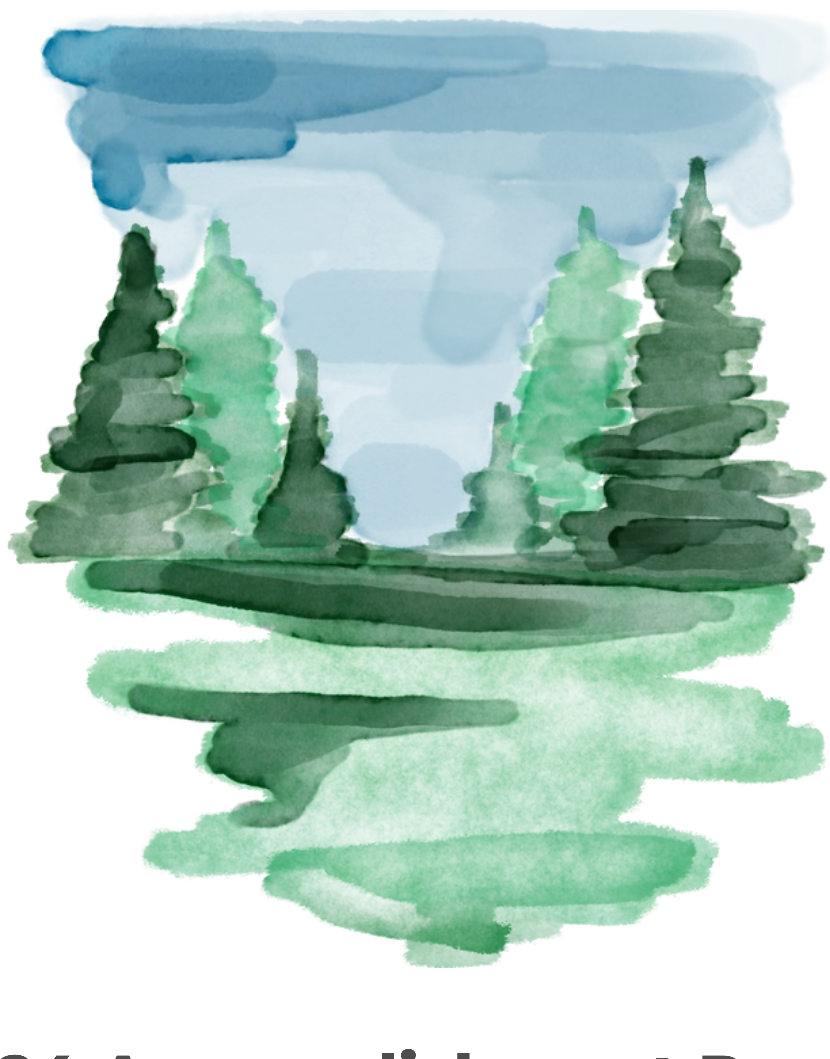
Animal and Plant Health Inspection Service
U.S. DEPARTMENT OF AGRICULTURE

Forest Pest Methods Laboratory



2024 Accomplishment Report

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

Forest Pest Methods Laboratory

2024 Accomplishment Report

Forest Pest Methods

Laboratory

2024 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: Amanda Davila-Flores, Everett Booth, and Angela Hoover

It was designed and formatted by: Amanda Davila-Flores, Everett Booth, and Angela Hoover

Contents

Publications

*FPML employees and cooperators are in bold

1. **Cooperband MF**, **Murman KM**. Spotted lanternflies respond to natural pheromone lures for mate-finding and oviposition. Insects. 2024;15(6).

2. Clare A. Rodenberg CA, Hajek AE, **Nadel H,** Stefanski A, Reich PB, Haynes KJ. Rising temperatures may increase fungal epizootics in northern populations of the invasive spongy moth in North America. NeoBiota. 2024;95; 291-301.

3. Cui M, Roe AD, Boyle B, Keena M, **Wu Y**, Braswell WE, Smith MT, Gasman B, Shi J, Javal M, Roux G, Turgeon JJ, Hamlin R, Porth I. Tracking the North American Asian longhorned beetle invasion with genomics. Evol Appls. 2024;1-17.

4. **Crook D**, Maynard E, **Furtado M**. Verification and evaluation of male-produced pheromone components from the citrus long-horned beetle, *Anoplophora chinensis* (Forster) (Insecta: Coleoptera: Cerambycidae). Insects. 2024;15(9).

5. **Crook D**, Wickham J, Ren L, Xu Z, Jones TH, **Warden M**, **Cosse A**. Further evidence that female *Anoplophora glabripennis* (Coleoptera: Cerambycidae) utilizes photo-degradation to produce volatiles that are attractive to adult males. Insects. 2024;15(12).

6. Dodds KJ, Sweeney J, **Francese JA**, Besana L, Rassati D. Factors affecting catches of bark beetles and woodboring beetles in traps. J Pest Sci. 2024;97(4);1767-93.

7. Faal H, **Cooperband MF**. Antennal sensitivity of spotted lanternflies, *Lycorma delicatula*: differential electrophysiological responses of males and females to compounds derived from host plants and conspecifics. Insects. 2024;15(3).

8. Gomes IV, Sobreira A, Aguilar JS, Carrera LG, Andrade JL, Caceres C, Verysen MJB, Stauffer C, **Myers SW**, Días V. Phytosanitary irradiation as an effective treatment for *Drosophila suzukii*. Sci Rep. 2024;14(1):22225.

9. **Gould JR**, **Losch C**, Sullivan L, **Wu Y**, Wang XY, Cao LM, **Broadley HJ**. Lifecycle of *Anastatus orientalis* (Hymenoptera: Eupelmidae) and synchrony with its host, the spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae). Environ Entomol. 2024;53(6):954-65.

10. Kamiyama MT, Takahashi M, **Broadley HJ,** Gomez-Marco F, Hoddle MS, Matsuura K. Evaluating the origin and spread of spotted lanternfly (*Lycorma delicatula*) in Japan. Population Ecology. 2024;67(2):1–18.

11. Santoiemma G, Battisti A, Courtin C, Curletti G, Faccoli M, Feddern N, **Francese JA**, **Franzen EKL**, Giannone F, Gossner MM, Kostanowicz C, Marchioro M, Nardi D, **Ray AM**, Rouques A, Sweeney J, Rooyen KV,Webster V, Rassati D. Testing a trapping protocol for generic surveillance of wood-boring beetles in heterogeneous landscapes. NeoBiota. 2024;95: 77-95.

12. Santoiemma G, Williams D, **Booth EG**, Cavaletto G, Connell J, Curletti G, DeGroot M, **Devine SM**, Enston A. **Francese JA**, **Franzen EKL**, Giasson M, Groznik E, Gutowski JM, Hauptman T, Hinterstoisser W, Hoch G, Hoppe B, Hughes C, Kostaniwicz C, Peterson DL, Plewa R, **Ray AM**, Salle A. Sucko K, Sweeney J, Rooyen KV, Rassati D. Efficacy of trapping protocols for *Agrilus* jewel beetles: a multi-country assessment. J Pest Sci. 2024;97(4):1795-810.

13. Scheff DS, Arthur FH, **Domingue MJ**, **Myers SW**. Combination insecticide treatments with methoprene and pyrethrin for control of khapra beetle larvae on different commodities. Insects. 2024;15(1).

14. Szymona K., Janowiak JJ, **Mack R**, Hamelin M, Gagnon M, Hoover K. Approach to validate ISPM-15 compliance for commercial treatment certification of dielectric standard heating of bulk solid wood packing materials using radio frequency. For. Prod. J. 2024;74(4): 318-330.

15. Zhang J, **Wang B**, Ren H, Chen J, Li J, Sun Y, Cui Y, Wang R, Liu M, Zhang F. Evaluation of the potential flight ability of the Casuarina moth, *Lymantria xylina* (Lepidoptera: Erebidae). Insects. 2024; 15(7) 506.

16. Zhang, J. **Wang B**, Wang L, Zuo C, Li J, Cui Y, Wen X, **Cowan D**, Wu S, Liu M, Wang R, Zhang F. Reproductive and flight characteristics of *Lymantria xylina* (Lepidoptera: Erebidae) in Fuzhou, China. Insects 2024; 15, 894.

First Report of Vibrational Communication in a Fulgorid

Miriam F. Cooperband[1], Aleksandra Dean[1], Isaiah Canlas[1], Rodrigo Krugner[2], Sam Stella[3], Kelly M. Murman[4], and Kyle Kaye[3]

[1] Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA; [2] Crop Diseases, Pests, and Genetics Research, USDA ARS, Parlier, CA; [3] Department of Biology, Xavier University, Cincinnati OH; [4] Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, East Stroudsburg, PA

Spotted lanternflies, SLF, (Hemiptera: Fulgoridae) are known to aggregate in large groups on host plants, even on inanimate objects, and aggregations can move as a group from one place to another (Figure 1A). This group movement likely results from species-specific communication (signals), as is the case in other aggregating and swarming insects like locusts. Developing effective survey, detection, and mitigation tools for any insect requires describing and understanding its communication system.

Many hemipterans use multimodal communication such as a combination of substrate vibrations and pheromones to communicate. Describing such signals has led to improved trapping tools for some species that are attracted from a distance by pheromones, then lured into the traps by vibrations. Understanding the most important signals used by SLF for aggregation, group movements, and mate location is essential to developing tools for detection and control. In 2020, we discovered that SLF were attracted to synthetic 60 Hz vibrations, providing the first experimental evidence that they may communicate through substrate vibrations. We subsequently used a sensitive laser vibrometer to investigate the subaudible vibrations of SLF of all life stages and to determine if and when they are generating substrate vibrational signals. We found that adult male and female SLF both generate several unique substrate vibrational signals which they use to communicate with conspecifics (Figure 1B, 1C).

This represents the first time any such signals have been documented for any member of the family Fulgoridae. Now that we know these signals exist, future work to describe their substrate vibrations, timing, and behavioral function will inform what drives SLF to aggregate, allowing us to incorporate key signals into improved detection, survey, and mitigation tools such as attract and kill, mating disruption, or mass-trapping technology.

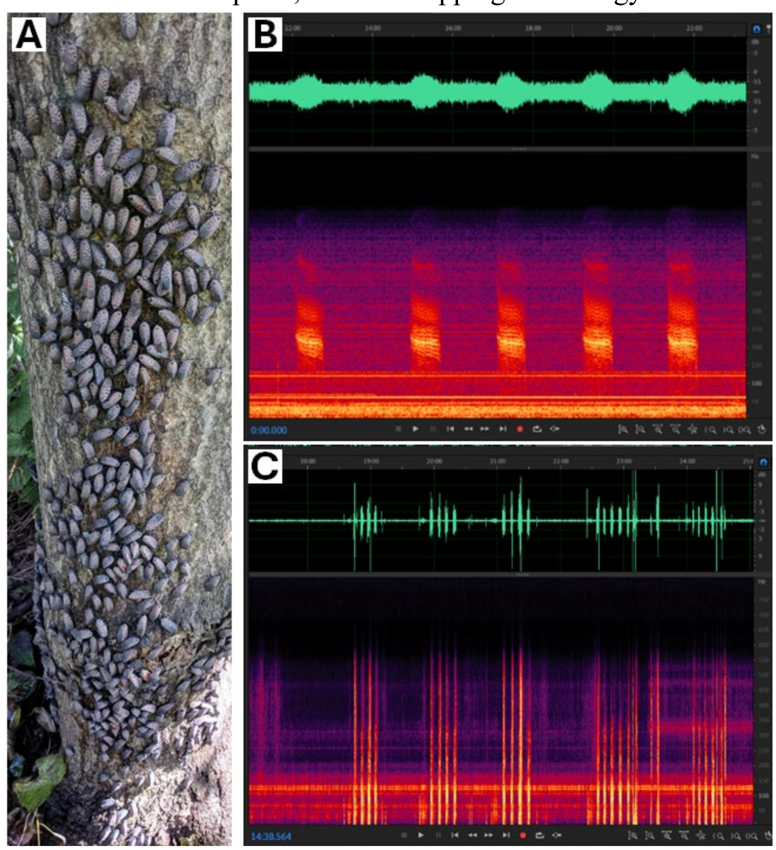


Figure 1. *(A) An aggregation of SLF on a tree. Laboratory laser vibrometer recordings of different types of substrate vibrational signals produced by (B) male and (C) female SLF. The x-axis represents 12 minutes of time. The waveforms shown in green represent intensity on the y-axis. In the corresponding spectrograms below that, the y-axis represents frequency (pitch), and increasing intensity is shown by brighter colors.*

Improved Trap for Spotted Lanternflies that Exploits Their Own Signals

Miriam F. Cooperband[1], Kelly M. Murman[2], Sam Stella[3], Aleksandra Dean[1], Rachel Braud[4], Dana Burton[4], Carly Heltzel[4], Emily Magnani[4], and Claire Martin[4]

[1] Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA; [2] Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, East Stroudsburg, PA; [3] Xavier University, Department of Biology, Cincinnati, OH; [4] Field Operations, USDA APHIS PPQ, DE/VA/WV

Our previous laboratory and field studies revealed that spotted lanternflies (SLF) have a complex multimodal communication system involving pheromones and substrate-borne vibrations. Understanding both the chemical and vibratory modes of communication is vital to incorporating those signals into a potent and species-specific lure for SLF. As work is ongoing to identify the key active pheromone components and describe their vibrational signals, we investigated a readily available option that used the trapped live SLF as the lure. We modified circle traps by replacing the plastic collection kill bag with a mesh bag (Figure 1A, B) which was attached to the tree trunk. Mesh bags allowed captured SLF to feed, produce body volatiles, release honeydew, and make substrate vibrations while inside, in essence using their natural signals to attract additional SLF.

To test these operationally, five volunteers from Field Operations in Delaware, Virginia, and West Virginia were recruited to help with the study. Experimental blocks consisted of a pair of *Ailanthus altissima* trees of similar size, spaced 3-7 m apart, one with a mesh collection bag and one with a plastic collection bag, and the positions of the two treatments were rotated at each servicing to control for any differences in the paired trees. Traps were deployed from early July to early November and serviced approximately every two weeks. Our results found that although 3rd instars could escape through the mesh, 4th instars were significantly more attracted to traps with mesh bags than those with plastic bags (Figure 1C), with up to a nearly 4-fold improvement. Adult males (Figure 1D) and females (Figure 1E) were both significantly more attracted to traps with mesh bags while aggregating before mating started (Early), but during peak oviposition time (Late-2) males were significantly more attracted to traps with plastic bags. When SLF are known to be present, the use of mesh collection bags can serve to significantly improve capture rates of 4th instars and adults prior to oviposition time, roughly from July to early October.

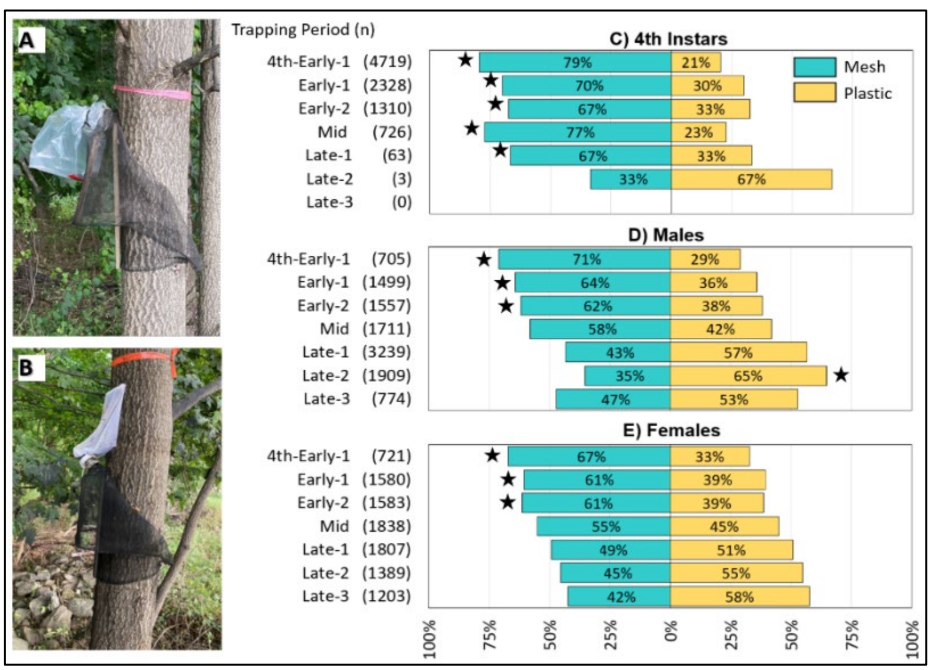


Figure 1. *Percent and number (n) of SLF caught in A) plastic or B) mesh collection bags in 2024 for C) 4th instars, D) adult males, and E) adult females. Significant differences are marked with stars (Wilcoxon 2-sample rank sums test, N=26, P < 0.05).*

Rearing an *Eriborus* sp. Parasitoid to Determine its Suitability as a Box Tree Moth, *Cydalima perspectalis*, Biocontrol Agent

Angela M. Hoover[1], Christine Dodge[1], Hannah J. Broadley[1], Connor O'Clair[1,2], Greg Simmons[1], Lukas Seehausen[3], and Marc Kenis[3]

[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] CAB International, Delémont, Switzerland

To combat the spread of box tree moth, BTM, *Cydalima perspectalis*, FPML is developing an *Eriborus* sp. wasp as a potential biological control agent (Figure 1). This undescribed species of *Eriborus* is a multi-voltine larval parasitoid of BTM and was discovered in South Korea in the BTM native range. Lab-reared cocoons were hand-carried to the FPML from CAB International (CABI) in Delémont, Switzerland in December 2023.

Eriborus sp. parasitizes all larval instars of BTM, with the highest rate of successful parasitism in L3-L5 instars. This species appears to be thelytokous with the capability of deuterotoky: unmated females typically produce clonal female progeny but will occasionally produce males. A single wasp develops within a BTM larva. Wasps kill and emerge from L5 larvae to spin their own small woolen cocoons. Larval wasp development spans 26-37 days at 25°C and is dependent upon the instar of the parasitized BTM larva. Wasp pupal development is relatively constant at 11-12 days at 25°C. Observations indicate that female wasps kept in isolation are more likely to produce male offspring, whereas female wasps kept in higher densities produce female offspring more consistently. Males emerge approximately one week before their sisters and are more short-lived, surviving on average for 1-2 weeks as adults. Female wasps live an average of three weeks, with some surviving 8-10 weeks as adults. There are no discernible characteristics to separate female and male cocoons, which are reared singly through to emergence to prevent unwanted mating.

In 2024, 10 generations of *Eriborus* sp. were reared at the FPML with a total of 780 cocoons produced. Wasps were reared continuously in a growth chamber (25°C, 65% RH; light cycle of 16:8 L:D), indicating that they do not experience an obligatory diapause. Adult wasps were provisioned with water and honey offered *ad libitum* and females were kept gregariously in groups of ~50-80 wasps in 1 cubic ft mesh dorms. All BTM exposed to wasps were stung, but not all larvae became wasps; 66% of larvae yielded *Eriborus* sp. cocoons, with remaining larvae becoming BTM pupae or else dying before completing development.

The establishment of this research colony has allowed for the year-round ability to conduct studies for the assessment of *Eriborus* sp. as a prospective biocontrol agent of BTM, including host range testing and studies investigating host-seeking behaviors. Observations and techniques learned during the development of our current rearing process will be beneficial in the event a mass rearing operation of *Eriborus* sp. is approved for augmented control of BTM.

Figure 1. *An Eriborus sp. wasp ovipositing into an L5 box tree moth larva.*

Determination of Host Cues Used by *Eriborus* sp. Parasitoids of Box Tree Moth, *Cydalima perspectalis*

Angela M. Hoover[1], Christine Dodge[1], Damon Crook [1], Hannah J. Broadley[1], Connor O'Clair[1,2], and Gregory Simmons[1]

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

Parasitic wasps often use volatile cues to locate appropriate hosts, either from the host directly, or those compounds produced by a damaged plant. We observed that *Eriborus* sp. wasps confined in arenas with box tree moth larvae, BTM, *Cydalima perspectalis*, exhibited probing behaviors on larval frass. We hypothesized this was likely due to an important chemical cue used by the wasp to identify suitable hosts. If volatile cues used by *Eriborus* sp. to locate BTM larvae in the field are host-specific, it would indicate that this wasp is specifically adapted to utilize BTM as a host and would likely have minimal to no negative impacts on non-targets. We investigated potential host cues used by *Eriborus* sp. to locate BTM using chemical ecology and behavioral assays.

We ran a series of Y-tube assays to determine whether BTM larva-damaged host plant material and frass were attractive to *Eriborus* sp. Teflon Y-plate olfactometers were set up with a control arm and treatment arm. The separate treatments tested included BTM-damaged boxwood leaflets, frass collected from host-fed BTM, and frass collected from BTM reared on a 30% boxwood diet developed by the FPML Rearing group. Individual wasps were given five minutes in the Y-tube to make a selection. We recorded the wasps' first and final choices as well as the time spent in each section of the Y-tube (control arm, treatment arm, midzone). *Eriborus* sp. were significantly attracted to both the host-fed and diet-fed BTM frass (Figure 1). They were not significantly attracted to BTM-damaged host plant material. For the host-fed frass trials, wasps selected the treatment zone and spent more overall time in the treatment arm than in the midzone and control arm combined. The diet-fed frass attracted wasps as well, but wasps spent more time moving between zones and their final choices were more varied.

In addition to behavioral assays, we isolated several compounds of interest from fresh host-fed frass using GC-EAD and GC-MS methods. Wasps gave clear antennal responses to benzaldehyde, benzyl alcohol, beta-linalool, and the compound phenol 2-methoxy, also known as guaiacol. Guaiacol is a documented component of insect frass and is also reported as a potential repellent to BTM adults ovipositing on boxwood. Guaiacol may be useful in the development of push/pull management strategies to repel BTM from boxwood while simultaneously attracting natural enemies. These results also help inform the suitability of diet-fed BTM larvae in host range testing studies, allowing for reduced spending on costly boxwood plants for colony rearing.

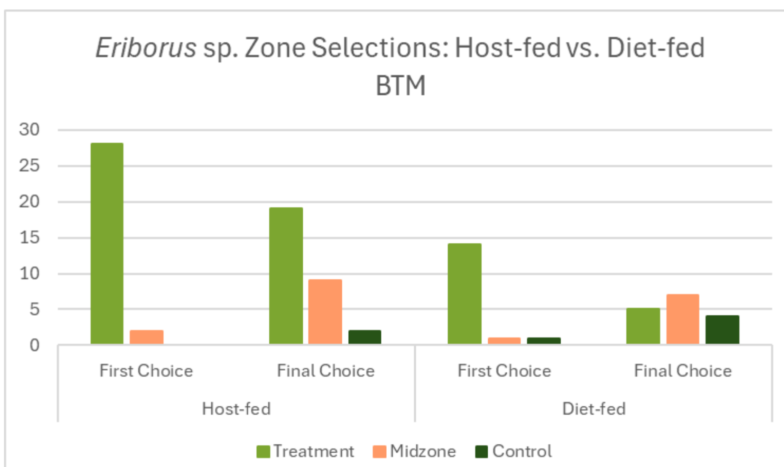


Figure 1. *A comparison of Eriborus sp. first and final choice frequencies in Y-tube olfactometers in the presence of host-fed BTM frass (left) and diet-fed BTM frass (right).*

Host Range Testing and Life History Studies of *Aprostocetus* sp. 7., an Adventive Biological Control Agent of Roseau Cane Scale

Connor D. O'Clair[1,2], Mike Martinson[1,2], Jeremy C. Andersen[2], Tanner C. Sparks[3], Lisa A. Tewksbury[4], Alexandra Johnson[4], Rodrigo Diaz[3], Scott A Schneider[5], and Hannah J. Broadley[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, Agricultural Center, Baton Rouge, LA; [4]University of Rhode Island, Plant Sciences and Entomology, Kingston, RI; [5]Henry A. Wallace Beltsville Agricultural Research Center, Systematic Entomology Laboratory, USDA ARS, Beltsville, MD

Roseau cane scale, *Nipponaclerda biwakoensis*, 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 its stress and dieback. 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 contributes to estuary habitat, which makes protection of this plant from roseau cane scale vital. There are four known parasitoids of roseau cane scale present in the Mississippi River Delta. Of these species, the parasitoid *Aprostocetus* sp. 7 was detected most recently. We evaluated the impact this parasitoid may have both on roseau cane scale populations and on native scale and mealybugs Specifically, our current set of studies aimed to quantify its potential spread to other native scale and mealybug species in the region via host range testing, and measure life history characteristics such as longevity, fecundity, and reproductive potential.

For host range testing, 14 scale insect and mealybug species were collected, reared, and evaluated (Table 1). Host range testing was triplicate, no-choice testing performed via introducing 15 female wasps into a cage with a potted plant that was infested with a non-target scale insect or mealybug. A control was simultaneously run with 15 female wasps introduced to a cage with roseau cane scale. After a month, the material was sampled to evaluate parasitism and scale density, and the rest was kept for further observation over eight weeks. The only non-target to date that *Aprostocetus* sp. 7 has shown some parasitism on *is Aclerda holci*, which is the most closely related species to roseau cane scale. This testing shows that *Aprostocetus* sp 7. is highly host specific.

Life history studies included evaluations of longevity of *Aprostocetus* sp. 7, sex ratio, host preference, development time, and fecundity. Under our rearing conditions, we found that *Aprostocetus* sp 7. females live an average of 19 days and males live 13 days, with a sex ratio of 3:2 female to male. Females will parasitize non-sclerotized roseau cane scale larger than ~2mm, and time from oviposition to emergence is typically 28 days. A single scale can host up to 11 *Aprostocetus* with a single female being able to produce upwards of 100 offspring in her lifetime. Overall, this work informs the evaluation of *Aprostocetus* sp. 7 as an important adventive biological control agent of invasive roseau cane scale.

Family	Scientific Name	Host Plant
Coccidae	*Saissetia coffeae*	*Chlorophytum comosum*
Coccidae	*Coccus hesperidum*	*Nerium oleander, Ficus elastica*
Coccidae	*Parasaissetia nigra*	*Ficus elastica*
Pseudococcidae	*Phenacoccus madeirensis*	*Coffea arabica*
Pseudococcidae	*Chaetococcus phragmitis*	*Phragmites australis*
Pseudococcidae	*Pseudococcus dolichomelos*	*Helianthus annuus*
Pseudococcidae	*Pseudococcus longipinus*	*Cycas* sp.
Pseudococcidae	*Saccharicoccus sacchari*	*Saccharum officinarum*
Pseudococcidae	*Antonina graminis*	*Phragmites australis*; *Cynodon dactylon*
Diaspididae	*Chrysomphalus aonidum*	*Ficus elastica*
Diaspididae	*Duplachionaspis spartinae*	*Spartina alterniflora*, *S. cynosuroides*
Monophlebidae	*Icerya purchasi*	*Citrus limon*
Aclerdidae	*Aclerda sp.*	*Phragmites australis, Miscanthum sp*
Aclerdidae	*Aclerda holci*	*Phragmites australis*

Table 1. *Family, scientific name, and host plants of all scale and mealybugs that we used for host range testing.*

Research on Spotted Lanternfly and its Associated Natural Enemies in its Invasive Ranges in Japan and South Korea

Hannah J. Broadley[1], Matthew T. Kamiyama[2], Seunghwan Lee[3], and Duk-Young Park[3]

[1]Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA; [2] Kyoto University, Graduate School of Agriculture, Kyoto, Japan; [3] Seoul National University, Department of Agricultural Biotechnology, Seoul, Republic of Korea.

Spotted lanternfly, SLF, *Lycorma delicatula*, has invasive populations in the Republic of Korea (South Korea), Japan, and the United States of America. In its native range, egg and nymphal parasitoids are known to be important in regulating populations in China. Prior work in South Korea on management and application of biological control methods have successfully introduced the egg parasitoid, *Anastatus orientalis* to suppress invasive SLF populations there. However, few follow-up investigations into the impact of *A. orientalis* or exploration into the presence of other parasitoids had been completed. Additionally, in contrast to the rapidly expanding populations of spotted lanternfly during the initial introduction in South Korea and in the U.S., SLF populations in Japan initially spread slowly and remained isolated to two prefectures (Ishikawa and Fukui) for 8 years post-invasion without any reports of agricultural damage. It was unclear if this slow establishment of spotted lanternfly in Japan was due to biological effects by natural enemies or other biotic or abiotic factors. Therefore, between 2021 and 2024 in South Korea and between 2022 and 2023 in Japan, we surveyed both regions extensively for additional parasitoids of interest, investigating both the egg stage and nymphal stages of spotted lanternfly across the known establishment range in both countries (Figure 1). Our studies aimed to evaluate the presence of native or introduced parasitoids to identify the parasitoids and evaluate their role on the invasion dynamics of spotted lanternfly in these countries.

We did not find any notable parasitoid species and found that *Anastatus orientalis* is important, but no other parasitoids were detected attacking spotted lanternfly populations there. However, through host range testing of *A. orientalis*, we have found this species to be insufficiently host specific to consider further use in North America.

While it was important to investigate the populations of spotted lanternfly in South Korea, where populations have largely been brought under control after initial severe outbreaks and in Japan, where populations of spotted lanternfly have been slow to establish, unfortunately no new candidate biological control agents were discovered through this work.

Exploration for new candidate biological control agents in these two regions is complete at this time and the focus for foreign exploration efforts continues to be on the regions of Eastern Asia where spotted lanternfly is native, including China and Vietnam.

Figure 1. *Evaluating emerged spotted lanternfly egg masses in South Korea in 2022.*

Development of Regulatory Treatments for Box Tree Moth, *Cydalima perspectalis*

Phil Lewis[1] and Amanda Davila-Flores[1]

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

Little information is available concerning pesticide efficacy for the recently introduced box tree moth, BTM, *Cydalima perspectalis*, a pest of boxwood plants. Insecticide products with five different modes of action were applied in 2023 and 2024 to boxwood foliage and tested against BTM eggs and young larvae at FPML's quarantine facility. Boxwood foliage is very glossy and repels water, so a wetting agent (CapSil) was used to facilitate spreading and adhesion for all spray applications. Efficacy screening of BTM life stages was done in 2023 and the following year the 3rd instar stage was tested to investigate persistence of low and high labeled rates of nine products. Products known to be effective against leaf-feeding caterpillars tested were Acelepryn, Mainspring, Intrepid, Conserve, TriStar, Mavrik, Perm-Up, Scimitar, and Talstar.

Mortality among the control groups was very low, with 95-100% larval survival. None of the hatchlings that emerged from treated egg masses survived. There were two products, Perm-Up and Scimitar, that resulted in significantly less hatch from the egg masses. Neonates were indirectly exposed to treatments by attaching a leaflet with an untreated egg mass with a pin to treated foliage. Neonates then migrated to the treated foliage. All larvae that consumed treated foliage died after seven days of exposure, except those exposed to Conserve. While it was effective on the same day of exposure to treatment, some larvae were observed feeding normally after seven days, indicating the product breaks down and loses activity over time.

To gauge longevity of the treatments, treated boxwood foliage was left outdoors for 1, 2 and 3 weeks prior to exposure to 3rd instar larvae. The highest labeled rates generally resulted in complete efficacy for at least three weeks post-treatment. Exceptions to this were TriStar and Mavrik which had 50 to 60% survival after seven days of feeding. Talstar had a small amount of survival at three weeks following seven days of feeding.

After two years of laboratory and semi-field testing, we were able to identify a variety of insecticides that can be used by regulators to prevent the movement of BTM outside of infested areas. Two products, TriStar and Mavrik, were not effective against BTM and should not be utilized. Applications of Conserve broke down quickly and were not very effective several days after treatment. The products that were effective for several weeks after treatment are Acelepryn, Mainspring, Intrepid, Perm-Up, Scimitar, and Talstar (Figure 1).

As a result of our work, the National Plant Board has updated the BTM compliance agreement to adjust the requirement of treatment of boxwoods prior to any shipments from 7 days to 14 days using approved products. The pesticide lab at FPML will continue to work with PPQ program managers and cooperators to refine the list of available products, rates and treatment timing to support the BTM program.

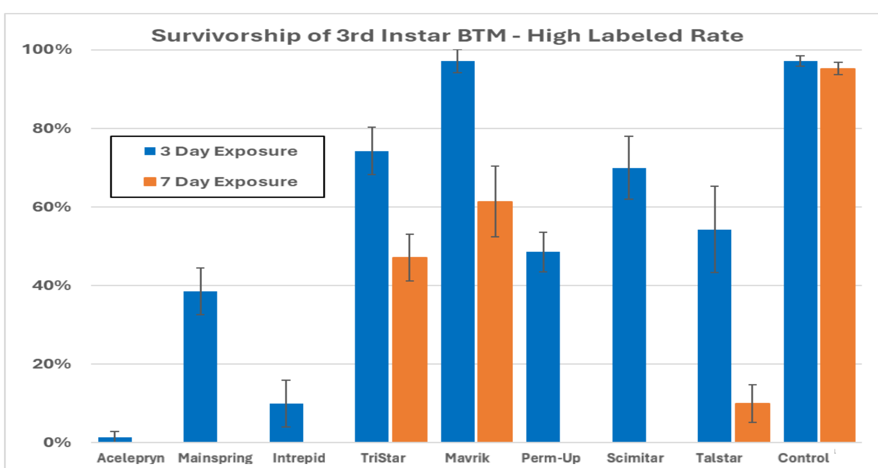


Figure 1. *Effectiveness of insecticides on 3rd instar box tree moth larvae three and seven days after treatment.*

Assessing Long-term Viability of *Lymantria dispar asiatica* Eggs Under Constant 24ºC

Baode Wang[1], David Cowan[1], Hannah Nadel[1], and Christine Mccallum[1]

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

The flighted spongy moth complex (FSMC) includes *Lymantria dispar asiatica*, *L. dispar japonica*, *L. umbrosa*, and *L. albescens* (syn. *L. postalba*). Current FSMC-free vessel certification programs require vessels to provide a two-year port-of-call history. This requirement aims to identify potential sources of FSMC egg mass deposition and facilitate targeted inspections. The USDA's APHIS FSMC program was asked to investigate the claim that FSMC eggs can survive for more than one year, thus justifying the two-year history requirement. In natural settings, FSMC moths typically lay eggs between May and September, depending on species, population, and weather. While egg hatching within 6-10 months is well-documented, reliable data on viable eggs surviving 1.5-2 years is lacking. *L. dispar asiatica* (LDA) eggs typically require low-temperature exposure (e.g., 5°C for 60 days) to hatch. This study investigates the hatch rate of LDA eggs under constant temperature to address this data gap.

Freshly deposited LDA egg masses (n=180) were placed in an environmental chamber and maintained at 24°C ± 0.7 and 57.1 ± 12.1% relative humidity. Egg masses were periodically checked for hatching, and the number of hatched larvae was recorded. No chilling period was provided.

Hatching commenced approximately five months after egg placement. As expected, hatch rates were low under constant temperature without chilling. Out of the 180 egg masses only 55 had any egg hatch. Egg hatch was assessed periodically until 25 months (Table 1).

The observed low hatch rate of un-chilled eggs aligns with expectations (<0.001%). The hatching of a small number of eggs after 21 and 25 months does provide partial evidence supporting the two-year port-of-call history requirement. This suggests that a very small percentage of eggs can remain viable for extended periods, even without chilling.

This study provides preliminary data supporting the two-year port-of-call history requirement. Ongoing research, including tests on LDA eggs exposed to infrequent chill periods and normal chilling regimes, will provide more comprehensive data to validate this requirement.

	L. dispar asiatica egg hatch over 25-month observation period at 24ºC							
	5 mo	6 mo	12 mo	18 mo	20 mo	21 mo	24 mo	25 mo
# Eggs Hatched	6	48	69	0	0	2	0	1

Table 1. *Total number of eggs hatching during a 25-month observation period with 180 L. dispar asiatica egg masses kept at a 24º C under constant temperature with no chill period.*

2024 Insect Rearing and Outreach

Lara Day[1], Cori Carson[2,3], Carrie Crook[1], Jie Fang[1,3], Kristine Grayson[3], Julia Kardos[1, 4], Hannah Landers[1], Susan Lane[1], Erica Martin[2, 3], Christine McCallum[1], Christine Swank[1, 3], Mary Watson[2, 3], and Mauri Hickin[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; [4]University of Rhode Island, Department of Plant Sciences and Entomology, Kingston, RI

At FPML, the rearing department completes studies to improve lab rearing techniques, develops diet formulations, and produces insects in support of various projects including the Cooperative Agricultural Pest Survey (CAPS) and Agricultural Quarantine Inspection. The insects reared are the Asian and citrus longhorned beetles, ALB, *Anoplophora glabripennis*, and CLB, *Anoplophora chinensis*; spongy moth, SM, *Lymantria dispar*; box tree moth, BTM, *Cydalima perspectalis*; Old World bollworm, OWB, *Helicoverpa armigera*; and emerald ash borer, EAB, *Agrilus planipennis*.

ALB and CLB colonies were reared to support attractant research for the CAPS program (182 adults), genetic research projects (over 300 insects), and training USDA detector dogs (30 larvae). Additionally, over 600 preserved specimens of various life stages were shipped for outreach to federal and state programs.

The SM colony provided the National Spongy Moth Slow the Spread project with 51,600 pupae. An additional 3,700 larvae and 3,300 egg masses were sent to federal, commercial, and academic institutions to support training, ecology research, and virus production. Spongy Moth Riker displays were provided to state plant health directors for outreach and training. Smaller colonies of the flighted SM complex also provided specimens to national identifiers.

The BTM colony provided over 8,100 insects for ongoing studies on developing an artificial diet for mass rearing. Over 11,000 larvae and 3,500 eggs supported pesticide research, and several adults were used to help develop an SIT program. Preserved BTM specimens and prepared Riker displays supported outreach, training, and identification.

An OWB colony was reared, and 140 insects were utilized in an ongoing integrated pest management project with external collaborators. Over 200 preserved specimens of varying life stages were provided to federal scientists and identifiers in support of studies on identification and comparing morphometrics of OWB and related species.

EAB were reared at the EAB facility in Brighton, MI to support the development of a wood-free rearing system for EAB and its parasitoids. Monthly artificial diet batches were prepared at FPML using a twin-screw extruder and sent to Brighton for testing and rearing (Figure 1).

These services support PPQ's mission to develop methods to detect, prevent entry, and control invasive insect pests. Insects produced by the rearing department are used in important collaborations with federal, state, international, commercial, and academic organizations.

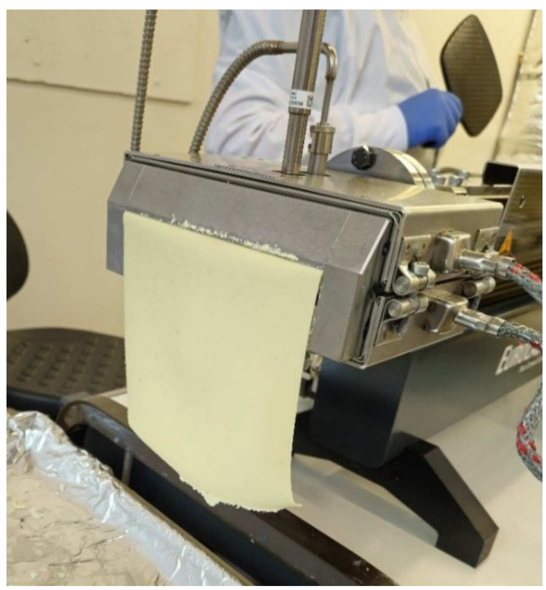


Figure 1. *Thin sheets of diet are extruded, packaged, and sent to the EAB rearing facility for testing.*

Improving Traps for the Box Tree Moth, *Cydalima perspectalis*, with a Focus on Excluding Pollinators in Suburban Settings

Joseph Francese[1], Everett Booth[1], Sarah Devine[2], and Allard Cosse[1]

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

Currently, the recommended trapping method for box tree moth, BTM, *Cydalima perspectalis* is a green bucket trap. The traps are usually made of either a combination of white and yellow or can be solid green in color. While the combined white and yellow traps are attractive to BTM, they can also attract pollinators, causing problems in areas where honeybees, *Apis mellifera*, are kept and in areas where the range of BTM overlaps that of endangered pollinators (ie., the rusty patch bumblebee, *Bombus affinis*). This work supports efforts to detect and delimit populations as well as to aid in determining the phenology of BTM as it moves into new areas.

In 2024, we conducted field assays at cemeteries, industrial parks, and private residences all within known areas of BTM infestations in Barnstable County, MA to compare four commercially available moth traps. Trap types included: *Heliothis* traps, milk carton traps, clear bucket (Unitrap) traps, and green bucket traps (as a control since yellow and white traps were restricted). Halfway through the season, we widened the entry ports of the milk carton trap by removing the center tab. Our goal was not only to compare catch of BTM, but also to compare non-target bycatch for each trap type. Over the entire field season there was no difference in BTM catch among the *Heliothis* or either bucket trap, but all caught significantly more than the milk carton trap. Removing the tab and widening the entry hole in the milk carton trap did significantly affect trap catch, improving BTM trap catch for the second flight period. The *Heliothis* traps produced ten times the amount of bycatch of all the trap types, with the majority of bycatch being Diptera, Lepidoptera, and Hymenoptera.

Based on these findings, the *Heliothis* trap is not recommended for BTM surveys due to the expense of the trap (per unit costs 8x that of the bucket trap), the time required to service the trap (3x longer than the bucket trap), and the increased non-target bycatch. Furthermore, while bycatch rates were low for the modified milk carton trap, it was not as effective at catching/detecting BTM throughout the season. The green bucket trap is still recommended over the other traps tested in this study for BTM trapping. In 2025, we will expand on this concept with a goal towards comparing additional trap types to provide the BTM Program with an effective, more species-specific trap.

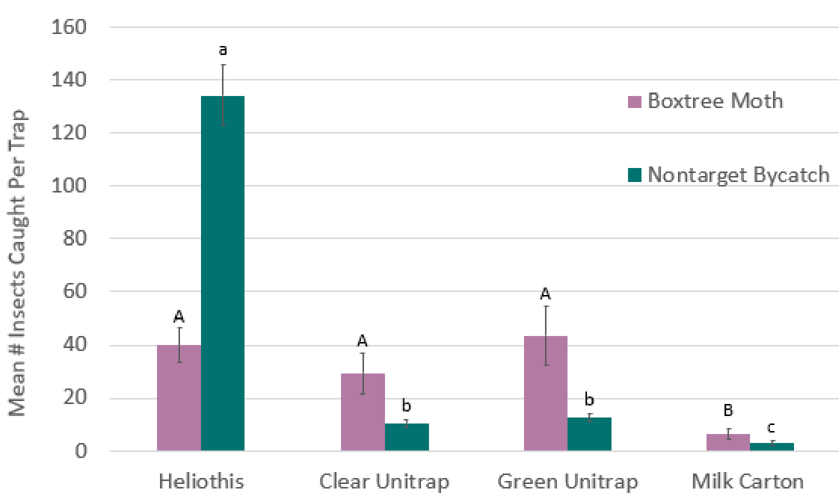


Figure 1. *Mean catch (SE) of box tree moth and non-target insects caught in four moth traps in Barnstable County, MA. Capital letters above error bars represent differences in box tree moth catch among traps, while lower case letters represent differences in catch among non-targets.*

Forest Pest Methods Laboratory CAPS Lure Support for the Detection and Survey of Pest Insects in 2024

Allard Cosse[1] and Anna Stevenson[1]

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

For the last few decades FPML has supported the Pest Detection/Cooperative Agricultural Pest Survey (CAPS), Survey and Supply Program with formulations and productions of specialized non-commercial insect lures. In 2024 we formulated and shipped 87,576 lures to CAPS stakeholders throughout the United States, for 27 different insect species (Figure 1).

Most of the pheromone-based lures produced by FPML are mixtures of two to five chemical components in ratios that are specific to the target insect species. In addition to the ratio-specificity, the 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 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 of FPML-produced lures and those that are commercially purchased through the Pest Detection/CAPS program, ensuring that lures made available through the Survey and Supply Program attract the target insect species.

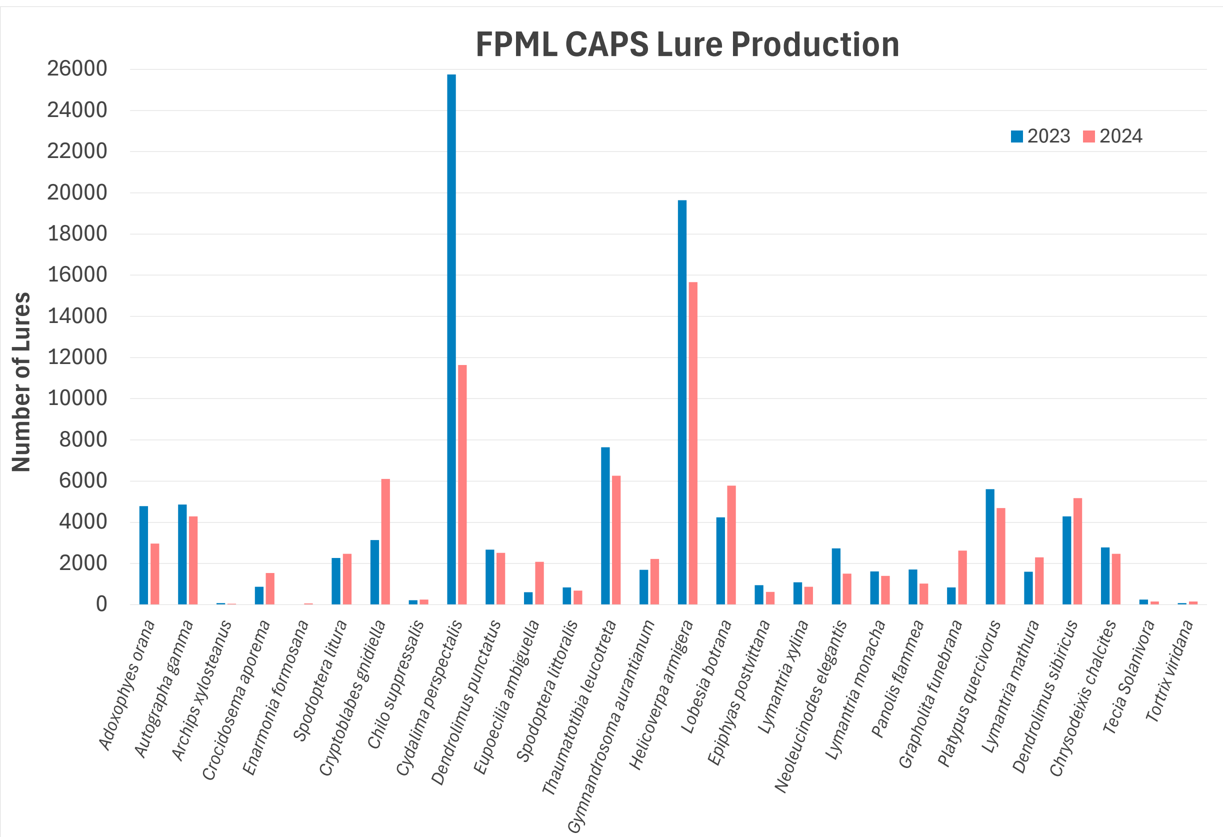


Figure 1. *Forest Pest Methods Laboratory CAPS lure production in 2023 and 2024 for 28 different insect species.*

Inheritance of Female Flight Capability in Spongy Moth Hybrids

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

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

Introduction of the flighted spongy moth complex, FSMC, *Lymantria dispar asiatica*, *L. d. japonica*, *L. albescens, L. umbrosa,* and *L. postalba* into the U.S. has been heavily monitored, with FPML providing molecular identification of *Lymantria* specimens in support of PPQ's spongy moth programs. This includes large scale trapping within the federal quarantine where non-flighted *Lymantria dispar dispar* populations are already established. If FSMC were to be introduced within the federal quarantine, hybridization with *L. d. dispar* would likely occur. Testing the flight capabilities of the hybrid and subsequent backcrossed offspring using a flight mill to measure speed, total flight time, and flight bouts (as a proxy for flight capability) would provide more insight into the risk level of FSMC being introduced in federally quarantined areas.

A group of flightless *L. d. dispar* females from New Jersey, flighted *L. d. asiatica* females from Korea, the hybrid female offspring of *L. d. dispar* males and the *L. d. asiatica* females (F1), and the backcrossed female offspring of the F1 generation and *L. d. dispar* males (BC1) have been tested on the flight mill. As expected, the flighted Korea moths had the fastest average flight speed, while NJ moths had the longest flight bout and the longest total flight time, although this was not significantly different from the Korea moths (Figure 1A and 1B). Based on these results and given that the flight mill only simulates flying where body weight is supported, we would predict that average flight speed may be a better proxy of flight capability.

The F1 and BC1 generations performed the worst in all measures of flight capability, suggesting that flight capabilities diminish in most offspring with continued backcrosses (Figure 1A and 1B). However, there are outliers with average speeds, total flight times and longest flight bouts that equal or exceed those of some Korean and NJ individuals (Figure 1A and 1B).

Further testing on the backcrossed offspring of the outlier BC1 moths is underway to see if they also exhibit downward trends in flight measures. If flight capabilities are lost within these backcrossed generations, PPQ's method for monitoring FSMC introduction and the established spongy moth population within the federal quarantine area could be reassessed.

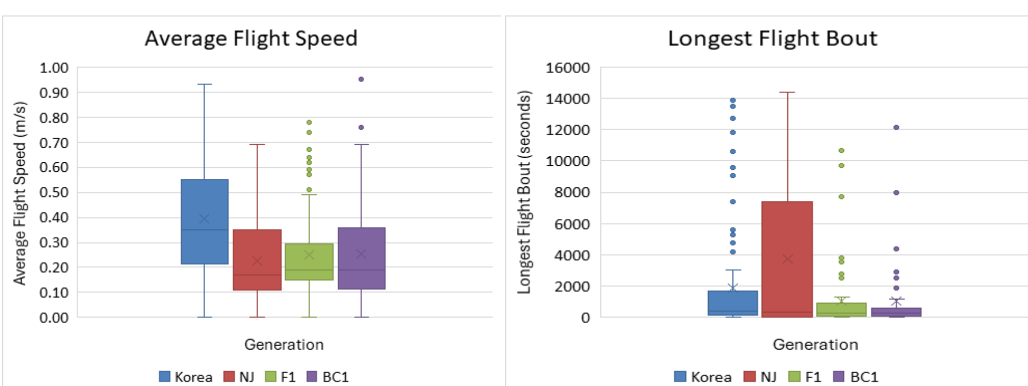


Figure 1. *(A) Average flight speed (m/s) of Korea, NJ, F1 and BC1 moths on the flight mill. The average is indicated by the X inside each box. (B) Longest flight bout (seconds) of Korea, NJ, F1 and BC1 moths on the flight mill. The average is indicated by the X inside each box.*

2024 Port and Domestic Spongy Moth Molecular Diagnostic Survey

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

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

The Forest Pest Methods Lab provides molecular diagnostic support for the annual PPQ spongy moth survey. The goal of the survey is to monitor and identify the introduction of flighted spongy moth complex, FSMC, *Lymantria dispar asiatica*, *L. d. japonica*, *L. albescens, L. umbrosa,* and *L. postalba* into the U.S. and account for spongy moth populations inside and outside the federal spongy moth quarantine. In recent years, two real-time PCR (qPCR) assays have been utilized to identify suspect FSMC to provide quick and accurate detections of suspect *Lymantria dispar asiatica*, *Lymantria dispar japonica*, and *Lymantria umbrosa*.

In 2024, 8,430 suspect *Lymantria* specimens trapped domestically were processed from 27 states (Figure 1). Many of the specimens were sent from three states within federal quarantine, PA, NY, and NJ, accounting for 2,819 of the submissions. North Carolina was the only state outside of the federal quarantine that submitted a larger number of specimens with 1,700 submissions. Only 41 submissions were received from several west coast states where there is a higher likelihood of FSMC introduction. Of the domestic survey specimens, only one was identified as a member of the FSMC (*L. d. asiatica*), which was trapped in a high-risk introduction area of California.

Ninety-three suspect *Lymantria* specimens were intercepted from 10 U.S. ports of entry and sent to FPML for molecular diagnostics. These resulted in 11 *L. d. asiatica*, 16 *L. d. japonica*, 13 *L. umbrosa*, three unknown, and 49 non-target species determinations. *L. d. japonica* and *L. d. asiatica* historically have been the most abundantly intercepted FSMC specimens from U.S. ports of entry. Only two other *L. umbrosa* identifications have been recorded from port interceptions throughout the survey's history.

To confirm this year's influx in *L. umbrosa* identifications, a separate qPCR assay targeting the ND1 genome segment was engineered and utilized. The current diagnostic assay targets COI genome segments that only differ slightly between the FSMC targets. Use of the ND1 genome segment for the *L. umbrosa* assay reduced the possibility of a false *L. umbrosa* positives that could be caused by mutations of COI genome of the closely related moths. The implementation of qPCR technology has allowed for enhanced assay development capabilities increasing the accuracy, speed and success rate of FPML's diagnostic assays for the annual survey. This allows FPML to provide prompt molecular diagnostic results to PPQ's Spongy Moth Detection Programs for continuous monitoring of current spongy moth populations within the U.S.

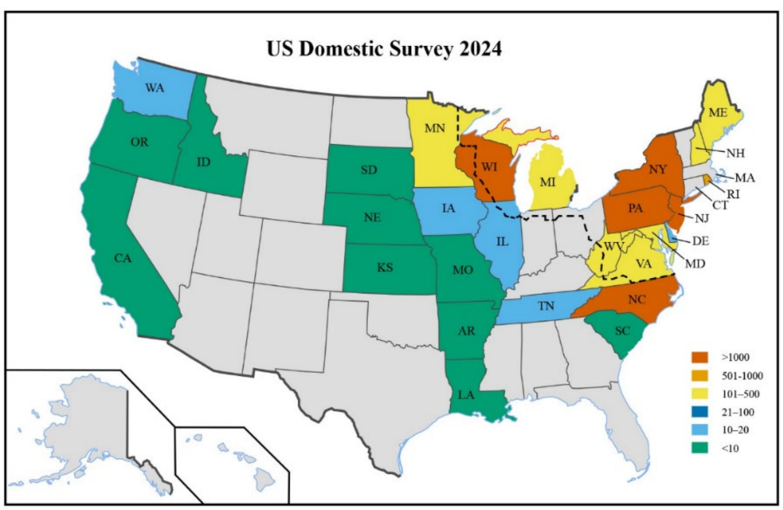


Figure 1. *Color gradient map of the U.S. showing the border of the federal spongy moth quarantine (dotted black line) and number of suspect Lymantria submissions received for the domestic spongy moth survey in 2024.*

Development of a Sample Pooling Method for the Spongy Moth Survey

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

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

Each year FPML supports PPQ's Spongy Moth Detection Program by utilizing qPCR to identify *Lymantria* specimens from around the United States as part of the flighted spongy moth complex (FSMC) survey. States submit thousands of domestically trapped specimens yearly. Currently these samples are analyzed individually, which is very labor intensive. To reduce the amount of time, money, and materials spent on this process, we have developed a new qPCR assay capable of analyzing pooled samples, allowing multiple samples to be processed at once.

The qPCR assay currently used to analyze FSMC survey specimens detects *L. d. dispar*, and three members of the FSMC: *L. d. asiatica*, *L. d. japonica*, and *L. umbrosa*. While this assay was able to detect pooled *L. d. dispar* specimens, it was unable to detect FSMC specimens pooled with other *Lymantria* samples. By combining assays presently used for secondary confirmation of suspect *Lymantria* specimens, we developed a new assay that works effectively on pooled samples containing DNA extracts of up to 40 individual specimens (Figure 1). This composite assay provides the additional benefit of detecting cryptic *L. d. asiatica* and *L. d. japonica* and detecting *L. umbrosa* with higher confidence than our initial assay. To expand our ability to detect FSMC, we also added an assay that detects *L. albescens*.

The new assay is in the process of being validated on native specimens with known identifications to assess its accuracy. Further optimizations will include increasing the number of DNA extracts in a pooled sample, and possibly pooling tissue samples to be extracted together, instead of pooling DNA extracts from individual specimens, which would increase the efficiency of the DNA extraction process.

Based on testing done to date, qPCR analysis using this assay can be performed on 40 times more specimens than at present without requiring additional materials or time. Even further increases in efficiency may be possible with more testing.

Also, the new assay detects a broader scope of the genetic diversity of the FSMC, increasing our ability to quickly provide high confidence results. Overall, this advancing detection method contributes to USDA's mission of regulating the movement and establishment of *Lymantria*.

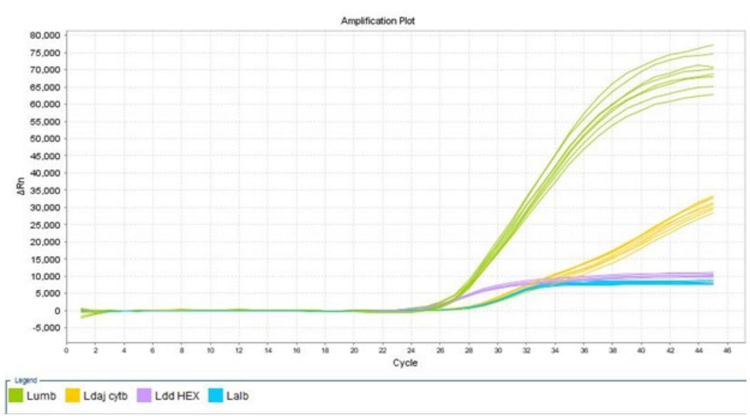


Figure 1. *Real-time PCR amplification plot for 8 replicates of a pooled sample containing 40 specimens (1 each of L. d. asiatica, L. umbrosa and L. albescens, and 37 L. d. dispar) on the new assay. All four sub assays, Lumb (detects L. umbrosa), Ldaj cytb (detects L. d. asiatica and L. d. japonica), Ldd HEX (detects L. d. dispar) and Lalb (detects L. albescens), show positive exponential curves, indicating that the target of that sub assay was detected in the sample.*

Development of an LED Array to Improve Insect Trapping for Coconut Rhinoceros Beetle, *Oryctes rhinoceros*

Damon Crook[1], Mohsen Paryavi[2], Keith Weiser[3], Michael Melzer[3], Chandrika Ramadugu[4] and Daniel M. Jenkins[2]

[1]Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA; [2]University of Hawaii, Department of Electrical & Computer Engineering, Honolulu, HI; [3]University of Hawaii, Department of Plant and Environmental Protection Sciences, Honolulu, HI; [4]University of California, Department of Botany and Plant Sciences, Riverside, CA

The current trapping method for Coconut rhinoceros beetle, CRB, *Oryctes rhinoceros,* a pest native to Southeast Asia, is a black cross vane trap with a lure. However, this trap/lure combination is not that efficacious. Most insects have a trichromatic color vision system, with UV, blue, and green photoreceptors, though many also have sensitivity well into the red portion of the spectrum. Since CRB is a nocturnal insect, research efforts have been focused on using artificial light to enhance trapping. However, light traps are typically cumbersome to deploy, require bulky batteries, must be waterproof, making them cost ineffective. Preliminary visual response wavelength data from retinogram studies conducted at FPML showed that CRB responds to the UV spectrum. The purpose of this project was to work with engineers at the University of Hawaii to develop and test a programmable LED array that would be cost effective and easy to deploy and validate efficacy against CRB (Figure 1A).

The resulting LED array is compact, solar powered, and waterproof. It is programmable through a graphical user interface on an Android app using wireless communication. Users can swiftly program the operating parameters including wavelength selections with intensity and modulation, and timing of operation throughout the day. These devices were then deployed in field sites in Hawaii for testing on CRB. The diffuser was mounted on either the top portion of the panel trap or in the bottom catch cup portion of the panel trap. Results indicated that beetles were significantly more likely to be caught in traps illuminated with UV wavelengths compared to other wavelengths or unlit traps (Figure 1B). In contrast, catches in CRB traps illuminated with any visible (non-UV) color LEDs were significantly less compared to traps without illumination.

This work validates the efficacy of the LED array for CRB trapping. The programmable nature of the array also makes it a good candidate for testing against other insect species, such as Asian Citrus Psyllid. Our research group also provided hardware design recommendations to improve energy efficiency of similar devices for more widespread deployment in insect trap surveys.

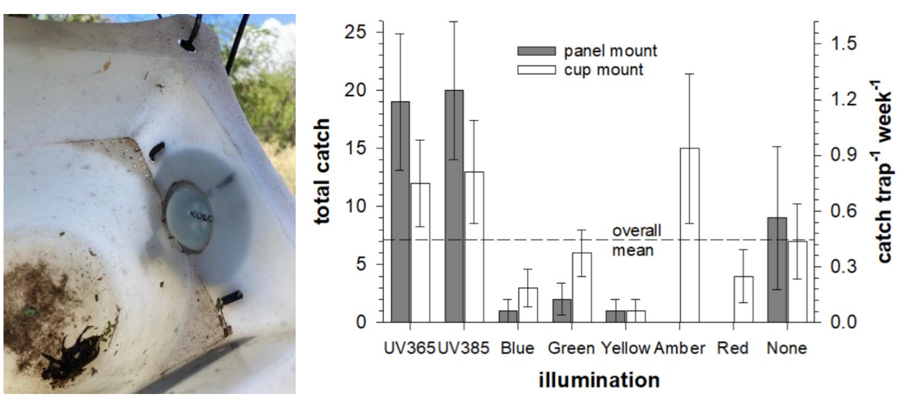


Figure 1. *The left image shows a panel trap for CRB with the attached diffuser to the cup mount. Graph show tests for coconut rhinoceros beetle (Oryctes rhinoceros): Total trap catch for each light treatment (first vertical axis), also expressed as catch per week (second vertical axis) with error bars equivalent to the standard deviation.*

Visual and Chemical Ecology of Lime Swallowtail

Damon Crook[1]

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

In support of the Citrus Health Response Program, FPML examined the visual and chemical ecology of a new invasive pest, the Lime Swallowtail, *Papilio demoleus*. It was detected in October of 2022, for the first time in the U.S., when a live caterpillar was collected from a Citrus sp. tree at a residence in Key West, Monroe County. The purpose of this study was to investigate visual and chemical attractants in an effort to develop future potential monitoring tools.

Visual color capabilities of adult butterflies were examined using electrophysiological methods. Ten replicates of both male and female citrus swallowtail heads were tested on the Electroretinogram (ERG) system based at FPML (Figure 1). Females were most sensitive to 380nm (violet – bordering on UV/Visible) and 440nm (violet). ERG measurements generally dropped off towards 700nm. Males were also sensitive to 380nm but also appeared sensitive to 460nm (blue range). As both sexes showed very similar responses, we can assume they both respond to the same visual components (UV reflection and pattern of wings).

Coupled gas chromatography / Mass Spectrometry (GC-MS) analysis did not identify any specific differences between male and female chemical composition. Male hind wings did appear to have higher amounts of n-hexadecanoic acid and linoleic acid. Linoleic acid is associated with being emitted from insects after death. No body wash components were seen to be antennally active when tested via coupled gas chromatography / electroantennographic detection (GC-EAD). Based on these results, we would therefore assume that mating is more visually orientated for this insect.

The two main components in lemon foliage (4 hr long aerations) were identified via GC-MS as E-Citral (Geranial) and Z-Citral (Neral). Both major components were seen to be antennally active for both male and female adults (Figure 2).

Our results indicate that lemon host volatiles such as Geranial and Neral could potentially be used as a lure for *Papilio demoleus* should a monitoring survey be required in citrus growing regions of the US.

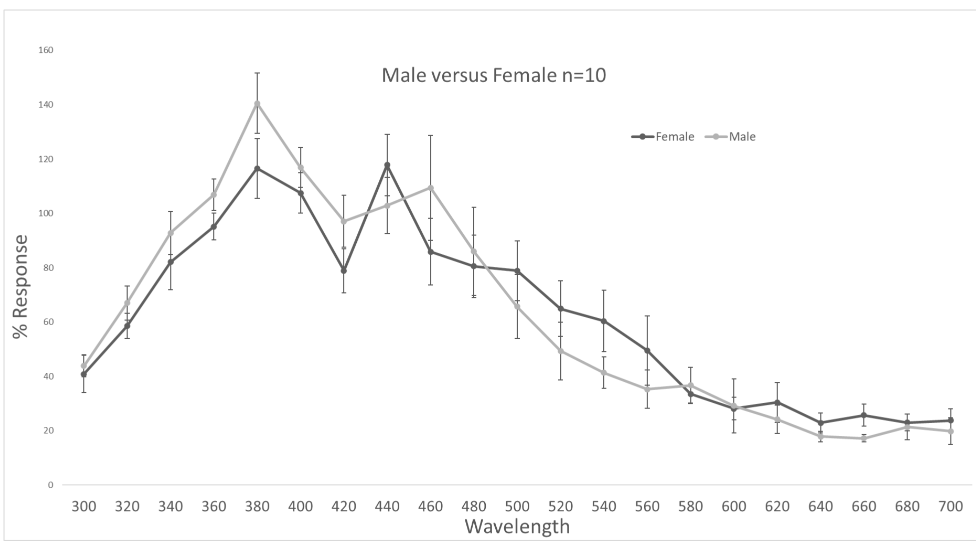


Figure 1. *ERG responses of male and female Citrus Swallowtails.*

Evaluation of Area-Wide Control of Box Tree Moth, *Cydalima perspectalis*, in Urban Areas of Western New York State

Gregory Simmons[1] and Alejandro Del Pozo[2]

[1] Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Salinas, CA; [2] Virgina Technical University Virginia Beach, VA

From 2023-2024, USDA and Virginia Tech scientists conducted an area-wide Box Tree Moth, BTM, *Cydalima perspectalis* control trial in Western New York. Applications of mating disruption (MD) pheromone (BTM-MESO, Trece), and BtK were made on three BTM infested landscapes. The goal of the study was to demonstrate the potential for BTM control using an area-wide strategy. Positive results could allow the adoption of an area-wide control program for local eradications of newly detected BTM as part of a "slow the spread" program to limit movement across the U.S.

A total of 51 acres were treated with MD and BtK. In both years, we observed reductions in BTM life stages in treated plots compared with untreated areas (Fig. 1). There was a 100% decrease in adult moth captures, 92-100% decreases in larval infestations, and 76-100% reductions in box tree moth damage during the last two sampling dates.

Results suggest that BTM in newly infested residential and commercial landscapes could be locally suppressed or eradicated using an area-wide control strategy. While having an MD product available would be an important component to include in a BTM control or nursery protection program, the BTM-Meso MD product for BTM control is not yet registered by EPA for use in the U.S. However, BTM is relatively easy to control with timely applications of Btk and several other insecticides. The most important factor is to make sure applications are made when BTM larvae are most susceptible, specifically as the overwintering larvae break diapause in the spring, and with applications timed to target early-stage larvae after peak flight periods.

It is not known how soon after a new detection that active control measures need to be applied to ensure a successful local eradication, but we estimate within the first 2-3 seasons after detection. We have observed that the BTM natural spread rate is relatively slow from infested areas which makes local eradication more likely. It is also unknown how long it would take to achieve nationwide eradication of this species if it were attempted. Experience from other moth eradication programs, and based on our treatments, a BTM eradication effort should plan on 3-4 years of making active treatments, followed by post control monitoring. Other factors that are important to consider include the size of the infested area and the distance or degree of isolation from nearby areas with boxwood.

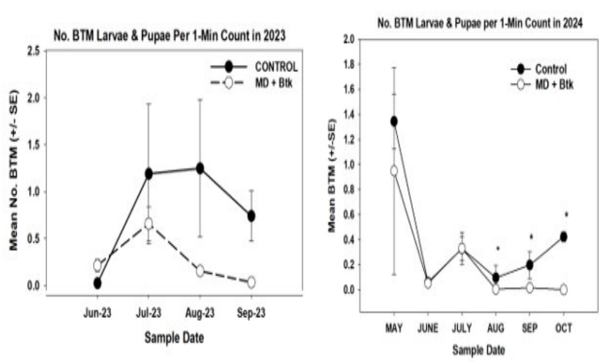


***Figure 1**. Number of BTM per one-minute visual survey on boxwood in treatment & control areas in 2023 (L) & 2024 (R). In 2023, there was a reduction of BTM life stages by 95% to 99% on the last two sample dates but this was not significant. In 2024, there was a significant reduction of BTM life stages on the last 3 sample months by 92-100% (X^2 = 10.61 d.f. = 1, P < 0.001).*

Box Tree Moth, *Cydalima perspectalis*, Trap Evaluation Study, Western New York, July 2024

Gregory Simmons[1], Mackenzie Wahl[1], Treya Gough[1], and Eugenia Garcia[1]

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

A green unitrap baited with a 3 mg pheromone lure with a Dichlorvos (DDVP) kill strip is the current recommended box tree moth, BTM, *Cydalima perspectalis* detection trap in the U.S, though it has some limitations requiring extra handling to count and recover moths -moths may become wet and rot with rain and are costly compared to alternatives. Additionally, to protect the endangered rusty patched bumble bee, unitraps of any color are not permitted by the US Fish and Wildlife Service in sensitive habitat areas due to the risk of bycatch. Last, the DDVP strip may be restricted in some states due to future changes in insecticide regulations. In 2021-2022, we showed that the unitrap is more attractive than several other traps, though we found that a large plastic delta trap (LPD) is the next best trap.

In 2021-2022, we first evaluated several traps for BTM capture efficiency in Croatia and we showed that the unitrap is more attractive than several other traps, though we found that a LPD had the next best capture rate. Given the limitations of the current standard BTM trap, in 2024, we returned to evaluate the standard green unitrap with DDVP kill strips to compared to several other options including: a unitrap with a round sticky insert (instead of a kill strip) baited with two 3 mg lures, and green LPDs baited with either one, two, or three, 3 mg lures. Results from the 2024 field season show there was a trend for greater trap catch on LPDs baited with 6 mg total pheromone (two 3 mg lures) compared to the other traps, though this was not a significant difference. While we have previous data that shows the standard unitrap is the most attractive trap type for BTM, in this case there were not any measurable significant differences in trap catch. However, because there is a need for trapping alternatives in areas where the unitrap cannot be used, or where the use of DDVP is restricted, we recommend the use of the green LPD baited with two 3 mg lures as the next best option for BTM detection in these areas.

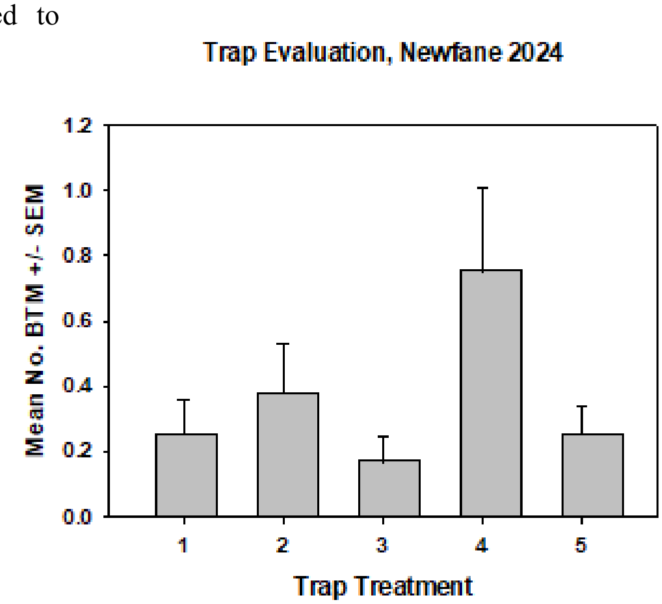


Figure 1 *Male BTM capture on five trap treatments in Newfane NY in 2024. Treatments: 1= Unitrap standard; 2 = Unitrap with no DPVM kill strip, sticky insert and two 3 mg lures; 3 = Green LPD with one 3 mg lure; 4= Green LPD with two 3 mg lures; 5 = Green LPD with three 2 mg lures. By Proc Genmod (SAS) there are no significant differences between treatments.*

Acronym List

ALB – Asian longhorn beetle

APHIS – Animal and Plant Health Inspection Service

BTM – Box tree moth

CABI – CAB International

CAPS – Cooperative Agricultural Pest Survey

CLB – Citrus longhorn beetle

CRB – Coconut rhinoceros beetle

DDVP – Dichlorvos

DNA - Deoxyribonucleic acid

EAB – Emerald ash borer

ERG – Electroretinogram

FPML – Forest Pest Methods Laboratory

FSMC – Flighted spongy month complex

GC- AED – Gas chromatography with atomic emission detector

GC- MS – Gas chromatography with mass spectrometry

LDA – *Lymantria dispar asiatica*

LED – Light emitting diode

LPD – Large plastic delta

MD – Mating disruption

OWB – Old world bollworm

PCR – Polymerase chase reaction

PPQ – Plant Protection and Quarantine

SM – Spongy moth

SLF – Spotted lanternfly

USDA – United State Department of Agriculture

UV – Ultraviolet

Forest Pest Methods Laboratory

1398 W. Truck Rd.

Buzzards Bay, MA 02542

508-563-0900