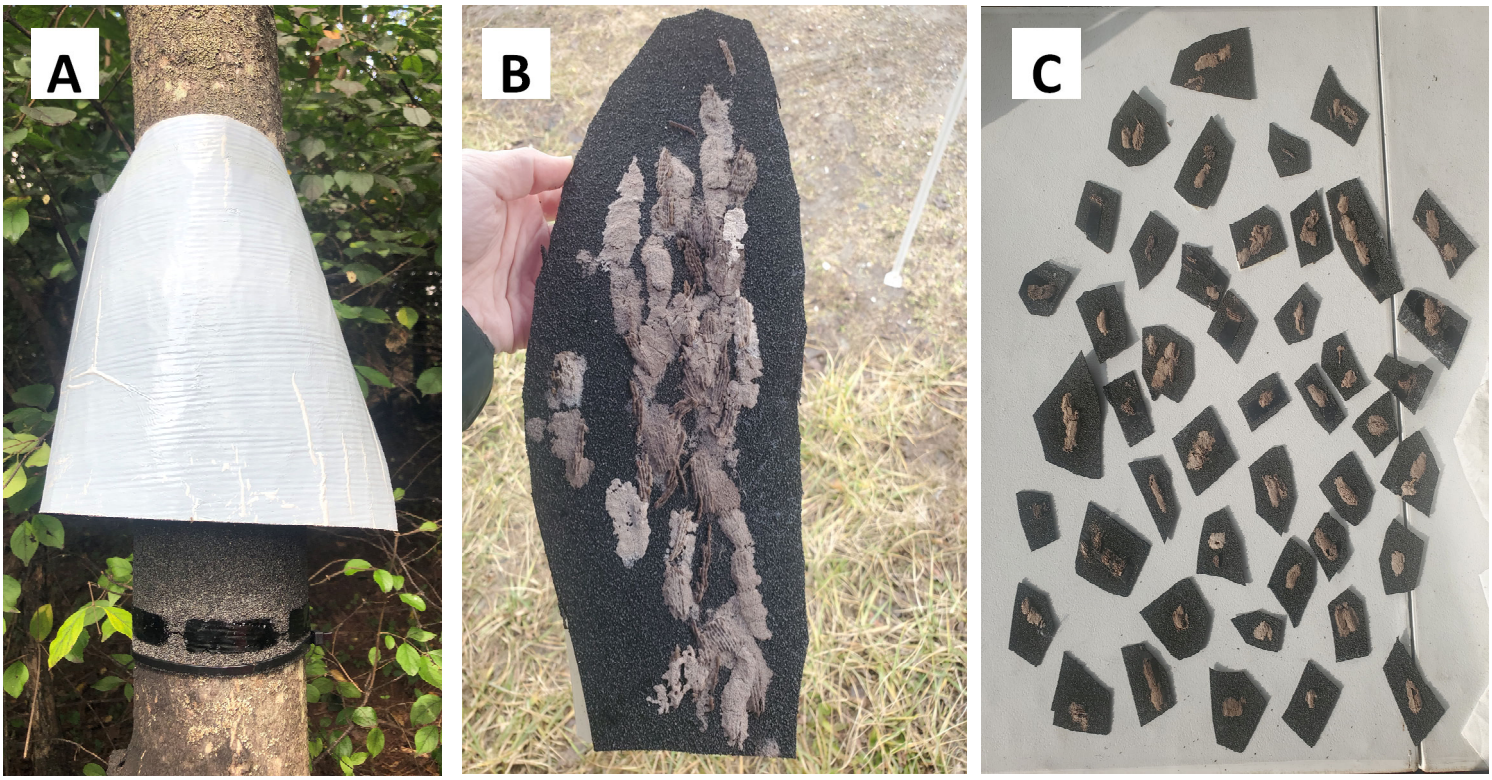

Figure 1. A) Lampshade trap setup on *Ailanthus* tree. B) Clusters of egg masses on the inner substrate of the trap. C) Cut pieces of roofing materials with egg masses on each piece.

Air cargo treatments for hitchhiking insects

Phil Lewis[1]

[1]Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA

Insects that inadvertently attach themselves to cargo pods or fly into cargo holds during air cargo loading operations can get rapidly transported to western states where they pose a threat to agricultural resources. There are very few products that can be used to control insects on an aircraft and the primary aerosol product, 10% d-phenothrin, used by USDA-PPQ and the Department of Defense (DOD) is no longer registered. Testing was conducted to identify an effective aerosol insecticide that could be used to prevent the transportation of hitchhiking insects by the air cargo industry and during military flight operations.

In 2021, three available products were tested at various application rates in a simulated air cargo space using 20 ft shipping containers at Dover Air Force Base (AFB) in Delaware. Japanese beetle adults were tested in July and spotted lanternfly adults were tested in August and September. These two species were used as surrogates because they are common species of concern for being transported via air cargo. The following year final testing was conducted for both insect species using the rates and products that had been identified from the 2021 container tests. Confirmatory testing was done within a large military cargo plane located at a museum adjacent to Dover AFB.

A suitable replacement for 10% d-phenothrin was identified for air cargo areas and baggage holds following testing. 1-Shot Aircraft Insecticide (EPA# 83795-1; Callington) applied at a rate of 1.05 oz / 1,000 cubic feet (triple the lowest rate of 0.35 oz) is very effective for any necessary insect mitigation efforts of hitchhiking insects that may be present in military and civilian air cargo spaces.

The Plant Protection and Quarantine Treatment Manual has been updated with the product and rate information identified in this study. It is anticipated that the Armed Forces Pest Management Board will adjust their protocols and product listings to make 1-Shot available for all control needs within military cargo planes.

Figure 1. Aerosol testing on a C-5A Super Galaxy airframe located at Dover Air Mobility Command Museum, Dover, DE.

Table 1. Summary results of aerosol trials of 1-Shot on insects in a C-5A airframe, 2022.

Species Tested	Treatment	No. Tests	No. Insects Tested	% Dead / Moribund @24 hrs	% Alive @24 hrs
Japanese beetle	1-Shot; 3X	6	720	100%	0%
Japanese beetle	Control	6	720	9.0%	91.0%
Spotted lanternfly	1-Shot; 2X	6	360	98.1%	1.9%
Spotted lanternfly	Control	6	360	36.4%	63.6%

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

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

[1]Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA
[2]Cornell University, Department of Ecology and Evolutionary Biology, Ithaca, NY
[3]Kansas State University, Department of Entomology, Manhattan, KS
[4]University of Massachusetts, Department of Environmental Conservation, Amherst, MA

For the second year, the Forest Pest Methods Laboratory has utilized molecular tools in support of PPQ's biological control effort to mitigate the spread of the Japanese beetle (*Popillia japonica)* using the pathogen *Ovavesicula popilliae.* This pathogen has been introduced to target sites to prevent the spread of Japanese beetle to western states such as California and Oregon, as well as other non-infested states through commerce and air cargo transport. In the summer of 2022, Japanese beetle specimens were collected and analyzed using a real-time PCR assay to detect the presence of *O. popilliae* DNA within the beetle. The results of this survey determined whether *O. popilliae* was successfully established in the various locations or if re-introduction of the pathogen was to be recommended for the following year.

Over 1,300 Japanese beetle were analyzed from 16 sites in seven states. Of these 16 collection sites, 11 (69%) from four states (Arkansas, Illinois, Ohio, and Virginia) contained at least one group of beetles that tested positive for *O. popilliae* infections, indicating *O. popilliae* establishment (Figure 1). Samples tested prior to inoculation at the Illinois and Virginia sites demonstrated presence of *O. popilliae* infections in the Japanese beetle groups tested. In contrast there were no detections of the pathogen in collections from Iowa, Missouri and Nebraska and only one sample group from Arkansas demonstrated establishment at sites from annual introductions of *O. popilliae* over several years.

The presence of the pathogen at the new sites in Illinois and Virginia are in contrast to the lack of pathogen presence following multiple annual reintroductions at several of our biological control locations. Factors that are likely involved include the influence of harsh climate and the need to identify inoculation locations that are suitable for the pathogen. These results have led to changes in site inoculation locations and trapping guidance for collaborators and will ideally result in a higher percent of establishment for all sites in the upcoming year.

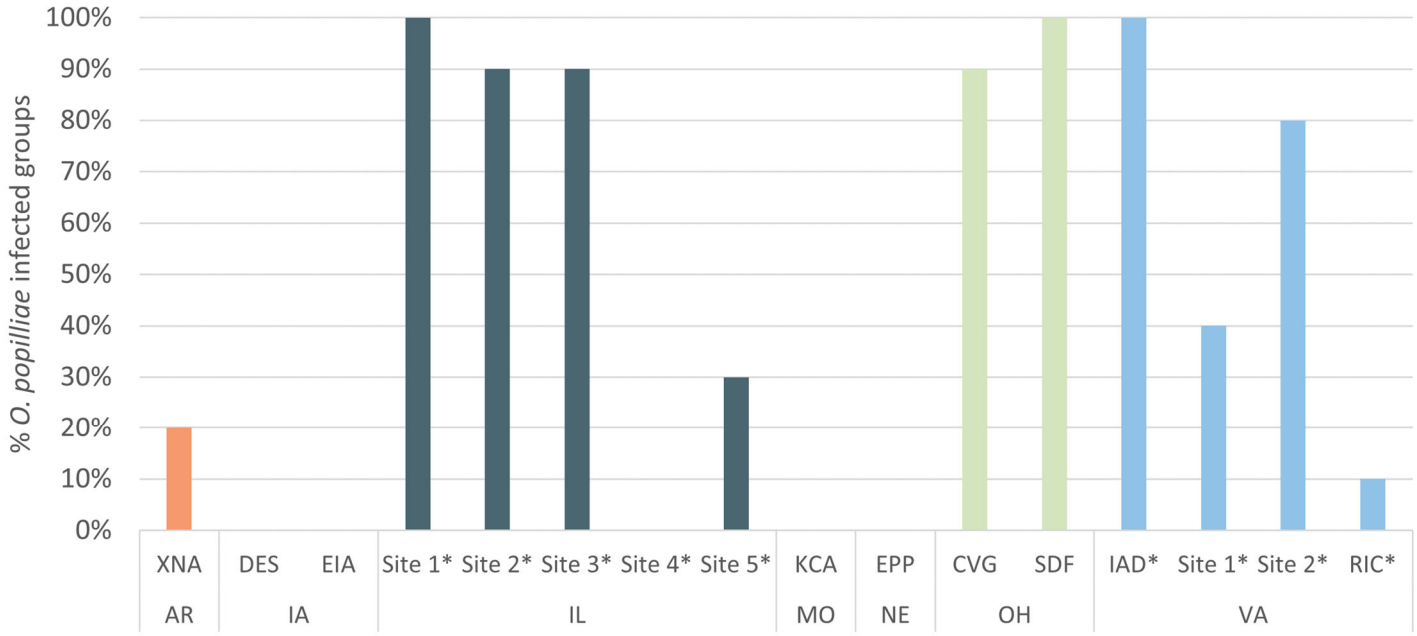

Figure 1. The percentage of Japanese beetle with *Ovavesicula popilliae* infections at introduction sites. Sites are FAA airport codes or alternative introduction sites. Sites where *O. popilliae was n*ewly introduced in 2022 is marked with an asterisk.

Flighted spongy moth complex trapping at U.S. military bases in Japan and the Republic of Korea

David Cowan[1], Baode Wang[1], and Ingrid Asmundsson[2]

[1]Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA
[2]USDA APHIS PPQ, Riverdale, MD

The Forest Pest Methods Lab (FPML) collaborated with PPQ's Preclearance and Offshore Program to conduct flighted spongy moth complex, FSMC, *Lymantria sp.*, trapping at U.S. military bases in Japan and the Republic of Korea. Trapping was conducted to investigate the duration and intensity of moth flight in support of NAPPO RSPM 33. These guidelines define the Specified Risk Period as the time during which additional regulations are imposed on vessels that have called on areas regulated for FSMC, and that are destined for ports in North America. During the Specified Risk Period there is an increased risk of hitchhiking adult moths and egg mass deposition on the vessels.

The Forest Pest Methods Lab provided scientific guidance, traps, and logistics for trap deployment and collection. Lures were also manufactured and/or procured by FPML personnel. Traps were deployed in two sites in the Republic of Korea and five sites in Japan, with three sites on the main island of Honshu and two on Okinawa. Milk carton traps were used for FSMC trapping in Korea whereas delta traps were used in Japan. A few wing traps were also deployed for *Lymantria xylina in* Okinawa. Deployment and servicing of the traps were performed by cooperators in the U.S. Army and samples were subsequently shipped to the FPML for identification and enumeration.

In the Republic of Korea, 508 FSMC were caught with numbers remaining consistent with what was observed in 2021 [1] (Figure 1). Moth flight began in late June and tapered off by mid-August, which is within the Specified Risk Period of June 1st through September 30th [2].

In Honshu, 66 FSMC were caught with numbers decreasing slightly from 2021. Moth flight began in late-June and ended by early August. In Okinawa, 67 *Lymantria albescence* were caught with overall trap catch increasing slightly from 2021. *Lymantria albescence* flight began before the start of the Specified Risk Period on May 25th; however, the majority of *L. albescence* were caught within this period — May 25th to June 30th [2].

Seventy-one moths were caught in *L. xylina* traps deployed in Okinawa and flight began before the middle of June and tapered off by the end of June. The Specified Risk Period does not apply to *L. xylina* as it is not a FSMC species.

Population levels of FSMC at trapping sites in the Republic of Korea and Japan were at low to very low levels in 2022. Population levels of *L. xylina* were at very low levels at trapping sites in Okinawa, Japan. This trapping survey provided PPQ with valuable information that aids in the determination of relative risk and timing of FSMC from these geographic areas and provided additional data on population levels of *L. xylina.*

References:

1. Cowan, D., Wang, B., Asmundsson, I., Mackay, Viera, K., J. and Leva, N. Asian gypsy moth trapping at U.S. military bases in Japan and the Republic of Korea. 2022. pp. 24. In: Viera K, Booth, and Davila-Flores A (eds). 2022. 2021 Accomplishment Report Forest Pest Methods Laboratory. United States Department of Agriculture, Buzzards Bay, MA.
2. NAPPO, North American Plant Protection Organization, RSPM 33, Guidelines for Regulating the Movement of Vessels from Areas Infested with the Asian Gypsy Moth. 2017. Accessed from RSPM_33-01-08-17-e.pdf (nappo.org).

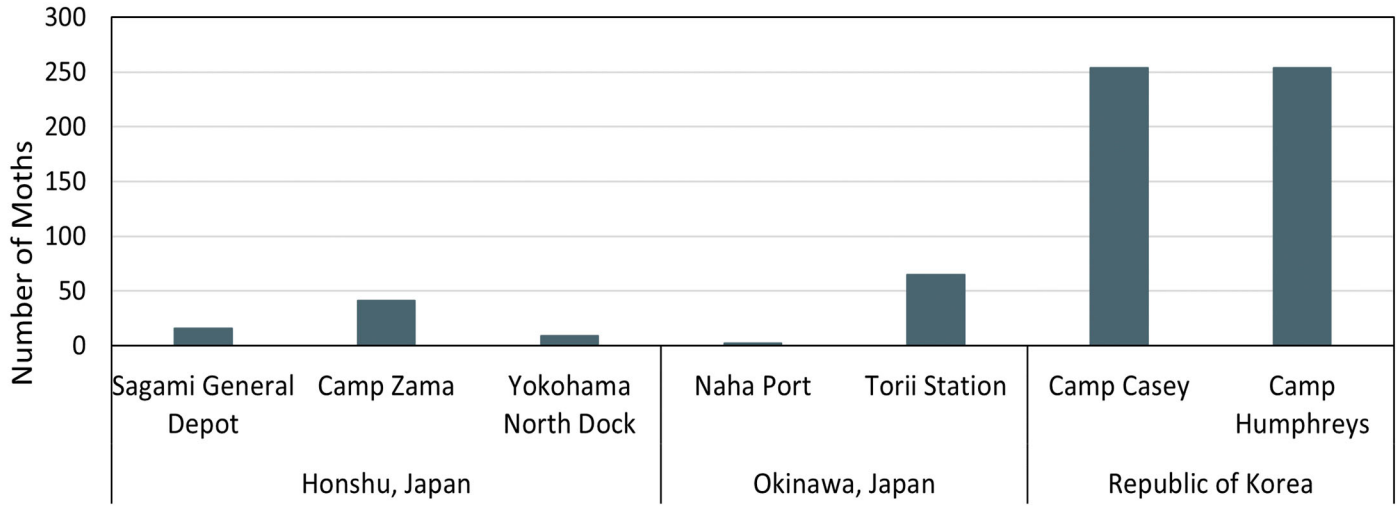

Figure 1. Flighted spongy moth complex trapping results for Japan and the Republic of Korea in 2022.

2022 Forest Pest Methods Laboratory insect production

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

[1]Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA
[2]Emerald Ash Borer Biocontrol Rearing Facility, USDA APHIS PPQ FO, Brighton, MI
[3]University of Richmond, Department of Biology, Richmond, VA

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

Chemical ecologists used 60 ALB specimens to research attractants for support of the CAPS program. Over 3,100 live larvae were produced for a biological control study that planted ALB larvae in sentinel logs to monitor for native parasitoids. Over 500 preserved ALB specimens were provided to federal and state outreach programs, and over 600 live eggs, larvae, and adults were provided to support domestic and foreign academic research. Emerald ash borer was reared at the Brighton, Michigan, EAB facility to support development of a wood-free rearing system for EAB and its parasitoids.

The USDA program to Slow the Spread of *Lymantria dispar* was provided over 11,000 *L. d. dispar* pupae for research on integrated pest management, focusing on developing a system to detect pheromones, evaluate mating-disruption techniques, and evaluate trapping methods. In addition, over 4,500 egg masses were provided to federal and academic institutions in support of biological control, pathology, ecology, pesticide efficacy, virology, and molecular diagnostics research. Commercial production of *Lymantria* virus was supported by the provision of 1,900 egg masses to Andermatt Canada. Fifty-one *L. d. dispar* displays and 24 displays comparing *L. d. dispar* with *L. d. asiatica* were prepared and provided to U.S. State Plant Health Directors for outreach use (Figure 1).

The FPML box tree moth colony provided thousands of research insects to develop mass rearing and integrated pest management techniques, and dried specimens were used to provide states with 43 moth displays in support of outreach and identification (Figure 1). In support of Commodity Treatment research done at the FPML, 96,660 eggs, 2,000 larvae, and 35,913 pupae of European grapevine moth, and 97,163 eggs of Old-World bollworm were provided for studies to test ethyl formate as an alternative to methyl bromide for fumigation of grapes and other produce imported at U.S. ports of entry.

Figure 1. Riker mounts displaying the full life cycles of *Lymantria dispar dispar* and *Cydalima perspectalis*

Box tree moth radiation biology

Hannah Nadel[1], Christine Swank[1,2], Gregory Simmons[3], and Kristine Grayson[2]

[1]Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA
[2]University of Richmond, Department of Biology, Richmond, VA
[3]Forest Pest Methods Laboratory Salinas Field Station, USDA APHIS PPQ S&T, Salinas, CA

The Box Tree Moth (BTM) Mitigation Program supports safeguarding of nursery production by developing tools to control or eradicate the pest. Among potential mitigation tools is the inherited sterile insect technique (ISIT), in which insects are mass produced, partially sterilized, and released in large numbers to reduce reproductive capacity and size of wild pest populations. An appropriate sterilizing radiation treatment for BTM was investigated. Effects were examined in the treated moths and in their offspring.

Doses of radiation appropriate for ISIT were found to lie between 130 and 200 Gy. At and above 130 Gy, females were rendered completely sterile; none of their eggs hatched when they mated with fertile males (Figure 1). Doses of 120 and 200 Gy were high enough to partially sterilize males (Figure 1), but their offspring (not shown) were far more sterile (fewer than 6% of their eggs hatched). Additionally, doses below 200 Gy were low enough to avoid affecting male capacity to mate with fertile females in laboratory cages. This suggests that radiation doses between 130 and 200 Gy would not hinder the released male's ability to fly and mate with fertile wild females, and therefore would promote the success of the SIT program.

The study provided the radiation dose appropriate for partially sterilizing BTM for deployment of ISIT, one of several strategic options to slow the spread or eradicate incursions of BTM in the United States.

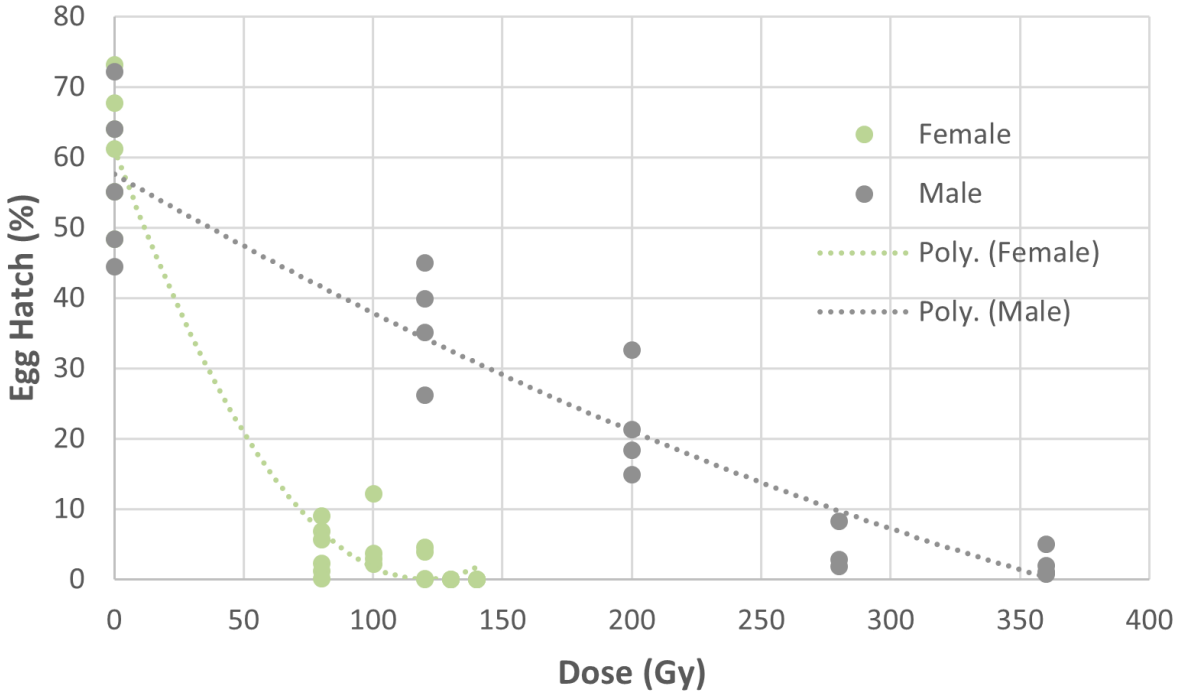

Figure 1. Percentage egg hatch (fertility) of male and female box tree moth exposed to a range of radiation doses and mated to unirradiated moths. Each dot represents the result of a cage with six pairs of moths. The dots on the left represent results from normal, non-irradiated moths. Females became completely infertile from exposure to 130 Gy and above. Males exposed to radiation were partially fertile after exposure to all tested doses.

Increasing egg hatch of long-term stored spotted lanternfly egg masses

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

[1]Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA
[2]University of Rhode Island, Department of Plant Sciences and Entomology, Kingston, RI

Thousands of spotted lanternfly, SLF, *Lycorma delicatula*, egg masses are stored for biocontrol research at the Forest Pest Method Laboratory. Egg masses and nymphs are needed for host specificity testing, life cycle studies, and rearing of SLF parasitoids. Because egg masses are not available in the field throughout the entire year, optimal cold-storage and emergence regimens are needed to keep egg masses viable for up to eight months in a laboratory setting. The goal of the present study is to increase nymph emergence from cold-stored (5℃, 0:0 L:D) egg masses throughout the year. A post-cold storage emergence temperature treatment emulating Pennsylvania spring (PA spring) conditions was tested, and nymph emergence was compared to the commonly used 25℃ (16:8 L:D) emergence condition.

Over an 8-month period, emergence of SLF nymphs was higher in egg masses that experienced the PA spring treatment than those that went directly into 25℃, except for egg masses stored for five months. After seven months of storage, there was a significant decrease in percent emergence of nymphs from egg masses in both treatments, but emergence was still higher for egg masses placed in PA spring temperatures compared to 25℃ (Figure 1). Post-emergence egg dissections revealed that irrespective of treatment, most eggs that did not emerge halted in a late stage of development, indicating that despite a prolonged chill period, developing nymphs can break diapause in both PA spring and 25℃ conditions. The percent of late-stage, unemerged eggs increased gradually as 5℃ cold-storage time increased. The number of desiccated and undeveloped eggs was low and not affected by treatment or length of storage.

The PA spring emergence regime significantly increased SLF nymph hatch. The improved storage and emergence regime allows for more efficient use of egg masses and a prolonged supply of nymphs throughout the year. Dissections showed that as cold storage time increased, eggs were able to break diapause, but were unable to exit the egg. This information can be used to guide future emergence optimization studies. Future studies will examine fitness traits of nymphs in these two emergence regimes. In addition, early season egg masses will be collected to determine a step down into cold storage procedure that can be used in conjunction with the PA spring emergence regime to obtain the highest percent of nymph emergence throughout the year.

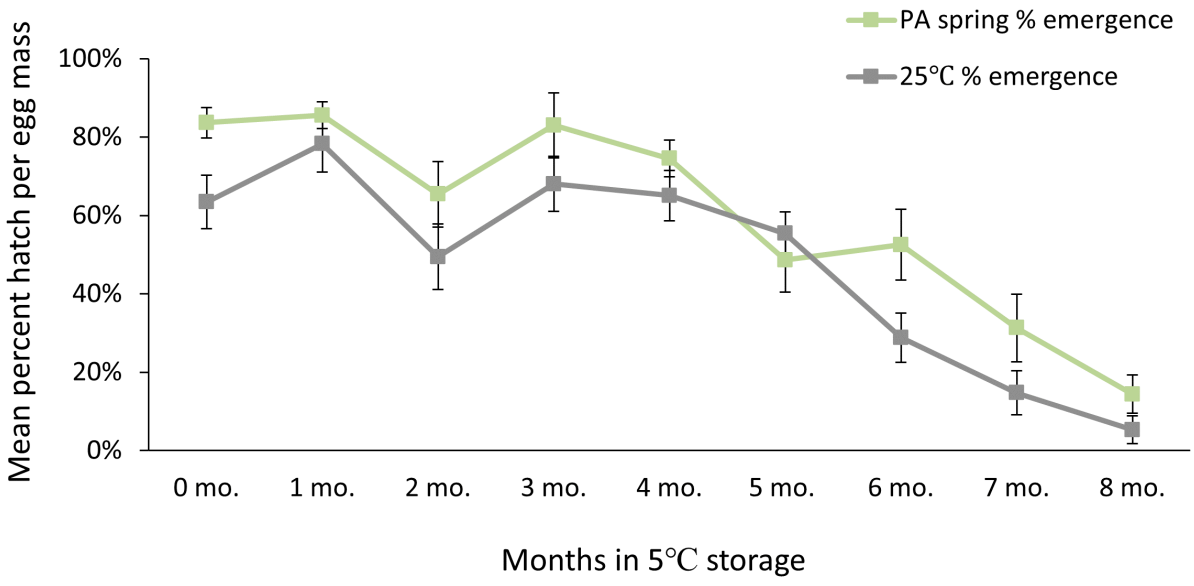

Figure 1. Mean percent monthly emergence of SLF nymphs from cold-stored egg masses that experienced PA spring and 25℃ emergence regimes over an 8-month period.

Influence of alternative hosts on trap catch and detection of spotted lanternfly

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

[1]Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA
[2]Xavier University, Department of Biology, Cincinnati, OH
[3]Forest Pest Methods Laboratory Bethel Field Station, USDA APHOS PPQ S&T, Bethel OH

Previous trap development work has shown circle traps to be an effective survey tool for spotted lanternfly, SLF, *Lycorma delicatula*, when placed on its preferred host, tree-of-heaven, TOH, *Ailanthus altissima*. However, little work has been done to investigate the efficacy of utilizing alternative host trees for trap placement despite SLF's highly polyphagous feeding habits. In 2021, a multistate assay was setup showing SLF detection rates to be comparable on black walnut, *Juglans nigra*, and TOH, expanding the available trapping and surveying options [1]. In 2022, an assay was conducted to compare SLF trap catch and detection rates on another potentially attractive tree genus, maple, *Acer* spp. (Figure 1). Trapping plots consisting of both maple and TOH were placed in SLF-infested sites in New Jersey, West Virginia, and Indiana to compare trap catch and detection across all SLF life stages. Results showed there was no significant difference in trap catch or detection between the two hosts for either nymphs or adults (Table 1). Having maple as an available host to use for trap placement provides alternative options for surveying areas where TOH are not available or accessible and will increase current surveying options for the spotted lanternfly program.

Figure 1. Spotted lanternfly nymphs feeding on new growth of a maple tree.

References:

1. Francese, J., Booth E., Devine S., Cooperband, M., and Murman, K. Improved survey methodology for spotted lanternfly: influence of host, pesticide strips, and trap check frequency on circle trap catch and detection. Pp. 26. In Viera, K., Booth, E., and Davila-Flores A. (eds) 2021. Accomplishment reports Forest Pest Methods Laboratory. USDA Buzzards Bay MA.

Table 1. Detection rates of spotted lanternfly nymphs and adults from circle traps set on *Acer* and *Ailanthus altissima* at three population densities (low, medium, and high).

Life Stage	Maple Low Density ≤ 30 SLF (n=7)	TOH Low Density ≤ 30 SLF (n=7)	Maple Medium Density 30-100 (n=5)	TOH Medium Density 30-100 (n = 5)	Maple High Density 100+ (n = 15)	TOH High Density 100+ (n = 15)
1st-3rd Instar	71.4%	57.1%	100%	100%	100%	100%
4th Instar	42.8%	28.6%	60%	60%	80%	73.3%
Adult	85.7%	100%	80%	100%	86.7%	93.3%

Forest Pest Methods Laboratory CAPS lure support for the detection and survey of pest insects in 2022

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

[1]Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA

For noncommercial insect lures, the Cooperative Agriculture Pest Survey (CAPS) relies on the support of Forest Pest Methods Laboratory (FPML) to produce survey-important insect lures. Lures produced at the FPML are a crucial component in helping PPQ safeguard U.S. agriculture and natural resources against the entry, establishment, and spread of economically and environmentally significant pests, and facilitate the safe trade of agricultural products. Additionally, these lures support insect survey efforts by State and academic partners.

Most of the pheromone-based lures produced are mixtures of two to five chemical components in ratios that are specific to the target insect species. In addition to the ratio-specificity, release rate, field longevity, and shelf-life are all important constituents of a lure formulation. Since improperly formulated lures can result in false negative detections, it is of the upmost importance that the FPML formulated lures can attract the target insect species. Lure formulation is based on the latest scientific literature, in-house research, and PPA 7721 funded research with collaborators in the U.S. and throughout the world. In addition to lure production, FPML performs Quality Control (QC) of FPML-produced lures and those that are commercially purchased through the CAPS program, ensuring that lures made available through the CAPS Survey and Supply Program attract the target insect species.

In 2022, the FPML produced 105,872 individual lures, an increase of 8.8 % over last year's output. Below is a graphical presentation of the FPML CAPS lure production for 2022 addressing lure productions for 29 different insect species. In addition to CAPS support, FPML continued to support the USDA Forest Service with QC analysis of *Lymantria dispar dispar* mating disruption formulations and supported various local and international research with experimental insect lure formulations in 2022.

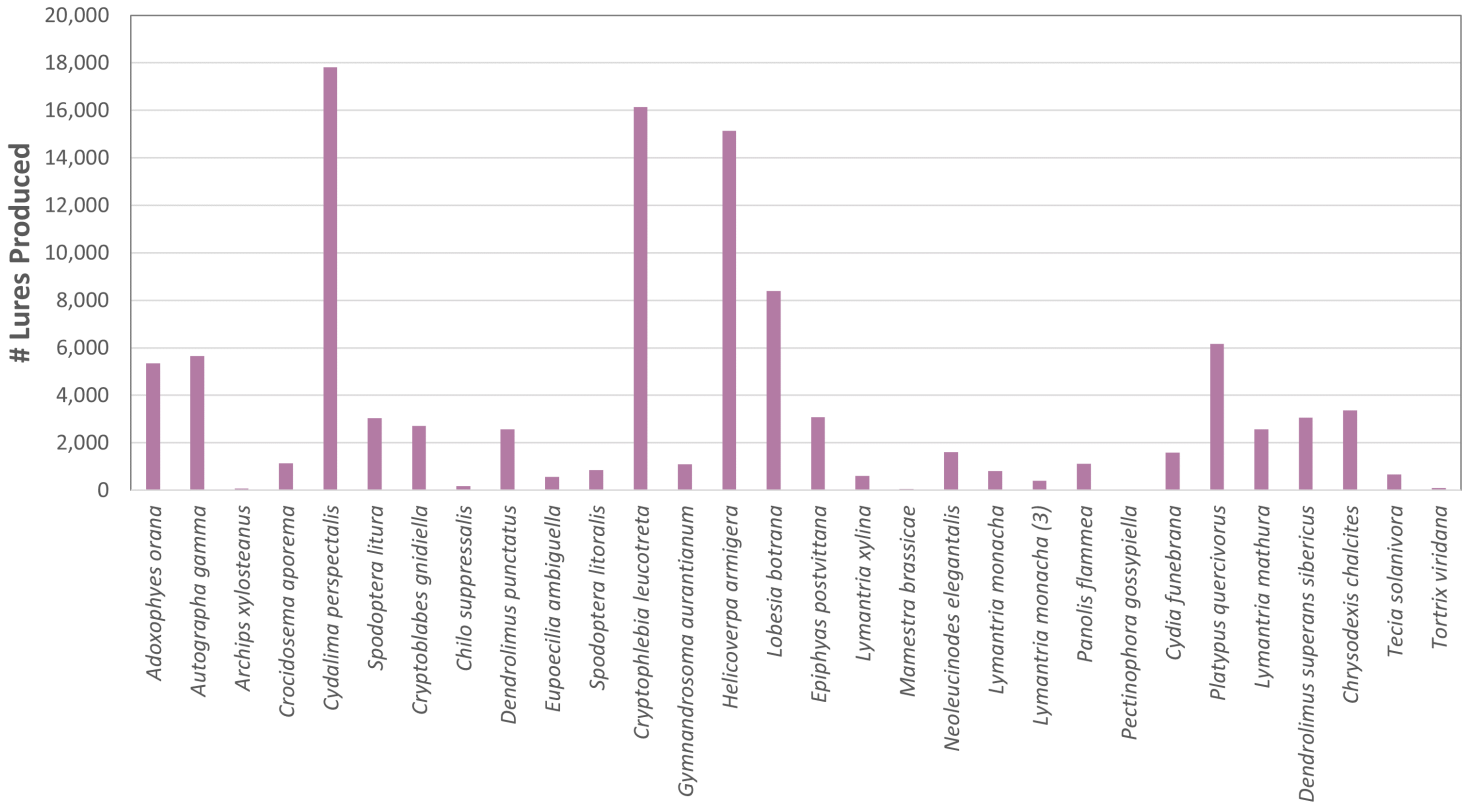

Figure 1. Forest Pest Methods Laboratory CAPS lure production in 2022 for 29 different insect species.

2022 Port and Domestic Spongy Moth Molecular Diagnostics Survey

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

[1]Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA
[2]University of Massachusetts Amherst, Department of Environmental Conservation, Amherst, MA
[3]Kansas State University, Department of Entomology, Manhattan, KS
[4]Cornell University, Department of Ecology and Evolutionary Biology, Ithaca, NY

To support PPQ's Spongy Moth Detection Programs, the Forest Pest Methods Laboratory (FPML) has utilized molecular tools to identify suspect *Lymantria* specimens both intercepted at U.S. ports of entry and trapped domestically for 30 years. Previously, a well-established standard PCR assay was used to distinguish between European and Asian spongy moth subspecies based on analysis of nuclear and mitochondrial DNA. In 2022, the standard assay was fully replaced by a newly validated real-time PCR (qPCR) assay, which utilizes fluorescent probes so that DNA amplification can be monitored in real-time. The assay requires lower amounts of DNA to function and is quicker compared to the standard PCR assay. Furthermore, the spongy moth qPCR assay is comprised of four sub-assays that allow for the identification of *Lymantria dispar disar*, *L. d. asiatica, L.d. japonica*, and *L. umbrosa,* thus increasing diagnostic capabilities.

In the 2022 calendar year, 29 specimens were intercepted from eight U.S. ports. Analysis showed 14 *L. d. asiatica*, 1 *L. d. japonica*, 2 *L. d. dispar*, and 12 non-spongy moth specimens. For the domestic survey, spongy moth specimens were received for molecular analysis from 33 states. A total of 11,723 specimens were processed, an 88% increase from 2021 (Figure 1). The assay identified 11,468 *L. d. dispar*. Twelve samples were determined to be a species other than spongy moth via DNA barcoding. No *L. d. asiatica*, *L. d. japonica* or *L. umbrosa* were detected. Two hundred and forty-three specimens remain unknown due to diagnostic assay failure, indicating a 2% failure rate with the qPCR assay. In comparison, the 2021 failure rate with the standard PCR assay was 9%.

Due to this year's increase in samples submitted, FPML is attempting to conserve storage space by instituting a new storage policy for spongy moth survey samples. The previous policy was to keep all materials analyzed, which led to a collection of more than 55,000 specimens and 30 years' worth of DNA extracts. The new policy will limit the long-term storage of processed domestic samples to 196 specimens per state, per year. For port samples and DNA extracts, all tissue vouchers and DNA extracts will be kept.

Overall, the molecular diagnostic results of this survey provide invaluable information to PPQ's Spongy Moth Detection Programs, which aims to monitor current spongy moth populations and the possible introduction of invasive subspecies into the U.S. Implementation of the qPCR assay has resulted in increased diagnostic capabilities, improved assay success, and faster processing.

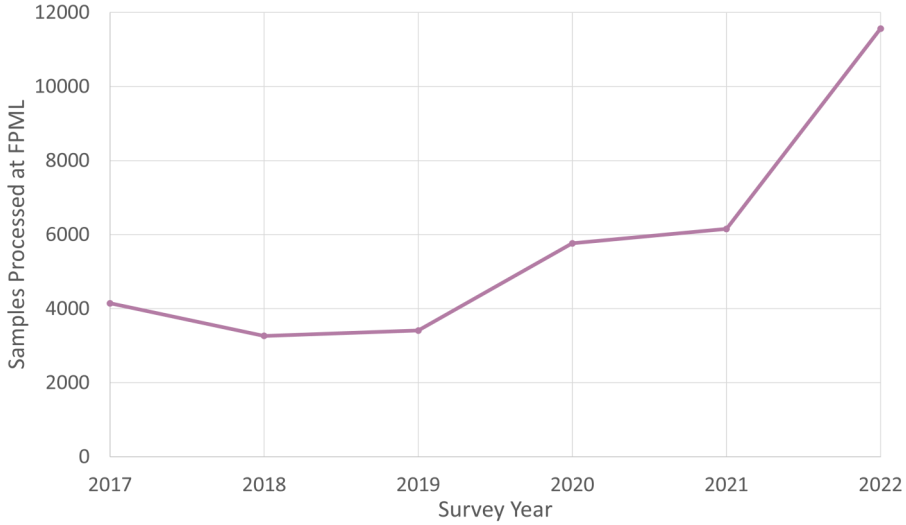

Figure 1. Graph showing number of samples processed at FPML every year from 2017 to 2022.

Cryptic genetic diversity and associated ecological differences of *Anastatus orientalis*

Yunke Wu[1,2], Hannah Broadley[1], Kendra Vieira[1], John McCormack[1,3], Hyeban Namgung[4], Yeongmo Kim[4], Alana McGraw[1,5], Marjorie Palmeri[1,3], and Juli Gould[1]

[1]Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA
[2]Cornell University, Department of Ecology and Evolutionary Biology, Ithaca, NY
[3]University of Massachusetts Amherst, Department of Environmental Conservation, Amherst, MA
[4]Kunsan National University, Department of Biological Science, Gunsan, Republic of Korea
[5]Kansas State University, Department of Entomology, Manhattan, KS

Molecular tools were utilized to assess genetic diversity of *Anastatus orientalis*, an egg parasitoid of spotted lanternfly (SLF), collected from China (Beijing and Yantai) and South Korea. Iso-female lines (originating from a single female) of *A. orientalis* were established based on genetic results and showed different responses in diapause behaviors to rearing conditions. Work was conducted at the Forest Pest Methods Lab to understand the genetic basis that underlies the observed discrepancies in important life history traits of *A. orientalis*.

Molecular analysis of two mitochondrial DNA segments recovered six haplotype groups (A–F) among 160 Chinese wasp specimens. Some haplotypes are widespread, and others only occur in certain locations (Figure 1). In Beijing, most specimens belonged to Haplotype C or D and their derivatives. Collections made in different years had similar genetic composition. Haplotype B and E seemed to be unique to the Yantai population. Haplotype B was the most abundant haplotype among all Yantai specimens at a frequency of 44.12%, double the frequency of Haplotype C or D in the Yantai collections. The 134 Korean specimens exhibited lower genetic diversity compared to the Chinese populations, and only possessed Haplotype C and D, and a few derivatives. Uncorrected genetic distances between haplotype groups range from 0.44% to 1.44% after controlling for within-group variation. The Chinese *A. orientalis* is characterized by high local genetic diversity and low wide-range genetic differentiation.

Additionally, significant differences in diapause behaviors were observed between the iso-female lines when reared in different conditions, indicating that haplotype had the strongest effect on diapause behaviors followed by rearing conditions. Rearing conditions that mimicked Beijing in mid-September resulted in high numbers of Haplotype C and D progeny wasps, but very few Haplotype B were produced. When reared in constant 25°C temperatures and with a long day condition (17.5 h light: 6.5 h dark), a high number of Haplotype D, a few Haplotype C, and no Haplotype B wasps were produced.

Our results suggest a genetic component in determining the life history traits of *A. orientalis*, which provides critical information for wasp rearing and subsequent tests of host specificity necessary for evaluating this parasitoid as a biological control agent for the SLF Program.

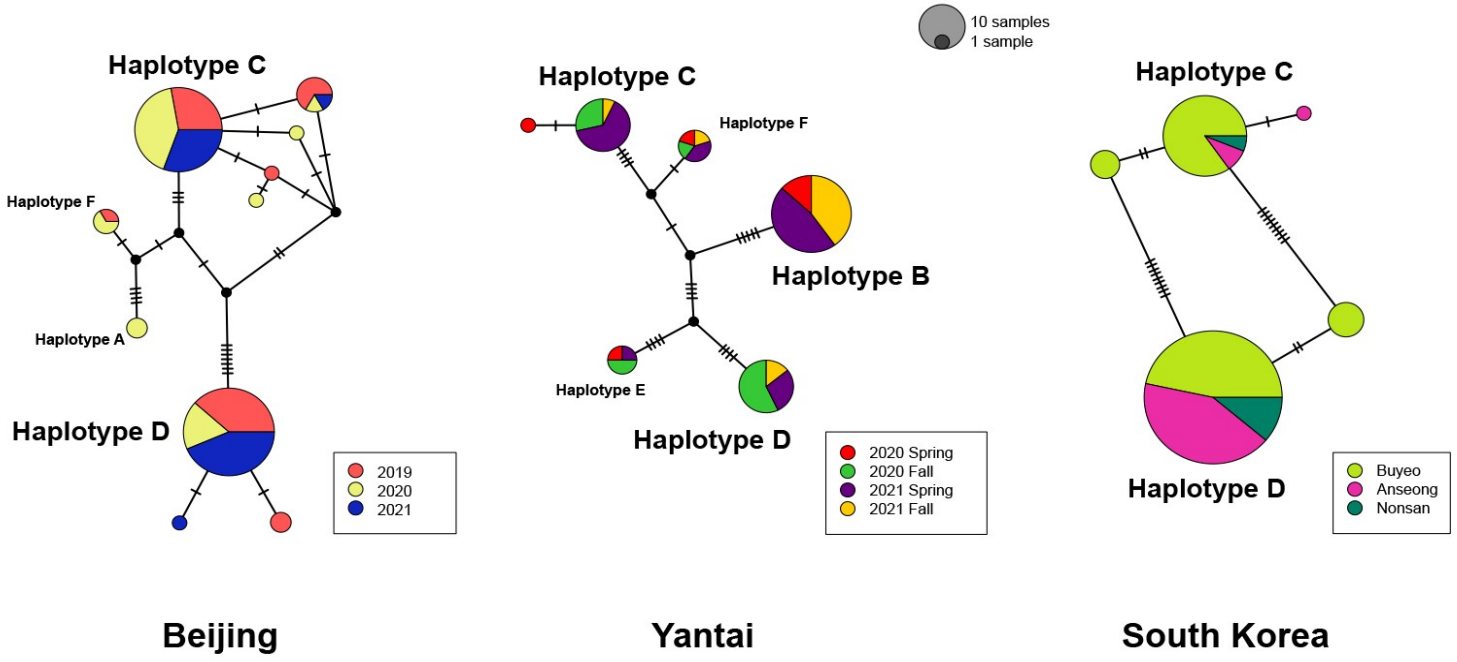

Figure 1. Genetic network of haplotypes in *A. orientalis* collected from China (Beijing and Yantai) and South Korea. Haplotypes are represented by pie charts, which size is proportional to the number of specimens. All three networks are on the same scale. Specimens are color-coded by collection year, emergence season, or location.

Assessing the accuracy of the spongy moth real-time PCR assay using specimens collected from Asia

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

[1]Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA
[2]Cornell University, Department of Ecology and Evolutionary Biology, Ithaca, NY
[3]University of Massachusetts Amherst, Department of Environmental Conservation, Amherst, MA
[4]Kansas State University, Department of Entomology, Manhattan, KS

To better support PPQ's Spongy Moth Detection Programs in the identification of spongy moth (*Lymantria dispar*), including flighted subspecies (*L. d. asiatica* and *L. d. japonica*), as well as *Lymantria umbrosa*, a real-time PCR (qPCR) molecular assay was fully implemented in the 2022 survey season to replace the obsolete agarose gel-based diagnostic assay used at the Forest Pest Methods Laboratory. During the survey season, results not matching expected patterns prompted the necessity to thoroughly reassess assay accuracy using moths with known collection localities from Asia.

Because spongy moth subspecies are generally restricted to respective geographic areas, assay accuracy was defined as correctly matching the molecular identification to its known collection location. It must be noted, however, that human-mediated moth translocations may lead to incorrect assumptions on species based on geographic location, leading to false "errors" in assay performance.

We sampled 100 specimens each from China, Japan, South Korea, and Russian Far East aiming to sample as many locations as possible (average 2-3 moths per location). Two sources of error were identified which resulted in a mismatched assay result: 1) point mutation (change of a single nucleotide) that hinders the binding of primer or probe to the target DNA, resulting in negative amplification when positive results are anticipated; 2) *L. d. asiatica* or *L. d. japonica* subspecies possess DNA segment that closely resembles *L. d. dispar*, resulting in the assay erroneously identifying the two former subspecies as *L. d. dispar*. The two sources of error were present in the Chinese and Korean samples but not found among the Japanese and Russian samples.

Results from the present study as well as original qPCR validation work is summarized in Table 1. A total of 507 specimens with known collection localities in Asia have been used to validate the qPCR assay to date. The Korean population resulted in the highest level of inaccurate identifications (12%), whereas moths from Japan and the Russian Far East were consistently identified correctly. Presence of European-like DNA among Asian populations occurred at a frequency about 1.5% on mainland Asia. The total assay error rate from both sources was estimated to be 5%, therefore assay accuracy was 95%.

Our reassessment work presents the first quantification of assay accuracy in processing samples from the annual spongy moth survey. This work provides survey confidence levels for detecting spongy moth subspecies originating from different parts of Asia.

Table 1. Combined data showing qPCR assay accuracy (error rate) for spongy moth specimens collected from Asia. There are two sources of error: point mutation and European-type DNA. The total error rate was estimated to be 5%.

	Moth with point Mutation	European type DNA	Number successfully amplified by qPCR
China	4	3	119
Korea	17	2	158
Russian Far East	0	0	63
Japan	0	0	149
L. umbrosa	0	0	25
Total	21	5	514

Optimization of the spongy moth real-time PCR assay for increased efficiency

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

[1]Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA
[2]Cornell University, Department of Ecology and Evolutionary Biology, Ithaca, NY
[3]University of Massachusetts Amherst, Department of Environmental Conservation, Amherst, MA
[4]Kansas State University, Department of Entomology, Manhattan, KS

With the full implementation of the real-time PCR (qPCR) molecular assay in the 2022 spongy moth survey season, we were able to process a record high number of over 11,000 moths. Such an increase in specimens poses challenges for technician workload and reagent cost. To meet the growing demand from survey stakeholders, we explored options for assay optimization and tailored it for use under different circumstances.

The original assay comprised of four sub-assays and can identify a spongy moth specimen to subspecies. Given that the Asian subspecies are extremely rare or not present at all in samples collected domestically, it may not be necessary to run every specimen with all four sub-assays. Instead, a first round of screening using two modified sub-assays could be sufficient to separate the subspecies of Asian origin, from *Lymantria dispar dispar*. If the former is detected, the original version of the assay can be used to further identify the specimen to subspecies.

Several modifications were made to the qPCR primer and probe DNA sequences of the AGM and Ldd sub-assays, which now forms a new duplex. Performance of each modification was initially tested with DNA standards, and the most promising candidate was subsequently tested with 320 field-collected, previously identified specimens (30 of which belong to Asian subspecies). Our results showed that only two specimens originating from Asia failed to be detected by the new duplex. A second test with 234 samples of known origin in Asia resulted in the unsuccessful detection of seven samples. Therefore, assay accuracy of the newly developed duplex was estimated to be 96.6%, which is a conservative estimation because upon close examination of the qPCR amplification plot most failure samples showed weak positive signals.

Standard curves were established for the new duplex and compared to the original assay (Figure 1), confirming that the modified sub-assays had comparable performance to the original sub-assays in terms of linearity and efficiency.

When the number of sub-assays is reduced from four to two during the initial screening, diagnostic efficiency is doubled by significantly reducing sample processing time and reducing the reagent cost by half. Therefore, implementation of the modified duplex for initial screening of domestic spongy moth samples will be benefit the Spongy Moth Program's efforts to screen for Asian subspecies through the annual survey.

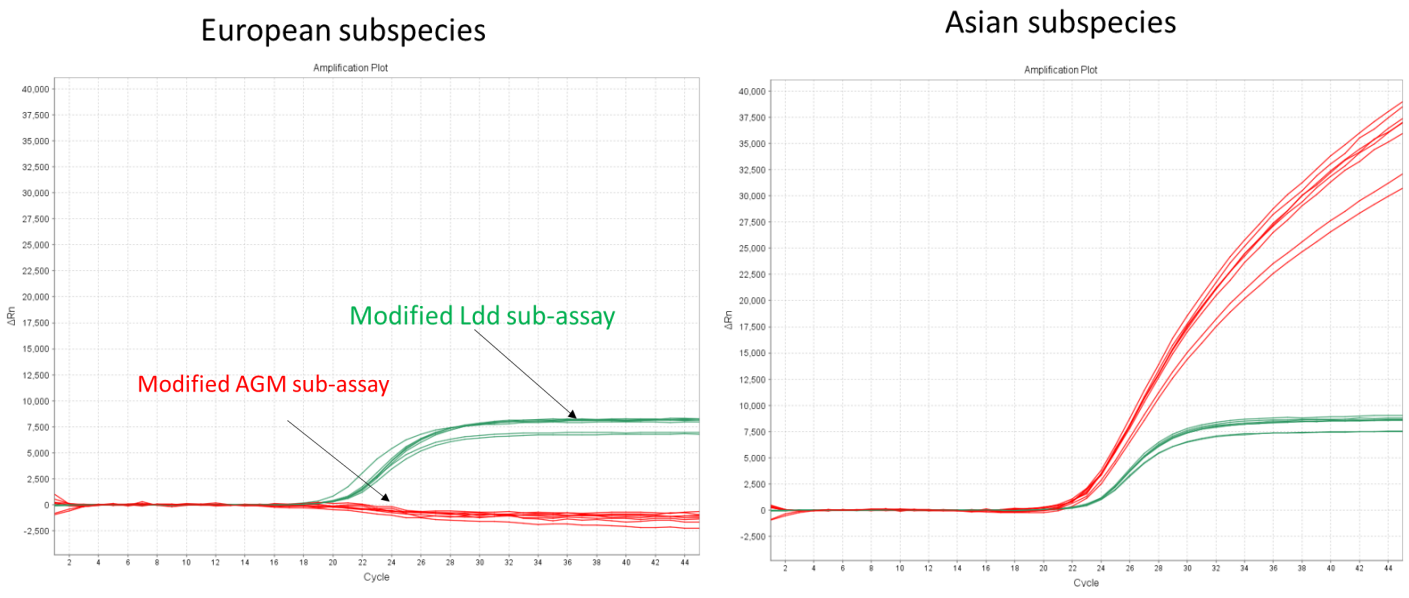

Figure 1. Two qPCR amplification plots when the sample is a European subspecies (left) and an Asian subspecies (right). Red lines are produced by the modified AGM sub-species and green lines are produced by the modified Ldd sub-assay. When the line elevates into a curve, the sub-assay is positive. A European subspecies is only positive for the modified Ldd sub-assay, whereas an Asian subspecies is positive for both sub-assays.

2022 *Lymantria dispar dispar* national risk assessment: Updating spongy moth risk model with 2021 trapping data

Melissa Warden[1] and Gericke Cook[2]

[1]Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA
[2]USDA APHIS VS Strategy and Policy, Fort Collins, CO

The *Lymantria dispar* risk assessment supports the Spongy Moth Program goals of 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* 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 2022 was updated with 2021 trapping data and USPS address forwarding data. In areas of short-range spread, about 2,400 more traps were set in 2021 than in 2020, and about 2,800 more traps had at least one positive detection. Some notable changes to the model included high-risk spread predictions increasing slightly farther to the west in Minnesota and to the south in the Carolinas and coastal Georgia. The risk model will be updated again in 2023 to reflect trapping data from the 2022 survey season.

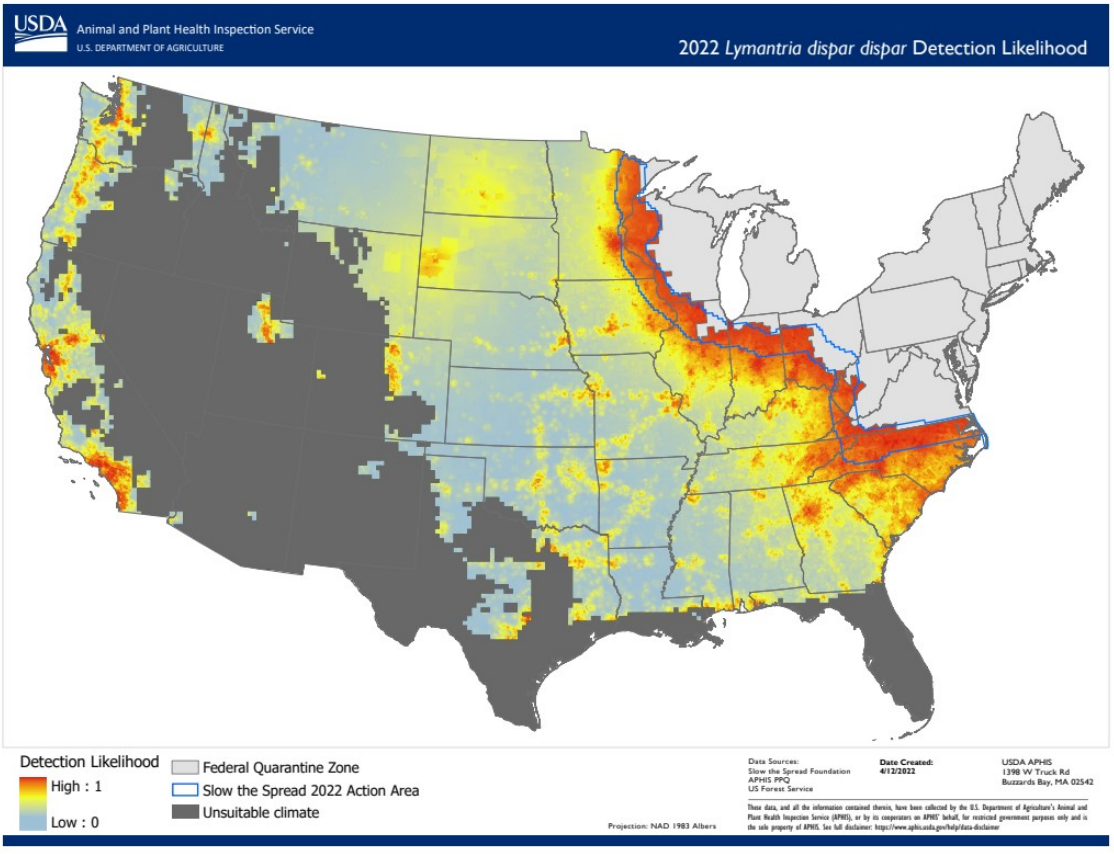

Figure 1. The 2022 *Lymantria dispar dispar* detection likelihood, with areas of unsuitable climate for establishment masked out.

Production of *Tamarixia radiata* and contributions to biological control of Asian citrus psyllid in California and Arizona

Ruth Henderson[1], Gregory Simmons[1], Raju Pandey[2], David Morgan[3], and Paul Rugman-Jones[4]

[1]Forest Pest Methods Laboratory Salinas Field Station, USDA APHIS PPQ S&T, Salinas, CA
[2]Citrus Research Board, Riverside, CA
[3]California Department of Food and Agriculture, Riverside CA
[4]University of California Riverside, Department of Entomology , Riverside CA

In 2013, a multi-agency team was established in California to support biological control of Asian citrus psyllid, ACP, *Diaphorina citri*. The biological control agent *Tamarixia radiata* is reared by the California Department of Food and Agriculture (CDFA) and the Citrus Research Board (CRB), with support from the Citrus Health Response Program. Agents are released in California and Arizona and used in an area-wide insect pest management (IPM) study in Hemet, CA.

A total of 3,356,538 *T. radiata* were produced in 2022. CDFA produced 2,711,595 *T. radiata* and CRB produced 445,290 in field cage insectaries and 199,653 in their isoline propagation colony. A total of 3,179,600 *T. radiata* was released in California. Another 90,000 were provided for classical biological control in Arizona and 3,700 for the Area-Wide IPM experiments in Hemet. The remaining *T. radiata* were used as starter material in production.

This year CRB contributed 372,726 *T. radiata* for release in California, 56,800 *T. radiata* to Arizona, and 2,100 *T. radiata* to the Area-Wide IPM studies. CDFA provided 34,900 *T. radiata* to Arizona and 1,600 to the Area-Wide IPM project. Over 26 million *T. radiata* have been released in California since 2013.

Tamarixia radiata release has been credited with a decline in observed ACP population density in California (Figure 1), and presence of Pakistani-origin *T. radiata* haplotypes were confirmed at non-release sites in two regions in Arizona where releases of *T. radiata* began in 2019, suggesting successful establishment. CRB has established 17 new isolines from *T. radiata* collected in the field in California to capture genetic diversity of field-adapted *T. radiata*.

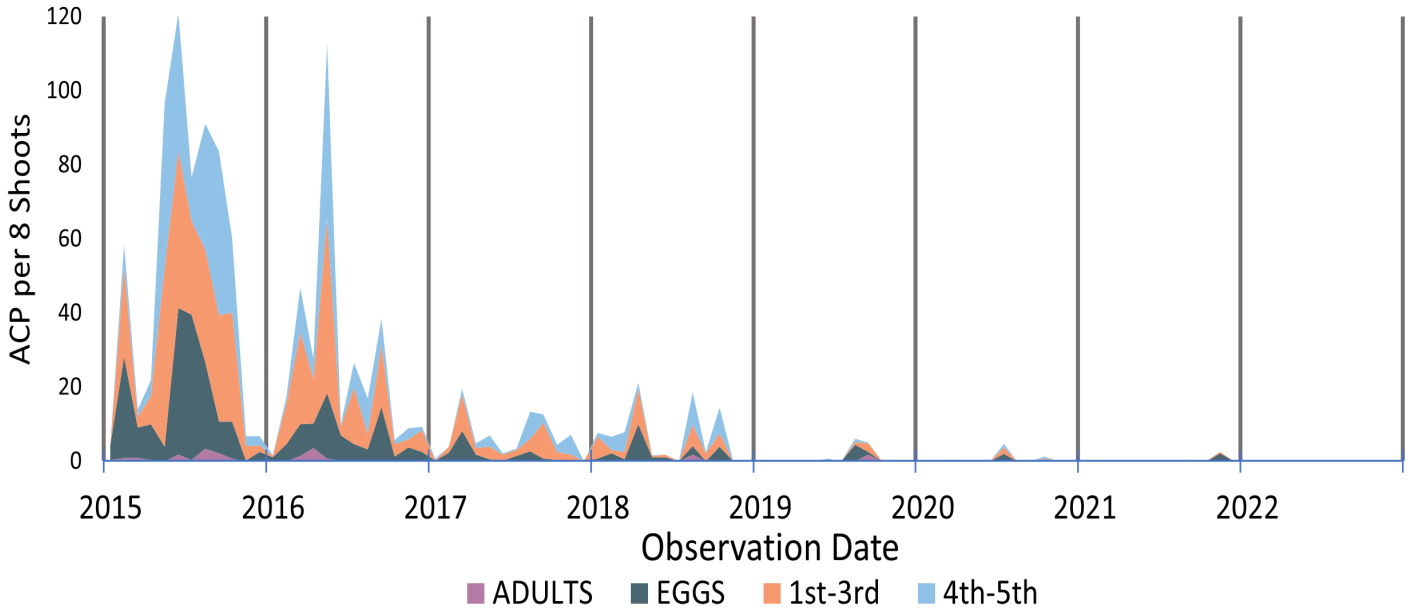

Figure 1. Monthly mean ACP life stages (adults, eggs, 1st-3rd instar nymphs, and 4th-5th instar nymphs) observed per 8 sampled shoots from 2015 to 2022.

Tool development for a systems approach to managing light brown apple moth in California nurseries

Ruth Henderson[1], and Gregory Simmons[1]

[1]Forest Pest Methods Laboratory Salinas Field Station, USDA APHIS PPQ S&T, Salinas, CA

The light brown apple moth, LBAM, *Epiphyas postvittana*, was deregulated as a federal quarantine pest but remains a concern for the export of live nursery plants grown in California. To minimize risk of LBAM spread and reduce the regulatory burden on nursery growers, we have developed a Treat and Ship protocol, a pre-shipment component of a systems approach to eliminating LBAM from shipments, which includes a list of pesticides fit for this purpose. Due to interest from nursery growers in less expensive pesticide options, we tested two low-cost pesticides, acephate and permethrin, for the treatment of LBAM.

Mean corrected mortality of eggs and neonate larvae from direct application of permethrin was 97.7%, and mortality from acephate was 59.5%. Permethrin residues caused 100% mortality to eggs and neonate larvae at two and seven days after treatment (DAT) and 93.7% at 14 DAT. Acephate caused 66.5% mortality at 2 DAT, 62.0% at 7 DAT, and 48.9% at 14 DAT. LBAM laid 82% fewer egg masses on permethrin treated plants than on control plants even 14 DAT. When applied to plants with 3rd-5th instar larvae in leafroll nests, permethrin caused 82.1% mortality, while acephate caused 69.4%.

While acephate was not effective enough for use in a Treat and Ship approach, permethrin performed similarly to current program pesticides (Figure 1). One gallon of Astro (active ingredient permethrin) is half the price of one quart of Scimitar GC (active ingredient lambda-cyhalothrin), a significant saving for growers. Efficacy data will be used to calculate risk of live LBAM in shipments of various sizes and create guidance for LBAM management.

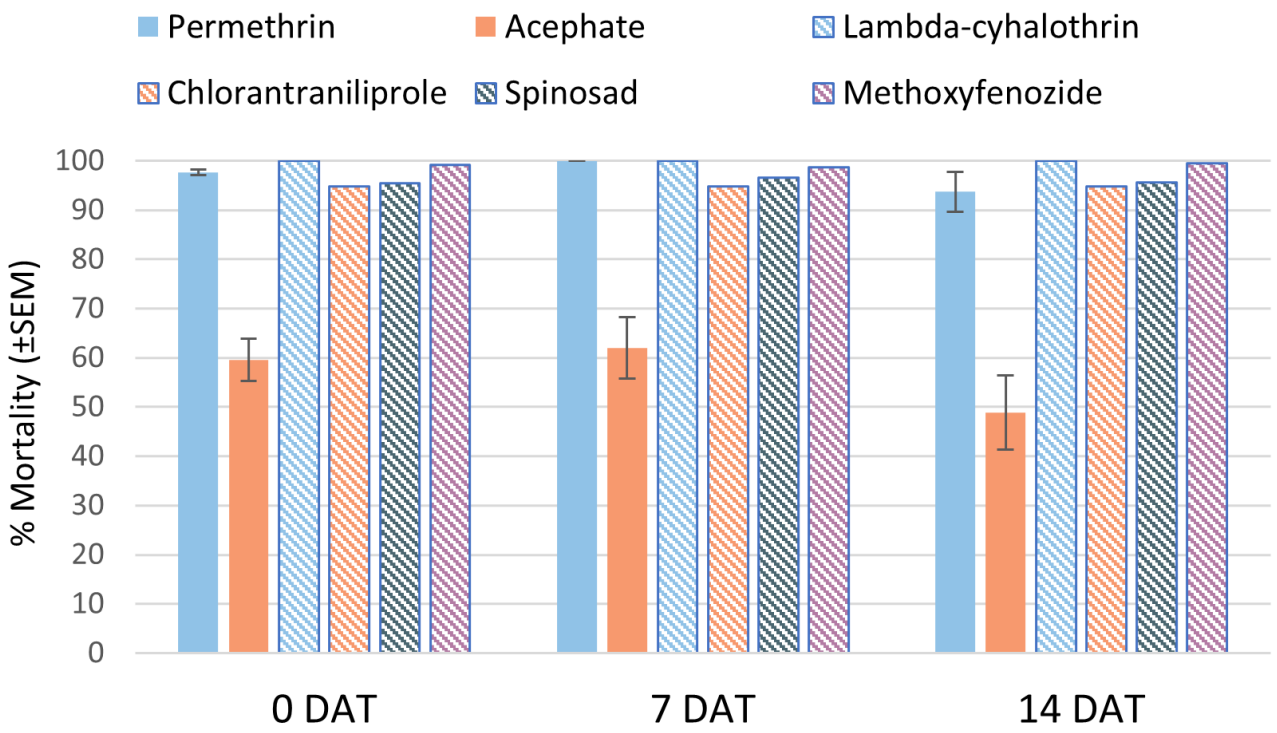

Figure 1. Mean corrected mortality (±SEM) of eggs and neonate larvae from permethrin and acephate (solid bars) and other program pesticides (shaded bars) from direct treatment (0 DAT) and residues (7 and 14 DAT).

Forest Pest Methods Laboratory

1398 W. Truck Rd.

Buzzards Bay, MA 02542

508-563-0900