USDA Animal and Plant Health Inspection Service
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2020 Accomplishments

Forest Pest Methods Laboratory

Animal and Plant Health Inspection Service
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Forest Pest Methods Laboratory

2020 Accomplishment Report

Buzzards Bay MA
Salinas CA • Bethel OH

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Animal and Plant Health Inspection Service

Plant Protection and Quarantine

Science and Technology

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Contents

Foreword

Commodity Treatment and Pest Management

Behavioral and Chemical Ecology

Biological Control

Commodity Treatment

Insecticide Technology

Pest Management

Rearing

Survey, Detection, and Analysis

Behavioral Ecology and Survey Technology

Chemical Ecology and CAPS Lure Support

Survey, Detection, and Analysis

Molecular Biology

Quantitative Risk Analysis

Salinas Field Station

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*FPML employees and cooperators are indicated in bold

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Radiotelemetry and harmonic radar for tracking spotted lanternfly in the field

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Introduction

In 2019 and 2020, we developed and evaluated techniques for investigating the movements of spotted lanternfly, SLF, *Lycorma delicatula.* Two available tracking technologies were developed and evaluated: harmonic radar and radio telemetry. In each year, wild SLF were captured and affixed with either a harmonic radar tag or a radio telemetry antenna. The captured SLF were subsequently released, tracked daily, and their movements were recorded along with host records and other parameters. Developing tracking technology for SLF will provide new insights into the movement patterns of wild SLF and inform the SLF Program's survey and control efforts.

Harmonic radar

Harmonic radar (Recco) utilizes lightweight passive tags (made in-house), which reflect a signal produced at a certain frequency by a hand-held transmitter/receiver. The receiver "listens" for its signal to be reflected by the tag. Since it is passive and does not require a power source, the tag has an unlimited shelf- and field-life but is limited by the distance that it can reflect the signal. Usually, these tags are not detectable when they are more than 25-50 m from the signal source, meaning a signal for a fast-moving insect may be lost when it travels beyond the tracking range. In addition, if the tag is heard but not seen (e.g., if the insect is high in the canopy), it is difficult to distinguish between different tagged insects because they are simply reflecting the signal without any other specific identifiers. Therefore, individual parameters such as distance traveled would be difficult to determine when multiple insects are released in the same area. For this reason, SLF with harmonic radar tags were also marked with a number, and their positions were only confirmed when they were visually observed. The harmonic radar tag consists of a diode (purchased from Recco) soldered to an antenna of the precise length to optimally reflect the signal wavelength being emitted. The commercially available antennae were too large for attachment to SLF, so we had to custom-build them. After evaluating several options, we decided on a fine, lightweight, but highly conducive wire that successfully reflected the signal from 50 m in preliminary trials. Unfortunately, when attached to SLF and released during experimentation, the wire tangled easily on foliage and bent, reducing its reflective surface and weakening the signal. In 2020, we tested a new type of wire that, although very fine and lightweight (~11 mg), held its form better under field conditions (Figure 1A). In addition to tracking SLF adults, in 2020 two small HR studies were conducted with SLF nymphs (Figure 1B). Tagged nymphs were released either in a cornfield or on a forest edge bordering the same field. These studies allowed

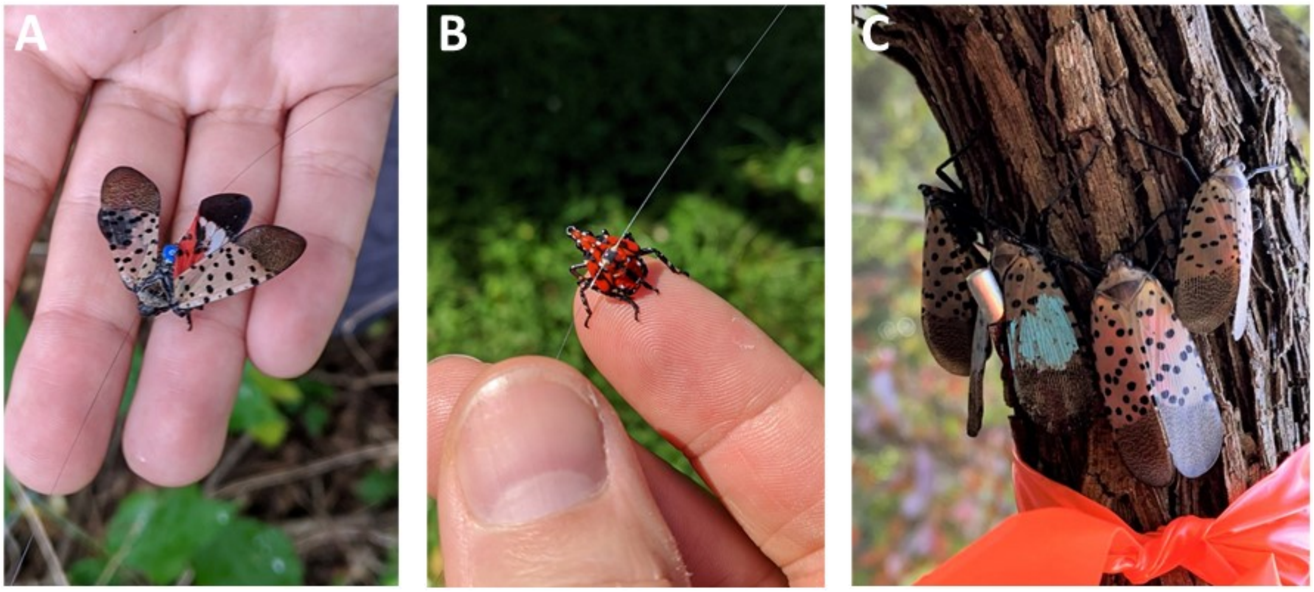

Figure 1. Insects located using tracking technologies in 2020. A dead adult SLF with a harmonic radar tag (A); a live fourth instar SLF with a harmonic radar tag (B); a live blue-marked SLF adult on grape with a radio telemetry tag (C).

both the collection of nymphal movement data and an opportunity to test new antenna wire types early in the season.

Radio telemetry

Radio telemetry involves a small radio transmitter (purchased from Lotek) attached to an antenna that is affixed to the insect (Figure 1C). The transmitter is powered by a very small battery and produces a strong signal that a receiver can detect up to 500 m away (under optimal conditions). This longer range helps the tracker locate tags from farther away, however, because it requires a battery, these tags are much heavier (we used 150 mg tags which were the smallest available), and can weigh down the insect and affect their movement. Because of the size and weight, the radio transmitter could only be used on SLF adults. Also, the battery life is limited to about three weeks once activated, after which it no longer produces a signal. If not used, the battery will last only a few months, so the shelf-life is limited, and batteries cannot be replaced, so tags are single-use. The 150 mg battery-powered nanotags became commercially available in 2019, and we pioneered their field testing on SLF that summer. During testing, we observed that tag failure accounted for the loss of about 10% of tagged SLF. After providing feedback to the company, no tags malfunctioned in 2020. Because each technology has unique advantages and disadvantages, we attempted to track SLF using both technologies. Perhaps the greatest advantage of radio telemetry technology is that each tag produces a unique signal that can be used to identify that individual insect. Therefore, even when the insect is out of sight, we can still identify the individual insect and determine how far it has moved, even if other tagged insects are in the area. This capability allows us to release multiple tagged males and females in the same location, at the same time and track each of their individual movements.

Results

The rate of tagged SLF locations using the different technologies in 2019 and 2020 is graphed in Figure 2. Both technologies had improved rates of discovery in 2020 compared to 2019, but radio telemetry had a better discovery rate overall. Harmonic radar tags were detected, but without seeing the SLF, it was difficult to determine exactly which insect was being detected. Harmonic radar tags that were detected high in a tree but never visually located were considered lost, which is reflected in the low recovery rate. The longest time we were able to locate live SLF with harmonic radar tags was 18 d after their release. Because harmonic radar signals do not provide any unique information about which insect is detected, movement information could not be recorded for an individual unless it was seen. When locating a radio telemetry signal, even if the insect was high in the tree and unable to be seen, the signal was unique for each insect so its location could be recorded. The maximum distances traveled by tracked insects in 2019 and 2020 were 60 and 50 m with harmonic radar and 434 m and 157 m with radio telemetry, respectively. Insects could be tracked more efficiently and for a longer time and distance with active radio telemetry tags than with passive harmonic radar tags.

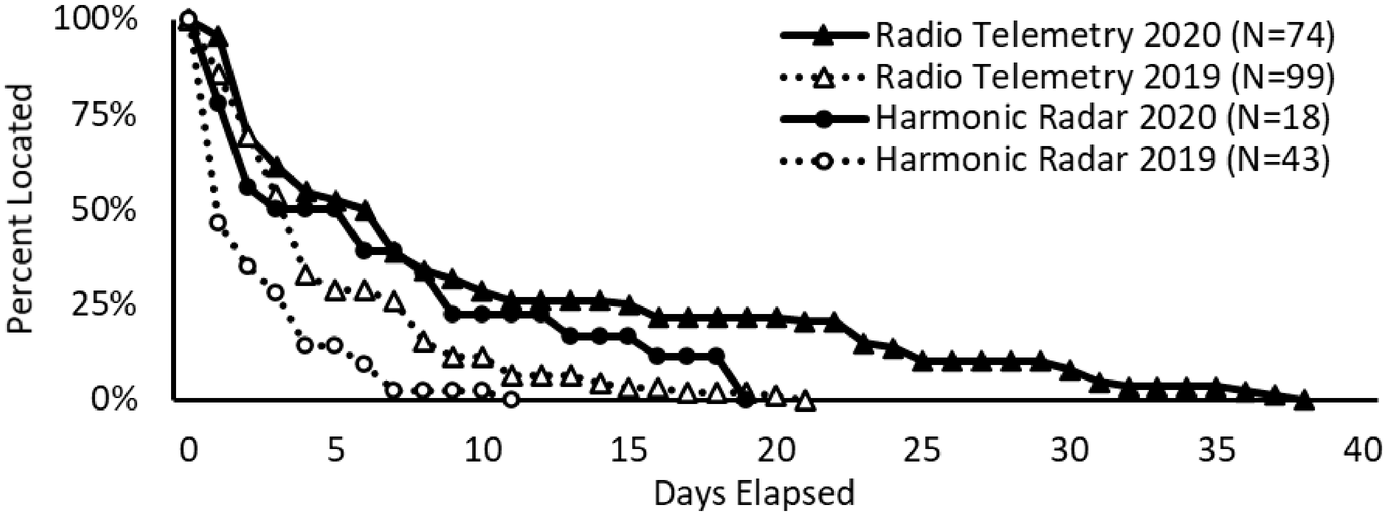

Figure 2. Rate of discovery and location of tagged SLF using different tag tracking technologies.

TRACKING SLF MOVEMENT PATTERNS

The elevation of SLF was calculated by triangulation of the signal using range finders. In 2020, the average elevation of both males and females was approximately 9 m high in the canopy. Early-1 adults (from emergence until Aug. 21, 2020) were evenly distributed at different elevations up to 12 m. Early-2 adults (Aug. 22 to Sept. 14, 2020), started to show a preference for elevations between 6-15 m. Mid adults (Sept. 15-28, 2020), the time at which mating was taking place, showed a sharp preference for elevations between 6-9 m. Late adults (Sept. 29 and later), when oviposition was underway, were again more evenly distributed at all elevations (Figure 3).

Adult tagged SLF were found on 27 species of plants (Figure 4). They were predominantly found associated with grape, or on grape climbing on other species. In addition, they were found on large trees. Tree-of-heaven ranked sixth among the most frequently visited species SLF were found on.

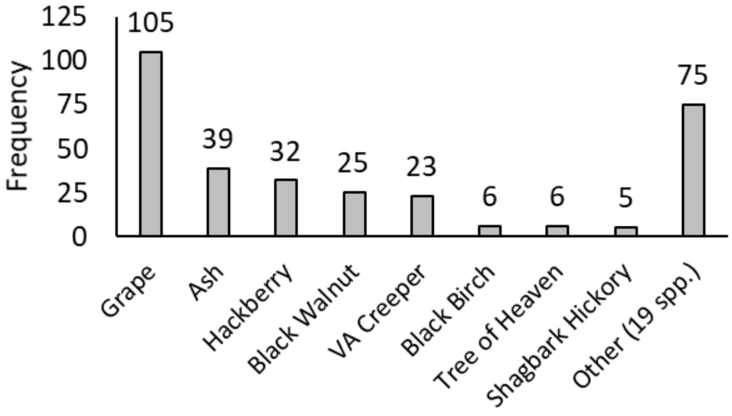

Figure 4. Excluding their release trees, SLF were found predominantly on or associated with wild grape, or grape wrapped around other species of trees.

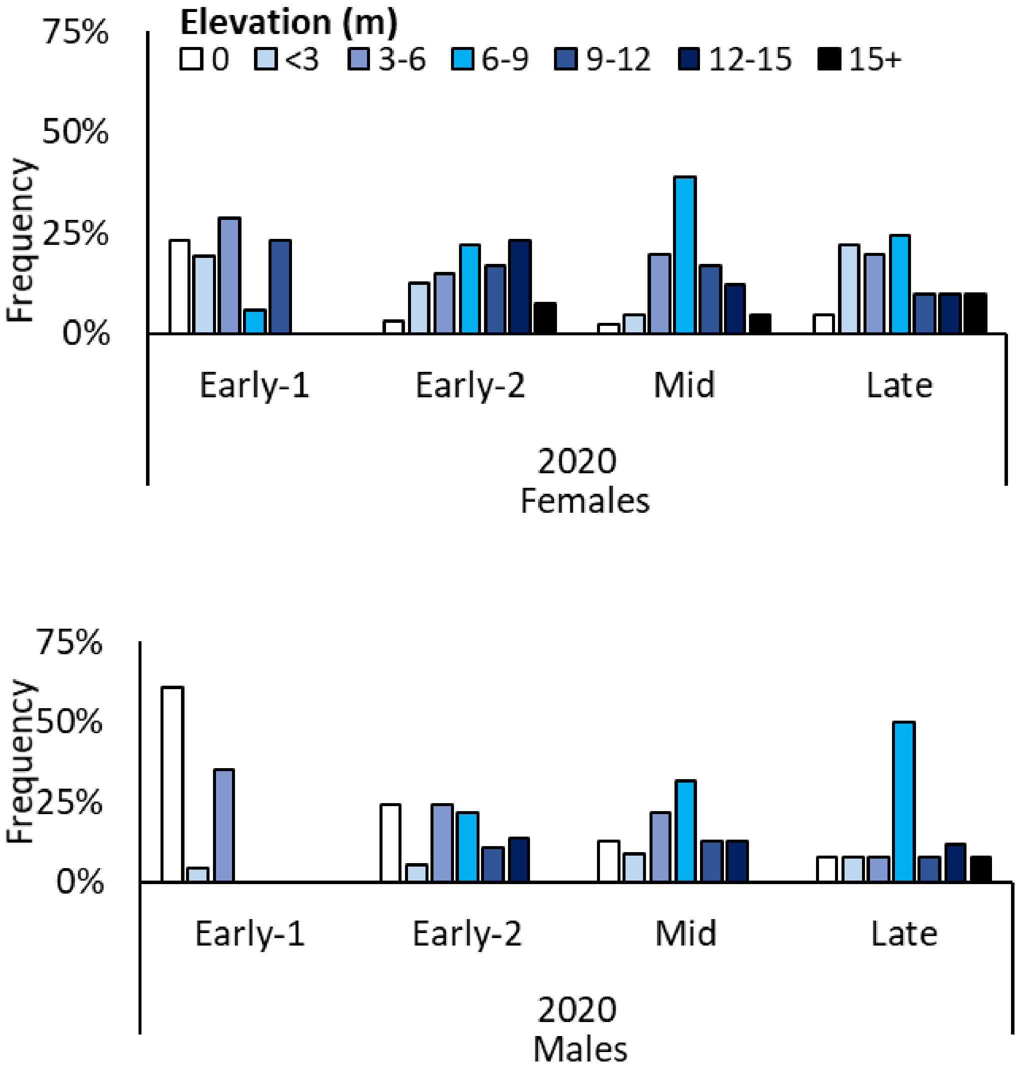

Figure 3. Height above ground (m) and frequency at which tagged SLF were found.

Distance moved by SLF appeared to increase in September during “Mid” when courtship and mating were taking place (Figure 5). On average, females moved considerably farther than males in both years (Figure 5). At the high-density site in 2019, there was a higher frequency of larger movements and a lower frequency of SLF staying in the same place than observed at the low-density site in 2020 (Figure 5).

Nymphal SLF studies in 2020 using harmonic radar showed that the more flexible antenna wire led to the ability to track them for a longer time (mean 3.3 ± 0.6 days vs. mean 1.7 ± 0.6, P = 0.078) but did not show differences in total distance moved (mean 9 ± 3 m, max 27.5 m vs. mean 4 ± 2, max 17.7 m, P = 0.128), perhaps due to the relatively small number of nymphs used. The distance moved per day was nearly identical with both wire types (mean 2.7 ± 0.5 m/d vs. mean 2.7 ± 0.7 m/d, P = 0.973). Combining both wire types, SLF nymphs moved further (corn: mean 12 ± 3 m, max 27.5 m, edge: mean 1.9 ± 0.4 m, max = 4 m, P = 0.001), at a greater rate (corn: mean 3.3 ± 0.4 m/d, max 5.4 m/d, edge: mean 2.1 ± 0.5 m/d, max = 5.5 m/d, P = 0.032), and were tracked longer (corn: mean 3.8 ± 0.6 d, max 5.1 d, edge: mean 1.2 ± 0.2 d, max = 1.8 d, P = 0.014) in corn vs. the forest edge. The higher movement observed in corn likely reflects both issues related to ease of tracking and the nymphs trying to move away from a non-preferred host.

Conclusion

Radio telemetry and harmonic radar both had benefits and drawbacks, trading off the ability to follow SLF over long distances with the impact of the weight of the tag on their movement. However, radio telemetry still provided more insight into the movement of SLF in the field because this method can track more specimens over a longer period. The majority of SLF were found to reside higher than 6 m in the canopy, and their capacity for movement over several hundreds of meters in a few days was revealed, despite carrying a heavy tag. Such movement may be associated with higher density populations and depleted food resources.

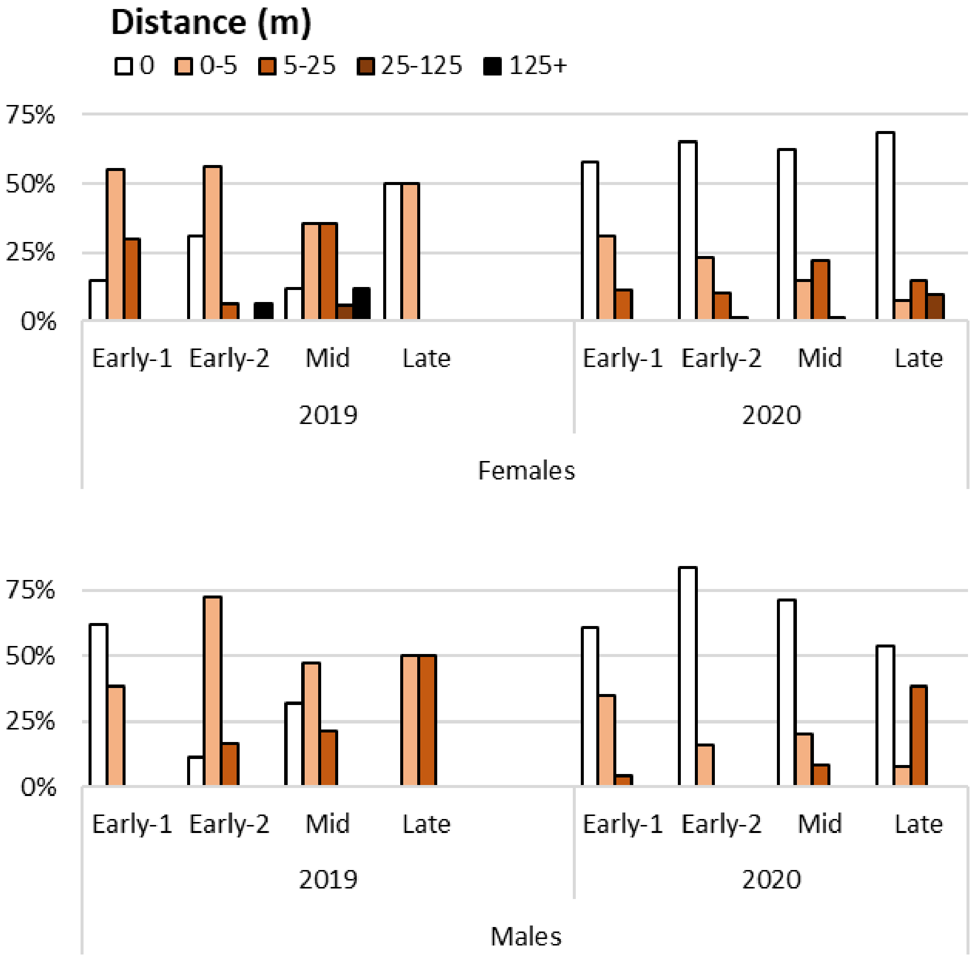

Figure 5. The frequency of movements of different distances (m) for females (top) and males (bottom) in 2019 at a high density site, and in 2020 at a low density site, using radio telemetry.

TRACKING AGH TO ERADICATE NEST

Using radiotelemetry to eradicate Asian giant hornet

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The Asian giant hornet, AGH, *Vespa mandarinia*, was discovered in 2019 for the first time in the United States around Blaine, Washington (Figure 1). The Asian Giant Hornet is a predator of honeybees and may have a significant impact on apiculture and the pollination of crops if it becomes established in the United States [1]. Additionally, it is also highly venomous and can be harmful to humans.

The Washington State Department of Agriculture (WSDA) made it a priority to locate the AGH nest associated with the recent detections in Blaine to protect honey bee populations in the area.

The technology and techniques developed at the Forest Pest Methods Laboratory (FPML) for tracking spotted lanternfly, SLF, *Lycorma delicatula*, were transferred to the Washington State Department of Agriculture, to serve as a tracking tool for the AGH nest. Radio telemetry uses a small transmitter (purchased from Lotek) attached to an antenna that is affixed to the insect. The transmitter is powered by a small battery and produces a strong signal that a receiver can detect up to 500 m away (under optimal conditions). Because the radio telemetry tags performed better on SLF in 2020 than in 2019, at the end of the 2020 season, extra tags were available.
In October of 2020, FPML offered WSDA the extra tags, the use of our Lotek tracking system, and provided personnel with video tutorials, so that WSDA could track live-captured wasps back to their nest. Within two days of receiving the system, an AGH was trapped. The AGH was chilled so that the Lotek tag could be attached to the live insect and was fed prior to its release. The tracking system was used to track the captured AGH back to its nest about 225 m away. This was the first AGH nest found in the U.S., and it was successfully eradicated a few days later. The WSDA has now acquired a radio telemetry system of their own, which can be used to locate more nests if more wasps are trapped in the future.

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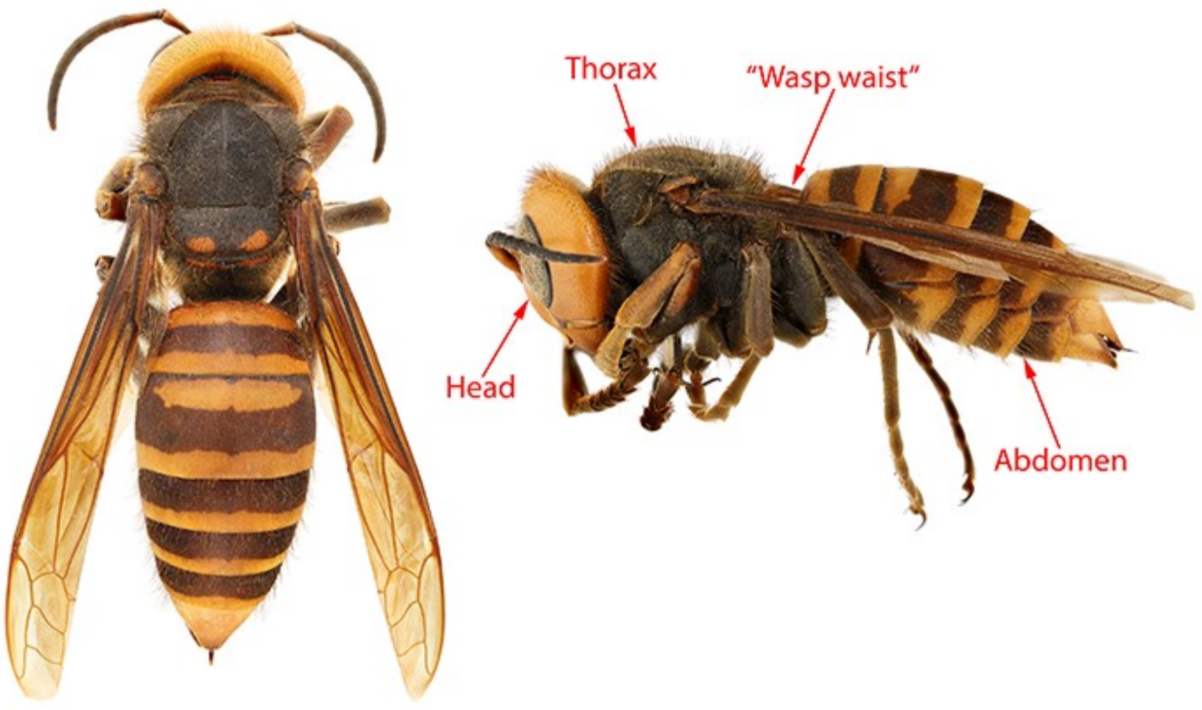

Figure 1. Asian giant hornet, *Vespa mandarinia.* Photo credit: Hanna Royals and Todd Gilligan.

Mortality of emerald ash borer in saplings: our next generation ash trees

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Introduction

Emerald ash borer, EAB, *Agrilus planipennis*, has been moving through American forests since it was accidentally introduced into Michigan in the early 1990s, leaving a trail of dead and dying ash trees in its wake. In 2007, the USDA began releasing several species of small wasps (parasitoids) that specifically target and kill EAB eggs and larvae. The released parasitoids have established in many states and have killed an increasing percentage of EAB over time. However, it has not been possible to rear and release enough parasitoids to halt mature ash tree mortality. Whether or not ash remains a viable component of North American forests depends on these small natural enemies' ability to prevent a resurgence of EAB populations after the outbreak subsides as the next generation of ash begins to grow in the forest. The present study was designed to support the EAB Program by quantifying the density and mortality of EAB in sapling ash trees following the release of parasitoids and the collapse of EAB.

Methods and results

Parasitoid release (8 total) and control (4 total) plots were set up in six forest stands in eastern (Hudson River Valley) and southwestern (Randolph) New York. Two larval parasitoids, *Tetrastichus planipennisi*, and *Spathius agrili*, and one egg parasitoid, *Oobius agrili*, were released beginning in 2011 and continuing through 2013. *Spathius agrili* did not establish at any of the plots, and recovery of the egg parasitoid was spotty. However, *T. planipennisi* was recovered in large numbers in all stands (in both control and parasitoid release plots).

Beginning in 2012, we monitored the health of up to 48 sapling ash and 48 mature ash trees in release and control plots. Trees were tagged and visited every year, during which data was collected on crown class (on a scale of 1–5, with 1 = healthy and 5 = dead) and signs of EAB infestation (woodpecker holes, D-shaped exit holes, bark splits, and epicormic shoots). In fall 2016 and spring 2017, we collected 60 saplings in eastern and western New York (240 saplings total) and dissected them to determine all EAB larvae's fate. Saplings collected in the fall were collected before wintertime predation by woodpeckers.

Although 40–60% of mature trees died, even in plots where parasitoids were released (Figure 1), significantly fewer saplings died compared to mature trees in both release and control plots. Additionally, while > 90% of the dead mature trees showed signs of EAB, most of the dead saplings did not, meaning they died from other natural causes such as competition. In saplings collected in spring 2017, most early instar (1–3) EAB larvae survived, while most mature (4th instar) larvae did not (Figure 2). In western New York, mortality of 4th instar larvae by spring 2017 was complete—no larvae in 60 saplings in 6 plots survived attack by the parasitoid *T. planipennisi* or woodpecker predation. The data suggest no EAB went on to reproduce and perpetuate the pest population. EAB and ash tree mortality in control plots were not significantly different from release plots because parasitoids released in 2011 quickly spread to control plots. By 2016/2017, when this study was conducted, parasitism was similar across all plots. Analysis was completed in 2020.

Our results are encouraging as they indicate that saplings growing in these New York forests are well protected from EAB. Overall, EAB densities were low, ranging from 0–4 larvae/m^2, and 70% or more of the sampled saplings contained no fresh EAB galleries (produced in 2016) (Figure 3). Some contained old galleries (produced before 2016), but those trees were still healthy and contained no new galleries.

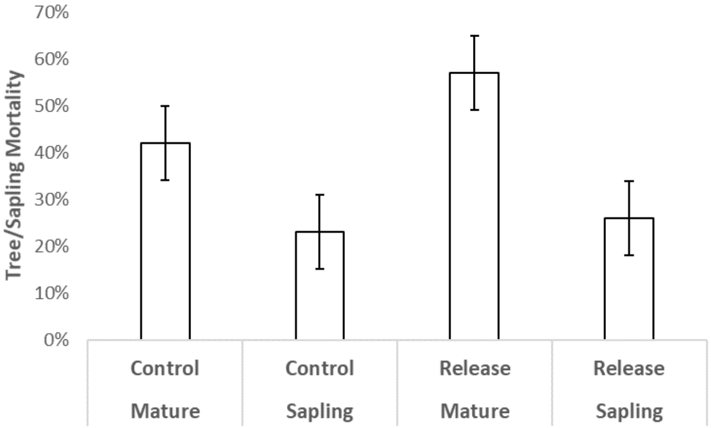

Figure 1. Mean (± SE) percentage of mature and sapling ash tree mortality in release and control plots between 2013 and 2017 in eastern and southwestern New York.

Conclusions

We found that after the initial onslaught of EAB, densities in saplings were low, many trees showed no evidence of EAB presence and the majority of larvae that did infest saplings were killed by *T. planipennisi* or woodpeckers. However, our work is not done; as the saplings grow, the bark will become too thick for the short ovipositor of *T. planipennisi*. Fortunately, since 2015 we have been releasing a congener, *Spathius galinae*, which has a long ovipositor, and is from an area climatically matched with the northern United States. *Spathius galinae* has already been documented as established in eight states. Our results contribute to a growing body of evidence that the EAB Biocontrol Program's decision to focus on controlling EAB populations in saplings which represent the future of ash in North American forests, is working.

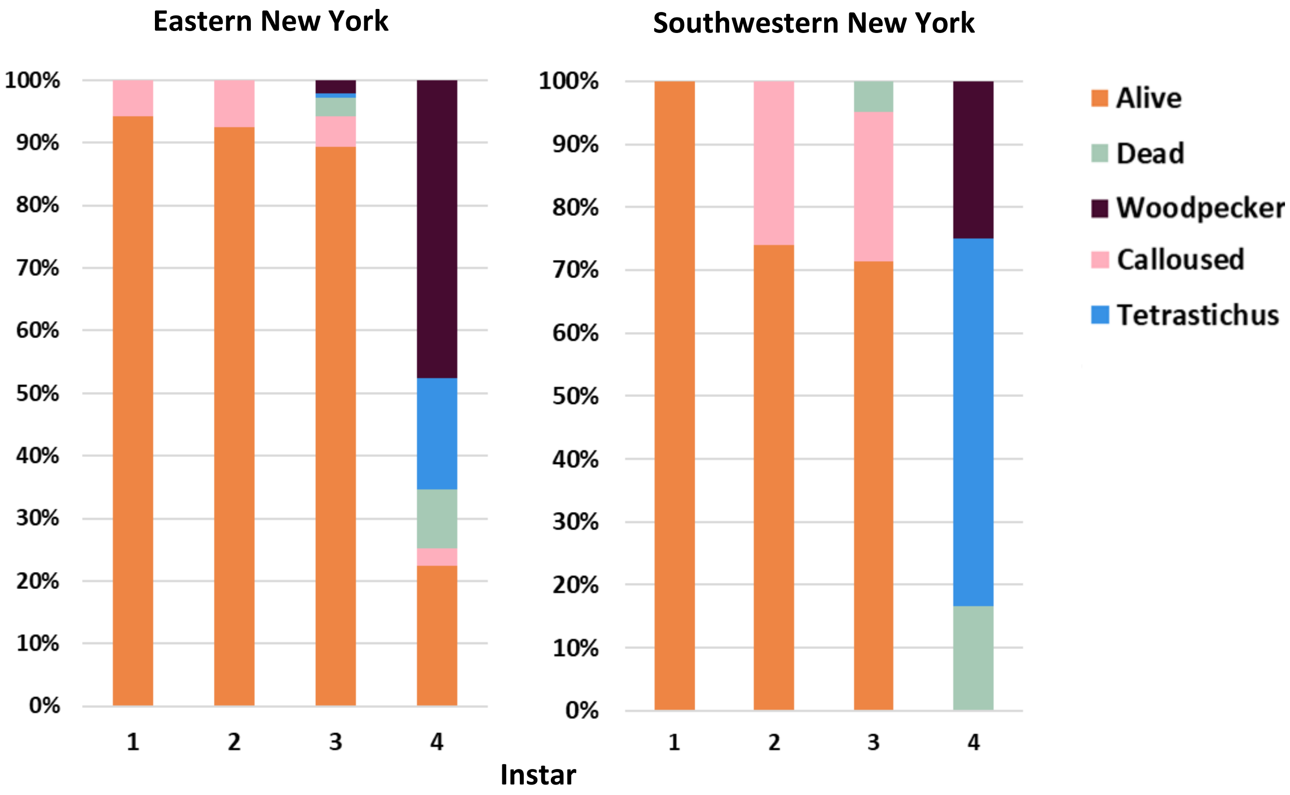

Figure 2. Mortality of 1st–4th instar EAB larvae in 120 ash saplings in eastern and western New York in spring 2017.

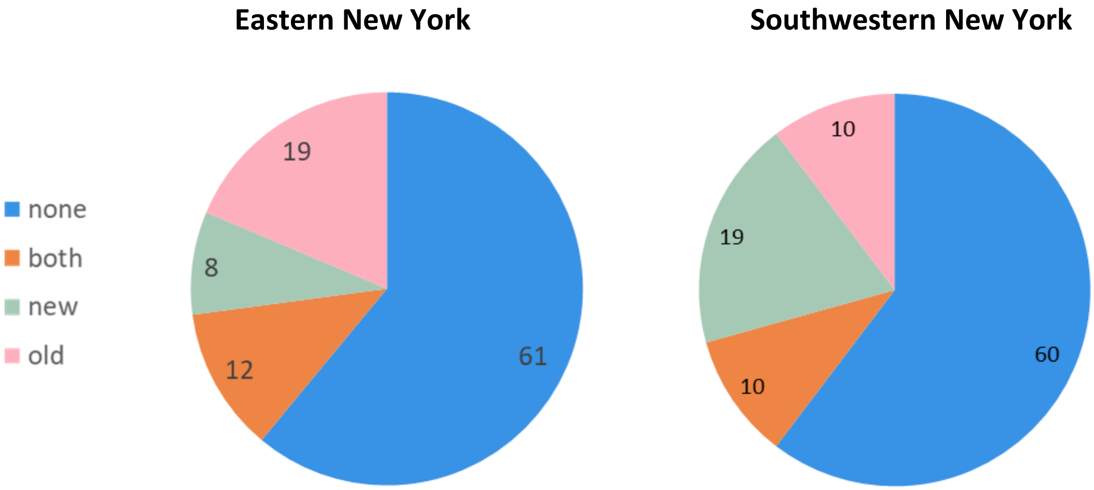

Figure 3. Percentage of 120 ash saplings collected in fall 2016 in New York forests containing 2016 EAB galleries, older galleries, both, or none.

Optimizing rearing procedures for *Anastatus orientalis*, a parasitoid of spotted lanternfly

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Introduction

Anastatus orientalis is an egg parasitoid of spotted lanternfly, SLF, *Lycorma delicatula*. It was discovered parasitizing SLF eggs in northern China in 2011 during exploration surveys for natural enemies to help manage an invasive population of SLF in South Korea [1, 2]. The *A. orientalis* colony maintained at the Forest Pest Methods Laboratory Insect Containment Facility (ICF) is being tested to evaluate its potential as a biological control agent of invasive SLF in the United States. This past year, a series of studies were conducted to better understand the life history of *A. orientalis* and the effects of rearing conditions [3]. The studies aimed to determine the longevity of *Anastatus orientalis* females and their fecundity over their life span to compare the production of wasp progeny when using stored SLF egg masses compared to newly collected egg masses, and to test the effects of different rearing and storage conditions of parasitized egg masses. These studies were designed to improve our methods for host range testing and enhance efficient rearing of *Anastatus orientalis*.

The results of these life history and rearing studies informed our rearing procedure for *A. orientalis.* Modifications were subsequently made to increase efficiency by using fewer females and less staffing resources to maintain the colony. Three modifications were made to the rearing method: (1) reduced the ratio of female wasps per egg mass from 5:1 to 3:2, (2) allowed wasps to parasitize egg masses for three weeks rather than just one week, and (3) minimized chill of parasitized eggs to less than three months when chill was needed to stagger emergence. The revised rearing method is outlined below and rearing outcomes are reported.

Materials and methods

The *A. orientalis* colony was maintained at the Forest Pest Methods Laboratory ICF. Laboratory colonies were established from parasitized egg masses collected from Beijing, China, from 2015-2019. The laboratory colony was genetically tested and found to be a genetically homogenous population (referred to as haplotype C) [4]. The SLF egg masses used to maintain the parasitoid colony were collected from various locations in Pennsylvania over two winter seasons (2019 to 2020 and 2020 to 2021). The egg masses were stored at 5°C and 65% RH with no light until used.

Up to 30 female or 10 male emerged wasps were placed in a rearing container with the sexes kept separate under conditions that were selected to simulate environmental conditions in mid-September in Beijing, China. Honey was streaked on the lid of each rearing container for feeding, and the wasps were held without access to SLF egg masses for a one-week preoviposition period. The laboratory colony was maintained by setting up groups of three females and one male in a small rearing container and providing two SLF egg masses per container to parasitize for one week. The egg masses were replaced the following week and again the week after for three exposure weeks total. After exposure, the egg masses were held for 30 days to allow time for their development before being moved to an environmental chamber set at 25°C constant temperature and 16:8 (L:D) to promote emergence. If the progeny were not needed immediately, the egg masses were moved into chill conditions at 5°C and 65% RH with no light following the 30-day developmental period. Any chilled egg masses were rotated in and out of chill so that any one set was in chill for as short a period as possible.

We started applying this protocol July 2, 2020. Prior to that, the previously published rearing protocol was used [3]. After implementing these modifications (reducing the ratio of female wasps per egg mass from five females to one egg mass to three females to two egg masses, keeping these females for new exposures each week for three weeks rather than just one week, and not storing parasitized eggs in chill for longer than three months), the production of wasps using the modified protocol was compared to the original protocol. The total number of wasps produced per egg mass and the female proportion of the progeny was also compared between rearing methods. Additionally, the effect that duration in chill had on the production of wasps and the timing of emergence of these wasps was analyzed. The following data were recorded: date of oviposition onset, date removed from oviposition, oviposition week, date into chill, date into 25°C, days in chill, emergence date, and number of SLF, *A. orientalis* males, and *A. orientalis* females that emerged.

Results

Four hundred and forty-three replicates applying the old protocol and 225 replicates applying the new protocol are included in these analyses. When applying the new protocol, the number of progeny produced declined slightly (Figure 1A). The data also showed a slight dip in the proportion of progeny that were female (Figure 1B) when comparing our old rearing protocol to the new rearing protocol. The number of progeny produced per egg mass decreased each exposure week (Figure 2). This is likely because a female would occasionally die during the three-week-long exposure. If they died partway through an exposure, production would decrease. It was relatively common for females to die in the third week (about one in every ten), resulting in low or no production of wasps from the set of two egg masses. We found no significant change in the sex ratio across the exposure weeks.

Duration of time spent in 5°C chill had a statistically significant effect on the timing of wasp emergence (Figure 3A). The longer egg masses were in chill, the longer until the first wasps started to emerge. However, the change was biologically very small (only about 3 days slower when held in chill for 3 months). Additionally, fewer wasps emerged the longer the egg masses were held in chill (Figure 3B), but the effect was small with an average decrease of only six wasps after three months. Broadley et al. 2020 [3] found that there was not a significant effect when holding egg masses in chill for two months but there was after holding them for four months. The findings in the current study further refine those earlier results and show that holding egg masses up to three months in chill does not have a significant effect. If possible, egg masses should not be held longer than three months.

Conclusion

From the results presented, we conclude that the modified rearing protocol is effective. The modified protocol takes less space in the growth chambers because two egg masses are exposed to wasps in the same rearing cup rather than just one per rearing cup. Using fewer rearing cups saves the time needed to wash the cups. Furthermore, using wasps for multiple exposures cuts down on the amount of time spent on acquiring new wasps each week and leaves more wasps available to use for other studies. The prior protocol used five females to create one parasitized egg mass, while the

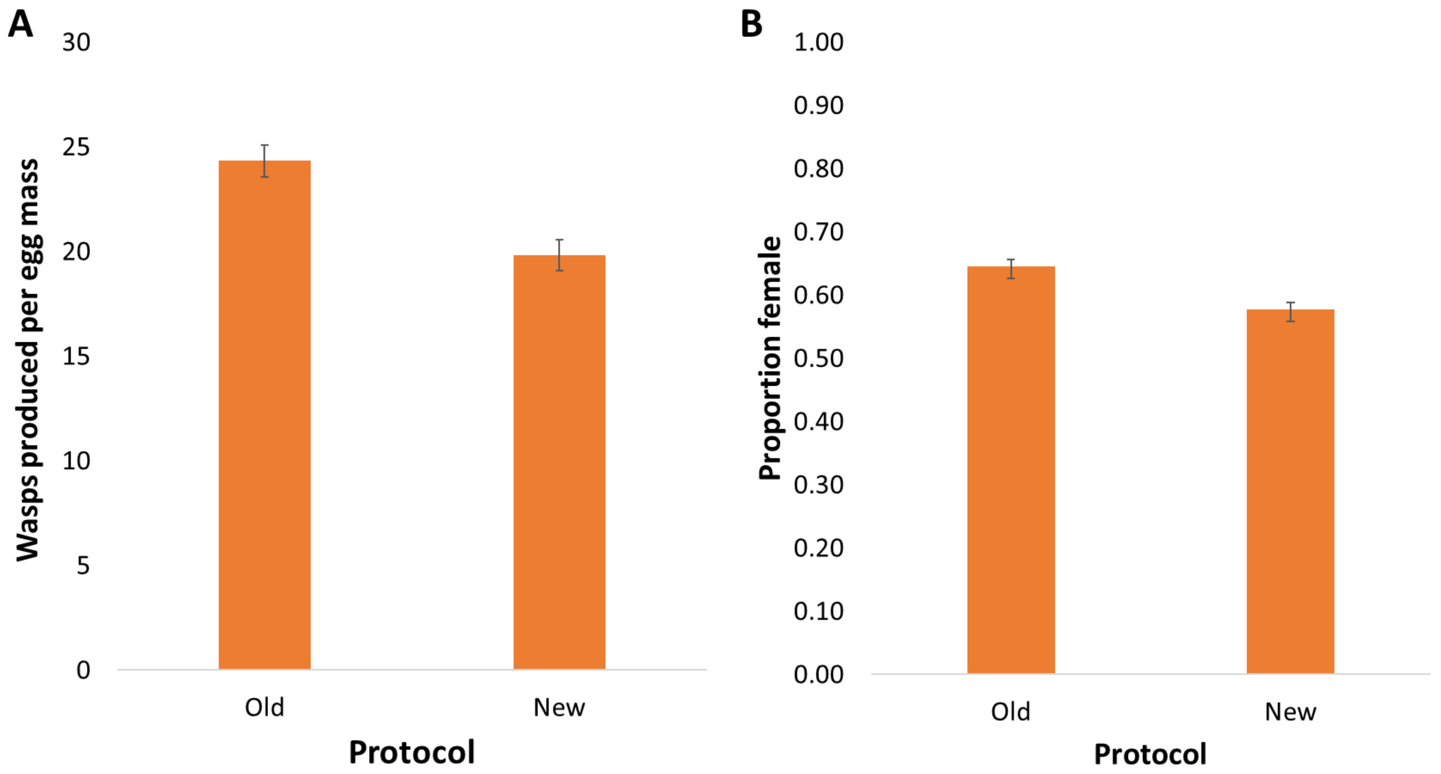

Figure 1. A) The average number of wasps produced per egg mass when applying the previously used protocol (old) as compared to the modified protocol (new) and B) the proportion female of the progeny produced using these two protocols. The bar represents means ± SE.

new protocol uses only three females to create six parasitized egg masses (a 1:2 ratio rather than 5:1). In addition, we estimate that the amount of time spent to complete this process is about a 20% reduction. While the total number of wasps produced per egg mass and the proportion of females produced using this modified protocol are both slightly reduced, we believe that the reduction in wasps, space, cups, and time needed to maintain the wasp colony outweigh these decreased outputs. Overall, the modified *A. orientalis* rearing procedure is more efficient, using fewer female wasps and less personnel time to maintain the colony, which supports research for the development of a biological control method for the SLF Program.

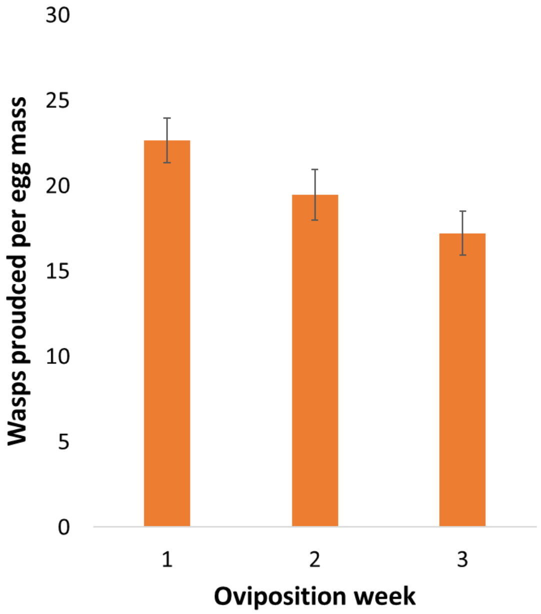

Figure 2. The average number of wasps produced per egg mass by oviposition week. The bar represents means ± SE.

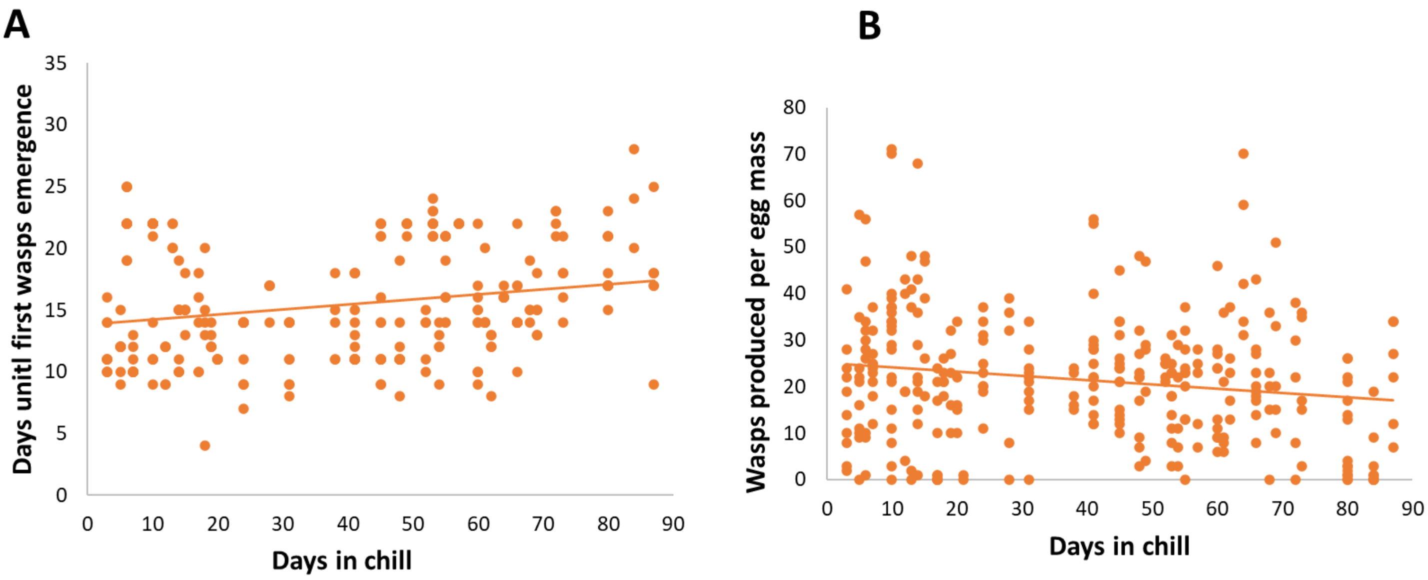

Figure 3. A) The number of days until initial wasp hatch after being removed from various durations of 5°C chill and B) the number of wasps produced per egg mass after different durations of time spent in 5°C chill.

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Determining the distribution, source population, and natural enemy complex of the roseau cane scale, *Nipponaclerda biwakoensis*, in Asia

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Introduction

Roseau cane or common reed, *Phragmites australis,* is the dominant plant species and a critical component of the Mississippi River Delta in Louisiana. The community of *Phragmites* in the Mississippi River Delta is comprised of different varieties, which together protect the marsh ecosystem from erosion and storm-related impacts, maintaining shipping channels and oil infrastructure. Widespread dieback and thinning of roseau cane stands in the Delta were noted in the fall of 2016 [1]. These diebacks coincide with observations of heavy infestation by roseau cane scale, RCS, *Nipponaclerda biwakoensis*a, a non-native insect originating from Asia. The infestation is the first record of a live population of RCS in the United States and, to date, RCS is the only member of its genus to be considered a pest [2]. Studies have found that severe infestation of the non-native scale RCS on *P. australis* can significantly reduce plant height, plant biomass, and proportion of the stem's length containing green leaf tissue, leading to premature senescence [3, 4].

Management options of the scale in the Delta are limited. Controlled burns to reduce scale infestations are not possible due to the extensive oil infrastructure. In addition, pesticides cannot be used because the estuary environment is the breeding habitat of numerous organisms. Classical biological control methods are being explored to provide a safe, cost-effective, and long-term approach for the management of this invasive scale. Prior research has shown that natural enemies play an important role in suppressing populations of RCS in their native range, and at least six different species of parasitic wasps have been documented attacking RCS in China and Japan [5-8]. Three of these wasps are in the family Encyrtidae (*Astymachus lasallei*, *Neastymachus japonicus*, and *Boucekiella depressa*) and have been found parasitizing the scale in Louisiana [1, 9]. However, Louisiana populations remain at outbreak levels with as many as 2,000 scales per stem. Additional mortality factors are needed to reduce population growth.

Developing biological control methods against the invasive RCS supports APHIS' goal of protecting American agriculture and PPQ's goal to optimize domestic pest management and eradication programs. Our methods development research team is a partnership of multiple institutions in the United States and across countries in Asia, including: Japan, South Korea, Taiwan, China, and Vietnam. We completed two years of surveys of RCS in its native range in order to: 1) identify the genetic source of the invasive population of scale in Louisiana, 2) compare population dynamics of scale across sites in Asia with the invasive population in Louisiana, and 3) rear and identify parasitoids across these populations.

Methods

Mature and immature RCS samples were collected for identification. Twenty-eight collection sites were identified in Asia and Louisiana (Figure 1), and collections were made through the growing season for two years. Using a standardized protocol, we collected data on host plant health, host plant identity (using molecular analyses), scale density and life stage, environmental conditions including water depth and salinity, parasitism rate, and parasitoid composition.

Representative scale specimens from each site were slide-mounted, and DNA was extracted. To date, 120 samples have been prepared, and 49 samples have been sequenced from 33 sites in Louisiana and Texas. Additionally, 317 samples were prepped and 195 sequenced from across Asia (three sites from China, two sites from Hong Kong, six sites from Taiwan, 11 sites from Japan, and six sites from South Korea).

To identify the variety of *Phragmites* utilized as host plants by the scale across Asia, we are using molecular markers to analyze DNA from host leaf material. Samples are still in the process of being analyzed. We also have accessed *Phragmites* species data from the Global Biodiversity and Information Facility (GBIF) database to evaluate which *Phragmites* host plants are most prevalent at our study sites.

Wasps that emerged from parasitized scale were stored in alcohol. A representative subset of parasitoid samples from each site and collection period are being characterized using morphological and genetic identifications. The parasitoids that are already present in Louisiana include *Astymachus lasallei*, *Boucekiella depressa*, and *Neastymachus japonicus*. We are comparing collections of these parasitoids from Louisiana to material collected from Asia. Molecular analysis of the parasitoids is underway to compare the haplotype network generated for the host aclerdid to assess the geographic origin of parasitoids recovered from Louisiana.

Results

Our foreign explorations have resulted in a new record for RCS in Taiwan and broader geographical distributions within Japan, Korea, and China (adding Hong Kong) than were previously known. When comparing the survey data from 2020 collected across locations in Asia to the data collected in Louisiana, some notable trends become apparent. In Louisiana, the density of live stems was lower (Figure 2A), the proportion of stems with scale was higher (Figure 2B), and, for the stems that had scale, the number of scales per stem was higher compared to those in Asia (Figure 2C). These findings show that scale infestations are more severe in Louisiana. The percentage of mature scales that were parasitized across the sites was variable, with some locations showing higher percentage parasitism and some locations showing lower parasitism (Figure 2D). Future work will include two years of collections for each site and an evaluation of the scale densities and the parasitoid community composition.

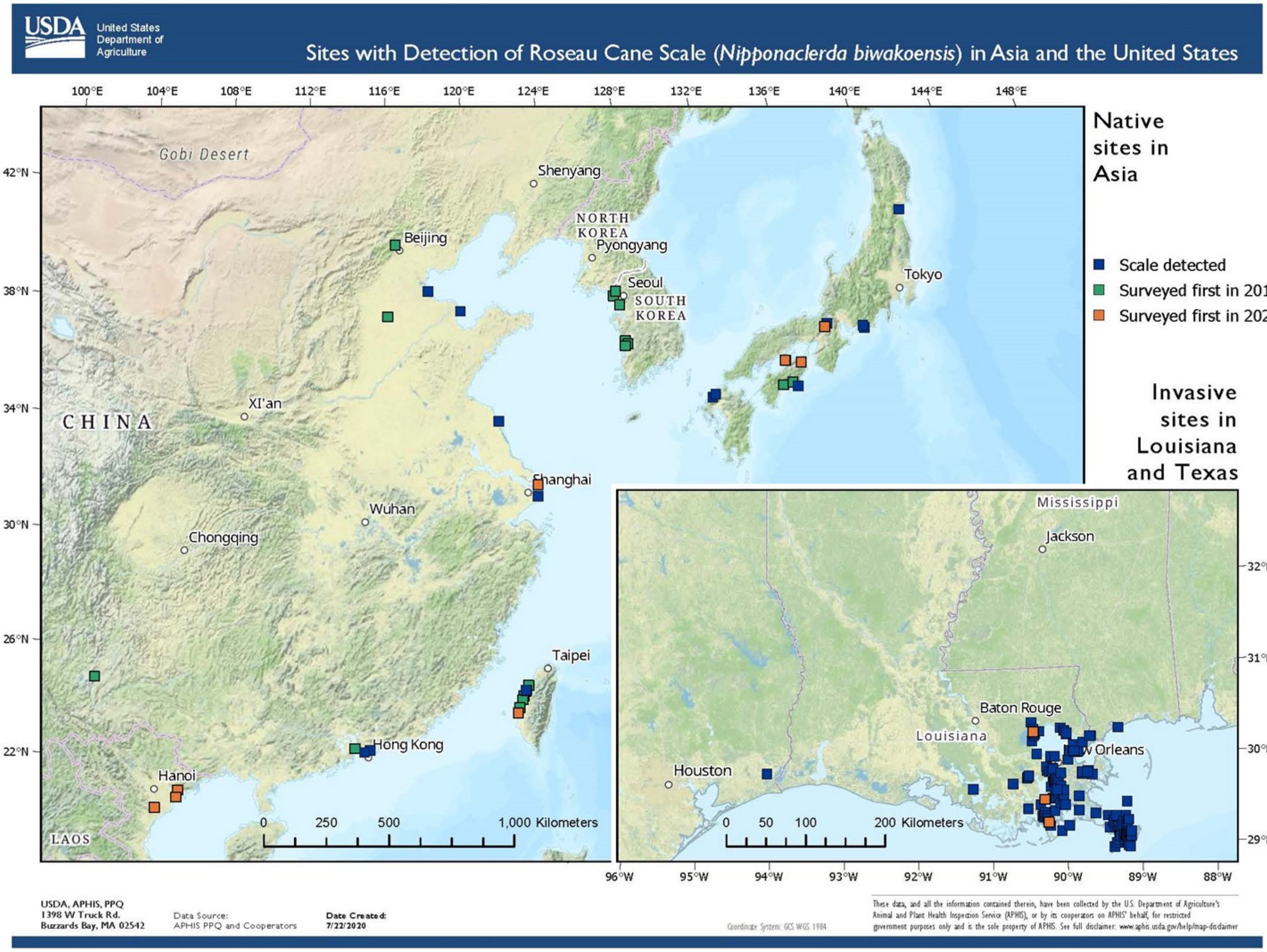

Figure 1. Sites with roseau cane scale (*N. biwakoensis*) including 2019 study sites in Asia (green), sites that were added in 2020 (orange), and all other locations with known detections of *N. biwakoensis* (blue). Additionally, we have made one-time collections of scale both in Asia and in Louisiana from these blue sites as part of our scale population genetic analyses.

Genetic source of the invasive population in Louisiana

Our estimated haplotype network (Figure 3) of scale populations shows a rich diversity of haplotypes across the sampled regions in Asia. The haplotype network confirms that specimens collected from the United States all belong to the same haplotype, except for one specimen that appears to have acquired a novel mutation post-introduction. This suggests a single introduction event of scale to the United States. The U.S. haplotype matches specimens collected from Beijing and Guangzhou, China, strongly indicating that the invasive population found in the U.S. originated from China. Interestingly, this haplotype is widespread throughout China, sharply contrasting with the general pattern observed across most sites, for which there is a strong connection between locality and haplotype (Figure 3). This suggests human-mediated transportation of this haplotype (USA1 in Figure 3) has already been occurring within mainland China. Similarly, the relationship between specimens from Hong Kong and Korea suggests human-mediated transportation may be involved as well, with an introduction of scales from Korea to Hong Kong at some point in the past. Additionally, two undetermined species of *Nipponaclerda* were collected, one from Vietnam and one from Japan; each formed their own disconnected networks (Figure 4). At this time, their identities are undetermined but they could be *N. leptodermis* or *N. triumpha,* both of which are known to occur in China [2, 8].

Population dynamics across sites in Asia and invasive population in Louisiana

From *Phragmites* host plant collections we have initial analyses underway to determine which species of *Phragmites* the scale is utilizing across the Asian collection sites and whether plant biotype affects scale population density. Based on the maps constructed from data downloaded from the Global Biodiversity and Information Facility (GBIF) database (Figure 5), we expect the scale collections from Japan and South Korea to be from either *P. australis* or *P. japonicus*. Collections from mainland China, Taiwan, and Vietnam will likely also be from *P. australis* or *P. japonicus*, but RCS may also be using *P. karka* in these regions. These results will be confirmed when genetic analyses are complete.

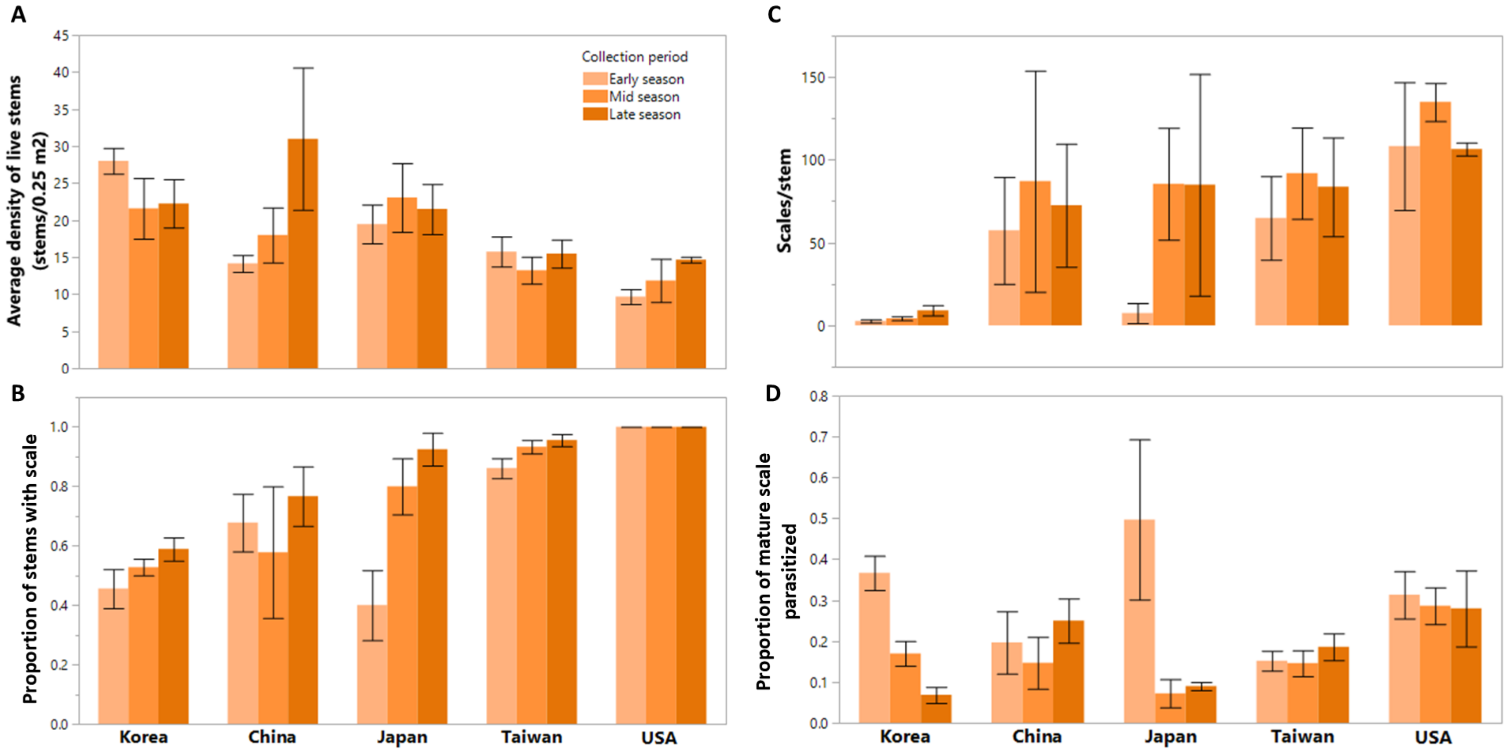

Figure 2. A) The average density of live *Phragmites* stems, B) proportion of stems with *N. biwakoensis* scale, C) the number of scales per stem for the stems that had scale, and D) the proportion of mature scale that were parasitized from the 2020 collections (except for the parasitism data show form Taiwan, which is the 2019 data). Bars represent means ± SE.

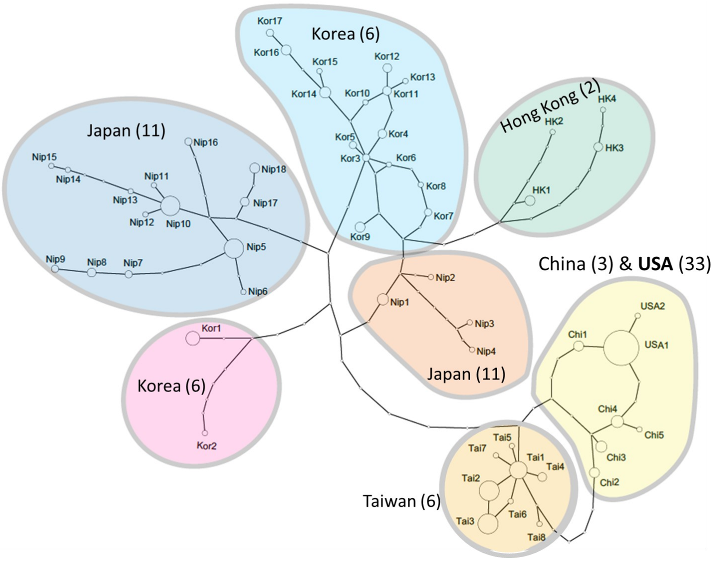

Figure 3. Haplotype network of *N. biwakoensis* collections estimated using 95% statistical parsimony in TCS 1.21.

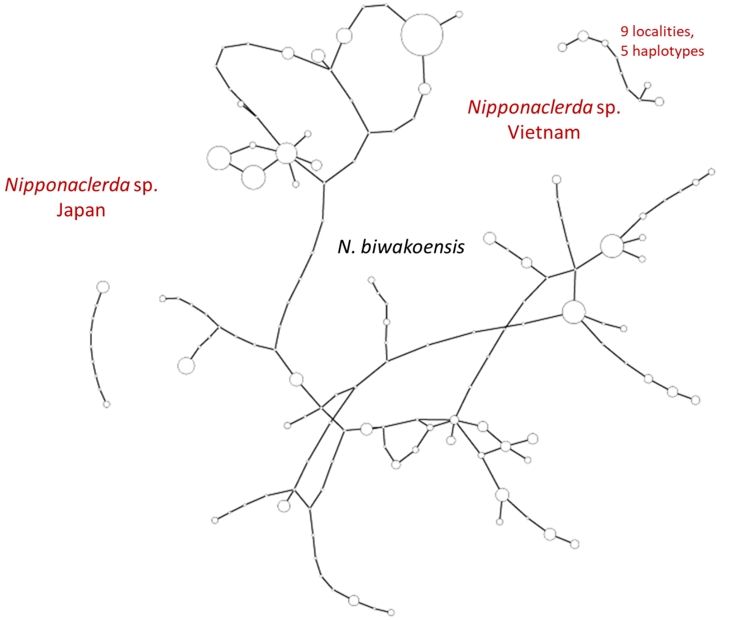

Figure 4. The unknown species of *Nipponaclerda* show with the *N. biwakoensis* COI haplotype network.

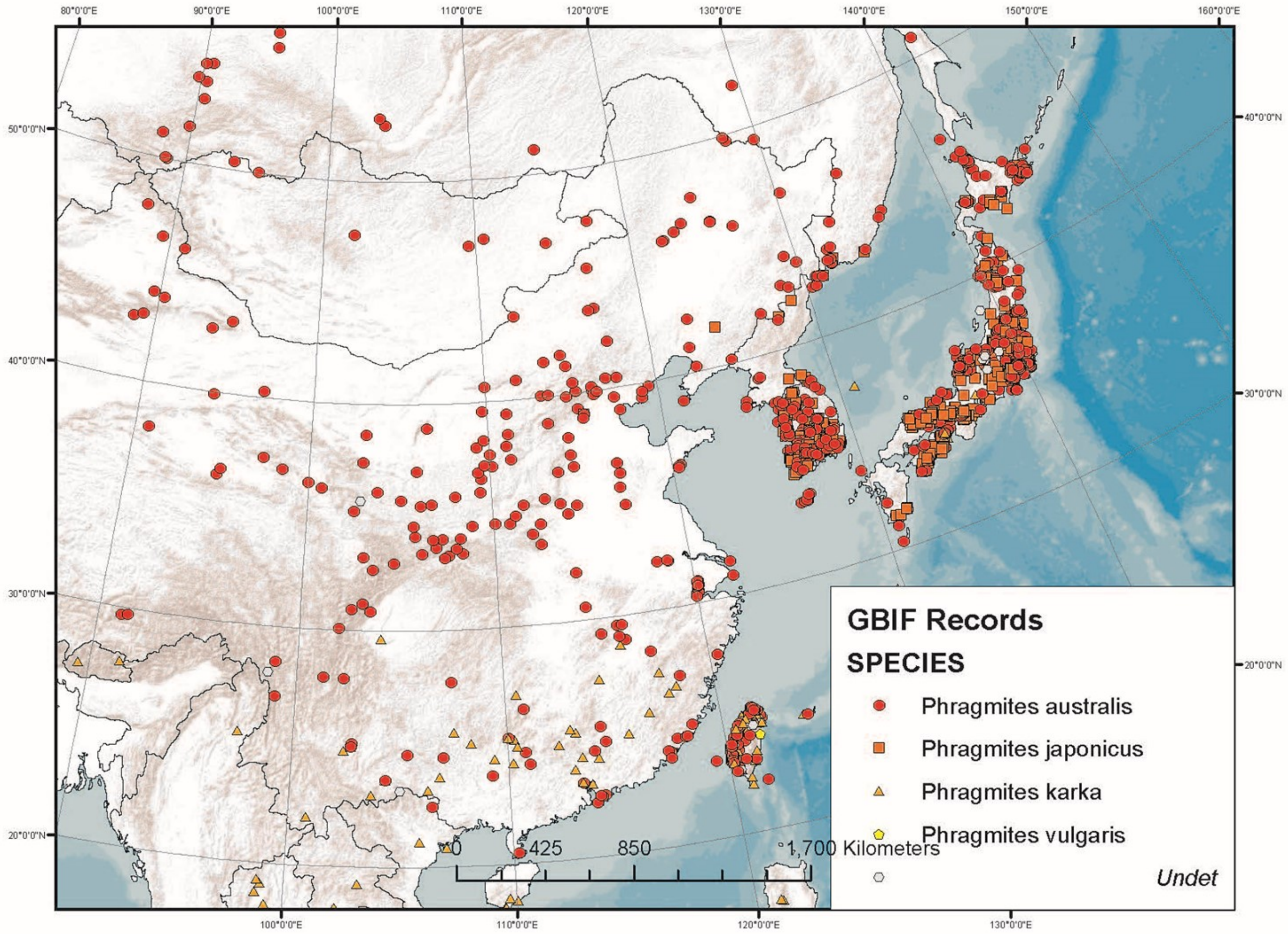

Figure 5. Phragmites records from the Global Biodiversity and Information Facility (GBIF) database across collection sites.

Rear and identify parasitoids across populations

To date, parasitoids not yet recovered from Louisiana but reared from Asia include *Astymachus japonicus, Aprostocetus* sp., and an additional four unidentified species of encyrtid wasp. We also have recovered the three species of parasitoid that have already been found parasitizing the invasive populations of scale in Louisiana (*Astymachus lasallei*, *Boucekiella depressa*, and *Neastymachus japonicus*). We've reared *Astymachus lasallei* from samples from Louisiana and Japan, and it is also possibly present in Korea, China, Taiwan, and Vietnam but further taxonomic work is needed to confirm [9]. *Neastymachus japonicus* was recovered from Japan and Louisiana. Roseau cane scale is the only known host of *A. lasallei, A. japonicus,* and *A. lasallei,* making these species of particular interest for further investigation [6, 7, 11, 12]. Planned morphological and molecular analyses will help to determine if the recovered collection is *A. japonicus* or *A. lasallei* and if the collections from Louisiana are the same as the collections from across Asia. *Boucekiella depressa* was found across all sampling sites in Asia but it is likely a generalist, as it has been documented from several aclerdids and pseudococcids and has been reported as a hyperparasitoid of Encyrtidae [13]. Work is underway to obtain identifications of the encyrtids wasps and to identify the species of *Aprostocetus* that has been recovered.

Conclusion

Data collection and analysis are ongoing but show some notable preliminary results. There is a lower density of live stems, and the scale densities are higher in Louisiana than in the scale's native range. Population genetic analyses of the scale show that the likely source population of the scale is China. Ecological niche modeling work that is underway suggests that southern China is the closest likely match. Further evaluation of the parasitism data and collected parasitoid samples will be completed and candidate parasitoids will be imported to begin rearing at the Forest Pest Methods Laboratory Insect Containment Facility. Future goals include delving deeper into the genetic composition of the parasitoid collections, evaluating mortality factors affecting scale population density in the native range as compared to the invasive range, and beginning host range testing of promising biological control agents. This work represents essential research towards the development of an integrated management program for control of the RCS insect pest infestation.

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Integrated methods of behavioral control of khapra beetle

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Introduction

Khapra beetle, *Trogoderma granarium*, is a pest originating in south-central Asia, which has become a worldwide concern for its potential damage to stored agricultural products (Figure 1). The warm, humid environments of facilities such as grain storage and production facilities provide conditions for economically impactful rapid outbreaks [1], even in climates where the establishment of endogenous outdoor populations cannot occur. Khapra beetle has not been established in the United States; therefore, early detection of khapra beetle introductions is a key goal of PPQ's khapra beetle program. Small populations may be challenging to detect because larvae can go into diapause when stressed by starvation or cooler temperatures and persist for long periods. Therefore, the potential exists for recontamination of treated facilities after the reemergence of diapausing larvae [2].

Figure 1. Khapra beetle, *Trogoderma granarium*. Photo credit: Bugwood.org

The United States has quarantine restrictions on khapra beetle that are needed to safeguard domestic grain supply and in order to access to export markets. It is frequently intercepted by customs inspections associated with travel and importation. The range of damage can vary, with the most significant event occurring in Arizona in the 1950s when an expensive and successful eradication program was needed to address a growing established population [3]. At the Forest Pest Methods Laboratory a variety of integrated control methods are being developed, including improved lures and traps, use of natural repellents, and insecticide-treated barriers, to support the Khapra beetle program with attract-and-kill and early detection tools. Recent advancements towards the development of the tools are detailed here.

Lure development

An effective lure is needed for optimal trapping of *T. granarium* to create a detection tool that allows more efficient surveillance. The established trap (Figure 2A) and lure for *T. granarium* was developed 30 years ago. Since then, new lures containing food and/or pheromonal stimuli have become commercially available. Some of these newer products have been developed and tested for closely related dermestids already in the U.S. but not under quarantine, such as *Trogoderma variabile*. The degree to which these insects may be able to act as a legitimate surrogate species for the behavioral responses of *T. granarium* has been investigated by the Forest Pest Methods Laboratory. In one study, we evaluated the attraction to, arrestment by, and preference between different semiochemical stimuli for immature life stages of both species. For *T. granarium*, in most cases, the lures showed a positive upwind response in comparison to the controls, indicating some degree of attraction. One lure, the Pantry Patrol Gel (Figure 2B) from Insects Limited (Westfield, IN), exhibited the most consistent positive response by larval *T. granarium* [4]. This lure was generally preferred to others in the arrestment assay. The assays were generally not as effective for *T. variabile*, with responses not consistently correlated with that of *T. granarium*. It was also determined that a lure consisting of only the adult pheromone was attractive to older larvae, but repellant to younger larvae. Because the pheromone lure is used in APHIS wall traps, these results indicate that the traps may be more effective for monitoring older larvae and adults versus younger larvae.

Behavioral repellency

While examining semiochemicals of *T. granarium,* potential repellants were also screened. Repellants may be used to protect the grain itself or in push-pull trapping systems that maximize surveillance ability. Oleic acid is known to function as a necromone in many insect taxa. It is currently unknown if this phenomenon is prevalent in stored product systems, or if there are cases where there may be implications for pest management. We recently explored how death affects the oleic acid content of *T. granarium* extracts, and whether the compound causes a behavioral response [5]. If larvae were frozen before they were extracted, they had greater oleic acid content than if they were placed into hexane alive. It did not matter if they were slowly frozen to death over several days at -20°C, or if they were flash-frozen in liquid nitrogen. It seems likely that mortality induced by freezing causes tissue damage that results in the emission of oleic acid. When these natural extracts had higher amounts of oleic acid, they were repulsive in two-choice assays versus controls. These results were repeated in two other dermestid species, *Trogoderma inclusum* and *T. variabile*. The range of dose eliciting the response was determined to be 68–131 µg oleic acid. However, when oleic acid was undetectable or very low in natural extracts (~ 2 µg) there was no effect on movement in *T. granarium* and *T. inclusum*, and attraction to *T. variabile*. We also performed the assay using a large range of doses of synthetic oleic acid. At the lower doses, oleic acid had no effect on movement in *T. granarium*, but it became strongly repellant at higher doses, beginning at 100 µg. These results indicate that necromones may be an overlooked aspect of stored product insect biology, which could improve pest management if further researched. For example, applying the compound to surfaces such as bags or other barriers protecting grains may be effective. Furthermore, oleic acid may also be useful for push-pull trapping systems.

Insecticide-treated barriers

Similar to the use of repellants, insecticide-treated barriers may provide a tool to protect certain products. They also have a potential use within attract-and-kill traps if they can induce mortality or prevent the insects from exiting. Long-lasting insecticide-treated netting (LLIN) (Figure 2C) has numerous potential applications beyond commonly known mosquito control programs. Stored products may be protected from insects such as khapra beetle using such netting. We found that brief (<30 min) exposure of *T. granarium* larvae to LLIN, similar to what might be expected in field conditions as insect forage, does not affect the chance of eventual adult emergence [6]. We also observed how well the larvae moved upwind with respect to odor stimuli or netting for 10 min and then placed them in a wind tunnel and monitored for movement toward a stimulus. Both untreated netting and LLIN were used, as were insects never exposed to any netting. The wind-tunnel assay was performed either with or without the Insects Limited Pantry Patrol gel that was previously shown to be highly attractive to larvae of this species. As expected, larvae not exposed to any netting showed an increased likelihood of walking upwind if the semiochemical lure was provided. A similar pattern was observed when the untreated netting was used, but the larvae became more likely to remain stationary in the assay after acclimating to the net. When LLIN was used, the larvae became more likely to move and there was a baseline increase in the likelihood of moving upwind. However, upwind walking was no longer related to semiochemical presentation. These observations suggest that particular care should be used concerning the airflow patterns and semiochemical landscape of the warehouse settings in which LLIN is deployed. Larvae may also appear to be arrested by untreated netting, which may be useful for trapping designs.

Deltamethrin-treated, Zerofly™ storage bags (Figure 2D), Vestergaard Frandsen Inc., Lausanne, Switzerland, are another potential tool that could be used to limit infestations during storage of grain in bags. We investigated the efficacy of the deltamethrin-treated bags against *T. granarium* adults and larvae. Deltamethrin-treated and untreated packaging materials were affixed to the bottom of plastic Petri dishes to create bioassay arenas to assess the effects of exposure within 24 h. Adult *T. granarium* were knocked down in <60 min, and 100% of adults were knocked down or dead after 24 h. *Trogoderma granarium* larvae were exposed and removed for 0.33, 1, 2, 3, or 4 d and subsequently monitored for larval death and adult emergence. Exposures were also repeated for these durations continually without removal. Larvae exposed for 4 d had 50% mortality versus 97% if continually exposed. Utilizing this deltamethrin-treated packaging could cause disruptions in natural populations of *T. granarium* found in storage facilities, and the treated packaging is an effective tool that could be implemented into an integrated pest management program for bagged grain.

Conclusion

Several tools have been evaluated for use in the management of khapra beetle. The focus on larvae is extremely important to ensure that insects might be better detected and controlled at this critical stage where prolonged diapause can occur. The optimal semiochemical attractant among commercial alternatives has been determined, and a novel semiochemical repellant has been discovered. We have also determined the lethality and behavioral effects of novel insecticidal barriers. Such knowledge may have many potential management applications. The primary objective of our current work is the development of attract-and-kill formulations. The tools explored at this point have not yet been incorporated into a new khapra beetle management trapping devices . We expect that we will be able to create a more efficient surveillance tool for capturing and retaining larvae and adults of *T. granarium* at low population levels. These new tools will support the Khapra Beetle Program in excluding the pest at entry points and minimizing damage when it is detected by enabling more effective preventative remediation.

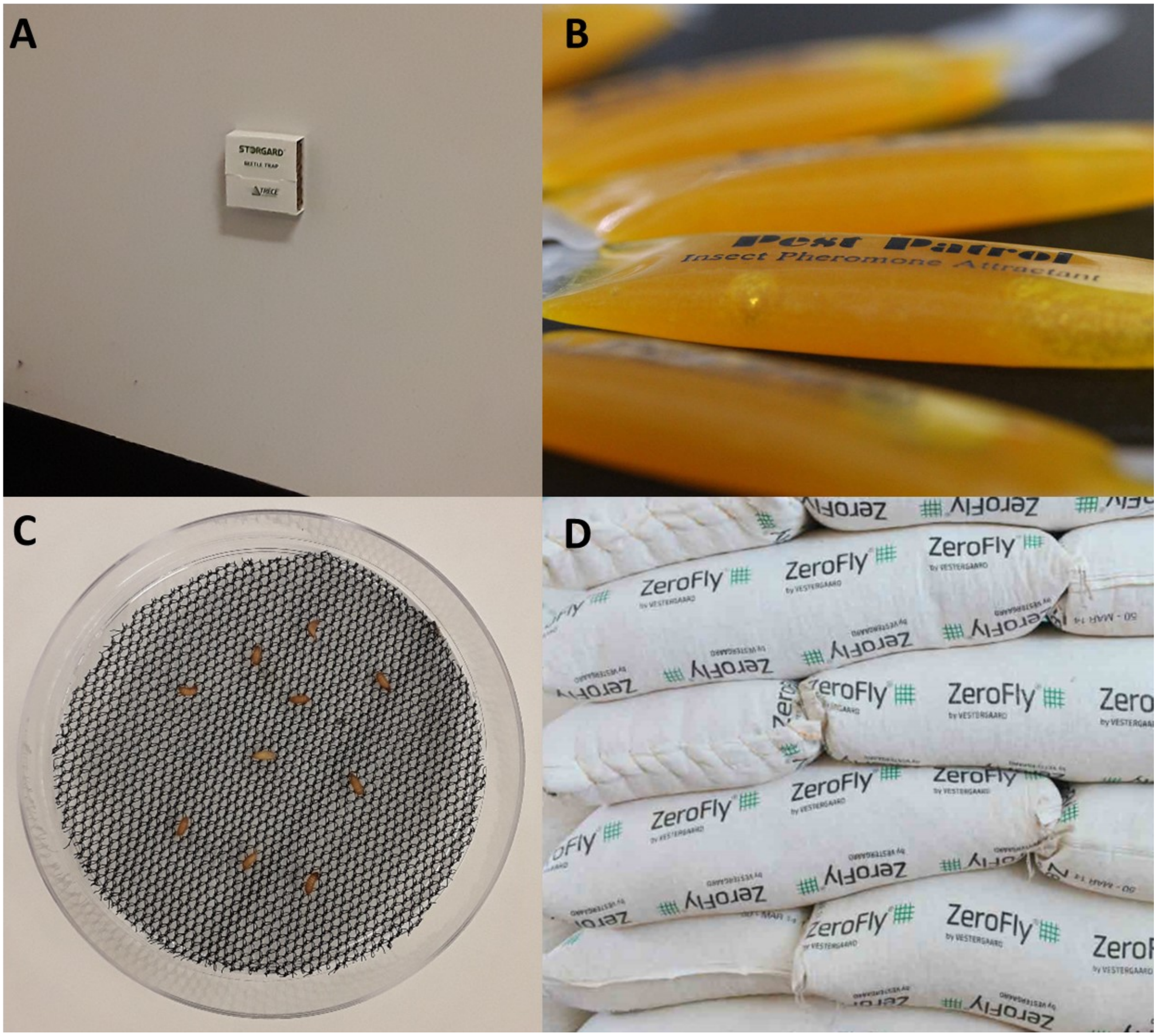

Figure 2. Tools used for khapra beetle management, including the APHIS program wall trap (A), Insects Limited Pantry Patrol Gel (B), long-lasting insecticide-treated netting (C), and Zerofly™ storage bags (D).

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Use of ground-based insecticide treatments for spotted lanternfly management

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Introduction

Spotted lanternfly, SLF, *Lycorma delicatula*, continues to spread into new areas since its first detection in eastern Berks County, Pennsylvania in 2014. Five states south and east of Pennsylvania declared SLF infestations by the end of the 2019 field season and during the 2020 field season, five additional states announced either the identification of established SLF populations (Connecticut, New York, Ohio) or new SLF detections (Maine, Massachusetts). The transportation of goods may pose a pathway for the human mediated transport of SLF. Additionally, the abundance of tree-of-heaven, *Ailanthus altissima*, the preferred SLF host plant, along transportation lines increases the risk that SLF can be transported out of the currently infested area and into new areas.

In order to mitigate the risk of SLF being transported out of an infested area via transportation routes, PPQ Field Operations staff will be spraying a contact insecticide along forested edges where SLF are present and when there is a risk of movement onto conveyances. To support this effort, a pilot study was developed to identify the best methods for treatment applications and for determining the impact of the treatments on SLF populations.

Methods

An arborist was hired to perform insecticide applications. A synthetic pyrethrin (bifenthrin, Avalon Insecticide) was applied at the maximum labeled rate (1 oz/1,000 ft^2) along a forest boundary using an industry-standard hydraulic spray rig fitted with a JD9 nozzle and 200 psi of hose pressure that yielded a spray height of up to 30-35 feet. Four applications were made to two treatment plots between late July to late September at three-week intervals.

Two locations in Pennsylvania were selected for the study and paired treatment and control plots were established at each. Plot requirements included the presence of the host tree, a road or boundary edge where the spray could be applied, and access to the site. To gauge population changes within the plots, six SLF circle traps [1] were deployed on *Ailanthus* (or cherry and black locust when not available) along the plot edge and extending into the plots (Figure 1). Trap bags were replaced after being deployed for one week for a pre-treatment population estimate and then again at three-week intervals for a total of five collections.

For mortality assessments of SLF, several ground collection methods were set up on the forest floor in a grid pattern within the plots. Twenty-five gallon watering tubs and survey stakes (nine of each per plot) were set up on the ground and prior to the second treatment, 2’x3’ tarps were added along the treatment edge. Circle trap bags were replaced every three weeks and all traps and monitoring points were checked

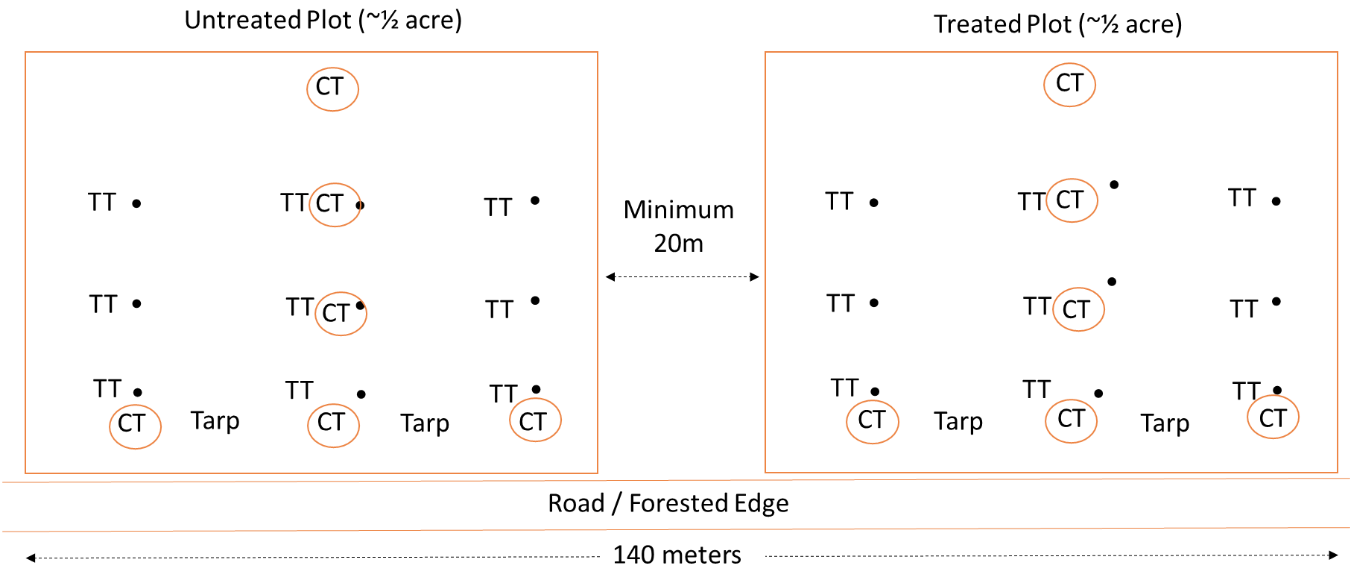

Figure 1. Plot layout and configuration of SLF monitoring tools. Tree traps: CT = Circle Trap (on tree trunk). Ground-based assessments: TT = Tub Trap, • = Survey Point, Tarp = 2x3ft.

and reset before each treatment. One to two additional checks of ground mortality were conducted between treatments. Non-SLF by-catch were placed in alcohol and identified to the Family level (hexapoda) or Order (other arthropods). The configuration of the field plots is noted in Figure 1.

Results

Circle trap data varied by site, trap location (edge vs. interior placement), and between treated and control plots. Variation between the two sites is to be expected, but catch averaged highest from the traps located on the edge versus the interior for all dates in both treated plots; however, none of the t-tests were significant due to the small sample size coupled with the highly variable trap data. Overall, the average number of SLF captured in circle traps within the treated plots was lower for all dates following the initial treatment, as compared to catch data in the control plots. A high amount of variability was present in this data comparison and none of the t-tests of the log transformed data were significantly different.

Comparison of the three methods used to assess SLF mortality on the ground indicate that the survey point method was superior for both treatment plots (ANOVA log of data; $F_{2,41} = 4.5$ and 33.9, $p < 0.02$ and $p < 0.001$). While there were only two tarps placed per plot, tarps were successful in collecting both SLF and non-target arthropods. At one of the sites, the average dead SLF found on the tarps did not differ from the survey point average.

Non-target insects and arthropods killed by the insecticide treatment (by-catch) were collected and set aside for later identification. There were 15 collection dates during the three months of this study and over 1,200 specimens were identified from the plots at one of the study locations (Appendix Tables 1 and 2). The abundance and number of non-target specimens collected correlated to the mortality numbers observed for SLF and a wide variety of insect Orders and Families as well as non-insect arthropods are represented in the Tables. The number of by-catch specimens gathered at the Neshaminy High School treatment plot (n=294) was much lower than by-catch collected at the other treatment plot at Lebanon Valley College (n=1,227). Differences in site characteristics may be one of the reasons; the site at the high school was next to a large manicured grass sports field whereas the site at the college butted up to a cornfield and in general there were more insects present at the site.

A significant portion of the by-catch specimens observed at the college treatment plot (30.2%) were gathered by examining and collecting individuals on the dirt-packed access road in

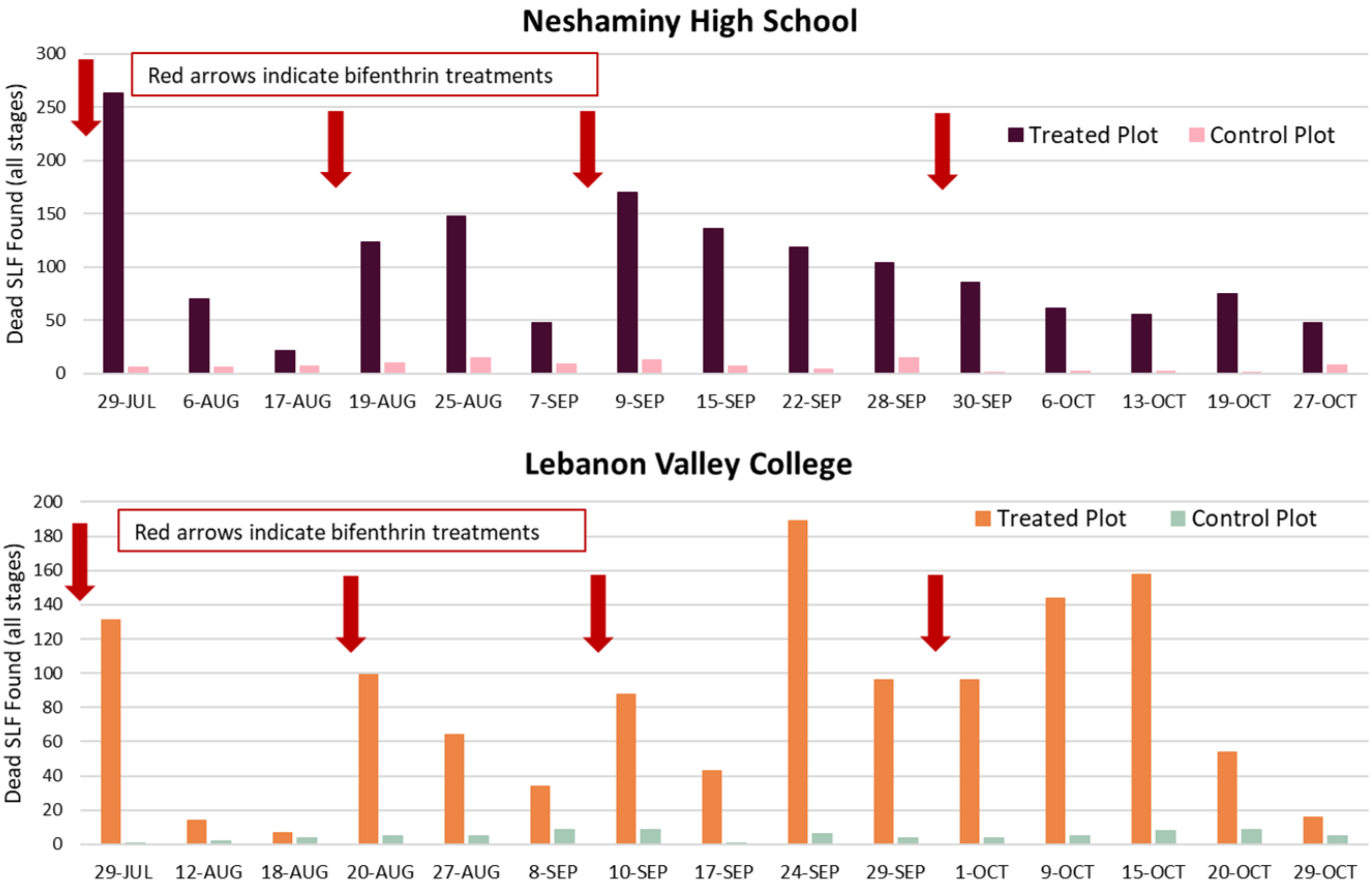

Figure 2. SLF mortality observed on the forest floor following bifenthrin treatments in two locations.

front of the plot. Half of this road was underneath the canopy of the first row of trees and the light color of the road's substrate made it easy to spot dead and moribund insects and other arthropods. The same "road-sweeping" technique was used to collect by-catch from the front of the nearby control plot at the college and resulted in finding a total of six individuals throughout the whole study. At the high school the presence of a fence and taller grass behind a guard rail before the sports field made sweeping the front of the plot for by-catch extremely difficult and was not productive.

Treated and control plots were separated by at least 100 meters, so mortality seen in the control plots is likely due to natural factors. There is a spike in SLF mortality immediately following the insecticide treatments and subsequent assessments found declining levels of mortality until the next spray (Figure 2, red arrows). Following the third and fourth treatments, however, SLF mortality seems to be more sustained and greater numbers of dead insects continued to be collected more than three weeks post-application.

Discussion

This study evaluated the application of a ground-based insecticide spray for control and suppression of spotted lanternfly populations. Additionally, information was collected on several methods employed to determine the impacts of the treatment on SLF populations. It was expected that data from the circle traps could be used to gauge SLF population levels during the study, but a combination of factors including a long sample period (three weeks), small sample size (six traps per plot), and the random but preferential selection and/or clustering behavior of this insect did not make this possible. Circle traps along the edge of the treatment plots ended up recording higher numbers of SLF than traps located in the interior and furthest away from the sprays. Highly variable data prevented statistical separation between treated and control plots. Migration of SLF into the treated areas likely kept treatment plot trap data elevated.

Survey points and tarps were easy to deploy and were useful in assessing SLF mortality following treatments. The watering tubs are expensive and bulky and did not present any advantage in this study as a sampling tool. The treatments impacted a significant breadth and number of arthropod specimens, which is a concern if more than just the edge of the forest boundary is being treated during SLF suppression activities. If a significant stretch of a rail line or an extensive length of property is to be treated, it would be advisable to set aside buffer zones at regular intervals where sprays are not applied. The use of buffer zones has been shown to assist in the reestablishment and recovery of arthropod communities when broad-spectrum pesticides need to be applied [2].

The insecticide sprays killed many SLF throughout the treated plots. A decline in mortality was noted over time following the first two applications, but subsequent treatments seemed to have had a cumulative and longer-lasting effect; a larger number of dead SLF was observed for longer periods of time in the treated plots. Circle trap and observational data indicate that SLF were actively migrating into the treated plots over time, especially during the adult stage in late August to early September. To better assess the impact of these types of treatments on SLF populations it is important to regularly conduct intensive visual surveys within treated plots to better gauge if sufficient control is being achieved. Conducting such surveys will help in determining whether re-treatment is warranted.

Conclusions

This project evaluated efficacy of an SLF insecticide treatment applied along the edge of forested plots like that being employed by PPQ Field Operations. The maximum labeled rate of bifenthrin is recommended for treatment longevity. An increase in SLF mortality was observed with repeat applications. Circle trap data were not useful in characterizing SLF populations in this study due to SLF clustering behavior and a three-week sampling period. Survey points and tarps are easily deployed and should be used when tracking SLF mortality following treatments. Intensive visual surveys are necessary to determine if re-treatment of plots is needed. There were negative impacts on non-target arthropods as would be expected with the use of a broad-spectrum pesticide. Extending applications beyond just the forested edge would likely have deleterious impacts on the arthropod community and greatly hinder the recovery of these communities following treatments. Untreated buffer zones are advised when large contiguous areas are to be treated. Evaluation of treatment methods and determination of best practices presented in this study will inform future SLF control efforts and treatment monitoring activities.

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Detecting new spotted lanternfly populations with sentinel trees and season-long catch comparison of sentinel trees and circle traps

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Introduction

The infestation of spotted lanternfly, *Lycorma delicatula*, has continued to spread since it was first detected in late 2014 in eastern Berks County (northwest of Philadelphia). This invasive pest is now established throughout southeastern Pennsylvania, as well as multiple counties in New Jersey, Delaware, Virginia, and Maryland that either border or are associated with the Pennsylvania infestation. There is great potential for this insect to spread beyond its current boundaries. During the 2020 field season three additional states announced the discovery of established SLF populations (Connecticut, New York, and Ohio) and two additional states reported positive identifications of SLF (Maine and Massachusetts).

The preferred SLF host is 'tree-of-heaven', *Ailanthus altissima*, which is found in disturbed sites and is common along rail lines, roadways, and median strips in areas where SLF is currently found. By treating select host trees with an effective systemic insecticide and placing a collection tub around the trunk, dead SLF can be collected. The treatments result in season-long toxic sentinel trees that can potentially be used as an early detection tool [1]. Key transportation routes, railways, truck stops, rest areas, and state DOT facilities are all ideal locations in which to set up sentinel trees. Other areas of concern (vineyards and nursery industry) can also be readily monitored using this technique. This report will detail the results of a three-year, area-wide effort to implement and utilize sentinel trees to detect SLF in high-risk areas nearest to the known range of the insect. Additionally, we report on a season-long trap catch comparison of SLF between sentinel trees and the most widely used SLF detection trap, the circle trap.

Sentinel trees for detecting SLF populations

The protocol for setting up a sentinel tree was reported previously, additional details can be found in the 2018 Otis Annual Report (pgg 33-34) [1]. Establishment of a sentinel tree is simple and consists of a bark spray treatment of the systemic insecticide dinotefuran and a collection tub placed around the trunk at the base of the tree. Data on trap catch results were reported by all cooperators via the PPQ SLF Otis Insecticide Trapping 2020 Collector Map, an ArcGIS tool that was developed by S&T in collaboration with PPQ ArcGIS specialist. The tool is available to state cooperators and PPQ staff via an Apple device using the ArcGIS Collector application.

States that participated in this trapping method between 2018 and 2020 included Pennsylvania, New Jersey, Virginia, West Virginia, Maryland, Delaware, New York, Ohio, Connecticut, and Massachusetts. Servicing of sentinel trees varied by location, but cooperators were encouraged to check them every two to three weeks. Outlined in Table 1 is the deployment and detection information of sentinel trees that were established by state and over time, as this initiative and the SLF range expanded.

For the initial year of this project (2018), traps were placed outside the core infested area of Pennsylvania, along the western edge of the quarantine zone, and north and south of Harrisburg. Along the eastern and southern edge of the quarantine zone, traps were established along shared borders with New Jersey, Delaware, and Maryland (Figure 1).

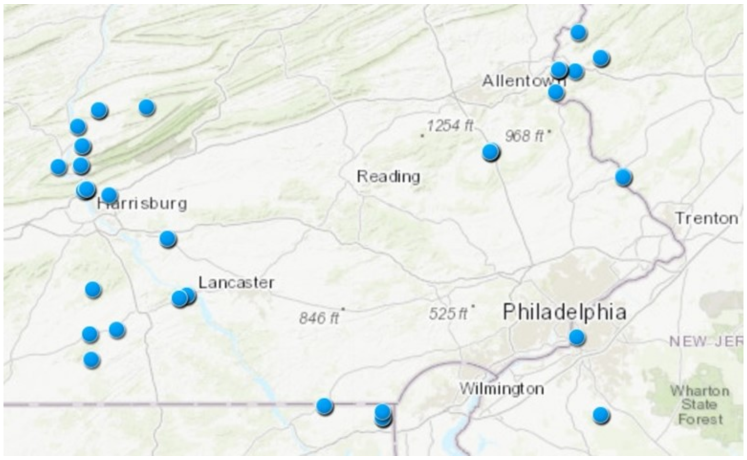

Figure 1. Sentinel tree locations in 2018 that were established along the periphery of the SLF infestation, which is centered in southeastern Pennsylvania.

Traps were also established within the satellite SLF population in Winchester, Virginia (not shown).

As the project evolved and state participation increased there was an additional focus placed on high risk areas. Particularly areas within the predicted spread of SLF from known locations, areas with previous SLF detections, or areas with a

Table 1. Sentinel trees deployed by state along with relevant trap catch and placement information.

		# Traps Deployed / Detections = √ (# SLF)			Trap information / Comments
		2018	2019	2020	
State and Location of Sentinel Trees	Connecticut			7	First deployed following SLF populations discovered in Fairfield County in 2020. Traps deployed in areas of intermodal pathways and around previous SLF incident locations.
	Delaware		7 √ (146)	11	Traps were placed along major transportation corridors and areas of concern.
	Maryland	4	12	8 √ (2)	Traps were placed along major transportation corridors and areas of concern.
	Massachusetts			3	The locations were chosen based on high-risk intermodal pathways in and around the cities of Springfield and Worcester.
	New Jersey	15 √ (11)		5 √ (306)	Traps were placed along major transportation corridors and areas of concern.
	New York			34	Six locations throughout the state at key areas of concern. SLF positives found near Ithaca and Staten Island late in 2020.
	Ohio			16	Five locations throughout the state at key transportation areas. First SLF positive in eastern Ohio found in late 2020 near a rail corridor out of Pittsburgh.
	Pennsylvania	28	27 √ (1)	45 √ (259)	Traps deployed in the vicinity of York and Harrisburg at the leading edge of the SLF infestation. Single positive in 2019 and 15 positive traps in 2020.
	Western Pennsylvania			20 √ (11)	Traps near diffuse SLF populations in the Pittsburgh area that were first identified in late 2019. Railyards and similar areas of concern.
	Virginia	5 √ (25)	4		Traps were placed along major transportation corridors and areas of concern.
	West Virginia		8	8	Traps were placed along major transportation corridors and areas of concern.

high-risk of interstate movement. Trap deployment in 2020 expanded to include six major cities within New York, major cities and high risk intermodal pathways in Ohio, a 50 mile arc from the initial SLF detection in Connecticut, and two major cities in Massachusetts with high intermodal movement along the main interstate highway and rail traffic. In addition, traps were deployed at high-risk areas around waterways and rail traffic in Pittsburgh, Pennsylvania (Figure 2).

In 2018, no SLF were detected in sentinel tree traps in Pennsylvania. During 2019 the main SLF population in Pennsylvania was still mostly to the east of the originally established trapping line around the cities of York and Harrisburg. However, a single positive adult SLF detection was made in one of the sentinel traps located south of York. This new SLF incursion was verified by three additional positive visual survey detections in 2019 that were noted by PPQ field operations staff within the vicinity of the positive sentinel tree. In 2020, SLF continued to expand into York and Harrisburg and there were 15 positive sentinel trees with over 250 SLF captured in the collection tubs (Figure 2, Table 1). The quarantine zone has since been expanded to include these areas and are now considered to be generally infested with SLF.

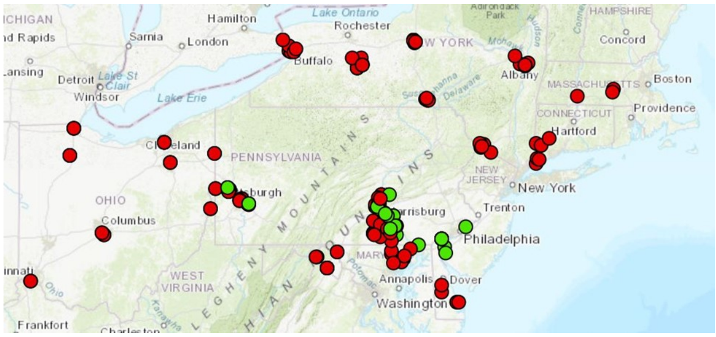

Figure 2. Sentinel tree locations in 2020; green dots denote traps that caught SLF.

Season-long catch comparison between sentinel trees and circle traps

A variety of trapping methods have been employed for SLF that take advantage of its climbing behavior up and down host trees and preferred plants. Sticky bands attached to tree trunks were widely used initially, but by-catch of birds and small mammals became a real concern with this method. One of the most efficient trapping tools and the most widely used for SLF detection is the circle trap, a modification of the pecan weevil trap [2]. The trap consists of an enlarged screen mesh cone that encompasses the tree trunk and funnels the SLF upwards into a one-way collection bag (Figure 3). A side by side comparison of SLF catch using the sentinel tree and circle trap methods was conducted in 2020.

Figure 3. Side-by-side pairing of a circle trap (left) and a sentinel tree at one of the study sites.

For the comparison study, three locations with abundant SLF populations in the vicinity of Allentown, Pennsylvania were chosen. Between four to six pairs of each trap type were established at each study location in late June 2020 on *Ailanthus altissima* trees, for a total of 15 sentinel trees and 15 circle traps (Figure 4). Trees were paired by size and randomly assigned a trap type; average tree diameter (± SD) for the sentinel trees was 7.3 ± 2.2 inches and circle trap trees averaged 7.4 ± 2.5 inches. Trap contents or trap bags were replaced every 2-4 weeks and a total of eight collections were made between early July and mid-November. About 108,000 SLF nymphs and adults were captured and tallied during the study.

SLF catch between the three trapping locations was not significantly different across all SLF life stages and the data were pooled for analysis. The first two collection dates are primarily composed of the black form of the first three nymphal instars while the red 4th instar immatures comprised over a third of the catch in the second collection and made up the majority of the third collection in early August. In the fourth collection, less than 8% of the catch were immature life stages. The vast majority of the final five collections were comprised of adult SLF (Figure 5, text boxes).

Both sentinel trees and circle traps are effective in capturing SLF, however there were three collection dates where the circle traps caught significantly more insects than those caught by the sentinel trees (Figure 5). The first difference was noted on the second collection date when the final SLF nymphal stage first appears in significant numbers. Circle

Figure 4. Sample of adult SLF catch by sentinel tree and circle traps (August 2020).

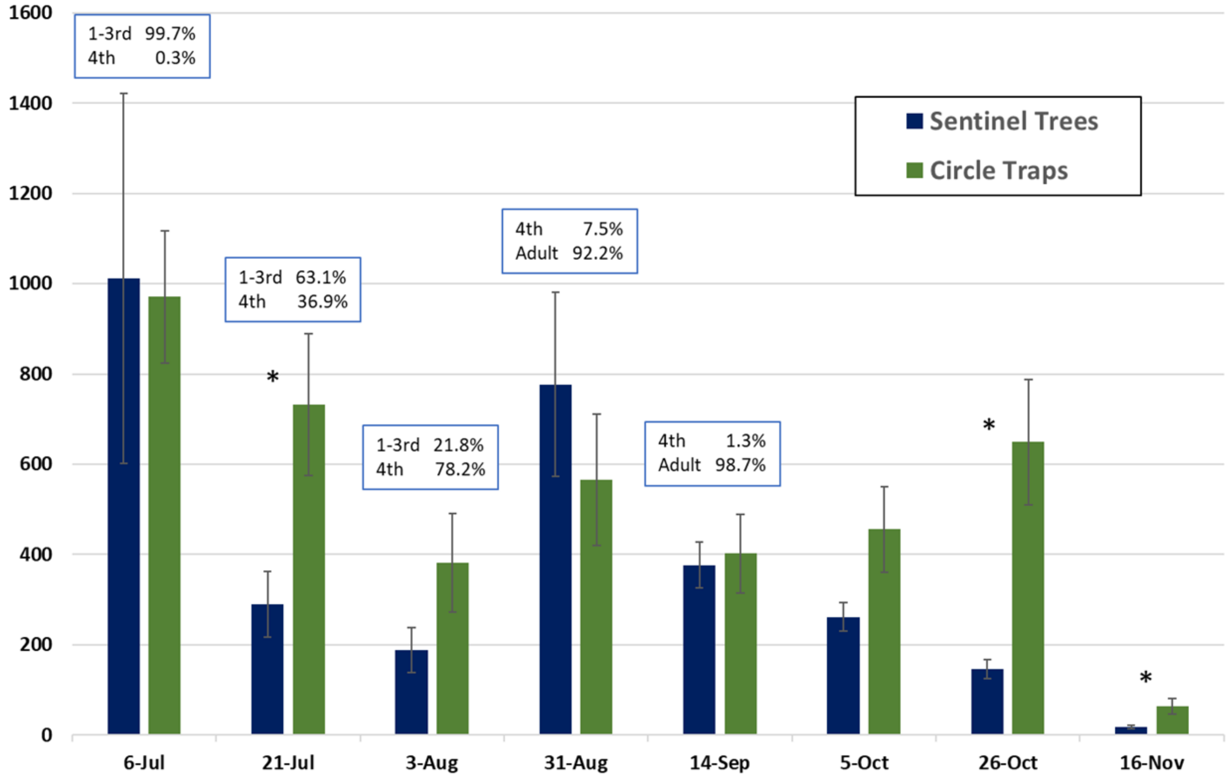

Figure 5. Trap catch by sentinel trees and circle traps of SLF nymphal and adult life stages during the summer (n=15). Significant differences between the traps on three collection dates are noted by an asterisk (t-test, log transformed data; $p < 0.01$).

traps also caught significantly more SLF adults on the final two collection dates in late October and mid-November. Trap catch was minimal for each trap type during the final collection, but circle traps had greater than a 4-fold increase in catch of adults in late October as compared to the sentinel trees.

Discussion

The use of sentinel trees as an SLF monitoring tool for new pest incursions was initiated in 2018 in a few locations around the edges of the main SLF infestation in southeastern Pennsylvania. Sites were selected based on either reports from the public, their close proximity to known infested areas, or additional circumstances that made them an area of concern. For example, West Virginia deployed sentinel trees along highways and state parks where there is a high risk of SLF being transported by the trucking industry, businesses, and people. West Virginia prioritized areas originating or associated with the nearby SLF infestation in Winchester, Virginia. Over time, participation expanded to include additional states and the number of sentinel trees and their locations were adjusted year to year as data became available and as SLF populations expanded.

The results show that sentinel trees can be a useful tool when surveying for the presence of SLF life stages where they are not known to be present. Delaware and New Jersey used sentinel trees to make decisions on whether to expand known areas of infestation. Alternatively, Virginia deployed trees near a dump site that had previous sightings of SLF and used negative results to help classify those finds as regulatory incidents. The single SLF detection by a sentinel tree in 2019 near the town of York in Pennsylvania was in an area where scattered positive visual detections and reports had been sent to the USDA by the public. Treating select host trees with insecticide to create a sentinel tree can be an effective tool to detect SLF presence or absence with minimal time and effort.

After three years of experience with sentinel trees, results demonstrate that this method is most useful when deployed on *Ailanthus* outside of known infested areas and has utility in capturing SLF when population densities are low. This method can be effective in investigating isolated incidents or detecting SLF range expansion, and is greatly augmented by the availability of a refined digital data collection tool that allows users to establish trees and enter trapping data throughout the field season (PPQ SLF Otis Insecticide Trapping 2020 Collector Map). Compared to intensive visual surveys and the use of sticky bands or circle traps that must be checked and replaced quite often, sentinel trees can greatly reduce the amount of time and personnel needed by an agency as they seek to identify SLF establishment and manage and prepare for an SLF infestation.

The trapping study comparing sentinel trees and circle traps was set up at three unique sites that generated surprisingly similar trap catch amounts. While the locations were within areas considered to have moderate to high levels of SLF, each had distinct characteristics. One site was an *Ailanthus* stand surrounded by a busy suburban shopping plaza and a road, the second was a row of *Ailanthus* between a busy road and a large corn field, and the third was a forested area alongside a water treatment facility along a quiet road with *Ailanthus* peppered throughout.

We determined that sentinel trees and circle traps are good methods for surveillance of SLF populations. However, neither method tallies all the insects present. Circle trap netting is circumvented, or the insects may hop and/or fly away before entering the trap. Individuals feeding on the main trunk and center branches of the toxic sentinel trees are more likely to fall into the tub and be accounted for so that tub contents are only a portion of the actual SLF mortality from a particular tree.

Trapping collections between the two methods revealed a few differences. When the final nymphal stage appeared in the collections, these 4th instars tended to be found in the circle trap collections by a 2:1 margin (second and third dates, Figure 5). Circle traps caught significantly more SLF than sentinel trees for the second collection. It is possible that behavioral differences in the 4th instar could have driven this result as has been noted previously in mark/release studies and preference by this SLF life stage for *Ailanthus* trees [3,4]. During peak adult activity there were no differences in trap catch between the two types of traps. For the final two collections, circle traps did catch significantly more SLF than sentinel tree tub collections. At this point in the field season (October and November) there are no dispersal flights, mating is complete, and egg laying and SLF populations are in decline.

Behavioral changes in late-season adults may have contributed to this difference which include a decline in adult flight activity and an increase in climbing behaviors due to their large body mass. The later may also impact SLF sensitivity to the insecticide (Lewis, unpublished data) and may be coupled by declining levels of insecticide in treated trees by October. It is also possible that late-season adults feed more sporadically and in lesser amounts compared to newly emerged adults. This would lead to sub-lethal doses of insecticide and a decline in catch by sentinel trees as the insects are not being impacted and/or are more capable of escape as their movements are only uncoordinated and not catatonic.

Conclusions

The use of sentinel trees (insecticide-treated *Ailanthus* with a collection tub at the base of the tree) is a valuable tool that can be used for season-long detection of SLF populations in new areas and when personnel resources are limited. A digital data collection tool is available and in 2020 sentinel trees were deployed at about 150 locations in 10 states. Results from this year confirm that these traps capture SLF at low population densities. The digital tool and trapping methodology are available to state and federal cooperators who have an interest in detecting SLF in areas of concern.

Sentinel trees and circle traps were compared for their ability to catch spotted lanternfly life stages throughout a field season. Trap contents and bags were collected every three weeks or so between early July and mid-November. Catch of adult SLF was not impacted by trap type during peak adult activity, although late in the season more SLF were captured in circle traps when trap catch was at the lowest level. Either trap is an effective tool that can be used to monitor SLF when there are moderate to high SLF populations.

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Confirmation of sea buckthorn as a host of Asian longhorned beetle

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Introduction

The Asian longhorned beetle, ALB, *Anoplophora glabripennis*, has been one of the most destructive invasive pests since its discovery in the U.S. in 1998. Currently, there are still limited infestations in several U.S. states. Current management measures include surveying trees to identify infestations and removing infested trees in ALB quarantine areas. Defining the ALB host range and excluding non-hosts is important for ensuring the ALB Program can amplify existing survey and management efforts.

The sea buckthorn, *Hippophae rhamnoides* (family: Elaeagnaceae), is a plant used primarily for soil and wildlife conservation but has also been used as food, traditional medicine, and a nutritional supplement [1]. Sea buckthorn has previously been listed as a potential ALB host [2]; however, there are no other references in the literature to support this assertion. Therefore, field caging studies were conducted in China to evaluate whether sea buckthorn is a true ALB host. Paired ALB adults (one male and one female) were caged with sea buckthorn trees in a field site in Inner Mongolia in July 2019. Ten paired beetles were caged among ten trees (one pairing per tree). The trees were checked periodically for egg sites, frass, exit holes (Figure 1), and the emergence of adults.

Results

A total of 121 egg sites were found on ten trees 50 days after caging, but only 24 contained active egg sites (determined by the presence of frass). Three months after caging, frass was seen at 51 egg sites, indicating approximately 42% of eggs hatched into larvae. Adult emergence from the caged section of trees began in July 2020, approximately one year after the cages were set up. A total of 17 ALB adults emerged from July to September 2020. Notably, all the offspring beetles were smaller in size than the caged parents. Additionally, male adults emerged earlier than females. Six pairs of the offspring beetles were caged with six trees of sea-buckthorn in August 2020. When checked in October 2020, four of the six trees had egg sites, but not all egg sites appeared to contain any eggs. The trees will be checked again in 2021 to determine whether it will take two years for a proportion of beetles to complete development.

Conclusion

The sea buckthorn is native to the cold-temperate regions of Asia and Europe and has been introduced in several Canadian provinces. In the U.S., sea buckthorn is found in botanical gardens [3] and has been grown for experimental purposes in Nevada, Arizona, and North Dakota [4].

Figure 1. ALB exit holes on sea buckthorn.

ALB completed full development from egg to the adult stage on sea buckthorn. It took approximately one year for most of the beetles to complete development in Inner Mongolia, China, indicating that the sea buckthorn is a suitable host for the beetle, and therefore, should be on the ALB host list. Further evaluation may be necessary to compare feeding preference and suitability to some known ALB preferred species such as red maple, *Acer rubrum*.

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TRAPPING AGM IN JAPAN AND THE REPUBLIC OF KOREA

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

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Introduction

The S&T Forest Pest Methods Laboratory collaborated with the USDA, Asian Gypsy Moth Preclearance and Offshore Mitigation Program to conduct trapping at U.S. military bases in Japan and the Republic of Korea. This second year of trapping was conducted to investigate the duration and intensity of the adult moth flight season for Asian gypsy moth, AGM, (*Lymantria dispar asiatica/japonica*, *L. albescens*, *L. postalba,* and *L. umbrosa)*. The trapping data was collected in support of NAPPO RSPM 33 (Guidelines for Regulating the Movement of Vessels from Areas Infested with the Asian Gypsy Moth) and provided PPQ with valuable information that aids in the determination of relative risk and timing of AGM translocation from these geographic areas. Nine sites were utilized in the study. Four sites were in the Republic of Korea. The five sites in Japan included three on the island of Honshu and two on Okinawa. Limited trapping was also conducted for *Lymantria mathura* and *Lymantria xylina* at some sites. The Forest Pest Methods Laboratory provided scientific guidance, traps, lures, logistics, as well as morphological and molecular diagnostics. Large green delta traps were deployed for all target species in Japan in mid-May. Traps were checked weekly through mid to late August. In the Republic of Korea, green milk carton traps were used for AGM and large green delta traps were used for *L. mathura* and *L. xylina*. Traps were deployed in mid-June and checked weekly through August. Trap deployment and servicing were done by cooperators in the U.S. Army in both countries. Samples were shipped to the Forest Pest Methods Laboratory for enumeration and identification.

Results

During the 2020 trapping season, 8,123 samples were collected. In Okinawa, 41 AGM and 36 *Lymantria xylina* were caught; several moths were caught when the traps were first checked on May 29th (Figure 1). In Honshu, 307 AGM and two *Lymantria mathura* were caught. In the Republic of Korea, 7,737 AGM specimens were captured, but no *Lymantria xylina* or *Lymantria mathura* were caught (Figure 2). Molecular analysis of a small subset (68) of the samples received indicated only one species was present in each location; *Lymantria albescens* was present in Okinawa, *Lymantria dispar japonica* in Honshu, and *Lymantria dispar asiatica* in the Republic of Korea. Molecular analysis also confirmed the morphological identification of *L. xylina* (Okinawa) and *L. mathura* (Honshu) and provided identification of incidental catch of *Asota ficus*, *Athetis reclusa*, *Nodaria cornicalis*, *Chrysorithrum amatum,* and *Hyphantria cunea*.

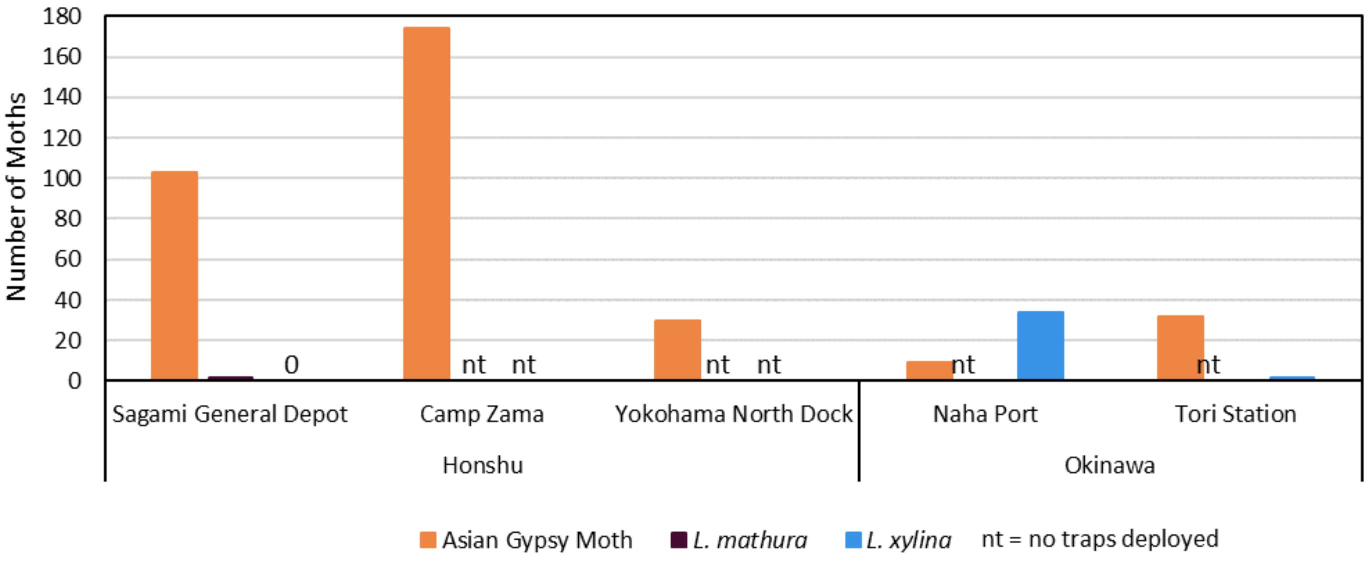

Figure 1. Asian gypsy moth, *L. mathura, and L. xylina* 2020 trapping results at U.S. military bases in Japan.

Conclusions

During the 2020 trapping season, trap catch of AGM at U.S. Military bases in the Republic of Korea were higher than in Japan. Population levels of *L. xylina* and *L. mathura* were at very low levels in Japan, and non-detectable in the Republic of Korea based on our results. In Okinawa, male moths of *L. albescens* were first captured on May 29, 2020, when the traps were first checked. Moth flight may have begun earlier as the traps were deployed two weeks prior. Future trapping in Okinawa should start earlier and include more frequent trap checks to ensure that the beginning of AGM flight is well documented. This trapping survey provides additional data to help confirm and refine the risk period associated with AGM flight and egg deposition and provides initial data on population levels of *L. xylina* and *L. mathura.*

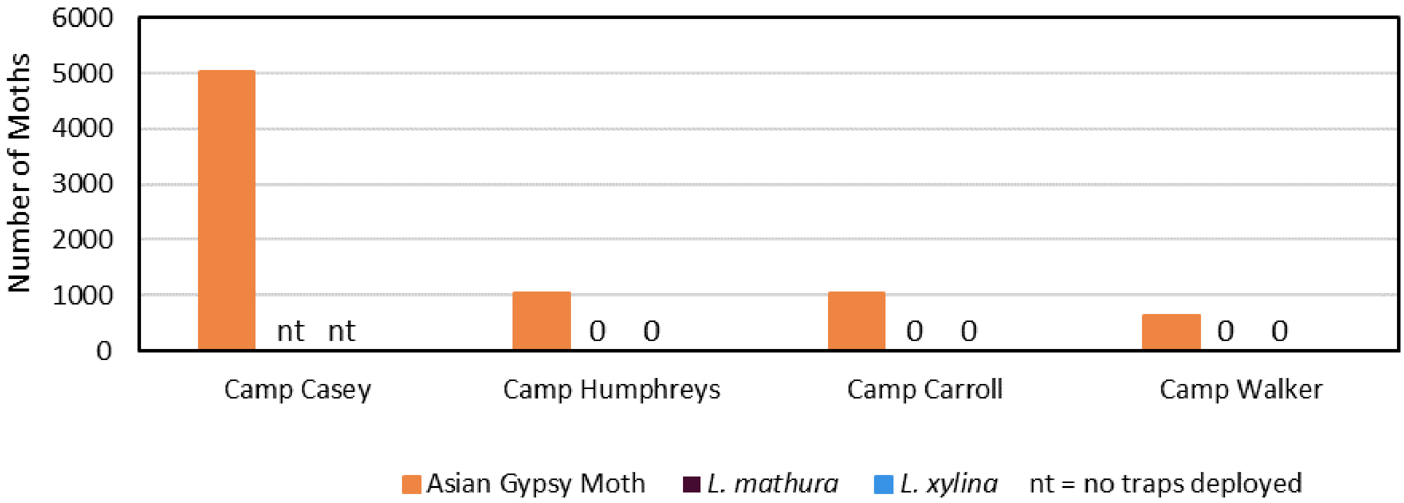

Figure 2. Asian gypsy moth, *L. mathura, and L. xylina* 2020 trapping results at U.S. military bases in the Republic of Korea.

Advances in mass rearing emerald ash borer: containerization and infestation

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Introduction

Mass rearing and release of parasitoids against emerald ash borer, EAB, *Agrilus planipennis*, is the most feasible strategy for controlling the invasive ash tree pest in the United States. However, parasitoid production is limited by reliance on ash tree bolts to rear host larvae. An artificial diet for EAB larvae was developed to replace host plant material (Keena et al. 2015), and attempts are still underway to improve EAB rearing performance by modifying the diet ingredients and texture.

In 2020 we focused on developing another aspect of mass rearing, a system that includes a rearing container for the unique needs of EAB larvae and their parasitoids, and a method to introduce EAB larvae into the diet efficiently. An ideal mass-rearing container must allow EAB larvae to make feeding galleries so that they can fully develop and enable the parasitoid wasps to drill through the diet to lay their eggs in the EAB larvae. The rearing container must also allow rearing staff to monitor the condition of the diet and insects periodically. This report details a prototype multi-larva rearing container that holds a thin sheet of diet and a method to introduce EAB eggs onto the diet. Studies were conducted at the EAB Biocontrol Rearing Facility in Brighton, MI. All rearing was done in an environmental chamber at 25 ± 2°C, 65 ± 5% relative humidity, and under 16 hours of light and 8 hours of dark per day.

Containers

The natural behavior of EAB larvae is to feed in serpentine galleries within the thin layer of inner ash bark (phloem). EAB parasitoids are adapted to locating and laying eggs in nearly fully grown EAB larvae buried within reach of their ovipositors; the hair-like ovipositors can reach about 2 – 2.75 mm into the diet. Based on these observations, we designed a prototype rearing unit composed of a pair of stiff transparent acrylic sheets that sandwiches a thin layer of leathery diet (Figure 1A). The rearing container is wrapped in plastic film to bind the unit together and retain moisture (Figure 1B). We utilized two sizes of experimental units, 20 x 25 cm and 13 x 20 cm, but the final dimensions for a production container are to be determined. The containers are held upright in wire office-file holders in an environmental chamber during larval development (Figure 1C).

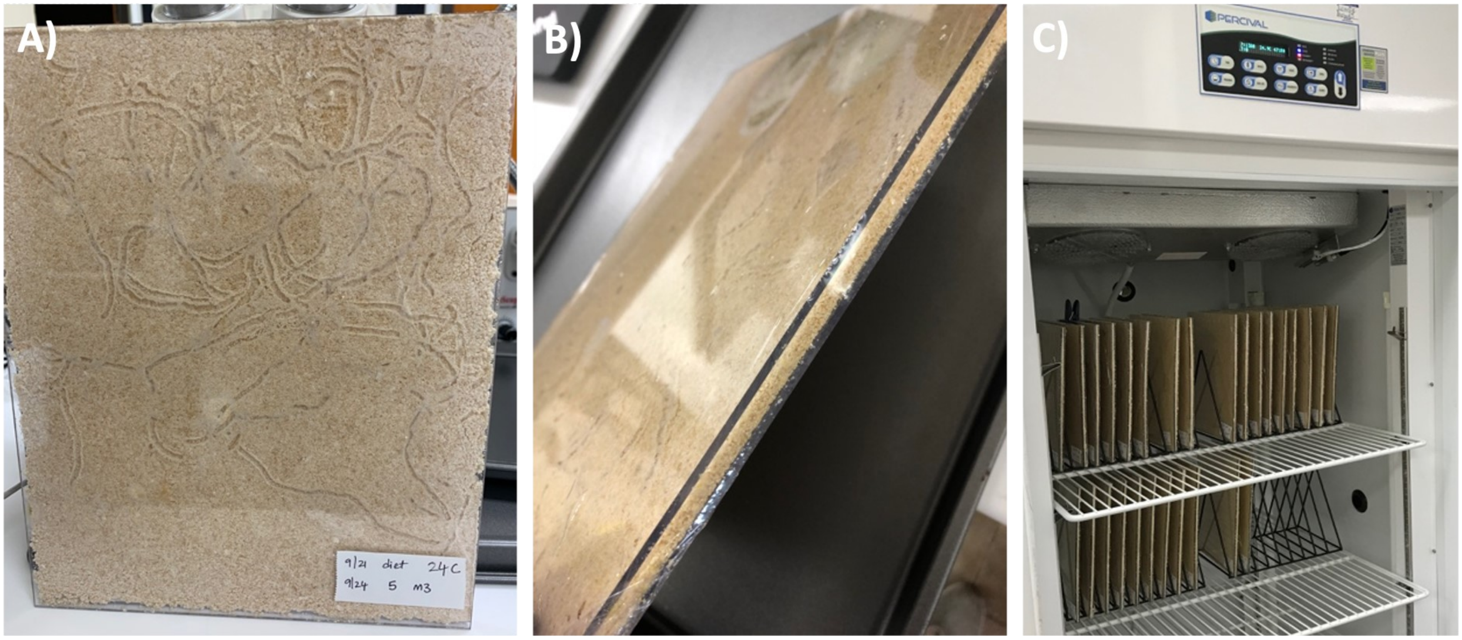

Figure 1. Prototype rearing unit composed of a thin layer of cohesive diet between a pair of stiff transparent acrylic sheets. A) Larval galleries in the diet formed by larvae infested vertically at the top. B) Side view of rearing unit with 3 mm-thick diet wrapped with plastic film. C) Rearing units held upright in wire office-file holders in an environmental growth chamber.

Cleaning of the reusable acrylic sheets can be achieved by dipping or spraying disinfectant. Staff can monitor the condition of the diet and insects through the transparent acrylic sheets. In 2020 we determined that diet sheets should be 3 mm thick based on EAB gallery measurements in bolts and the length of the parasitoids' ovipositors. This thickness should support EAB larval development to near maturity without hindering parasitoids' ability to parasitize them.

Diet infestation

Mass production requires an efficient method for infesting the rearing medium with multiple same-aged groups of insects. After a preliminary study, the egg stage was found to be more practical for introducing EAB into an artificial diet than young larvae. However, successful infestation has been limited because moisture is drawn onto eggs from the surroundings and tends to drown eggs in contact with diet. Current rearing practice relies on eggs laid on coffee filters, which wicks moisture from the diet onto the eggs. Earlier work by Allen Cohen (North Carolina State University, Raleigh, unpublished) suggested that a wax paper barrier placed between EAB eggs and the surface of the diet prevents drowning without affecting the larva’s ability to burrow into the diet.

We tested the efficacy of a wax paper moisture barrier to promote egg hatch on diet containing 50% moisture, which is preferred by EAB, and on diets with 45% and 60% moisture levels. We compared the percentage of eggs that hatched when pieces of filter paper with eggs were placed directly on the diet's surface and when they were placed on top of a strip of soy-wax-paper laid over the diet (Figure 2).

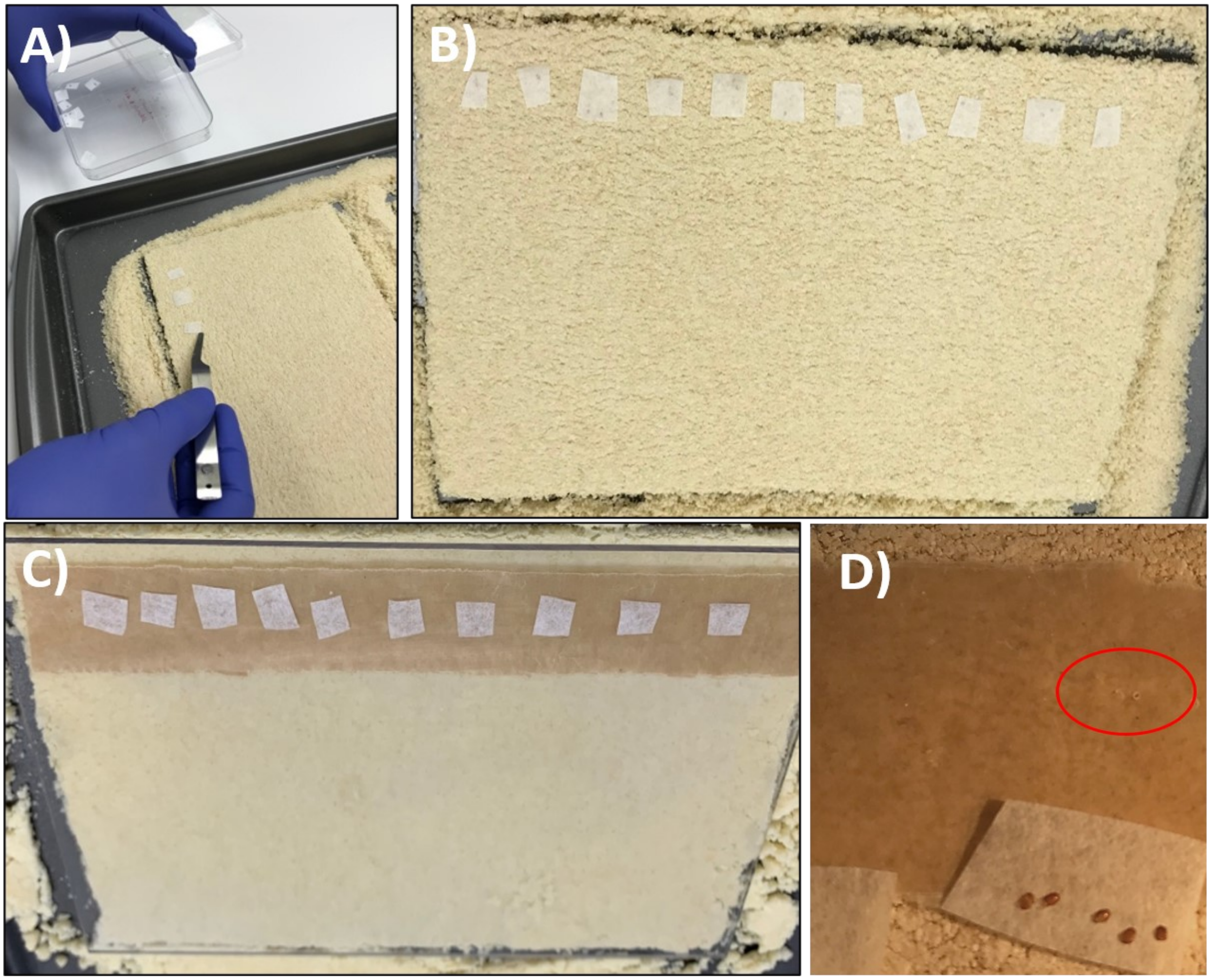

Figure 2. EAB egg infestation test using a moisture barrier to prevent eggs from drowning. A) & B) Pieces of coffee filter with eggs directly on diet. C) Brown soy-wax paper barrier between eggs and diet. D) Hatched eggs on filter paper moved aside to reveal minute holes (within the red oval) chewed by larvae through the wax paper.

The containers were wrapped in plastic film and incubated for at least six days in the environmental chamber. To examine whether the wax paper barrier hindered the entry of larvae into the diet, we counted the number of holes made by the larvae as they burrowed into the diet. We assumed that each hole represented the entry of one larva into the diet. We expected egg hatch to be higher when the barrier was employed, regardless of diet moisture content, and that when no barrier was used, a greater percentage of eggs would die on the moister diets.

The results for egg hatch and larval entry into the diet were mostly as expected. The moisture barrier promoted a higher egg hatch than no barrier in diets containing 45% and 60% moisture but had no effect on the intermediate diet (Figure 3); hatch on diet with 50% moisture without a barrier was higher than we predicted.

As expected, on the wettest diet, the hatch rate was low when the barrier was absent. The percentage of larvae that entered the diet was unaffected by a wax paper barrier. On the whole, the results indicate that a wax paper barrier can enable eggs laid on a coffee filter (the standard egg-harvesting technique used in EAB production) to be integrated into a mass-infestation protocol on artificial diet with minimal death due to drowning, even up to 60% moisture in the diet.

Conclusions

Major contributions to developing an EAB mass-production system in 2020 included a container system with features beneficial to rearing multiple EAB larvae to later instars and monitoring the rearing progress. Container dimensions are flexible and can be made to fit any specifications. A mass infestation system for EAB on an artificial diet was tested and is expected to enable integration of an existing egg collection method on coffee filters into the system with minimal loss of eggs due to moisture in the diet.

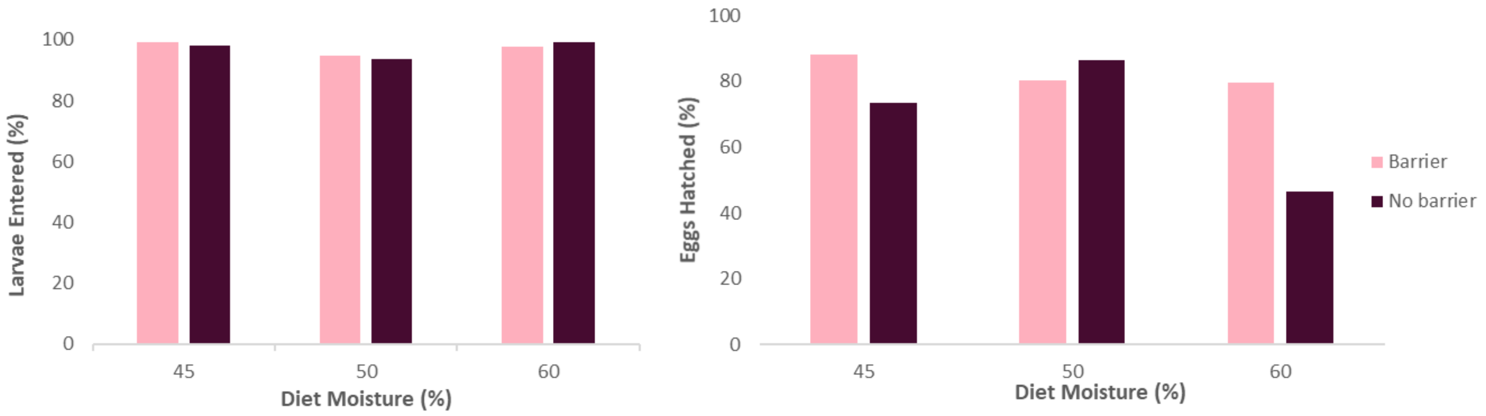

Figure 3. Percentage of EAB eggs that hatched (right) and percentage of hatched larvae that entered the diet (left), with and without a soy-wax paper barrier between the eggs and the diet. Each rearing container was infested with 10, 15, or 20 eggs.

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REARING LONGHORNED BEETLES TO SUPPORT METHODS DEVELOPMENT, RESEARCH, AND OUTREACH

Production of Asian longhorned beetle, citrus longhorned beetle, and velvet longhorned beetle

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Introduction

Three species of longhorned beetles were reared in support of the ALB Program, lure development for PPQ's Cooperative Agricultural Pest Survey (CAPS), risk mapping, and outreach.

Asian longhorned beetle

Colonies of Asian longhorned beetle, ALB, *Anoplophora glabripennis*, originating from New York and mixed U.S. sources, were reared to provided insects in support of PPQ, methods development, outreach (Figure 1), and research conducted by cooperative universities.

Citrus longhorned beetle

Colonies of citrus longhorned beetle, CLB, *Anoplophora chinensis,* originating from Italy and China, were established in 2020 to support lure development for CAPS. The development of rearing methods is in progress. Gauze-wrapped cardboard blocks used as oviposition substrates by ALB were deemed unsuitable for CLB. However, both striped and red maple wood bolts are successful egg substrates, with no notable wood species preference by CLB. Young larvae were reared within wood bolts until frass was observed, after which they were transferred to an artificial diet similar to ALB diet. Larvae were reared in Petri dishes and plastic cups, following methods used for ALB rearing. To date, 159 larvae were reared to two months of age, 102 survived 11 weeks of chill at 10°C, 12 pupated, and seven adults emerged. Two mating pairs have begun oviposition for the next generation.

Velvet longhorned beetle

Rearing methods for velvet longhorned beetle, VLB, *Trichoferus campestris,* are being developed to support host-range testing and refine risk mapping for this species in the United States. The beetle is frequently found in wood packaging material accompanying trade goods from China and appears to be established in at least four U.S. states. Studies conducted at the Forest Pest Methods Laboratory indicated that winter chill is required for pupation. The colony originating from China is in its 5th generation and is being utilized in studies to determine the winter chill requirement for life-cycle completion.

Pupation rates were similar across 5, 10, and 15°C chill temperatures and 3- and 4-month chill duration treatments, but larvae chilled at 15°C for four months had the highest (82%) pupation success. The study continues with the addition of a -20°C treatment; 52 larvae entered chill at about 150 days post-hatch, along with 10 larvae entering their second year in chill and one larva entering its third year. Results will enable us to expand the colony and refine projections of the beetle's host range and pest potential.

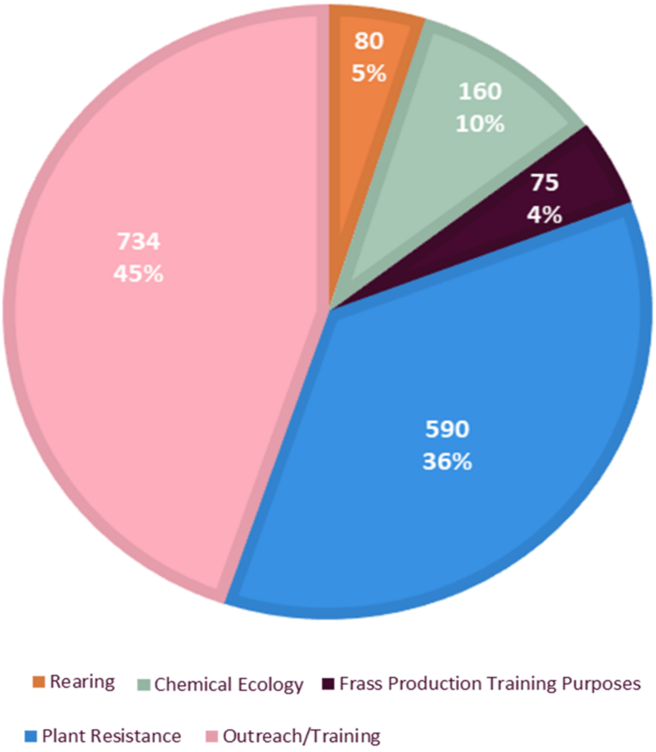

Figure 1. The number of Asian longhorned beetle specimens provided for research and outreach in 2020.

Production of invasive moths

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European gypsy moth

European gypsy moth, EGM, *Lymantria dispar dispar,* is a significant forest pest that was introduced into the northeastern United States in the late 1860s. The Forest Pest Methods Laboratory has reared gypsy moth for decades in support of the European Gypsy Moth Program's goals to define the extent of the gypsy moth infestation, eradicate isolated populations, and limit the artificial spread of gypsy moth beyond the infested area. Over 80,000 male pupae were reared for projects affiliated with the Gypsy Moth Slow the Spread Foundation to conduct research aimed at limiting its spread and testing the efficacy of mating-disruption products. Egg masses were regularly sent to the USDA Agricultural Research Service Lab in Beltsville, MD to research EGM pathogens. About 300 egg masses and 500 larvae of EGM were distributed to six academic institutions in the U.S. to support education and research on insect pathogens, including Cornell University, University of Chicago, Pennsylvania State University, University of Rhode Island, and the University of Richmond. The Great Lakes Forestry Centre in Ontario, Canada utilized 1,400 egg masses for various research projects. A total of 785 egg masses were provided to the University of Natural Resources and Applied Life Sciences (Vienna, Austria), The Max Planck Institute (Jena, Germany), and the Swiss Federal Institute for Forest, Snow, and Landscape Research (Birmensdorf, Switzerland) for research. Studies were conducted on the responses of EGM to changes in temperature, the resistance of trees to herbivore feeding, and the relationship between the timing of tree leaf flush and EGM populations. EGM specimens were provided to the Gypsy Moth Program for outreach and training programs in the U.S.

Asian gypsy moth

To support the Asian Gypsy Moth Program, six colonies of Asian gypsy moth, AGM, *Lymantria dispar asiatica,* collected from different locations, and one colony each of *L. d. japonica*, and of rosy moth, *Lymantria mathura*, were maintained for use in research, outreach, and identification training. The AGM colonies originated from egg masses collected in China, Mongolia, Russia (two colonies), and the Republic of Korea (two colonies). Each colony is divided into three groups that are hatched from eggs at different times of the year. About 300 larvae are reared in each group, totaling about 900 per colony per year. Important differences between Asian and European subspecies are that AGM has a broader host range and the females are capable of flight, whereas female EGM are generally unable to fly. These traits could allow AGM to spread rapidly into and through uninfested areas. If AGM arrives in an EGM-infested area, the flight genes may be lost over several generations of mating with EGM.

Table 1. EGM and AGM specimens reared in 2020 and provided to U.S. and foreign institutions.

Purpose	Life Stage					
	Egg masses	Larvae	Male Pupae	Female Pupae	Riker Mounts	Specimens*
EGM Colony	10,400					
AGM Colonies	4,500					
Research: IPM development			90,000	50		
Research: GM biocontrol	1,250					
Research: Pathology	1,255	500				
Research: Ecology	290					
Research: Unspecified	1,000					
Research: Pesticide assays	400					
Outreach, training, education	200		100		21	100
Total	**19,295**	**500**	**90,100**	**50**	**21**	**100**

* "Specimens" are insects preserved for outreach displays and as references for identification.

Research planned in 2021 will explore the flight ability question by mating AGM with EGM and tracking female flight ability over several generations, with each generation being mated back to EGM.

Old world bollworm

In addition to Asian gypsy moth colonies, two unrelated moth colonies were maintained in 2020 at the Forest Pest Methods Laboratory Insect Containment Facility: Old world bollworm, OWB, *Helicoverpa armigera,* and European grapevine moth, EGVM, *Lobesia botrana*. The OWB colony has been maintained since 2017 and supported the development of molecular diagnostic and mass-rearing methods. In 2020 weekly production rates were approximately 125-150 individually reared pupae and 9,860 eggs. A total of 50 frozen adult OWB specimens was provided to the S&T Ft. Collins Laboratory molecular group and their cooperators to test a new real-time PCR bulk extraction assay. Additionally, 16 hybrids of OWB and corn earworm, *Helicoverpa zea,* were also provided to the Ft. Collins team to support the development of diagnostic methods for distinguishing between the two species and their hybrids at both the larval and adult stages. Insects produced from the OWB colony were also used in rearing-density studies conducted at the Forest Pest Methods Laboratory, which aim to develop group rearing methods that could be applied to the mass rearing of this cannibalistic species. Baseline data on survival at five densities showed that percent survival to the pupa stage decreased from about 80% for one or five larvae to 12% for 200 larvae per rearing box. These data will be compared with survival in rearing boxes to which various items are added to increase surface area. We hypothesize that items such as plastic honeycomb on the surface of the diet can provide refuges for larvae and decrease encounter rates, resulting in lower rates of killing and cannibalism.

European grapevine moth

The EGVM colony has been maintained since 2010 and has supported PPQ’s Cooperative Agricultural Pest Survey by providing male moths to test trappers’ ability to correctly identify specimens placed in traps. Weekly production rates for 2020 were ~900 adults and ~37,000 eggs per week. Each year, 200 dead, internally marked males are provided to train pest-detection trappers in California. Different traps for several pest species are monitored by cooperative effort among federal, state, and tribal agencies to provide early warning if invasive pests are introduced and require immediate action to eradicate. Trainers secretly plant the EGVM specimens in EGVM pheromone traps to test trapper readiness to distinguish them from other trapped moth species that are not pests. The EGVM has been eradicated from the United States, so wild moths are not available for training. The moths are reared on a red diet that imparts a red color to their internal organs. Trainers squash trapped EGVM to reveal the color and determine whether trapped EGVM are lab-reared specimens used for training or introduced wild moths. Because this moth is easy to rear, the colony is also being retained for use in future equivalency studies on X-ray and cobalt-source radiation, which will provide insight into transitioning to a safer source of radiation for sterile insect production and release.

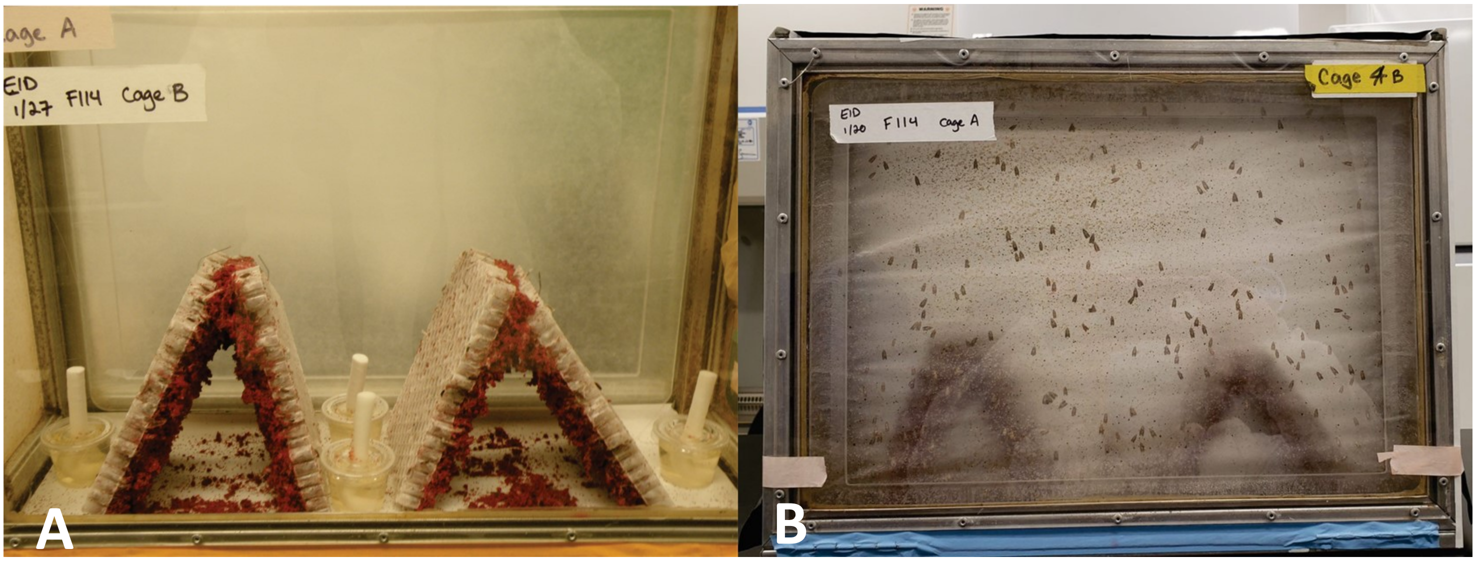

Figure 1. A) EGVM mating and oviposition cage. The inverted V structure is a plastic honeycomb containing pupae and a shredded red diet. Eggs are laid on the removable waxed paper walls of the cage. B) EGVM adults and eggs in a cage, viewed through the waxed-paper oviposition substrate.

A multi-state comparison of detection rates for three spotted lanternfly trapping tools

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Introduction

The spotted lanternfly, SLF, *Lycorma delicatula*, is an invasive, phloem-feeding fulgorid generalist with a high potential for environmental and economic impacts. Improving and developing trapping technologies that are effective at detecting and monitoring SLF populations is essential in supporting the SLF Program's goals. Our goal is to improve trap efficacy by optimizing trap designs to leverage SLF behavior. In 2020, the circle trap, a modified pecan weevil trunk trap fitted with a collection bag, was transferred to the SLF program for operational use. This trap provides a cost-effective, easy-to-deploy, and re-usable alternative to glue-covered sticky bands placed around trunks of host trees.

The key to a successful survey trap is its ability to detect insects at a very low population density. Before 2020, trap comparison assays used to develop new trap types were conducted in medium- and high-density sites in eastern Pennsylvania's generally infested areas. Trapping in high-density sites provides insight into the overall functionality and efficacy of one trap over another, which is important in assessing a trap's potential as a successful monitoring tool. However, high trap catch occurring in areas with relatively high densities provides little insight into the trap's ability to serve as an early detection tool — identify a particular target species when it is only present in low numbers. The present study aims to compare detection rates of three commercially-available traps at sites outside of the generally-infested area.

Methods and results

A field assay, in cooperation with PPQ, state, and university cooperators, was conducted in low-density SLF areas to compare detection rates of three currently available traps: 1) BugBarrier sticky band, 2) circle jar trap, and 3) circle bag trap (Figure 1). Seventy-one replicates of each set of traps were placed in eight states: CT (6), KY (4), MA (4), MD (10), NJ (14), NY (20), OH (3), and WV (10). For each replicate, traps were placed on adjacent or near adjacent *Ailanthus altissima* trees.

Figure 1. Three trap types tested in a multi-state SLF detection tool comparison. A) BugBarrier sticky band. B) Circle trap with a collection jar. C) Circle trap with a collection bag.

Replicates were placed at least 150 m away from each other. Traps were baited with a methyl salicylate lure and were checked every two or three weeks throughout the field season. Of the 71 placed replicates, 20 were found to be positive for SLF.

The circle trap with the bag collection method caught significantly more early instar SLF than both the BugBarrier and the Jar style traps. Circle traps with bags also caught significantly more adult SLF than the BugBarrier traps (Figure 2). Detection rates were not significantly different among any of the traps for any life stage of SLF (Figure 3).

Conclusions and plans for 2021

In terms of the number of SLF caught, the circle trunk bag trap caught more SLF than the other two traps, but detection rates were comparable among all three traps. Based on these results, we would recommend the use of the circle bag trap due to it being relatively inexpensive and easy to deploy. However, BugBarrier traps can still serve as an effective SLF detection tool. Additional trapping assays will be conducted to develop visual attractants and to address operational questions about the traps.

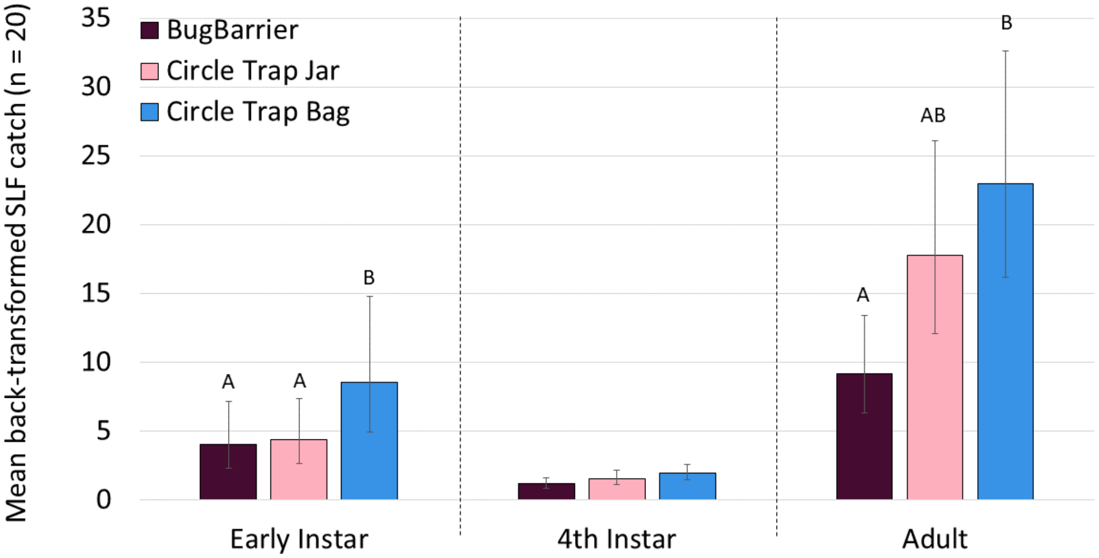

Figure 2. Mean back-transformed catch with 95% confidence intervals (n = 20) of three life stages of SLF on three trap types.

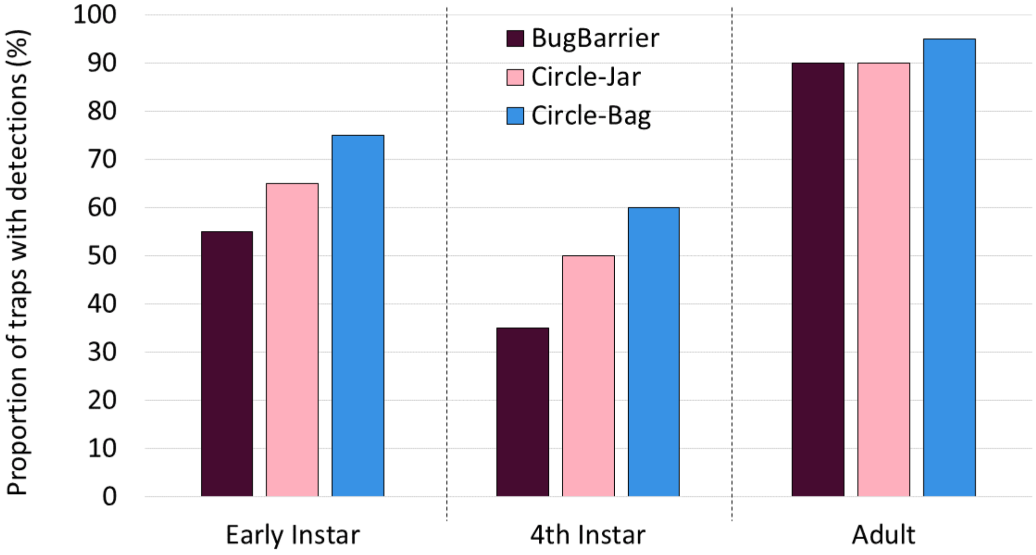

Figure 3. Percentage of traps (n=20) detecting three life stages of SLF on three trap types.

DEVELOPING HOST ODOR ATTRACTANTS FOR JAPANESE ORANGE FLY DETECTION

Host odor attractant for the Japanese orange fly, *Bactrocera tsuneonis*

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Introduction

The Japanese orange fly, JOF, *Bactrocera tsuneonis* is an important pest of citrus in Japan and China (Figure 1). It is distributed in the southern part of Japan including Kyushu Island and the Amami archipelago. Japanese orange fly has also been reported to have infested citrus in the Szechwan and Kweichow Provinces of southwestern China, and likely Taiwan. With increased global trade of citrus from areas infested with *Bactrocera* spp. to different parts of the world, there is a possibility that fresh, infested, citrus fruits from these areas will reach the U.S. market. Currently, there are insufficient data on effective lures and survey tools. The development of early detection tools is essential in ensuring a timely response by PPQ's Fruit Fly Program if Japanese orange fly is detected in the U.S. The present study addresses this lack of knowledge by investigating a host odor attractant. Results of this work demonstrate that the developed host attractant could be effective for the detection of male adult JOF infestations.

In Japan and China, there appears to be only one JOF generation per year. There, adult emergence begins in early June and lasts through July/August. Occasionally, adults may be found as late as October. Oviposition occurs primarily in July and August, and eggs are laid under the rind of immature green fruits.

Figure 1. Adult *Bactrocera tsuneonis*, frontal view.

Typically, a single oviposition puncture is made in a fruit; two to six eggs are deposited in the puncture from which only one larva emerges. Larvae appear in early October and devour the contents of one carpel after another, with two to ten carpels being infested by a single maggot. By early November, the larvae are mature, and about this time the infested fruit drops to the ground. Pupation occurs in the top two inches of soil.

This ongoing work is a collaboration between APHIS PPQ S&T, NSF Center for Integrated Pest Management at North Carolina State University, scientists from the Guangdong Institute of Applied Biological Resources, Hunan Academy of Agricultural Sciences, and Yong Shun County Department of Agriculture in China.

Insects used in experiments

In 2019, the Forest Pest Methods Laboratory received approximately 1,000 JOF pupae that were collected by our collaborators in southern China. These pupae were kept in moist sand and were placed at 25°C until adult flies emerged (after approximately 5 weeks). Emerging virgin flies were sexed and separately placed in groups on dry hydrolyzed yeast mixed with sucrose. Flies on this dry diet had access to a water wick.

Volatile collections, bioassay, chemical identification, and field testing

A vacuum system was used to trap (on Haye-Sep Q polymer) volatiles from green immature (~ 4 cm diam.) sweet orange fruits (*Citrus sinensis*) that were obtained from South Africa. Collected compounds were examined for biological activity by gas chromatographic-coupled-electroantennal detection (GC-EAD) and further analyzed by gas chromatographic-coupled-mass spectroscopy (GC-MS) for chemical identification. Those compounds that showed GC-EAD activity with male and female JOF antennae were identified by GC-MS and authenticated with commercially obtained standards. The antennal active compounds were formulated into polyethylene vials for the slow release of active ingredients to be tested in citrus orchards in southern China.

Field testing comprised of placing pairs of green sticky sphere traps (Figure 2; 20 replicate pairs, baited vs un-baited) 20 meters apart in a commercial sweet orange orchard for three weeks in August 2020 in Xuanwei, Yunnan Province, China. Trapped insects were counted weekly, however, attractants were not replaced during this period.

Figure 2. Baited green sticky sphere trap.

Results

The emerging JOF adults were able to survive at least three weeks on the provided dry diet, a period long enough to work with mature flies for the GC-EAD analysis. The GC-EAD analysis using antennae of male and female flies showed at least seven different compounds that showed antennal activity with both male and female adults. These compounds were identified by GC-MS as d-limonene, (+/-)-linalool, 4-terpineol, α-terpineol, (E)-citral, (Z)-citral, and geranyl acetate. The identified compounds were formulated as neat compounds (0.5 ml/vial total) into micro polyethylene vials, which gave a release rate of 100-150 µg/day at compound ratios that roughly approximated the ratios found in the volatile collections from immature sweet oranges.

The pair-wise field study of baited and un-baited green sticky spheres showed that the seven component host odor bait attracted significant (pair-wise t-test, *p* = 0.0002, n=20) numbers of male flies in China (Figure 3).

Conclusion

From this work we can conclude that male *B. tsuneonis* adults are attracted to a synthetic blend of host odor compounds and that this host odor attractant can be used for the detection of *B. tsuneonis* populations.

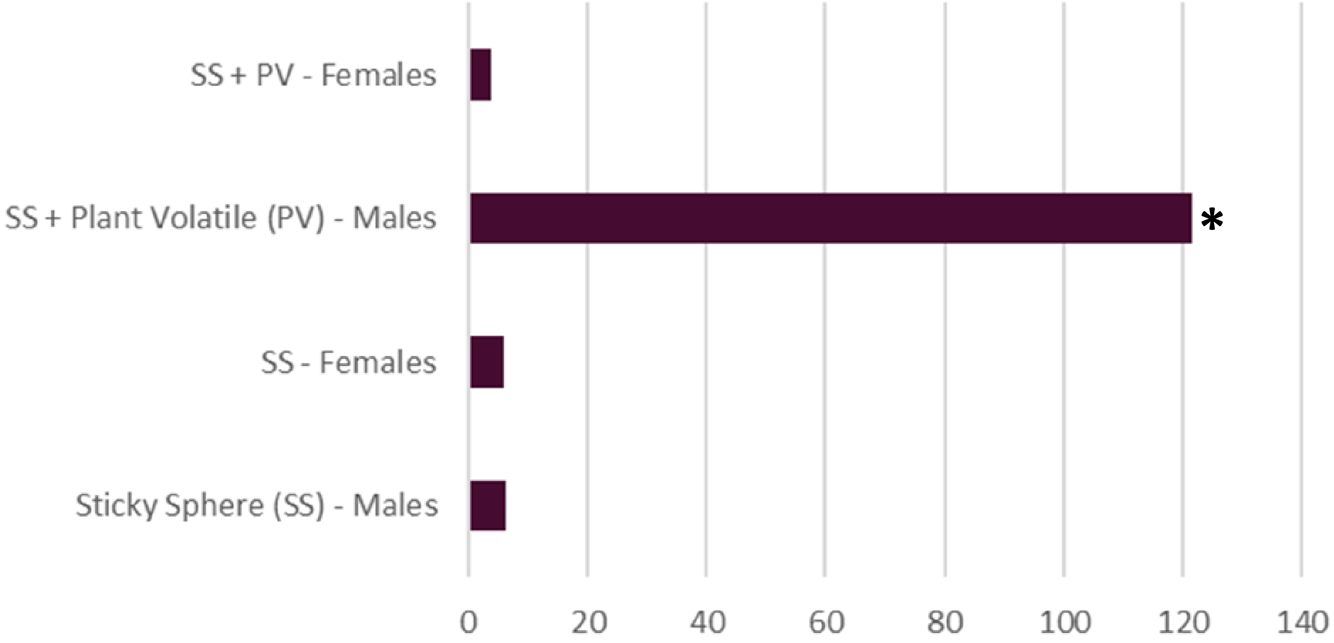

Figure 3. Total number of trapped male and female *Bactrocera tsuneonis* on baited (host odor) and un-baited green sticky spheres (* = pair-wise t-test, *p* = 0.0002, n=20).

FORMULATING LURES TO SUPPORT CAPS

Forest Pest Methods Laboratory CAPS lure support

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

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In 2020 the Forest Pest Methods Laboratory formulated 86,195 specialized insect lures for 33 targeted insect species. These lures were prepared in support of PPQ's Cooperative Agricultural Pest Survey (CAPS) program. The CAPS program is tasked with providing a survey profile of exotic plant pests in the United States deemed to be of regulatory significance. Additionally, CAPS coordinates early detection and surveillance activities in support of State Departments of Agriculture, tribal governments, and other cooperators.

The number of formulated CAPS lures increased by 4,768 compared to 2019. In collaboration with the CAPS Survey Supply and Procurement Program, the Forest Pest Methods Lab is responsible for the procurement and quality control of non-commercial pheromone chemical components. We also formulated 1,260 *Lymantria xylina* string lures for offshore PPQ monitoring programs for the 2020 season and supported the U.S. Forest Service by analyzing several gypsy moth pheromone formulations, including those used by the Gypsy Moth Slow the Spread Foundation, a program aimed at limiting the spread of gypsy moth from known infested areas to neighboring non-infested areas.

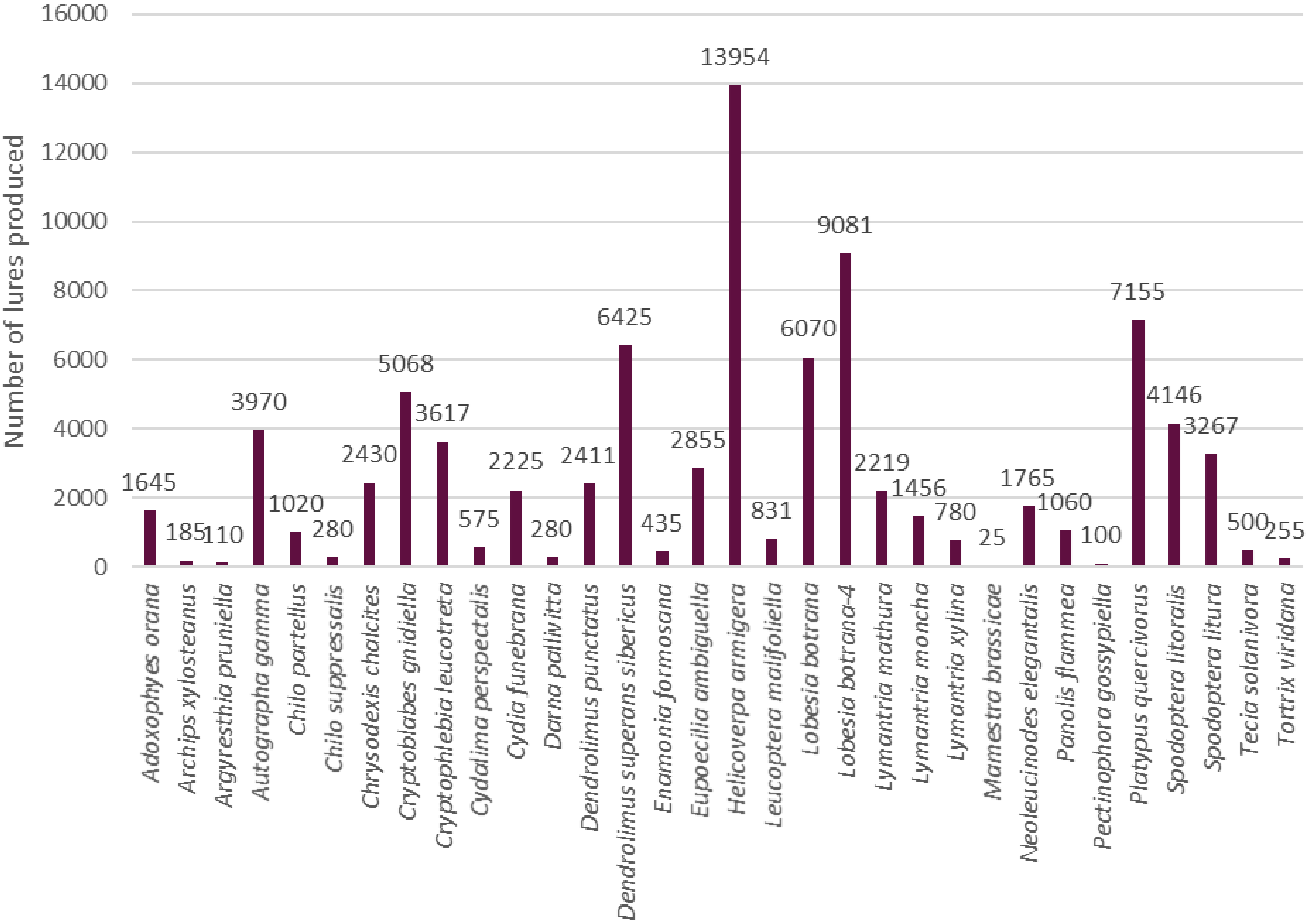

Figure 1. Summary of lures produced in 2020.

2020 Port and Domestic Gypsy Moth Molecular Diagnostic Survey

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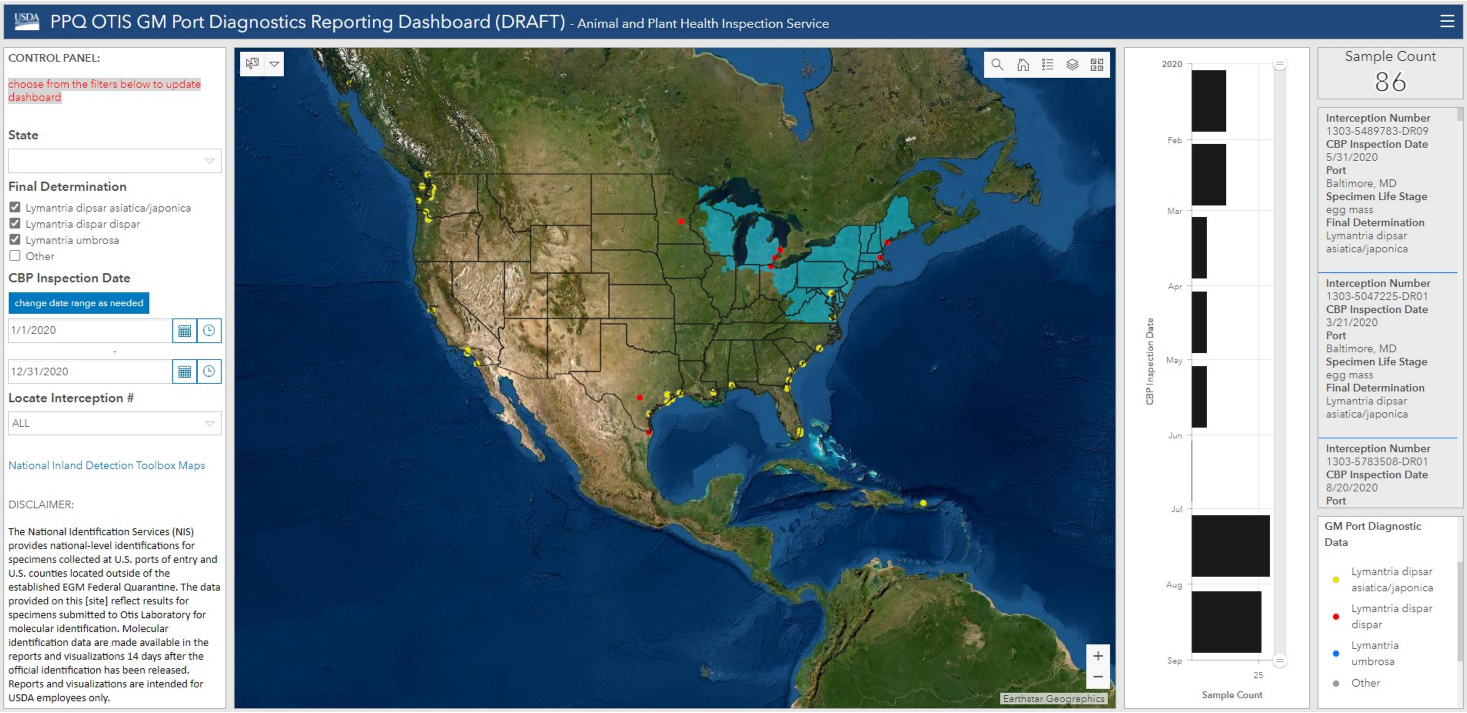
The Gypsy Moth Molecular Diagnostics Survey supports the Asian Gypsy Moth (AGM) and European Gypsy Moth (EGM) Programs. The port survey serves to identify potential AGM specimens intercepted on vessels entering U.S. ports. The domestic survey screens for potential AGM trapped by federal and state agencies while also monitoring the current EGM population inside and outside the established Federal EGM Quarantine.

In 2020, 230 total specimens intercepted at 20 U.S. ports were analyzed. Significant findings include 163 egg masses identified as *Lymantria dispar asiatica/japonica* and one egg mass identified as *Lymantria dispar dispar*.

Additionally, 5,664 specimens submitted from 32 U.S. states were analyzed as part of the domestic survey. Seven specimens were identified as *Lymantria dispar asiatica/japonica*. Three of these specimens were adult inland detections from Santa Clara County (California), Multnomah County (Oregon), and Cowlitz County (Washington). The other four specimens include three adults and one egg mass found in Indiana, Pennsylvania, on a cargo container originating from Shanghai, China. A similar incident occurred in 2019 when cargo containers from South Korea containing eight AGM egg masses were delivered to the Letterkenny Army Depot in Pennsylvania. As a result, increased trapping efforts in and around this area lead to a significant increase in the number of specimens analyzed from the state of Pennsylvania in 2020 (>1200%); however, no additional *Lymantria dispar asiatica/japonica* were detected in PA. For the 2020 survey season molecular analysis identified 4,533 specimens as *Lymantria dispar dispar*; 168 were detected in counties outside of the established EGM Federal Quarantine.

In an effort to make molecular diagnostics results more accessible and beneficial to the Gypsy Moth Program, a beta version of an ArcGIS dashboard was produced in collaboration with Field Operations Hub ArcGIS Team (Figure 1). The dashboard currently allows for the geographical visualization of all molecular gypsy moth port interception data available since 2016. Upon completion of the next phase of development, the dashboard will also include domestic survey data. To request access to the dashboard, please contact Nevada Trepanowski.



☑ Lymantria dipsar asiatica/japonica
☑ Lymantria dispar dispar
☑ Lymantria umbrosa
☐ Other

1/1/2020 - 12/31/2020

ALL

DISCLAIMER:

The National Identification Services (NIS) provides national-level identifications for specimens collected at U.S. ports of entry and U.S. counties located outside of the established EGM Federal Quarantine. The data provided on this [site] reflect results for specimens submitted to Otis Laboratory for molecular identification. Molecular identification data are made available in the reports and visualizations 14 days after the official identification has been released. Reports and visualizations are intended for USDA employees only.

Sample Count
86

Interception Number 1303-5489783-DR09
CBP Inspection Date 5/31/2020
Port Baltimore, MD
Specimen Life Stage egg mass
Final Determination Lymantria dipsar asiatica/japonica

Interception Number 1303-5047225-DR01
CBP Inspection Date 3/21/2020
Port Baltimore, MD
Specimen Life Stage egg mass
Final Determination Lymantria dipsar asiatica/japonica

Interception Number 1303-5783508-DR01
CBP Inspection Date 8/20/2020
Port

● Lymantria dipsar asiatica/japonica
● Lymantria dispar dispar
● Lymantria umbrosa
● Other

Figure 1. *Lymantria* spp. port interception confirmations displayed on ArcGIS dashboard.

IDENTIFYING GENETIC DIVERSITY OF SLF PARASITOID TO SUPPORT BIOLOGICAL CONTROL

Using molecular tools to detect cryptic genetic diversity within collections of the spotted lanternfly parasitoid, *Anastatus orientalis*

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[2]Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY
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Introduction

Anastatus orientalis is an egg parasitoid of the spotted lanternfly, SLF, *Lycorma delicatula*, and is being tested as a potential biological control agent for the pest. Live *A. orientalis* collected in China were reared under Beijing and Pennsylvania conditions at the Forest Pest Methods Laboratory Insect Containment Facility. These specimens exhibited different lifecycles, such as having an extra summer generation or skipping the fall generation, which contrasts with the previous observations that *A. orientalis* has two generations per year [1]. Observations varied from year to year, between collection locations, and under different environmental conditions. To understand the mechanism potentially driving these lifecycle inconsistencies, molecular tools were used to examine the genetic composition of the *A. orientalis* specimens collected in China. If multiple genetic lineages are found, they may represent separate biotypes or even cryptic species, which may correspond to different lifecycles and other related ecological characteristics.

Methods and results

First, attempts were made to amplify the mitochondrial COI barcode for *A. orientalis* using the universal primers LCO/HCO [2] as has previously been done [3]. COI barcodes were successfully generated for nine individuals. Upon examination of the remaining data, a 11-T (thymine) homopolymer region was identified that causes frame shift during DNA sequencing, resulting in noisy data for the remaining specimens.

Subsequently, a second pair of primers, NJ/MD [4, 5], worked very well with *A. orientalis*. This primer pair amplifies a 391 bp fragment that is downstream of the COI barcode. Preliminary analysis of the NJ/MD region revealed considerable genetic divergence among our samples, 30 wasps from Beijing and 30 from Yantai, which belong to four haplotype groups (referred to as A–D).

To generate longer sequences and confirm the NJ/MD region's divergence, the generated *A. orientalis* COI barcodes were aligned, and new primers were designed to circumvent the homopolymer region. Several new primer pairs successfully amplified in all haplotype groups and sequencing reactions produced clean data.

The new primer pair 192F/720R was chosen to work in conjunction with NJ/MD. The 192F/720R region accounts for 71% of the complete COI barcode and the two fragments overlap slightly, and therefore can be concatenated into a continuous 900 bp sequence.

Because only limited divergence was expected within each haplotype group, 2–4 representative specimens per group were included in the final dataset, along with three outgroup taxa (*A. gansuensis*, *A. meilingensis*, and *A. fulloi*) downloaded from GenBank. A HKY distance-based gene tree confirmed divergence among the haplotype groups (Figure 1). So far, Group A was only recovered in Beijing, and it appears rare in abundance within the samples collected. Group B seems to be endemic to Yantai. In contrast, Groups C and D occur in both Beijing and Yantai. The results suggest that in both Beijing and Yantai, at least three mitochondrial haplotype groups co-exist. Some distances exceed the conventional species identification threshold of 1% for the COI barcode in the Barcode of Life Data System (BOLD) [6].

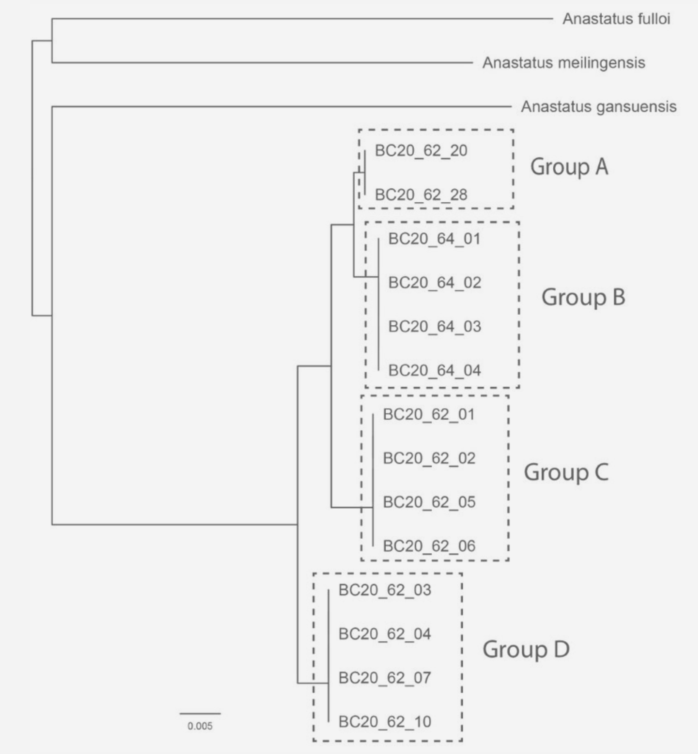
Given the cryptic genetic diversity discovered among *A. orientalis* specimens, it is necessary to assess how much of the anomalies in lifecycle observations can be attributed to genetic differences. We've already established a colony of Group C in the Insect Containment Facility, and plan to establish iso-female lines for Group B and possibly Groups A and D. An iso-female line is descended from a single gravid female, ensuring no mixture of haplotypes, and thus removing any confounding factors from genetics. Multiple iso-female lines will be established and lines within the same haplotype will be mixed to avoid a genetic bottleneck. Rearing and life history work has been conducted on Group C [7], and host specific testing is almost complete. Testing to date shows that this haplotype group is likely not sufficiently specific to be considered for release as a biological control agent; the other haplotypes need to be evaluated.

Extensive survey of the *Anastatus* spp. attacking spotted lanternfly throughout China has been planned for 2021. Genetic analyses of these samples will further determine each haplotype group's distribution and possibly uncover more genetic diversity. Field lifecycle studies are also being initiated to determine how parasitoid emergence patterns relate to haplotype diversity.

Once *Anastatus* parasitoids whose lifecycles are synchronous with the availability of host eggs are discovered, host specificity testing and development of rearing methods will begin.

Conclusions

We recovered considerable cryptic genetic diversity among *A. orientalis* collected from China. It is possible that different haplotype groups have distinct lifecycles, which would bear significant implications for biological control of spotted lanternfly. The development of molecular tools for *Anastatus* will enable us to identify additional candidate species in this genus, including those native to the United States.



Uncorrected genetic distance

	Group A	Group B	Group C
Group A	-	-	-
Group B	0.4%	-	-
Group C	0.8%	1.0%	-
Group D	1.3%	1.5%	1.3%

Figure 2. Phylogenetic tree of the four haplotype groups recovered among *A. orientalis* collected from Beijing and Yantai, China. Uncorrected genetic distance ranges from 0.4% (between Groups A and B) to 1.5% (between Groups B and D).

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IDENTIFYING GENETIC DIFFERENTIATION AMONG U.S. VLB POPULATIONS

Genomic data reveals cryptic genetic diversity among invasive populations of *Trichoferus campestris* in the United States

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Introduction

The velvet longhorned beetle, VLB, *Trichoferus campestris*, is native to East Asia but has been found across North America including many U.S. states. The beetle attacks weakened, stressed trees and occasionally healthy trees, particularly apple and mulberry. Our previous study, based on six mitochondrial protein-coding genes, revealed considerable genetic diversity among the invasive *T. campestris* specimens in the United States [1]. Multiple species delimitation analyses suggested the presence of cryptic species within those samples. To further our understanding of the genetic relationships between mitochondrial lineages, genome-wide SNP (single nucleotide polymorphism) data were generated for *T. campestris*. Characterizing the genetic structure of VLB can help understand the process of introduction.

Methods

With the advent of next-generation sequencing, SNPs have been widely used in population genetics because they can provide thousands of independent estimations for genetic diversity within and among populations. A total of 167 VLB specimens were sampled for ddRAD (double digest restriction-site associated DNA) genotyping following the protocol from Peterson et al. [2] with modifications.

The resultant library was sequenced and single 150 bp reads were quality-checked and assembled into contigs. The recontigs were used as the reference to re-align all raw reads to assess coverage and identify SNP loci. The resulting data were trimmed to remove all SNPs that had more than two alleles. Specimens with missing data greater than 30% were also discarded.

Results

The final dataset consists of 1,355 SNP loci and 96 VLB individuals, including 61 found in domestic states, 32 intercepted at U.S. ports of entry, and three from its native range in East Asia. Discriminant Analysis of Principle Components clustered the specimens into four groups (Figure 1). The optimal number of principal components was determined to be seven, and three discriminant functions were retained. A scatter plot with the four genetic clusters revealed considerable genetic divergence among *T. campestris* in the United States. (Figure 2), similar to the result from a previous study [1]. Because over two-thirds of specimens (n = 73) were shared between the two datasets, direct comparisons of relationships can be made between mitochondrial lineages and genetic clusters.

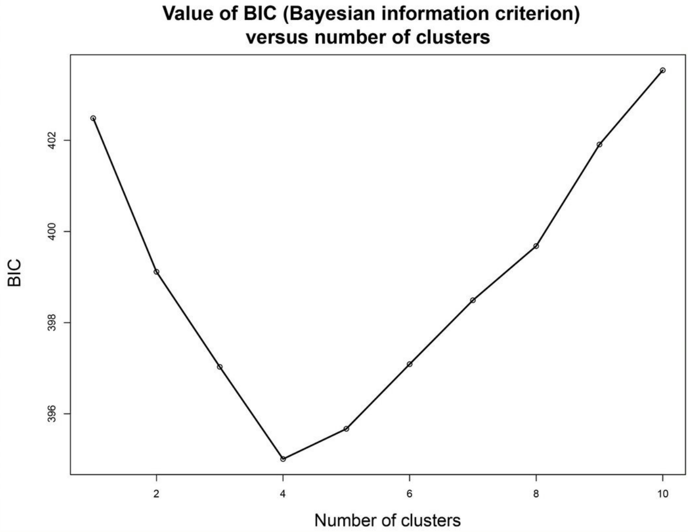

Figure 1. Bayesian information criterion (BIC) supports four clusters among *T. campestris* in the U.S.

Two clusters were from Utah, where a large *T. campestris* population has been found. Cluster 1 corresponds to the majority of specimens collected from the Holladay and Murray area, whereas Cluster 4 exclusively includes all specimens from Pleasant Grove. This clear phylogeographic pattern contrasts with the mitochondrial gene tree, in which Utah specimens were scattered in different lineages as a result of the five mitochondrial haplotypes present. Finding two distinct clusters bears significant implication for the introduction history of *T. campestris* because it suggests that the population from Holladay/Murray is genetically distinct from the one from Pleasant Grove. Additionally, the two clusters suggest that the Pleasant Grove population was likely founded through multiple introduction events followed by years of random mating, as evidenced by the occurrence of divergent mitochondrial haplotypes and a relatively homogeneous nuclear genome.

Among the four genetic clusters, Cluster 3 contains the largest number of samples with representatives from all three mitochondrial lineages, ranging from intercepted specimens, beetles from other U.S. states, and the three specimens from East Asia. Interestingly, one specimen from China, which has a unique mitochondrial haplotype and thus was considered a cryptic species [1], shows a genomic makeup like other *T. campestris* in this cluster. The contrast between nuclear and mitochondrial data exemplifies the complex evolutionary history of *T. campestris* and likely signals historical introgression from other unsampled lineages or closely related species [3]. Lastly, Cluster 4 contains six intercepted specimens from Washington and California. Their mitochondrial DNA belongs to Lineage I [1], but their SNP data are different from others in the same mitochondrial lineage.

Conclusions

Genome-wide SNP data were generated for *T. campestris* and confirmed large genetic differentiation among specimens in the U.S. Particularly, two distinct nuclear groups were present in Utah, which contains multiple divergent mitochondrial haplotypes. Conflicts between genomic data and previous mitochondrial data suggest a complex evolutionary history and complicated processes of invasion. The results provide important information useful for the mitigation of future introduction of *T. campestris.*

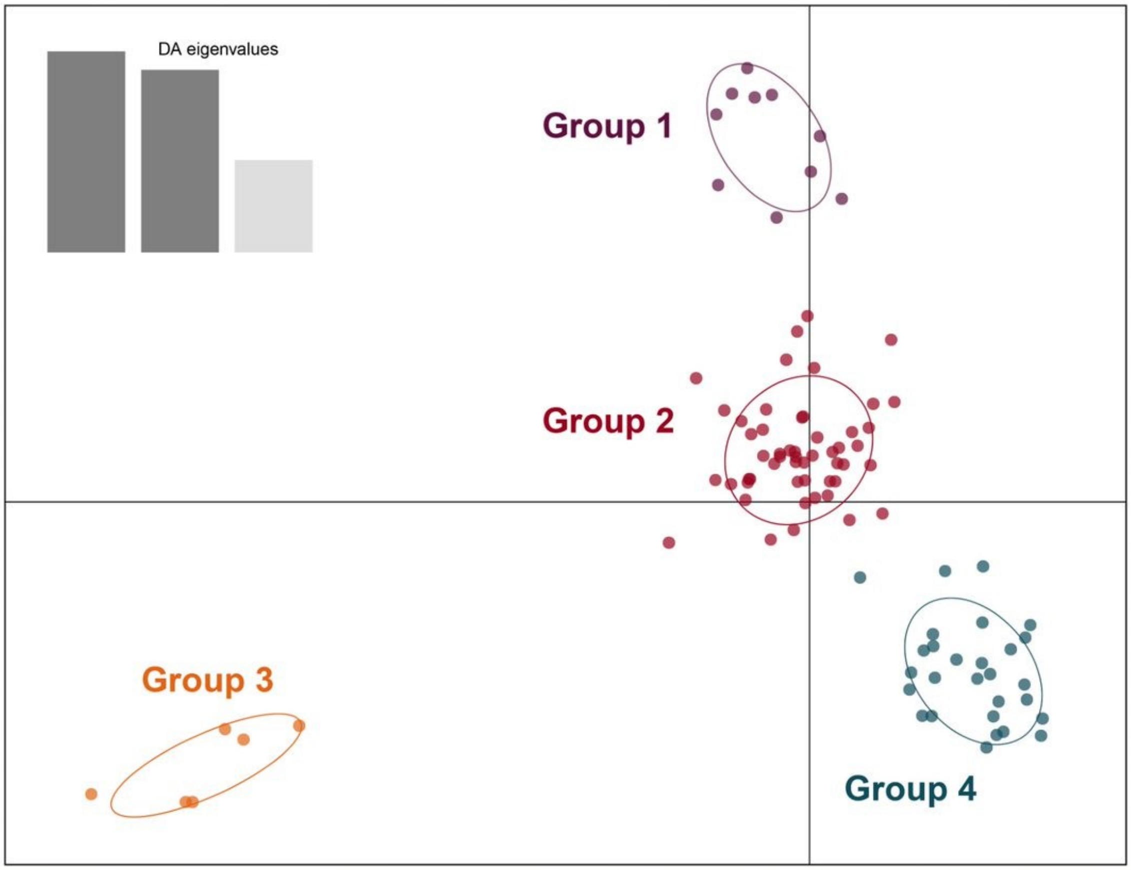

Figure 2. Scatterplot showing the separation of the four genetic clusters in first and second discriminant functions (x- and y-axis). Inset shows the eigenvalues of all three discriminant functions.

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UPDATING EGM RISK MODEL WITH 2019 TRAPPING DATA

2020 European gypsy moth national risk assessment

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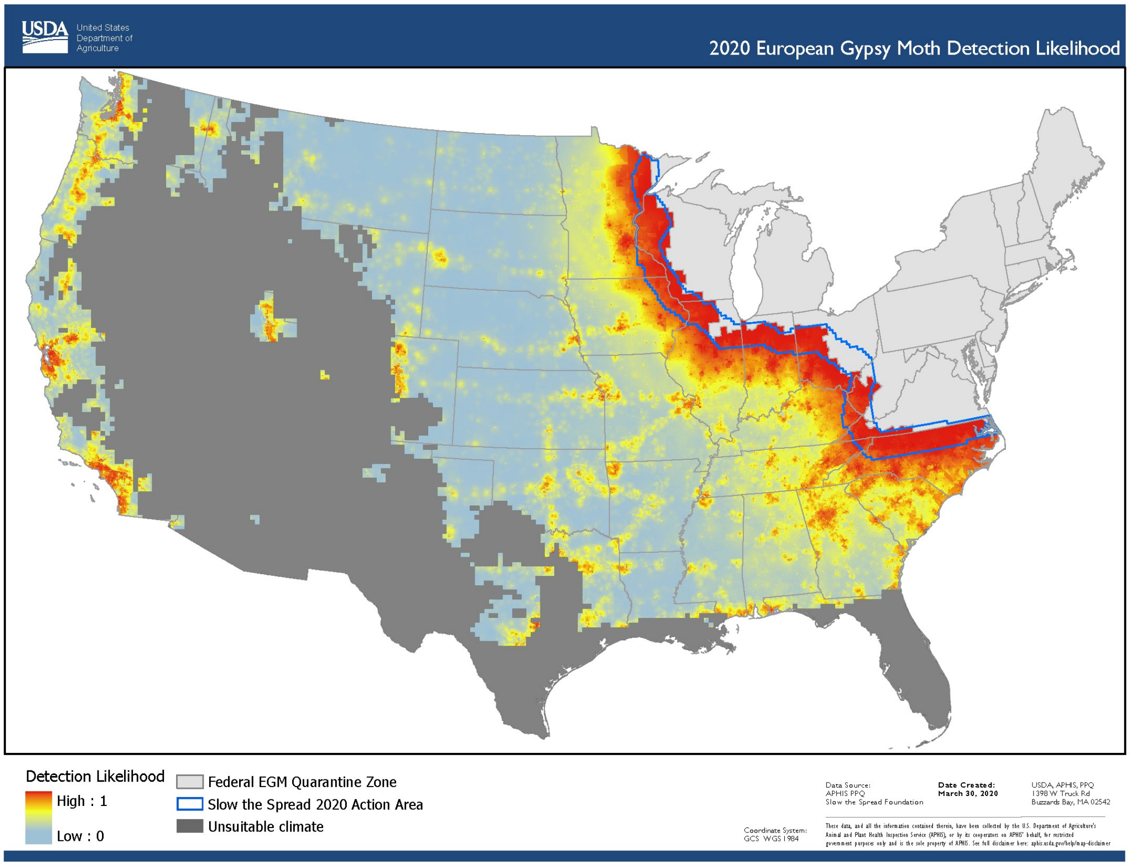
The European gypsy moth, EGM, *Lymantria dispar dispar*, risk assessment supports the Gypsy Moth Program's goals of detecting isolated EGM infestations and limiting artificial spread beyond the known infested area. The risk assessment evaluates spread mechanisms and predicts the likelihood of EGM detection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

The modeling framework is a species distribution model, which identifies areas with similar site characteristics to locations where the pest has been found and estimates a high likelihood of detection in those areas. The model considers mostly anthropogenic factors such as USPS address forwards from within the federal EGM 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.

In 2020, the model was updated with trapping data from 2019. The model does not consider environmental variables and relies on a post-hoc process of masking out areas without suitable hosts or climate for establishment. For 2020, the climate mask was revised based on a newly published climate suitability model [1] for Asian gypsy moth, AGM, *Lymantria dispar asiatica*, a related subspecies. The revised climate mask improves the model by addressing a previous issue in which too much risk was assigned to areas of the U.S that are too warm and dry for EGM establishment.

References

1) Paini DR, Mwebaze P, Kuhnert PM, Kriticos DJ. Global establishment threat from a major forest pest via international shipping: *Lymantria dispar*. Sci Rep. 2018 Sep 13;8(1):1–7.



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APHIS PPQ
Slow the Spread Foundation

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Figure 1. The 2020 EGM detection likelihood with areas of unsuitable climate for establishment masked out.

Classical biological control of Asian citrus psyllid in Arizona

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Residential citrus trees in urban areas provide a refuge to the Asian citrus psyllid, ACP, *Diaphorina citri,* and present an obstacle to its conventional control tactics. Since 2013, efforts have been underway to establish the parasitoid *Tamarixia radiata* as a classical biological control agent of ACP in urban Arizona. High-quality, genetically diverse biological control agents are reared at the University of California Riverside (UCR) and are shipped to Arizona weekly. Releases and monitoring occur in four regions: San Luis, Wellton, Ajo, and Nogales. Once every four weeks, *T. radiata* are released, the citrus flush growth is accessed, and ACP life stages and the presence of *T. radiata* are monitored. Program goals include examining regional ACP phenology and determining the establishment of *T. radiata* in release areas.

In 2020, 86,000 *T. radiata* were shipped to Arizona for release. Monitoring data were examined for each biocontrol region to compare flush growth and ACP population patterns. San Luis and Wellton have similar climates to each other and to Yuma, while Ajo and Nogales have less extreme heat in the summer and, in the case of Nogales, a cooler winter. Monitoring data suggest that these climatic differences have led to phenological differences in both citrus flush growth and ACP populations (Figure 1).

A sample of 30 *T. radiata* collected in Nogales in 2019, before official biological control releases, had been identified as a haplotype not reared at UCR, indicating that these *T. radiata* originated from another source. In 2020, 22 *T. radiata* specimens , 10 from Ajo and 12 from Nogales sites, were collected from yellow panel traps and sent to UCR. All usable specimens were identified as haplotypes that occur within the genetically diverse UCR colony, suggesting that they are related to UCR wasps. Specimens collected in Nogales in late 2019, prior to biological control releases, belonged to a haplotype not reared at UCR, and may be descended from *T. radiata* released in Mexico. No flush shoot samples were collected in 2020 due to low immature ACP infestation levels in all regions.

The Salinas Field Station supports ACP biological control efforts in Arizona by: 1) compiling and analyzing the plant and insect monitoring data gathered by Field Operations personnel in Arizona, 2) screening yellow panel traps for *T. radiata* and sending them to UCR cooperators for genetic analysis, and 3) examining immature ACP on flush shoot samples to determine the percent of *T. radiata* parasitism.

In 2020, a full year of *T. radiata* release, monitoring of flush and ACP populations, and *T. radiata* recoveries was completed in the four new release areas; this has allowed us to begin examining phenological differences in these regions. In 2021, we aim to gain more insight into the potential establishment of *T. radiata*, as new release sites have been chosen, and previous release sites will be used exclusively for monitoring purposes.

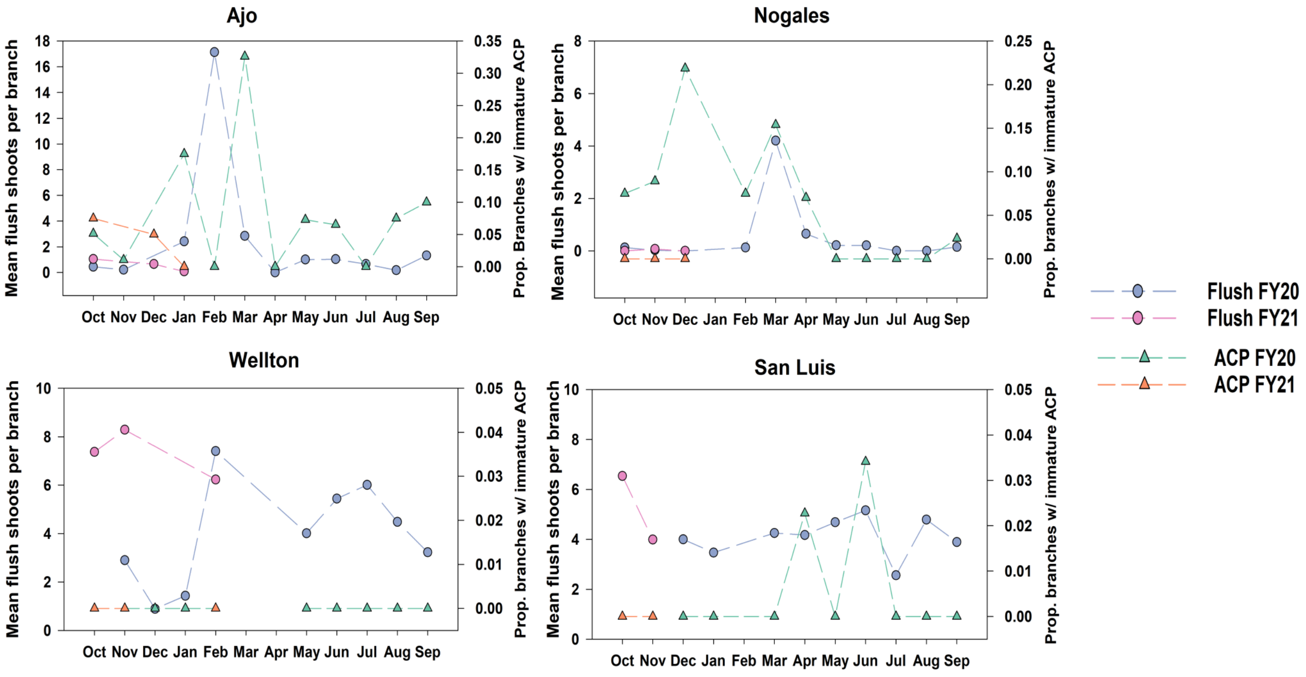

Figure 1. Mean number of flush shoots per sample branch (dots) and proportion of sampled branches with ACP eggs and/or nymphs (triangles) in each biological control region.

2020 *Diomius pumilio* production

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Diomus pumilio is a generalist predator reported to be one of the key natural enemies of Asian citrus psyllid, ACP, *Diaphorina citri*, in southern California. ACP is one of the principal vectors of *Candidatus Liberibacter asiaticus*, the causal agent bacterium of citrus greening disease. In 2019 the Citrus Research Board (CRB) began methods development for the mass production of *D. pumilio* (Figure 1).

Figure 1. *Diomius pumilio.*

The production of *D. pumilio* is part of a collaborative ACP area-wide integrated pest management (IPM) program in southern California. Suppressing ACP populations is one of the key strategies used to keep commercial citrus free of citrus greening disease.

Preliminary studies indicated using *Heteropsylla* sp. as a host, reared on *Acacia farnesiana*, would be the most efficient and economical way to mass rear *D. pumilio* under greenhouse conditions. As rearing methods have been refined, production has steadily increased. Current rearing methods utilize four *A. farnesiana* in a rearing cage inoculated with 200 psyllids. The cage is then inoculated with 20 *D. pumilio,* which produces adult beetles in approximately three weeks.

Following the above methods, rearing cages produced 21,553 *D. pumilio* in 2020 (Figure 2). Production cages averaged 330.5 beetles per cage from 40 cages. Of the beetles produced, 20,766 were released in Hemet as part of the area-wide IPM program for ACP.

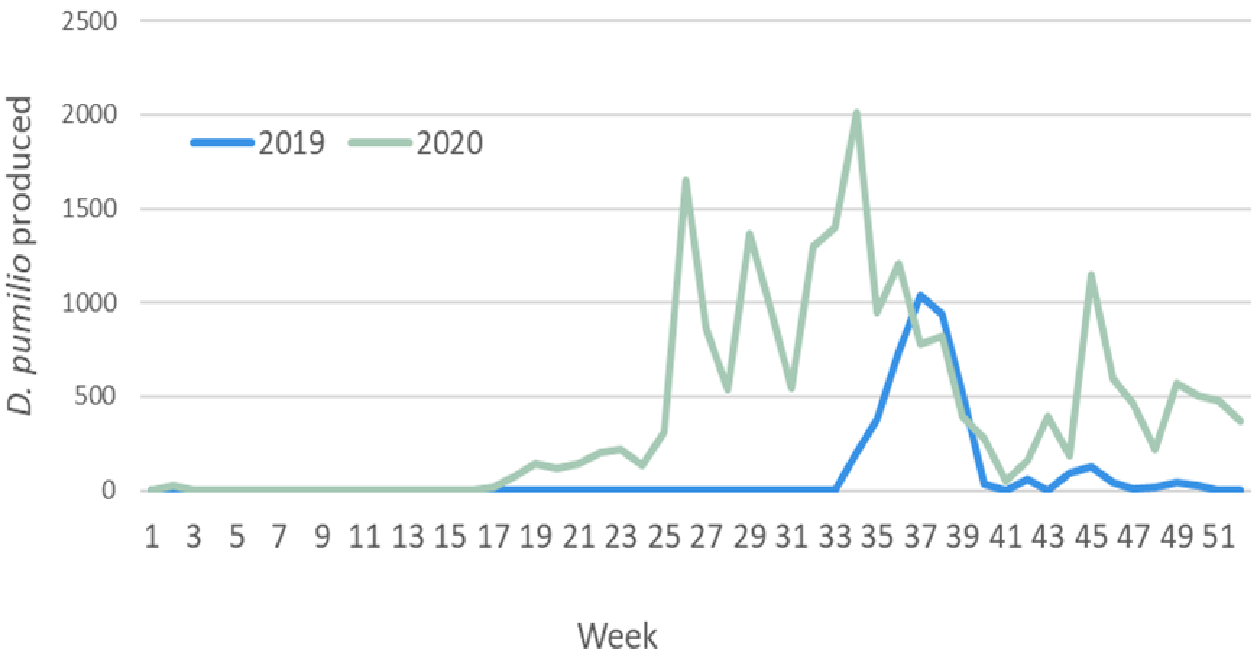

Figure 2. *Diomus pumilio* mass rearing production.

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