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2011 CPHST Laboratory Report Otis Laboratory

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Two New Organic Ground-Applied Mating Disruption Products for LBAM at Different Point Source Densities

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Contributors: D. Maxwell Suckling, Eckehard Brockerhoff, Lloyd Stringer, Ruth Butler, & Delyse Campbell (New Zealand Institute for Plant & Food Research)
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Two new, organic, ground-applied mating disruption products were evaluated against light brown apple moth (LBAM) at different point source densities in organic vineyards in New Zealand, in an area where high densities of LBAM were known to occur. The two new products, Iscatech SPLAT LBAM HD-O and Hercon Disrupt Bioties LBAM, were tested at label application rates of 500 points per ha against the industry standard, Isomate LBAM Plus Twist Ties. They were also tested at 322, 72, and 25 points per ha. Each point contained 183 mg of pheromone. Untreated plots were used for controls. Traps baited with 3 mg of LBAM sex pheromone were used to evaluate mating disruption, as well as traps baited with live females. All three products provided excellent mating disruption at 500 points per ha. The 322 point source level also provided good mating disruption. At the 72 and 25 point sources, mating disruption occurred at a lower level and tapered off earlier. All three products can be used effectively against dense LBAM populations at 322 or 500 points per ha, in plots as small as one ha. Lower dosages may still provide a low level of mating disruption.

Since the introduction of LBAM in California, there is a desperate need for environmentally and socially benign products that can be used to control the growing pest population. Mating disruption can be effective against LBAM, but broad scale aerial applications of mating disruption products are no longer being considered for political reasons. The only ground applied mating disruption product that has been in use by the program has been Isomate LBAM Plus Twist Ties (**Fig. 1a**), which are plastic dispensers containing the pheromone. Although effective when used properly, the drawback to utilizing this product is that they are labor-intensive to apply, must be hand-applied, and later must be retrieved after the pheromone has dissipated.

There is a strong need for alternative products which can be mechanically applied over large areas, which do not require retrieval, and organic mating disruption products which are considered environmentally friendly.

Two companies developing such products for use against LBAM are Hercon Environmental and Iscatech (**Fig. 1b & 1c**). Iscatech has marketed SPLAT for other pests and now has an organic version made of food-grade ingredients to be used against LBAM. Hercon, for use against LBAM, is developing Disrupt Bioties, long biodegradable organic fibers impregnated with pheromone, to be tied in a similar manner as the Isomate Twist ties, but without the need for retrieval because they degrade over time.

The Isomate Twist Tie label states that they should be applied at a rate of 500 points per ha, with 183 mg of pheromone being contained in each dispenser. This study also addressed whether mating disruption could be established with fewer point sources and less product applied.

Figure 1: *a) Isomate Twist Tie, b) Isca Tech SPLAT, and c) Hercon Bioties*

A cooperative agreement was established with principle investigator Dr. Max Suckling (Plant & Food Research, Christchurch, New Zealand) (**Fig. 2**) and his team of experts on mating disruption to test the two new products, Iscatech SPLAT BAM HD-O (hereafter referred to as SPLAT) and Hercon Disrupt Bioties LBAM (hereafter referred to as Bioties) against the industry standard, Shin-Etsu Isomate LBAM Plus Twist Ties (hereafter referred to as Isomate). LBAM pheromone, purchased by the USDA from Bedoukian, was delivered to the three manufacturers for formulation into their respective products. Chemist Lisa Mosser (USDA APHIS PPQ CPHST) developed analytical techniques for each of the products, and analyzed samples of each product prior to application in order to verify that the formulation of pheromone dose matched that listed on each product label.

Figure 2: *Maxwell Suckling (far left) and his crew who helped in LBAM mating disruption application in New Zealand.*

Experimental design. An organic vineyard (**Fig. 3**) was selected for the study because it used no chemical pesticides and had high LBAM densities. Also, because grapes are commonly grown in California, information derived from this study can be directly applied to use on this crop in California. Pre-trial trapping took place (from November 19, 2011 until December 16, 2011) in order to determine how to block the experiment based on population variation throughout the field sites. For pre-trial trapping, 174 delta traps with sticky liners were placed 100 m apart and baited with 3 mg 2-component LBAM sex pheromone lures.

For the experiment, a randomized block design was used. The vineyard was divided into 69 1-ha square plots, each separated by 50 m, located over three sites, and subsequently divided into five blocks plus a set of untreated plots. Each block contained 11 plots, 9 of which were assigned one of the 9 treatments, and the last two were untreated controls. There were a total of 10 treatments: the two new products (SPLAT and Bioties) were each applied at four point source levels (25, 72, 322, and 500 per ha), one level of Isomate (500 per ha), and the untreated controls. Attempts were made to standardize the size of each point source so that they each contained approximately 183 mg of pheromone.

Three weeks prior to treatment, pre-treatment trapping was conducted to establish baselines for each plot. Seven traps were placed N to S along a transect through the center of each plot. These 483 delta traps with sticky inserts were baited with the 3 mg lure and monitored 1 and 3 weeks prior to the product applications, the week of application, and the 10 weeks following application. Additionally, four delta traps, each baited with a caged LBAM female, were placed 25 m inside the edge of each plot. The experiment occurred from February 10 to May 19, 2011.

Products. Products were applied by a team of 18 people (**Fig. 2**) over two days on March 8 and 9, 2011. Due to grower's concerns, all products were applied to

Figure 3: *The Mudhouse Vineyard field sites in Waipara, Canterbury, NZ.*

posts rather than on or in the foliage. Isomate Twist Ties were each tied to the top wire next to a post. Using a 50 ml syringe, SPLAT was applied as two 1-ml dollops on the top of a post. Unexpected issues with the Biotie formulation caused them to be too brittle to bend, so each Biotie was broken into three or four pieces and stuck to the tops of posts using Bostik Blue-Tack.

In addition to the application of products in plots, 70 samples of each product were placed in an unused plot for aging. This provided five sample replicates for each product and sample date, for a total of 14 sample dates starting on the day of application and for 70 d thereafter. On each sample date, the samples were sealed and stored at -80°C until the end of 70 d. Then the samples were sent to chemist Lisa Mosser who conducted a quantitative analysis on them to determine the amount of pheromone remaining in each sample after different amounts of time.

Results & Discussion. All three products were found to be formulated as labeled, and resulted in successful mating disruption at the application rate of 500 points per ha (**Table 1, Figs. 2 & 3**). More moths were caught in traps baited with pheromone lures than with females, and mating disruption was higher with female-baited traps than with pheromone lure-baited traps. There was no significant difference in mating disruption between the three products at 500 points per ha (P=0.789). Also, with increasing numbers of points, mating disruption improved for both traps baited with lures and females (P<0.001). Mating disruption was about 50% successful at the lowest numbers of point sources, and about 94% successful at the highest numbers of point sources. Based on these results, these two new products offer effective organic alternatives that can be used for LBAM mating disruption, and application at 75% of the label rate would only minimally reduce disruption.

Table 1. Post-treatment percentage disruption (approx. 95% confidence limits). N = external control.

Rate	SPLAT	BIOTIE	Isomate	N
a) Lure traps				
25	48.4 (23.7,65.1)	51.9 (29.5,67.1)		
72	71.7 (58.3,80.8)	63.5 (46.6,75.1)		
322	90.5 (84.9,94.0)	87.1 (80.3,91.6)		
500	94.4 (90.4,96.8)	94.2 (90.2,96.6)	95.5 (92.3,97.3)	
N				9.0 (-20.7,31.4)
b) Caged female traps				
25	77.9 (52.8,89.7)	53.8 (13.7,75.2)		
72	90.5 (77.0,96.1)	71.5 (44.9,85.3)		
322	93.3 (81.9,97.5)	96.2 (87.4,98.8)		
500	95.9 (85.3,98.9)	97.1 (87.9,99.3)	97.7 (90.8,99.4)	
N				-128.0 (-333.0,-20.0)

Comparison of Glue Types for Moth Survey Traps, and Development of a New Re-usable Moth Trap

Project Lead: Joe Francese and Dave Lance

Contributors: Natalie Leva and Vic Mastro (Otis Laboratory)

Dozens of lepidopteran species are surveyed for by the CAPS program. The use of "Tanglefoot" and "stick-em" type traps for survey and detection is problematic. Survey crews must deal with messy surfaces during trap set-up and take-down as well as during periodic checks. Insects caught on these traps usually pick up some glue that coats wings, legs and other taxonomic characteristics making identification difficult. Alternatives that alleviate these problems are desirable. The work being undertaken in this project will:

- Identify ways to improve ease of identifying species of moths caught in traps
- Evaluate hot-melts/thin-layer "hard" adhesives
- Evaluate and/or develop non-sticky traps for monitoring invasive pests
- Improve the handling ability of traps for survey crews
- Develop new technology for sampling insects -- one size does not fit all

In 2011, we started to undertake this work by performing a pilot study with a local surrogate species, the gypsy moth, *Lymantria dispar*. In future years we will look at developing and testing materials for a wider range of species.

We conducted field assays to test trap inserts from four companies that manufacture moth traps. These included:

1. Two "hot-melt" adhesives
   a. Alpha Scents
   b. Great Lakes IPM
2. Two traditional "Tanglefoot"-type adhesives
   a. Scentry
   b. Isca

We used the same trap type, a large plastic delta trap from Alpha Scents, for all inserts. Traps were placed along the southern border of the Massachusetts Military Reservation (MMR) (a northern edge of the Crane Wildlife Management Area) From 15 July 2011 until 5 August 2011.

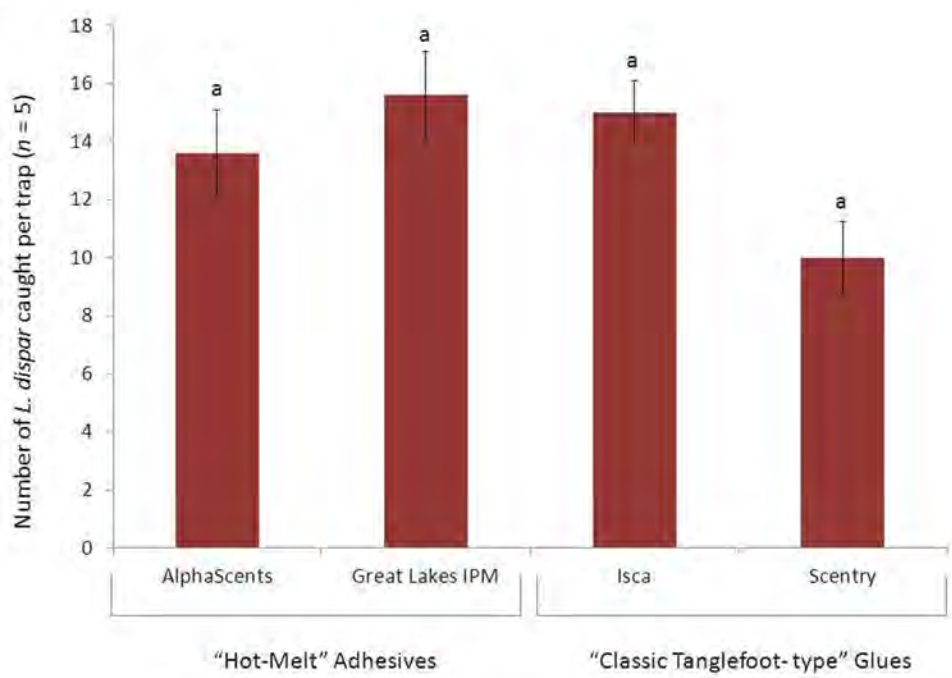

Figure 1: *Number of* L. dispar *caught per trap in a glue comparison study conducted on Cape Cod, MA in summer 2011 (n = 5).*

Numbers of moths per trap were recorded at the end of the study (**Fig. 1**). In addition, traps from the field study were later placed in cages (with groups of 7-8 moths) to confirm their ability to continue to catch moths after 3 weeks in the field. Used inserts were also provided to the molecular lab at Otis for their input on possible effects of the glues on DNA analyses. We will attempt to run similar assays to these with other species in 2012.

As part of this continuing work we are also developing a trap that is adaptable to multiple species including *Dendrolimus* spp., does not rely on sticky material to trap insects, is re-usable but has a disposable container to hold insects, is reasonably priced, and can potentially include modules for automated data acquisition.

A cooperative agreement has been set up with a company in North Carolina to begin developing a prototype using 3D CAD and 3D printing methods, followed by machining of injection molds for trap production. The trap is similar in concept to "Multi-pher" traps or "Uni-traps," but includes several detachable parts to provide the desired flexibility of function and ease of use. We hope to test it in 2012 for several species.

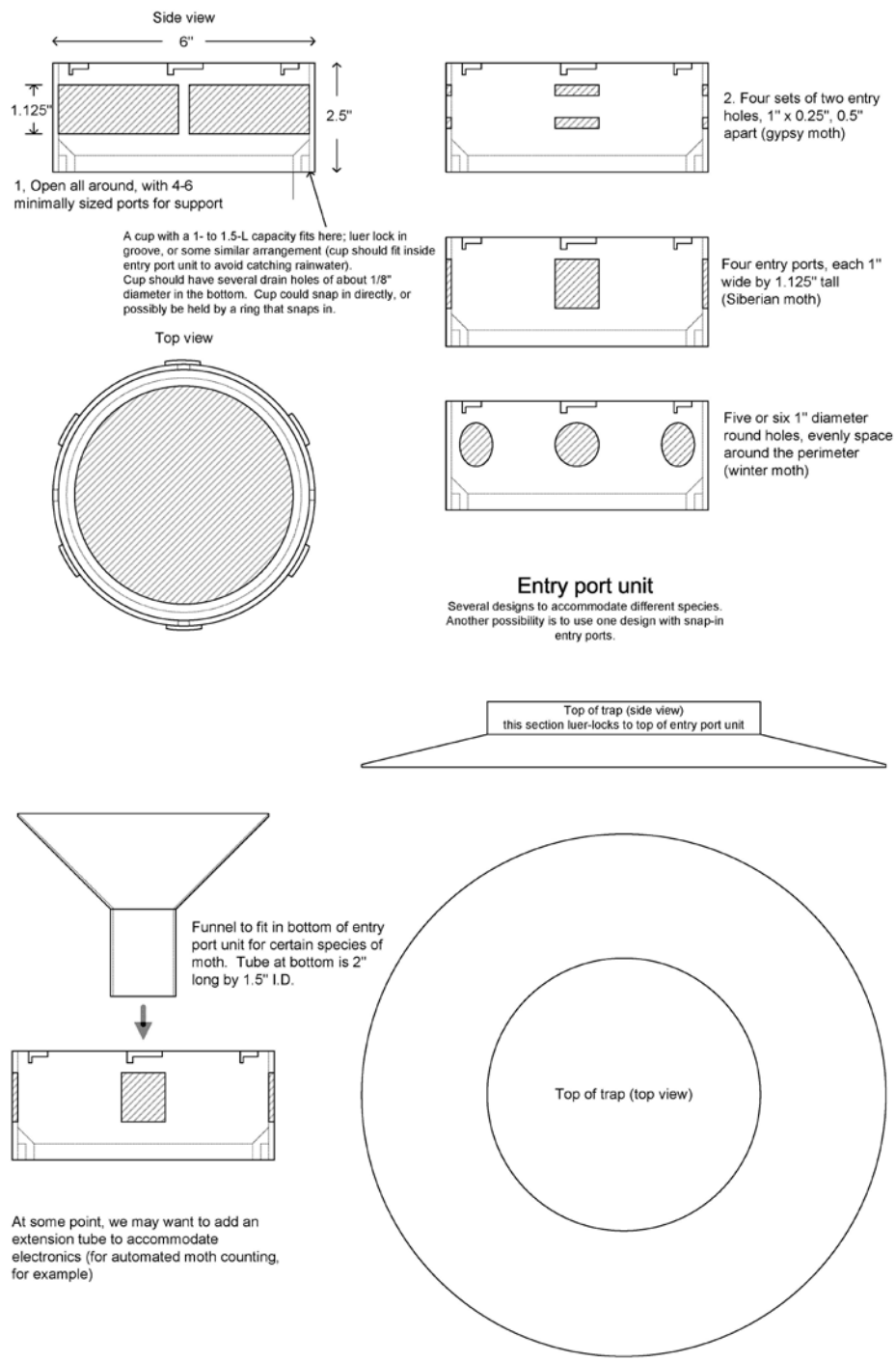

Figure 2: *Diagram of new trap design.*

Possibilities include:

- Gypsy moth
- Winter moth
- *Spodoptera*
- *Paralobesia* (and possibly *Lobesia*)
- *Dendrolimus* (*sibiricus* and possibly *superans*, *punctatus*, *pini*)
- *Helicoverpa armigera* and *punctigera*

Evaluation and Development of Survey Techniques for the Asian Gypsy Moth, *Lymantria dispar* in China

Project Lead: Baode Wang

Cooperators: Zhichun Xu (Beijing Forestry University)
Feiping Zhang (Fujian Agriculture and Forestry University)
and more than 50 Chinese college students and local collaborators

Contributor: Vic Mastro (Otis Laboratory)

Project goals and objectives.

- Development of survey techniques for the AGM
- Determine the distribution of AGM in China
- Evaluate disparlure for *Lymantria xylina* and monachlure for *Lymantria monacha* in southern China

Materials and methods. This study was divided into two sections primarily based on geographical regions in China, one is the region north of the Yangtze River, and another is the region south of the Yangtze River.

Information concerning the distribution and occurrence of AGM in China was collected from literature, newspapers, interviews, and the internet in 2011.

In the southern region, 24 field sites in nature reserve, seaport, airport, forests, orchards, and shelter belt were selected to place 230 *Lymantria* traps in the province (district) of Fujian, Hubei, Hunan, Sichuan, Chongqing, Guangdong, Shenzhen, Guizhou, Yunnan, Guangxi and Jiangxi in June of 2011.

The other 100 traps with monachalure were set at the field sites in Fujian province at the same time. All traps at each site were 50 (or 75) meters from each other. After placement at each site, traps were checked once every 1 week or 2 weeks until no target moths were captured in two successive checks or until the end of August. All specimens caught by the traps at each site were collected and regularly recorded.

In the region north of the Yangtze River, traps were placed in the following provinces and cities: Helongjiang, Jilin, Liaoning, Neimonggu, Hebei, Beijing, Tianjin, Shandong, Henan, and Anhui, with 1-5 sites at each individual province or city, and 5-10 traps at each site. Traps were placed as early as late May in some provinces and checked since until mid-September in northeastern China. All traps at each site were 50 (or 75) meters from each other. Traps were checked once every 1-2 weeks until moths were captured in two successive checks or until the end of August. All moths caught by the traps at each site were collected and regularly recorded for information of site, date and species. Flight period of moths were closely monitored at all areas.

Figure 1: *Traps were placed at several sites in provinces south of the Yangtze River in China.*

Summary. Based on the trapping results from previous years, we selected 11 provinces (regions) to place *Lymantria* traps in 2011. Zhejiang, Jiangsu and Shanghai were excluded in 2011 because we did not trap any AGM during the previous years. However, Guizhou, Guangxi and Yunnan (**Fig. 1**) were added because there were indications that AGM or closely related species may occur in these places.

In 2011, 1,178 *Lymantria* males were caught in traps at 10 field sites in Fujian, Sichuan, Chongqin, Guizhou, Yunnan and Hubei, but no months were captured at the other 14 sites. Among them, 782 male AGM adults were trapped at the sites of Enshi of Hubei, Beipei of Chongqing, Chengdu of Sichuan and Guiyang of Guizhou, 258 *Lymantria dissluta* were captured by the traps with disparlure at Lianjian and Xiapu of Fujian, 128 male *L. xylina* adults were found in the same traps with disparlure at Shishi of Fujian, and 1 *L. marginata* and 6 moths of an unidentified species were caught at Shaowu of Fujian. In addition, other two unidentified species were found at Kunming of Yunnan and Xiamen of Fujian, respectively. Most of the specimens were captured in June and July.

Figure 2: *AGM males captured in a trap at a site in Changping district, Beijing.*

The above results show that AGM occurs in Guiyang, Chongqing, Chengdu and Enshi. The disparlure is also attractive to various *Lymantria* species such as *L. dissluta*, *L. xylina*, and *L. marginata*. Our data also suggest that adults of AGM and other *Lymantria* species may emerge before June in southern China.

The results from the survey in the region north of the Yangtze River confirm that AGM occurs in most areas, especially, the region north of Beijing (**Fig. 2**) such as Northeastern China. No AGM was caught in traps in several sites such as Daxin, Mentougou (Beijing), Zhungeer (Neimeng), Huanghua, Baoding (Hebei), Weihai (Shandong), Dagang, Tanggu(Tianjin). No AGM was captured at the two sites in Henan province. The earliest trap catch was on June 8 in Liuan, Anhui province and Tengzhou (Shandong province) became the second earliest (June 11). The last catches were in Qinhuangdao, Hebei province (September 18), Zhangjiakou, Hebei province (September 13), and Jianping, Liaoning province (September 12). The number of moths captured per trap was generally still very high at these sites, e.g., 43 for Dalian (Liaoning province), and 236 for Jianping (Jilin province). AGM Population level in Dalian, Liaoning province was higher than it was in 2010. Larvae can be seen feeding on host leaves in a number of places (**Fig. 3**).

The highest trap capture remain to be located in Zhangjiakou of Hebei province (100-150 miles northwest of Beijing) with an average per trap capture of 795 from July 13 to September 13. Male flight period of the AGM can be roughly determined based on the results at each individual area.

Figure 3: *Larvae feeding on host leaves at a site in Dalian, Liaoning province, China in late August 2011.*

We also tested the number of male moth trap captures in relation to distance among traps (**Table 1**). It seems that traps that were farther apart caught more males. However, this increase in trap capture is easy to understand considering the increased area covered by traps that are farther apart.

In 2011, lights of different wavelengths were also compared for trapping AGM adults, especially, females. The test was conducted in Hebei province, in north China.

There were some differences in terms of catches by traps using light with different wavelengths. The results suggested that wavelengths within the UV range (345 nm – 368 nm) attracted more females than the 420 nm wavelength light did.

Table 1: *The number of AGM captured in traps placed in groups of different distances (July 18 to August 18, 5 traps in each group).*

Distances Among Traps (m)	No. Traps per Ha	No. Males per Trap	Est. Total Male Capture per Ha
25	25	4.6±2.1a	115.0±51.8a
50	9	10.9±3.8a	97.1±34.1a
100	4	21.0±7.9b	84.0±31.6a

Evaluation of Flight Capabilities and Orientation of Asian Gypsy Moth Females in their Native Range

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Contributors: Kwang-Su Lee, Chang-Ki Hong, Yeong -Joon Kwak, Keun-Su Kim (Sangji University, Korea)
Vic Mastro and Dave Lance (Otis Laboratory)

This work was done cooperatively with cooperators in two countries: Russia and Korea. AGM are native to both countries.

Goals and objectives in Russia. The purpose of this collaborative research project is to:

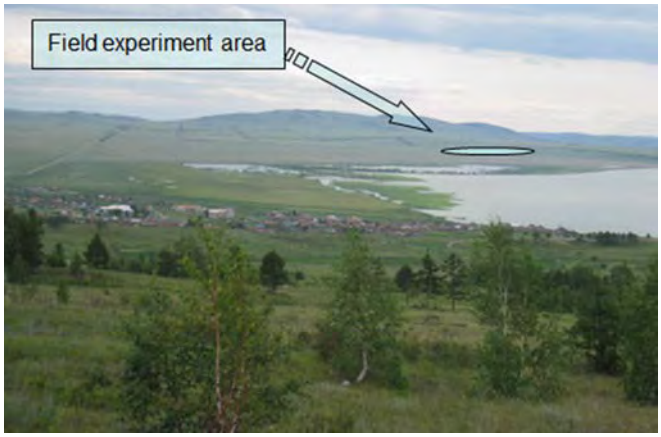
- Estimate in a direct field experiment the flight characteristics of the AGM female to lights
- Investigate the geographical origin of females, their weight, age and rearing density in female flight characteristics

Materials and methods. Studies were conducted in the forest steppe region of the Khakass Republic (Southern Siberia). There were no trees, but some steppe shrubs are occasionally present (**Fig. 1**). The valleys are unpopulated (no villages, camps or roads), so there were no light sources at night. Three groups of AGM were used:

1. Urals population 1: Pupae were collected in the field near the city of Kamensk-Uralskiy,
2. Trans-Urals population: larvae of the last instars were collected and were reared in the lab on leaves of *Betula pendula* till pupation,
3. Urals population 2: Egg masses collected from areas near Kamensk Uralskiy in previous fall and reared on the artificial diet.

Rearing was used to obtain insects from "dense" and "sparse" populations. Larvae from the 4th instar were

Figure 1: *Experimental area in Southern Siberia, Russia.*



reared individually and in groups of 10-20 per container. These pupae were preconditioned to the light cycle that they would experience in the field and were delivered to the field laboratory 2-3 days after pupation.

Lab experiments. One day after pupation, pupae were sexed and individual female pupae were weighted and kept in a separate container (vertical Petri dish) to let fresh emerged female moths to expand their wings. All females went through a "flip test" (Keena, 2009) one day after emergence.

Field experiment. The experiments on flight distance were set in a steppe in the period from sunset to 01:00 AM. A table with 6 vertical poles was placed in the bottom of a steppe valley as the flight starting points for AGM females. Each pair of poles had its own observer to document the insect flight behavior (**Fig. 2**). All manipulations at the starting point were made under red lights. Small gasoline power generators were used to power light sources. Females of known weights were individually marked.

Results. Average pupae weight of AGM female pupae collected in the field was 889 mg for Kamensk Uralskiy, and 714 mg for Chelyabinsk. The weight of female pupae reared on artificial diet was 1,090 mg for individual rearing, and 805 mg for group rearing.

Flip tests showed high flight predisposition in females of all four groups. The percentage of "non-flipped" females during the 30 minutes was 12%, 11%, 14% and 16% for Kamensk-Uralskiy, Chelyabinsk, "sparse" and "dense" populations respectively. Nearly 49%, 33%, 52% and 41% of females of these groups had flipped immediately. No correlation was found between female "flipping" possibility and their weights and origins.

Figure 2: *Set up of the AGM "Night Flight Test".*

Field experiments were conducted with lights for 5 nights. No females demonstrated straight flight. The vast majority of them did not leave the poles. There were only two cases of passive flight, in which females glided down to 1-2 m from the pole.

Subsequent "Pole" tests were conducted on the territory of field station before sunset. It was found that nearly half of females demonstrated active ascending flight in the evening (between 5 pm - 8 pm). No flights were recorded after sunset.

Preliminary conclusions.

1. The females of AGM population from all the territory of Russia from Urals to the Russian Far East have capabilities for active flight .
2. AGM females fly during first half of night in Western, South and Eastern Siberia, and at the Russian Far East. However, AGM females in Trans-Urals fly during the day.

Goals and objectives in Republic of Korea.

- Characterize female AGM mating and flight behavior in Korea provides us with a better understanding of when females mating, flight, and what they respond to for making better mitigation decisions.
- Determine the temperature dependent development of AGM
  - Eggs collected from nine different locations were tested to determine whether there is any differences among populations.
  - The test was conducted in three different degrees (15º, 20º, and 23º C), and 10 eggs (replicates) from each individual locations were used. More data is being collected.
- Dispersal of AGM larvae
  - Eggs were placed on trees under natural conditions in a net house and larvae were checked and recorded for their locations. The results indicate that a proportion of early instar larvae moved away from the tree they were placed on (**Table 1**).
- Determine the light preference and flight distance of released females.

Materials and methods. Pupae (>10 d old) were placed in the field in small protective cages to allow eclosion under natural conditions. Emerged females were marked with fluorescent ink on the dorsum of the thorax. The adult release site was devoid of light sources in the immediate vicinity. Released females were checked for their positions and behaviors hourly in

most cases. Once females begin to disperse, surrounding vegetation and light sources within 2 km (or longer) were regularly searched for the presence of marked females and egg masses subsequently during the female flight period.

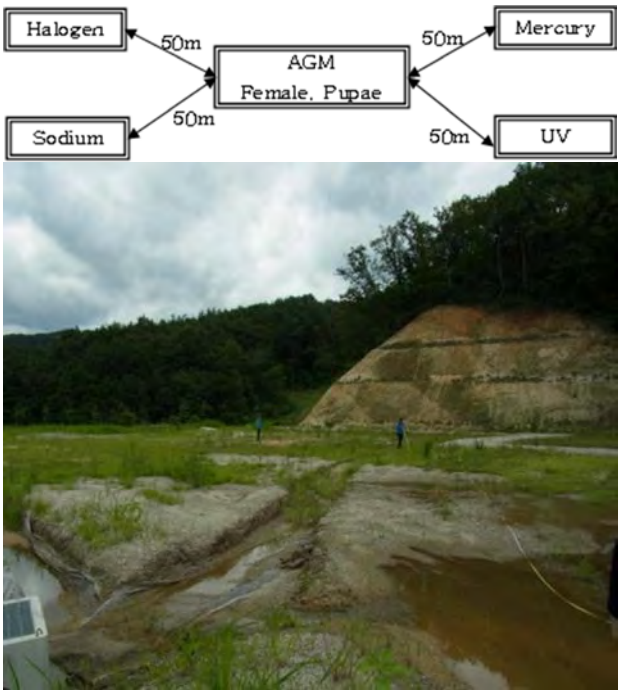
Table 1: The number (percentage) of AGM larvae at different locations (on August 20, 2011) in relation to the tree where eggs were placed.

Distance from tree (m)	No. Larvae
0 (on tree)	13 (36%)
>0 -2	3 (8%)
>2-4	5 (14%)
>4-6	1 (3%)
>6-8	4 (11%)
>8-10	6 (17%)
Not Found	4 (11%)

Pre-test trials were conducted 8 times from July 5-15 using fluorescent and UV light sources. It was determined that females are most active during 7:00 pm to 9:00 pm, and their activities are minimal after 10 pm. Among the 28 females that were tested in a corridor (the distance between the light source and the adult AGM release point was 62 m), only four moved further than 31 m.

Female light preference. Four different types of light sources (halogen, mercury, sodium, and UV) were placed 50 m from release point (**Fig. 3**).

Figure 3: Arrangement of light sources and landscape of the release site.



In the 4 tests with a total of 504 normally emerged female adults, only a few females flew to the tested light sources. Most of them flew to UV light, and a few to mercury light. No females were found at the Sodium and halogen light sources.

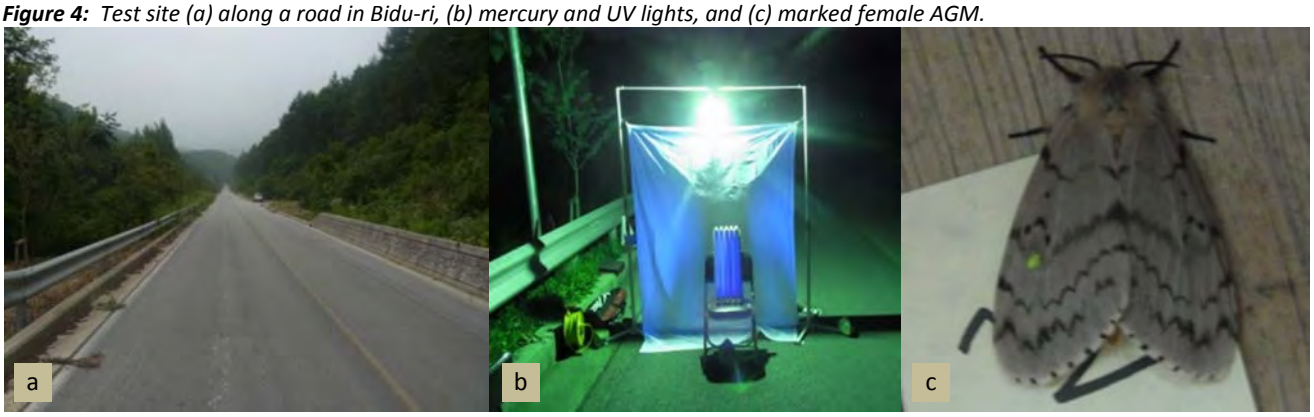
- Most females remained in the release cage during the testing period and they walked more than fanned wings or flew.
- A proportion (around 18%) of females did not emerge or emerged abnormally.
- No egg masses were found.

Female flight distance. The test was conducted along a road in Bidu-ri (**Fig. 4a**). The distance between female release points to the light source was 530 m (1st test), and 320 m (2nd test). Based on the results of the female light preference study, mercury and UV lights were used as the light sources (**Fig. 4b**). AGM females were marked with a dot on their forewings (**Fig. 4c**).

- Among the 105 females tested on the first test, only 23 flew, and 20 of the 100 females flew on the second test. However, most females flew short distances, and no marked AGM were found at the light traps.
- The only egg masses that were found in the area were laid by wild AGM females.

More females were tested in 2012 with similar settings, and data will be analyzed soon.

Figure 4: Test site (a) along a road in Bidu-ri, (b) mercury and UV lights, and (c) marked female AGM.



Comparing Electrophysiologically Determined Spectral Responses in Seven Sub-species of *Lymantria*

Project Lead: Damon Crook

Contributors: Emily Hibbard & Vic Mastro (Otis Laboratory)

Figure 1: *Pairs of female (left) and male (right)* Lymantria *moths.*

The Asian gypsy moth (AGM) is defined by the United States Department of Agriculture as "any biotype of *Lymantria dispar* (*sensu lato*) possessing female flight capability". Since 1991 there have been repeated incursions into North America by AGM. These incursions resulted mainly because gravid females were attracted to lights at Russian ports where they then deposited eggs on cargo and ships. These ships then headed to North America where the neonates hatched and dispersed. Controlling these incursions by state and federal emergency suppression efforts has cost many millions of dollars. Studying the visual capabilities of *Lymantria*, particularly the Asian varieties may help better understand their attraction to the lighting in ports and on ships. The main aim of this project was to make recommendations and modifications to lighting in ports and on ships to help reduce the risk of further incursions into other countries.

Studying the receptor sensitivity of an insect's visual system can help to evaluate the available stimuli that evoke a behavioral response. The simplest way to determine the spectral responses of a visual receptor system is to record the combined responses of several receptors, the electroretinogram (ERG). This information allows one to delimit the detectable range of colors to the insect being studied and lead to the possible evaluation of stimuli that might evoke a behavioral response. The aim of this years project was to survey the spectral responsiveness of several *Lymantria* using electrophysiological methods (ERG) and compare the results with reported behavioral responses.

Insects. Moths were reared at the USDA insect containment facility at the Otis Laboratory, Buzzard's Bay, MA (**Fig. 1**). Moths were laboratory-reared from egg masses collected from several different regions (**Table 1**). The ERG system used in this study (similar to that described by Crook[(1)]) consisted of a lamp that delivered light stimuli to the compound eye of a moth via a monochromator, liquid light guide cable and focusing lens.

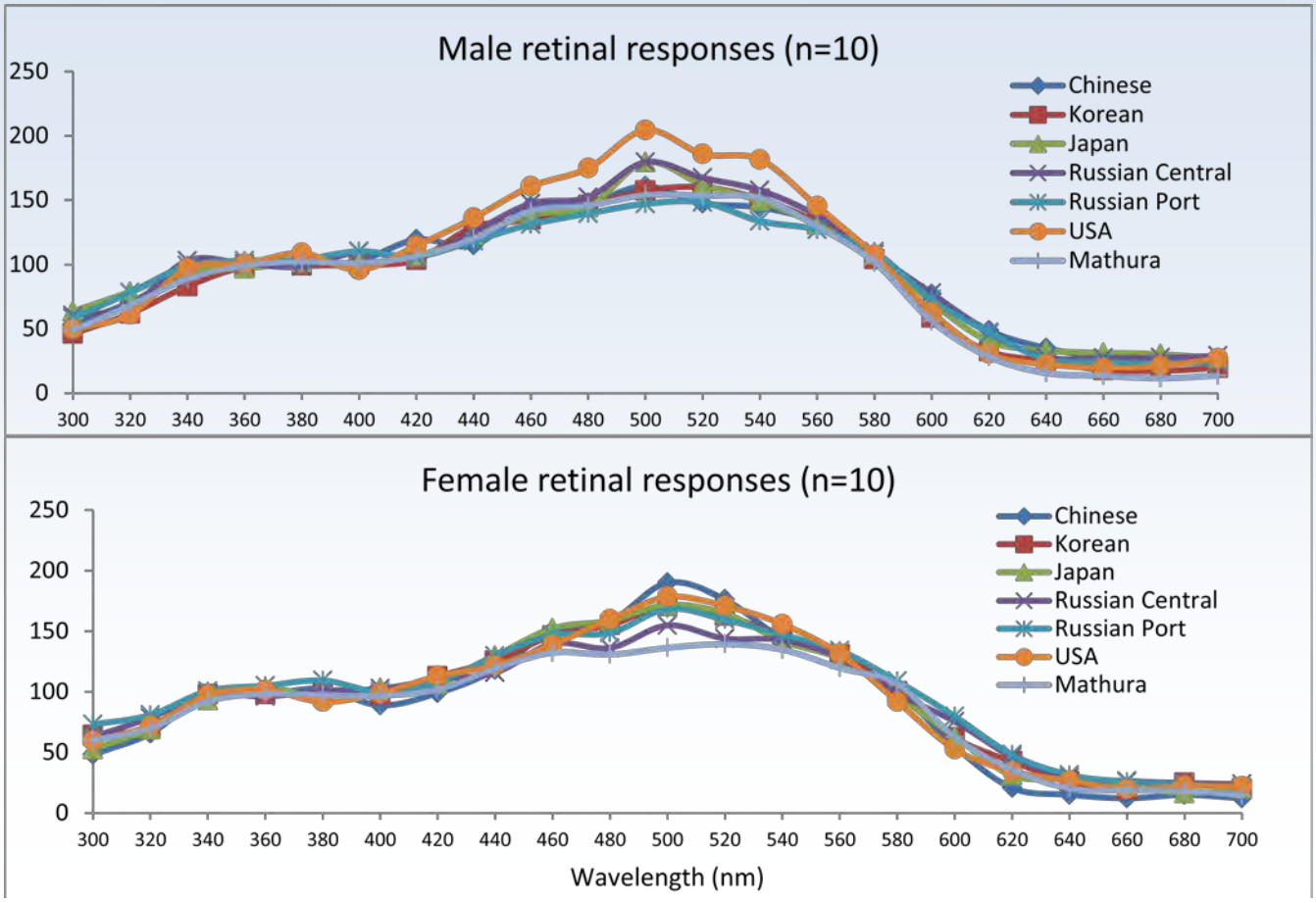
Two sets of 'flash stimulation runs' were done for each sex, of each moth species tested. The first test involved stimulating the insect with wavelengths between 300 and 700 nm in increments of 10 nm, presented randomly to the insect. A reference wavelength of 360 nm was flashed onto the moth preparation every four stimulations so data could be normalized against it later. This took into consideration the possible reduction (or in some cases, improvement) in responses over time as the insect life decayed.

The second test randomly stimulated the insect with wavelengths between 300 nm and 420 nm in increments of 5 nm. A reference wavelength of 600 nm was flashed onto the moth preparation, after four random stimulations (for data normalization).

Table 1: *Moth species and regions collected.*

Source	Acronym	Species
Chinese	AGM	*Lymantria dispar asiatica*
Korean	AGM	*Lymantria dispar asiatica*
Japanese	JGM	*Lymantria dispar japonica*
Russian Central	AGM	*Lymantria dispar asiatica*
Russian Port	AGM	*Lymantria dispar asiatica*
North American	NAGM	*Lymantria dispar dispar*
Rosy 'Gypsy' Moth	RGM	*Lymantria mathura*

Figure 2: *Male (top) and female (bottom) retinal responses of seven Lymantria* subspecies to light wavelengths between 300-700 nm.



The entire insect preparation (n = 10 for each sex, for each moth species) was allowed to adapt to total darkness for at least 10 min before a spectral sensitivity run commenced. The insect remained in total darkness between stimulations and was screened off from any external light sources in the room so as to minimize interference.

Results. All moth subspecies showed two main peaks in sensitivity between 300-700 nm (**Fig. 2**). A small peak in sensitivity was seen in the UV region at around 360 nm. A larger second peak in sensitivity was seen at around 500 nm (blue-green region). No distinct response to red was found. This supports retinogram data taken for *Lymantria dispar* in a study by Brown and Cameron[(2)]. All subspecies showed a similar trend in their visual capabilities. There were no obvious differences between male and female responses for each subspecies tested.

When all moth subspecies (except *L. mathura*) were screened between 300-420 nm (in 5 nm increments) we found responses to plateau at around 360 nm. Ports should try and avoid using lighting systems / bulbs that have a UV output of around 360 nm. Once the lab work is fully analyzed we hope to make recommendations on what lighting systems to avoid using around docks to help lower the risk of transportation to the USA. These results may also help improve light trap detections of the moth.

References

[(1)] Crook, D. J., Francese, J. A., Zylztra, K. E., Fraser, I., Sawyer, A. J., Bartels, D. W., Lance, D. R., and Mastro, V. C. 2009. Laboratory and field response of the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae), to selected regions of the electromagnetic spectrum. *J. Econ. Entomol*. 102:2160–2169.

[(2)] Brown, E. A. and A. E. Cameron. 1977. Studies of the compound eye of *Lymantria dispar* (Lepidoptera: Lymantriidae) males, and behavioral implications. *Can. Entomol*. 109: 255-260.

Molecular Analysis of Gypsy Moths Trapped or Intercepted in Ports and Other High-Risk Areas in the U.S.

Project Lead: John Molongoski

Contributors: Vic Mastro , Deborah Winograd, and Dyanna Louyakis (Otis Laboratory)

Lymantria dispar is a moth in the family Lymantriidae. Three subspecies of *Lymantria dispar* are recognized: *L. d. dispar* (L) or European gypsy moth, *L. d. asiatica* (Vnukovskij), and *L. d. japonica* (Motschulsky). For U.S. regulatory purposes, the latter two subspecies are considered Asian gypsy moths (AGM). *L. d. asiatica* is found in temperate Asia from the Ural Mountains east to China, Korea, and Far East Russia. *L. d. japonica* is found on several Japanese Islands including Honshu, Shikoku, Kyushu, and parts of Hokkaido. Unlike female gypsy moths of the North American strain, those of the Asian strain can fly great distances. In addition, Asian gypsy moths (**Fig. 1**) have a much greater host range than do North American gypsy moths and thus present a far greater threat to deciduous tree species if they should become established in the United States. Since 1992, adult gypsy moths trapped in U.S. ports during the annual moth flight season, as well as gypsy moth eggs, larvae, or pupae intercepted on vessels and containers at U.S. ports, have been sent to the Otis CHPST Laboratory for molecular analysis in order to determine whether the intercepted samples are of the Asian strain.

Two genetic markers are used to assess the genotype of submitted gypsy moth specimens: a nuclear marker designated FS1[(1)], and a mitochondrial marker utilized in conjunction with the restriction enzymes NlaIII and BamHI[(2)], respectively. Two alleles or variations occur at the FS1 loci, a 207 bp band designated as North American (NA) and a 312 bp band designated as Asian (A). As gypsy moths are diploid organisms, each moth contains two copies of each autosomal (non-sex) chromosome. The two copies or alleles of FS1 can be identical (homozygous) or different (heterozygous) in a given specimen. Thus, three combinations are possible: a moth can be homozygous North American (NA) possessing two copies of the North American allele, homozygous Asian (A) containing two copies of the Asian allele, or heterozygous (H) containing one copy each of the North American and Asian allele. As the designation implies, the North American FS1 allele is the allele most frequently found in gypsy moths from North America while the Asian allele is the only FS1 allele present in moths from far east Asia. The Asian allele is, however, also detected in the U.S. population at a low percentage (approximately 3-6% depending upon geographic location in the continental U.S.). Each of the FS1 alleles, on the other hand, is present in high abundance in gypsy moths from Europe and Western Russia.

Figure 1: *Male (left) and female (right) adult Asian gypsy moths.*

The mitochondrial marker is characterized by four possible haplotypes or variations defined by restriction fragment length polymorphisms (RFLP). After completion of the mitochondrial PCR, the amplified DNA fragment is incubated in the presence of the restriction enzymes NlaIII and BamHI, respectively.

For the North American haplotype (NA), neither of the enzymes digest or cut the amplified DNA (Nla and Bam negative). For the A1 haplotype (commonly found in moths originating in Europe and Western to Central Russia, but in recent years also detected in Asian populations), NlaIII cuts the DNA fragment into two pieces (Nla positive), but BamHI does not cut the DNA fragment (Bam negative). For the A2 haplotype (common to far east Russia and far east Asia), both enzymes cut the DNA fragment (Nla and Bam positive). The fourth possible haplotype (Nla negative and Bam positive) has been detected in only a very few moths from China and is extremely rare. This haplotype is designated A3.

Similar to the FS1 Asian allele, the A1 mitochondrial haplotype is also found in the US population at a low percentage. In both cases, the exact percentage varies depending upon location within the U.S. It is important to keep in mind that these designations for the nuclear FS1 marker and the mitochondrial marker are not meant to indicate the evolutionary origin of the moths tested, but rather implies the geographic region where they are found in

Table 1: *Possible gypsy moth haplotypes after analysis with FS1 and mitochondrial markers.*

Band Size (bp) Produced in FS1 PCR	FS1 Designation	Band Size (bp) Produced in Mitochondrial RFLP	Mitochondrial DNA RFLP Data		Mitochondrial DNA Haplotype	Distribution (Where found most frequently)
207	NA	500	Nla-	Bam-	NA	North America
207	NA	350/150	Nla+	Bam-	A1	Europe > North America
207	NA	300/150/50	Nla+	Bam+	A2	Very rare: Austria,Slovak Rep, Tunisia
207	NA	450/50	Nla-	Bam+	A3	Very rare, 1 moth in Delaware (2009)
207/312	H	500	Nla-	Bam-	NA	North America
207/312	H	350/150	Nla+	Bam-	A1	Europe > North America
207/312	H	300/150/50	Nla+	Bam+	A2	Rare: Austria, China, Korea, Tunisia
207/312	H	450/50	Nla-	Bam+	A3	Not found to date
312	A	500	Nla-	Bam-	NA	North America
312	A	350/150	Nla+	Bam-	A1	Europe > Asia
312	A	300/150/50	Nla+	Bam+	A2	Asia
312	A	450/50	Nla-	Bam+	A3	Rare: China, Japan

the highest abundance. The possible haplotypes that can result after a specimen is analyzed with both the FS1 and mitochondrial markers are summarized in **Table 1**.

The states from which gypsy moth life forms were submitted to the Otis Laboratory for molecular analysis in 2011 are shown in **Fig. 2** (shaded). In addition, the figure also shows the U.S. ports from which presumptive gypsy moth egg masses were intercepted from arriving vessels and containers/cargo, and the number of egg masses found in each port.

Table 2: *Eastern region states submitting gypsy moths to the Otis CHPST Laboratory for genetic analysis in 2011.*

State	No. Traps Received	No. Adults Received	No. Egg Masses Received
Alabama	1	1	
Connecticut	81	>1000	
Delaware	27	>1000	
Florida		1	2
Georgia			1
Illinois	17	55	
Indiana	9	41	
Maine	12	>1000	
Maryland	3	15	1
Massachusetts	49	>1000	
Michigan	12	97	1
Minnesota	57	148	
New Jersey	110	>1000	
New York	7	19	
North Carolina	8	23	
Pennsylvania	7	117	
Rhode Island	67	>1000	
Virginia	13	261	
West Virginia	16	139	
Wisconsin	128	>1000	
TOTAL	**624**		**5**

SUMMARY OF 2011 EASTERN REGION GYPSY MOTH SUBMISSIONS

In 2011, presumptive adult gypsy moths collected in ports and other sensitive areas were received from 20 states in the Eastern Region (**Table 2**). Egg masses submitted for identification were received from 4 states (Florida, Georgia, Maryland, and Michigan). A total of 624 traps and 4 egg masses were received.

No pure strain Asian gypsy moths were detected in the Eastern Region. In general, results from 2011 Eastern Region submissions were typical for the established gypsy moth population found in the generally infested area with the possible exception of two states (North Carolina and Minnesota). In certain areas of these two states, the percentage of occurrence of the FS1 "Asian allele" (A) is higher than in the rest of the generally infested area. This was true of gypsy moths analyzed from these same areas in 2010 as well.

SUMMARY OF 2011 WESTERN REGION GYPSY MOTH SUBMISSIONS

In 2011, various moth life stages (adults, pupae, larvae, and egg masses) intercepted in 10 states in the Western Region were submitted to the Otis CPHST Laboratory for genotype analysis (**Table 3**). A total of 368 traps were received containing approximately 502 adult moths. The majority of adult gypsy moths received were from Iowa. In addition, 92 egg masses, 1 pupa,

Figure 2: States(shaded) from which gypsy moths were analyzed in 2011 and intercept port locations showing the number of intercepted egg masses submitted for analysis from each port.

and 1 larva were received from interceptions on 41 vessels or containers in ports in California, Louisiana, Oregon, Texas, and Washington, respectively.

As the majority of the Western Region lies outside the generally infested gypsy moth area, all of the specimens received from these locations were analyzed with the exception of the samples from Iowa (due to the large number of moths received). A brief state by state summary of 2011 results for the Western Region is as follows.

California: One adult female gypsy moth and 17 gypsy moth egg masses were intercepted in the state of California in 2011, all from the vessel Aranda Colossus in Long Beach. All samples tested proved to be Asian gypsy moth (AGM).

Iowa: Three hundred forty five traps containing approximately 475 adult gypsy moths were received from Iowa in 2011. Approximately one third of the specimens were analyzed and have been found to be North American gypsy moths.

Louisiana: Ten separate egg masses were intercepted from 8 different vessels in New Orleans, LA during 2011. Eight of the egg masses were determined to be AGM, one was found to be an unidentified Lepidoptera sp., and the final egg mass could not be identified because of the lack of DNA amplification.

Missouri: Six gypsy moths were received from locations in Missouri during 2011. Each moth tested North American.

Montana: One North American gypsy moth was trapped in Montana.

Nebraska: One North American gypsy moth was trapped in Nebraska.

Oregon: Seventeen egg masses were intercepted on 2 vessels in Portland, Oregon during 2011. None of these were gypsy moth. Sixteen found on the vessel Santiago Express were determined to be an unidentified Lepidoptera species. The final egg mass, from the vessel Susaki Wing, could not be identified as it was in very poor condition and no DNA amplification could be achieved.

South Dakota: One North American gypsy moth was trapped in South Dakota.

Texas: Forty four egg masses and 2 adult moths were intercepted off 26 different vessels in 4 Texas ports (Houston, Brownsville, Port Arthur, and Corpus Christi) during 2011. Nineteen of the egg masses were determined to be Asian gypsy moth (AGM) while 19 additional egg masses were not gypsy moth, but were determined by DNA sequencing to likely be some species of Lepidoptera. Six egg masses did not amplify DNA when PCR was attempted and remain unidentifiable. Neither of the 2 adult moths tested were gypsy moth. One was determined by sequencing to be either *Orgyia definita* or *O. antiqua*. The second adult is awaiting DNA sequencing for further identification.

Washington: Fifteen adult gypsy moths and 1 larva were received from the Washington State Department of Agriculture. All 16 specimens tested North American. Four egg masses were collected from 3 vessels inspected in Washington during 2011, but none were gypsy moth. Two egg masses found on the vessel Iolite in Seattle were DNA sequenced and were presumptively identified as either *Euproctis similis* or an unidentified Lepidoptera species. One egg mass found on the vessel Mol Pace V/36E in Seattle also was identified as an unidentified Lepidoptera species. The final egg mass found on the vessel Zaliv Amerika in Blaine, WA was not gypsy moth and was identified by DNA sequencing as *Leucoma salicis*. Finally, a dead and desiccated pupa found on the vessel Csav Brasilia in Seattle was too degraded to obtain any DNA amplification and thus could not be identified.

Table 3: *Western region states submitting gypsy moths to the Otis CHPST Laboratory for genetic analysis in 2011.*

State	No. Traps Received	No. Adults Received	No. Pupae or Larvae	No. Egg Masses
California		1		17
Iowa	345	475		
Louisiana				10
Missouri	6	6		
Montana	1	1		
Nebraska	1	1		
Oregon				17
South Dakota	1	1		
Texas		2		46
Washington	14	15	1 each	4
TOTAL	**368**	**502**	**2**	**94**

Figure 3: *Asian gypsy moth egg mass typical of those intercepted at U.S. Ports.*

SUMMARY OF 2011 U.S. PORT EGG MASS INTERCEPTIONS

The interception and identification of Asian gypsy moth egg masses (**Fig. 3**) entering U.S. ports via vessels and/or cargo and containers is of great interest to the AGM Program and represents a primary line of defense against the introduction of the Asian strain of gypsy moth into the continental United States.

In 2011, egg masses were intercepted in the Eastern Region ports of Brunswick, GA, Baltimore, MD, Tampa, FL, and Jacksonville, FL (1 egg mass from each port). Only the intercept from Jacksonville was determined to be Asian gypsy moth (AGM). The majority of the egg masses received for identification originated in Western Region ports. The specific ports and number of egg masses received from each were as follows: Portland, OR (17), Seattle, WA (3 EM's and 1 pupa), Blaine, WA (1), Long Beach, CA (17), and New Orleans, LA (10). By far, the majority of the egg masses intercepted in 2011 (approximately 40%) were from ports in Texas: Houston (31), Brownsville (8), Port Arthur (5), and Corpus Christi (2). In total, 99 egg masses were analyzed: 47 (47.5%) were identified as Asian gypsy moth, 1 (1.0%) was North American gypsy moth, 44 (44.4%) were not gypsy moth or any other Lymantria species, but could only be categorized as an unknown Lepidoptera sp. or otherwise unidentifiable specimen, and 7 (7.1%) did not amplify via PCR likely due to the degraded condition of these samples.

References

(1) Garner, K.J., and J.M. Slavicek. (1996). Identification and Characterization of a RAPD-PCR marker for Distinguishing Asian and North American Gypsy Moths. Insect Molecular Biology. 5: 81-91.

(2) Bogdanowicz, S.M.,W.E. Wallner, J. Bell, T.M. Odell, and R.G. Harrison (1993). Asian Gypsy Moths (Lepidoptera: Lymantriidae) in North America: Evidence from Molecular Data. Ann. Entomol. Soc. Am. 86: 710-715.

Development of a Global Gypsy Moth Microsatellite Database

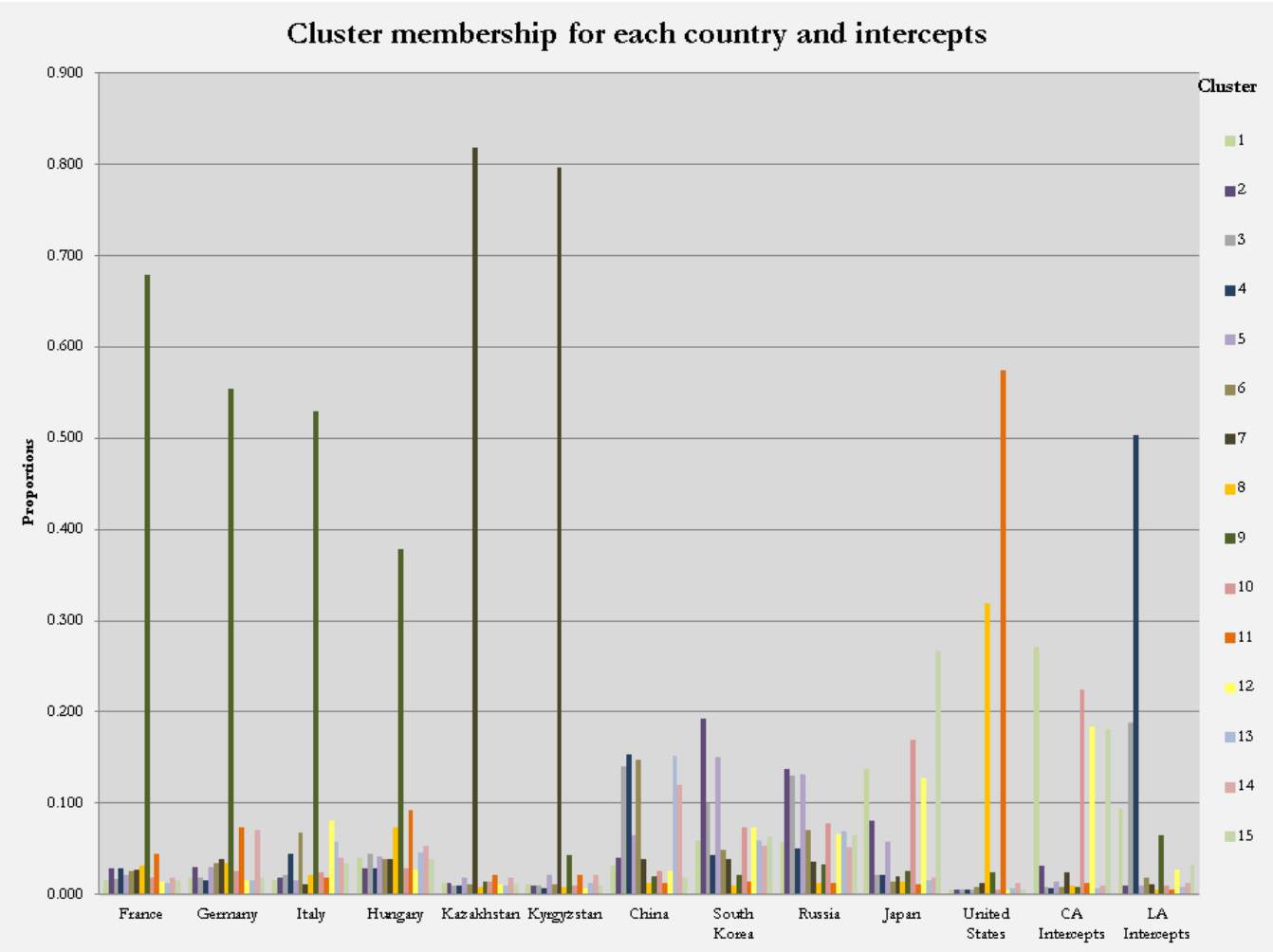
Project Lead: John Molongoski

Contributors: Vic Mastro , Deborah Winograd, and Dyanna Louyakis (Otis Laboratory)

In order to determine the likely origin of gypsy moth egg masses intercepted at U.S. ports with greater probability, microsatellite markers[(1)] have been evaluated in an effort to increase the ability to predict the source population or geographic origin of the egg mass. Microsatellites, also termed short tandem repeats or STR's, are tandemly-repeated DNA sequences having repeat unit lengths of 2-4 base pairs (for example, [GTT]n, [AT]n or similar). Their total length varies due to increases or decreases in the number of repeats found in different GM populations. In order for microsatellite markers to be of maximum utility in determining source populations, a microsatellite database or library for gypsy moths worldwide has been constructed. Gypsy moths collected in four Asian countries (China, Japan, Korea, Far East Russia), in four European countries (France, Germany, Hungary, Italy), in Central Asia (Kazakhstan and Kyrgyzstan), and in the continental United States (Connecticut, Michigan, Minnesota, New Jersey, and West Virginia) have been screened with 9 different microsatellite markers. Approximately 1800 gypsy moths distributed worldwide have been examined.

The data generated using multiple microsatellite markers will allow us to determine multi-locus genotypes for individual moths in order to determine whether the suspect moth is a recent Asian introduction into the

Figure 1: *Cluster membership of gypsy moth specimens from across the globe determined with the population analysis software STRUCTURE.*



continental United States. We have just begun to investigate worldwide gypsy moth populations using a software package termed STRUCTURE. This program implements a model-based clustering method for inferring population structure through the use of genotype data[(2)]. The software allows investigators to determine population structure, identify distinct genetic populations, and assign individuals to populations. **Fig. 1** shows the results of our preliminary calculations using STRUCTURE to identify genetically homogenous groups of gypsy moths, termed clusters, in the populations noted above, as well as for a set of selected egg masses intercepted from ports in California and Louisiana respectively during 2011.

As shown in **Fig. 1**, the global population of gypsy moths examined falls into four major geographic populations. Moving from West to East, the European population represented by France, Germany, Italy, and Hungary is distinct from the Central Asian population (Kazakhstan and Kyrgyzstan), which in turn is quite different from Far East Asia (China, Korea, Russia). Japan is also quite distinctive differing from the other Far Eastern countries. As seen in **Fig. 1**, the United States population is unlike that found elsewhere in the world. Finally, STRUCTURE analysis was applied to a group of gypsy moth egg masses intercepted in Long Beach, CA and in New Orleans, LA respectively in 2011 (**Fig. 1**). These data suggest that the California intercepts possibly originated in Japan while those intercepted in Louisiana may have their origin either in China, South Korea, or far east Russia.

Future efforts on this project will include expanding the global gypsy moth microsatellite database to include additional countries and geographic locations and testing additional recently developed microsatellite markers. In addition, the STRUCTURE analysis will be refined such that future gypsy moth intercepts in the United States can more easily be compared to the global microsatellite database and a determination of their probable origin be made with greater confidence.

References

(1) Bogdanowicz, S.M., V.C. Mastro, D.C. Prasher, and R.G. Harrison. (1997). Microsatellite DNA variation among Asian and North American gypsy moths (Lepidoptera: Lymantriidae). Annals of the Entomological Society of America. 90: 768-775.

(2) Pritchard, J.K., P. Stephens, and P. Donnelly. (2000). Inference of Population Structure using Multilocus Genotype Data. Genetics. 155: 945-959

Gypcheck Virus Production for US Forest Service

Project Lead: Hannah Nadel

Contributors: Sue Lane, Hannah Landers, Chris Lokerson, and Alyssa Pierce (Otis Laboratory)

Otis Laboratory has had long-standing annual interagency agreements with the US Forest Service to grow nucleopolyhedrovirus (NPV) for the production of Gypcheck, a bioinsecticide specific to gypsy moth, *Lymantria dispar* (L). In recent years, the typical amount supplied to USFS has ranged between 3,000 to 6,000 acre equivalents of Gypcheck. In 2011 the production target was between 3,000 and 4,000 acre equivalents.

To produce Gypcheck, gypsy moth larvae are reared at Otis on artificial diet, inoculated with NPV during the 4th instar, and harvested when over 75% of larvae are moribund or dead (**Fig. 1**). The cadavers are collected in plastic bags and stored at -20°C for twice-yearly pickup and transport by FS personnel to the USFS Laboratory in Hamden, CT, where the virus is isolated and frozen as a powder for eventual formulation as Gypcheck (development of the NPV production system is described in Podgwaite, 1981). The source of larvae used for NPV production is the gypsy moth colony, which numbered about 220,000 adults in 2011.

Figure 1: *Harvesting gypsy moth larvae infected with NPV for Gypcheck production.*

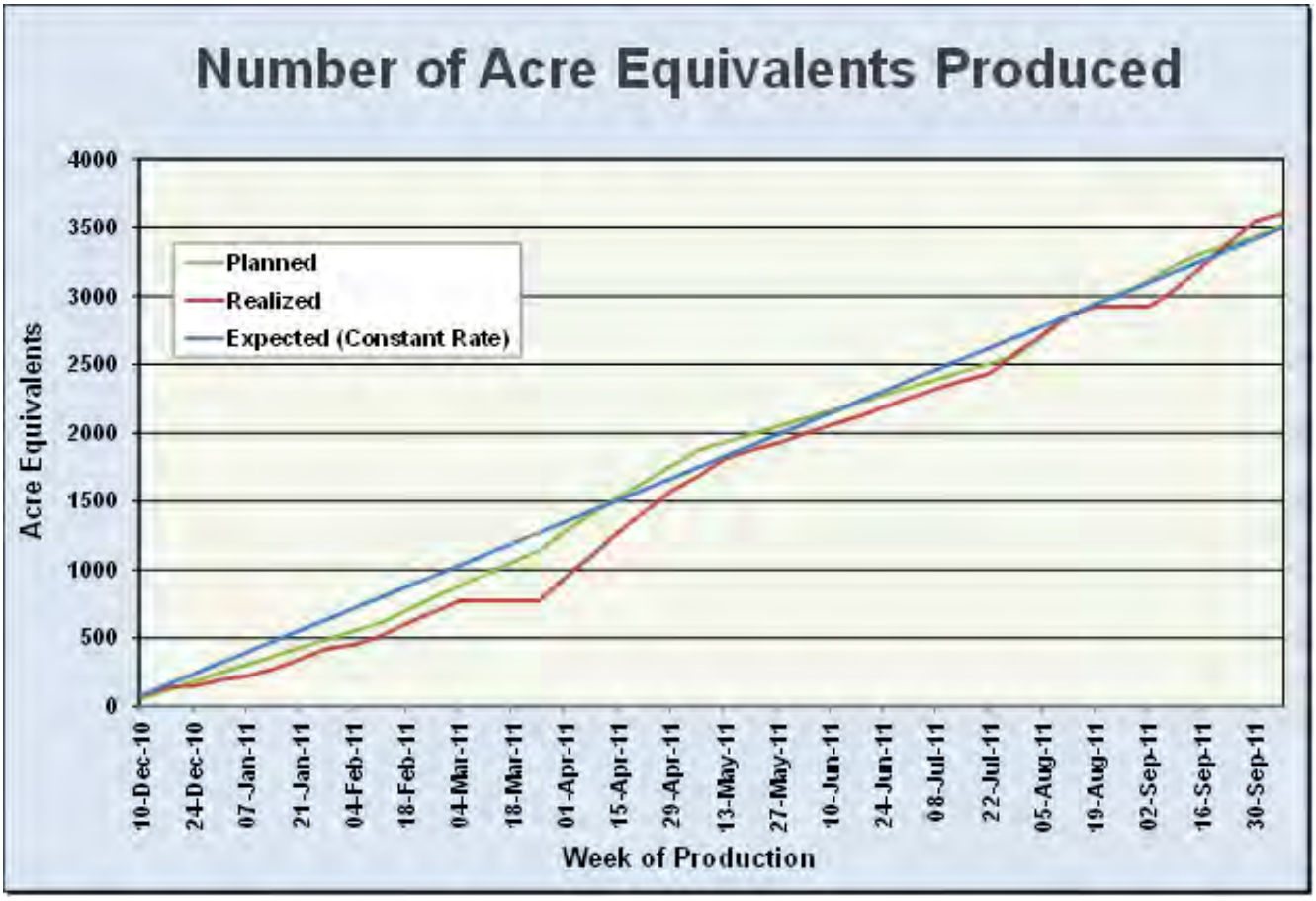
About 700 infected, late-instar gypsy moth cadavers are used to produce an acre equivalent of Gypcheck (John Podgwaite, USFS, Hamden, CT, personal communication). Annual NPV production planning is based on this figure, alongside costs of labor and supplies and the amount of expendable funding provided. In 2011 the virus rearing team consisted of three full time employees, one of whom was paid by funds provided through the agreement, with planning, oversight, supply orders and troubleshooting by a technician and the project leader. The technician also provided quality assurance with the part-time assistance of an additional technician.

NPV production for FY 2011 achieved a target of 3,500 acre equivalents (**Fig. 2**) with 2,450,000 reared infected larvae. A break in funding during March necessitated a shutdown of production for three weeks but the shortfall was recovered by the end of the fiscal year.

Reference

[1] Podgwaite, J.D. 1981. NPV production and quality control. Pp. 460-467. *In*: C.C. Doane and M.L. McManus (eds.). The gypsy moth: research toward integrated pest management. US Department of Agriculture, Washington, DC.

Figure 2: *Weekly planned and achieved Gypcheck acre equivalents produced in 2011. The expected rate is hypothetical, free of deviations caused by unforeseen problems or other sources of change. The planned rate includes expected shifts in labor allocation to other tasks and shutdowns for cleaning. The realized rate reflects slowed production due to unforeseen problems, planned allocation of labor to other tasks, and increased production to compensate for slowdowns.*



A Rearing System for European Grapevine Moth at the Otis Insect Containment Facility

Project Lead: Hannah Nadel

Contributors: Tanya Dockray and Susan Lane

1. Introduction. In the fall of 2009, after 13 years of surveillance for the European grapevine moth (EGVM), *Lobesia botrana*, throughout the United States, the moth was detected in vineyards in Napa County, California, and was reported in nine other counties in the state [(1)]. Originally from Europe, this tortricid moth has spread to the Mediterranean Basin, the Near East, eastern Africa, southern Russia, Japan, Chile, and Argentina [(2)]. Its distribution in California is shrinking due to mitigation efforts and was confined in 2011 to Napa, Solano, and Sonoma Counties [(1)]. In addition to the need to establish quarantine zones around invasion areas and to deploy integrated pest management practices, a need was recognized to develop regulatory treatments against the moth in grape commodities. Studies on fumigation and cold treatments were planned at CPHST Otis Laboratory starting in summer 2010, spurring the need to quickly establish a production colony of EGVM at the Otis Insect Containment Facility to supply sufficient numbers of insects for probit-9 analyses. A medium-scale rearing system was developed and studies were made to facilitate scaling-up in the event that the need arose for large-scale mass production.

About 1,000 diapausing pupae were collected on grapevines in California in early February 2010 to start the colony. An initial output target of 5,000 experimental insects per week for commodity treatment studies was set in April, with studies to begin as soon as possible. We aimed for a rearing system based on artificial diet, which is ideal for mass production and was successfully used for EGVM in the past [(3)]. To gather information to apply toward development of the rearing system, several biological parameters were recorded on the field-collected parental generation. Performance of F_1 larvae was tested on four available diets to determine which diets were most promising for the EGVM colony. As larval containers and adult oviposition cages were being developed, larval crowding was found to negatively affect survival and prompted us to seek ways to minimize its effects without increasing container size. Wax paper was selected as an oviposition substrate that was suitable for both the colony and for commodity treatment studies. Although some earlier workers assumed that water sufficed as an adult diet to achieve maximum egg production by EGVM [(4)], we compared performance of adults with various provisions to confirm this assumption in our rearing system. A full time technician was hired in May 2010 to rear EGVM and further develop rearing methods. This report describes the rearing system in use at the end of 2011 and some of the conclusions made during its development, and summarizes the history of colony productivity.

2. Rearing system development.

2.1. Rearing environment. Colony and research insects were held in a walk-in environmental chamber inside the Otis Insect Containment Facility at 25 ±2°C, 65 ±3% RH, and 16L:8D. Photoperiod included 1 hr of lower light intensity before scotophase to simulate dusk.

2.2. Parental generation.

2.2.1. *Biology and termination of diapause*. Of the 1009 diapausing pupae collected on February 3, 2010 in Oakville, Napa Co., California, 240 were sexed, weighed, and isolated in tubes for emergence. Emergence was also recorded for additional males that were not weighed. According to Deseő et al. [(5)], a photoperiod exceeding 13 hr is needed to break diapause in EGVM. Pupae were observed daily for emergence to confirm termination of diapause and to

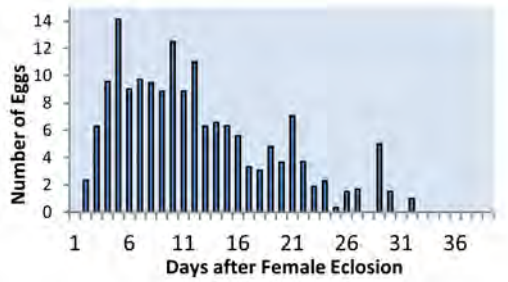
Table 1: *Comparison of biological parameters of the field-collected parental generation with and without food and mating opportunity. Fecundity studies were made with one female and two males placed in a mating cage and provided with a honey-water solution (grouped, fed adults), whereas unfed adults had no mating opportunity and no provisions.*

Sex	Days from Collection to Emergence	Weight (mg) of Live Pupae	Longevity of Grouped, Fed Adult (Days)	Longevity of Isolated, Unfed Adult (Days)	Number of Eggs per Female	Range of Eggs per Female
Male	24.7 (n=186)	6.4 (n=125)	28.3 (n=68)	12.5 (n=42)	--	--
Female	26.7 (n=95)	7.5 (n=100)	20.6 (n=28)	16.1 (n=58)	143.4 (n=26)	59 – 259

record date of adult death. Newly emerged females were each grouped with two males in oviposition cages and provided with a 10% solution of honey in water. Egg production and mortality were recorded daily.

As expected, diapause was terminated under the 16-hr photoperiod. Adults emerged from over 88% of pupae, and males tended to emerge a few days earlier than females. Females weighed more than males. Adults with access to honey-water lived longer than unfed adults, but, interestingly, although unfed females lived longer than unfed males, the reverse occurred in moths with access to honey-water. Note, however, that females with access to honey-water also had access to mates and usually laid eggs, which may have caused them to senesce earlier than their male counterparts. From a rearing perspective it was useful to note that a source of water and carbohydrate increased longevity compared with no food or water and that potential egg output with such provisions could reach about 260 per female. Laboratory-reared generations were later found to have higher pupal weights and egg output.

Figure 1: *Average daily egg output of the parental generation (n=26).*



Daily oviposition records showed that egg-laying was possible on the second day after eclosion but more commonly began on the third day (**Fig. 1**). About 70% of eggs were laid within the first 15 days, and 80%, 90% and 100% were laid by 19, 22, and 32 days, respectively. Based on these data we initially planned to hold colony adults in egging cages for 14 days before cycling them out of production.

2.2.2. *Parasitism*. Parasitoids emerged from about 1% of field-collected pupae and were sent for identification to John Luhman (retired, MN Department of Agriculture / University of Minnesota Insect Collection). He identified the following species: Ichneumonidae, *Pimpla hesperus* (Townes); Chalcididae, *Conura side* (Walker); Pteromalidae, *Dibrachys cavus* (Walker); and Eupelmidae, *Arachnophaga* (*Idoleupelmus*) sp.

2.3. Larval Rearing

2.3.1. *Larval diet selection*. To speed up diet selection four diets were chosen for comparison that were either already available at Otis Lab or were known to be suitable to EGVM or other tortricids. The first was a locally made diet regularly used at Otis Lab for gypsy moth (*Lymantria dispar* [Lymantriidae]) (GM) production. The second was diet for pink bollworm (*Pectinophora gossypiella* [Gelechiidae]) (PBW) made at the CPHST Pink Bollworm Lab in Phoenix, AZ and shipped to Otis Lab for rearing light brown apple moth, *Epiphyas postvittana* (Tortricidae) (LBAM). The third and fourth were Stonefly Heliothis Diet (Ward's Natural Science, Rochester, NY) (SH), which was successfully used for rearing EGVM in Israel, and a bean-based diet that was used but abandoned for rearing of LBAM at Otis Laboratory; it was originally developed for *Cryptophlebia* [(6)].

Survival and development rate of isolated F_1 individuals from egg to adult was observed in 1.5 ml Eppendorf tubes containing 1.0 ml of diet. A pinhole was punched in the lid for ventilation and a single egg was inserted a day before hatch. Instars were determined by the number of head capsules shed by larvae. Pupae were removed from cocoons, sexed, and weighed.

Performance of EGVM on the four diets was not consistently superior or poor across the measured parameters. However, the SH and GM diets appeared to be the most favorable in terms of survival, weight (**Table 2**), and development rate (**Fig 2**). PBW diet produced relatively heavy pupae but had poor survival and relatively slow development. The LBAM diet had high survival but low pupa weight and slow development. Males developed about 2.0 and 2.5 days slower on LBAM diet relative to GM and SH diets, respectively. Although females also tended to develop more slowly on LBAM diet, the differences among diets were not statistically significant. We therefore initially selected GM diet for rearing EGVM. LBAM and PBW diets were eliminated based on performance, while SH diet was eliminated based on cost comparison with GM diet. Materials cost per kilogram of dry diet was roughly

Table 2: *Average survival to adult and pupa weights (±SE) of F_1 EGVM reared individually on four diets.*

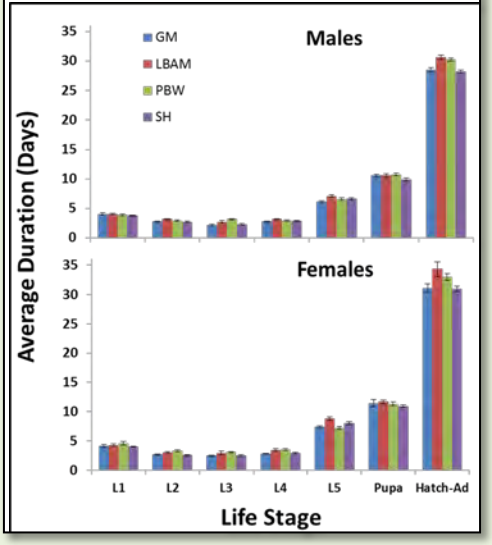
Diet*	Larvae (n)	Survival to Adult (%)	Female Pupa Weight (mg)	Male Pupa Weight (mg)
GM	44	91.7	14.4±0.33a	10.2±0.27a
PBW	45	80.4	13.7±0.68ab	9.5±0.45a
SH	50	98.0	13.4±0.35ab	9.8±0.37a
LBAM	45	97.8	12.0±0.45b	8.4±0.35b

*Abbreviations for diets are as follows: GM = gypsy moth; PBW = pink bollworm; SH = Stonefly Heliothis; LBAM = bean-based diet (Sinclair 1974). Different letters in the same column denote significant differences at α = 0.05 (ANOVA followed by Tukey-Kramer HSD for means comparison).

four times higher for SH diet than GM diet.

GM diet was initially adequate for EGVM production but later proved problematic due to its susceptibility to mold growth. Within a few months the incidence of heavy mold growth in rearing containers reached an unacceptable level with regards to potential effects on worker health and diet uniformity. Preliminary trials of SH diet indicated that it was similarly susceptible to mold growth. During this time and in the same room, a colony of LBAM was being reared on PBW diet with little or no apparent mold growth. Despite this diet's poorer survival rating during the initial comparison of four diets, we attempted to rear EGVM on PBW diet in rearing boxes, with good results and little mold growth. The reason for this diet's poor survival rating in the initial trial remained a mystery until recently when Eppendorf tubes were again used with PBW diet in an unrelated study. As in the diet trial, one pinhole was punched through the lid with a dissecting probe, but even more (>30%) larvae died. When much of the lid was cut away and replaced with mesh, survival on the PBW diet increased to >90%. We therefore suspect that a build-up of harmful gas or displacement of air may occur when ventilation in the tube is inadequate.

Figure 2: Comparison of duration (±SE) of each instar, pupa, and total development time from egg hatch to adult eclosion of F_1 EGVM reared individually on four diets. Diet abbreviations are explained in Table 2.



2.3.2. *Infestation method, larval rearing containers, and larval density*. The small to moderate weekly output target of 7,000 to 10,000 EGVM needed to ensure a minimum of 5,000 experimental insects did not require development of extremely space-efficient rearing trays. The most important requirements were minimal larval escape from containers to avoid risk of accidental transport out of the containment facility and exclusion of mold mites. We therefore chose an airtight locking box with a gasketed lid, and hot-glued a mesh window onto the lid with 50 µm mesh-openings for air flow and prevention of unwanted movement in and out of boxes (**Fig. 3**). These boxes are not tapered and therefore do not stack efficiently for storage. The boxes measure 15 x 10 x 7 cm. Each is lined with a 3 mil polyethylene sheet to facilitate box cleaning and to provide a pupation substrate. During the early phase of rearing-system development, about 300g of GM diet was poured over the sheet and wax paper egg strips were taped to the lid of the box. Contact between eggs and diet was avoided because we suspected that eggs died from moisture released by the diet and because mold grew at the contact points. However, taping the egg strips to the lid led to poor survival to the adult stage. We suspected that larvae reached the edges of the diet and settled there to feed, causing high larval density around the perimeter of the boxes. Because we observed biting and kill during encounters among larvae, we hypothesized that crowding is detrimental to survival. To test this we taped strips with various numbers of eggs in boxes with 300 g of GM diet and recorded the number of pupae and adults that developed.

Percent survival declined markedly as egg density increased on GM diet (**Table 3**). Shortage of food was unlikely to be a factor affecting mortality because pupal weight was not dramatically affected by larval density and excess diet remained in the boxes after pupae formed. Because pupal sex ratio remained similar across rearing densities, the sexes did not appear to be differentially affected by rearing density, i.e., male and female larvae appear to be equally likely to kill or be killed as a result of an encounter with another larva. Average sex ratio in this study, as with the colony in general, did not differ from 1:1.

At this point in the development of a larval rearing system the major problems appeared to revolve around mold susceptibility and uneven larval distribution in GM

Figure 3: EGVM rearing boxes with locking lids. Wax paper egg strips are laid over honeycomb on PBW diet (left). Pupation occurs in the diet, in honeycomb cells, and on the polyethylene sheet lining the box (middle). Box lids have windows for airflow (right); mesh opening size is 50 µm for containment of neonate moth larvae and exclusion of mold mites.

Table 3: *Effect of egg density on average (±SE) survival to the pupal stage, pupa weight, and sex ratio in rearing boxes with 300 g of GM diet. Each density was replicated 10 times.*

No. of Eggs	Unhatched Eggs	No. of Pupae	Percent Survival*	Female Pupa Weight (mg)	Male Pupa Weight (mg)	Proportion Male
100	2.5±0.8	44.0±4.2	45.4±4.3	13.8±0.1	9.3±0.1	0.51±0.02
200	3.0±0.4	62.8±3.8	31.9±2.0	13.4±0.1	9.3±0.1	0.49±0.03
300	2.4±0.8	86.1±5.0	29.0±1.7	12.9±0.1	8.8±0.1	0.47±0.01
400	2.6±0.8	66.0±10.5	16.6±2.6	13.4±0.1	9.2±0.1	0.51±0.02

* Percent survival was calculated from hatched eggs.

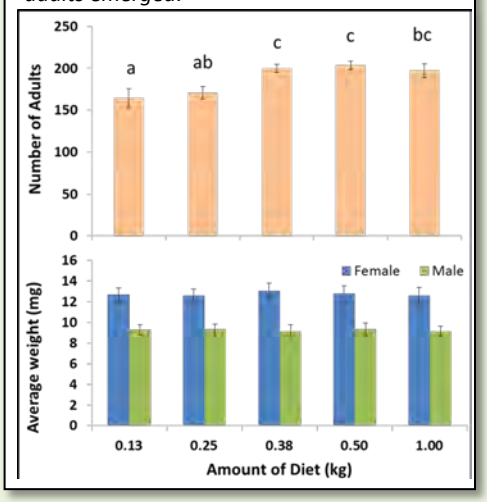
diet. Following the switch from GM to PBW diet, both of these problems were greatly reduced.

The small, firm nuggets of PBW diet appeared to increase surface area and improved dispersal of larvae throughout the diet compared with the softer, moister texture of GM diet, even when it was broken up with a fork or potato masher. Output of adults per box from 100 eggs doubled from an average of 26 on GM diet to 50 on PBW diet. Light-diffuser grids were placed on the diet as platforms on which to lay strips of eggs, and benefitted the system by improving neonate dispersal onto diet and by avoiding contact between the egg sheet and the diet, thus averting mold growth. The grids were soon replaced by 12.5 mm thick polyethylene honeycomb (8 mm cell diam) on the diet surface, which provides larvae with many feeding and pupation refugia (**Fig. 3**). Output of adults per rearing box with 400 g of PBW diet currently exceeds 500.

Our target productivity per rearing box in 2010 was 300 adults, which we estimated required 0.4 to 0.5 kg of PBW diet for food and refuge. However, to determine at what amount the diet was most efficiently utilized we tested productivity of 300 eggs on various weights of diet. Ten replicates were made of each treatment. Egg strips were placed on honeycomb over PBW diet weighing 0.13 to 1.00 kg and the number of reared adults was recorded. A random subset of 30 pupae from each box was sexed and 10 of each sex were weighed.

The study showed that we could reduce the amount of diet offered per insect in our rearing system. Number of eggs surviving to adult increased significantly between 0.25 and 0.38 kg of diet and plateaued at around 200 moths at 0.38 kg (**Fig. 4**). The most efficient amount of PBW diet for infesting with 300 eggs in our rearing system is therefore somewhere between 0.25 and 0.38 kg (without addressing cost-benefit ratio). Pupal weights for each sex were uniform both within and across treatments, suggesting that lower survival in smaller amounts of diet was not due to shortage of food, and may therefore be due to higher effective larval density in lower diet volume. Pupal sex ratios showed no trends and were male biased; however pupae to be sexed were selected from the polyethylene sheets lining the boxes, which we later found to have a higher concentration of males than other pupal substrates in the boxes. Pupation site preferences in boxes by males and females will be studied in future. Further tests will be made to pinpoint the optimal amount of diet for larval survival by integrating the effects of diet weight with volumetric dimensions.

Figure 4: *Mean (±SE) number of adults and their pupal weights reared from 300 EGVM eggs in boxes with various quantities of PBW diet. Different letters over columns denote significant differences at α = 0.05 (ANOVA followed by Tukey-Kramer HSD test to separate means). Note: two of the 0.13kg replicates were excluded from the results because the diet desiccated and few adults emerged.*



2.4. Adult Moths.

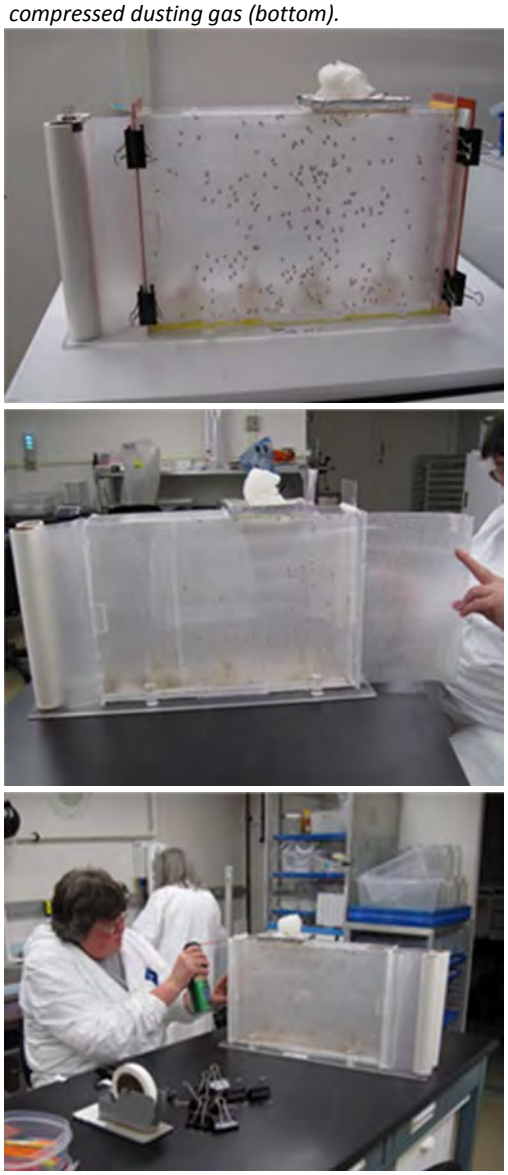
2.4.1. *Transfer of adults from rearing boxes to oviposition cages*. The current protocol for transferring adults to a single oviposition cage consists of collecting a total of 700 adults from five or more rearing boxes into vials utilizing a hand-held vacuum and tapping them into the cage through a mesh sleeve. Boxes are opened in a mesh dome cage and moths are vacuumed from the box, the box lid, and cage walls. Because males tend to emerge about 3 days earlier than females, we strive to equalize sex ratio per cage by collecting moths from two cohorts infested a few days apart. Moths are collected from any box for up to 5 days.

2.4.2. *The oviposition cage. EGVM oviposits readily on wax paper*. This substrate proved useful not only as a component of the rearing system but also for experiments, as its transparency allows eggs to be viewed easily from both sides, especially against a dark background, and the paper is easy to mark and cut.

Earlier observations of adults in paper cans suggested that they prefer to rest and oviposit on the sides of the can rather than on the top or bottom. Oviposition cages were therefore designed to maximize vertical surfaces for egg deposition (**Fig. 5**).

Cage material is clear acrylic to allow light entry, and a mesh (50 µm openings to prevent neonate escape) window and sleeve are used for air flow. Cages have two broad walls, 30.5 cm high (to accommodate standard rolls of household wax paper) x 45.7 cm wide, and two narrow walls that are 7.6 cm wide, which accommodate a vertically sliding door at the front and a fixed wall at the back. A detachable acrylic base holds the cage and a pair of dowels for seating rolls of wax paper. Vertical slits at the front and back of the cage allow the wax paper to be pulled for egg harvest. Acrylic panels with silicone gaskets are clipped onto the front and back of the cage to prevent escape by neonates through the slits. A sheet of paper lines the floor of the cage, and adult diet is presented on the floor in four small reservoirs of 10% honey in water with dental wicks. Moths are added from vials through a mesh sleeve at top of the cage. Eggs are harvested at 3 to 4 day intervals by removing the panels and gaskets and pulling out the portion of wax paper exposed to moths. Egg sheets are cut off the wax paper rolls and held in plastic bags at 6°C until use, usually within 3 days. Adults are killed after 10 days with a spray of isopropyl alcohol and the cages are cleaned via the removable sliding door at the front.

Figure 5: An oviposition cage (top) and an egg sheet being harvested on a wax paper egg sheet pulled through the cage (middle). Moths are deterred from escaping through slits by bursts of compressed dusting gas (bottom).



The oviposition cages are adequate in the short-term but need several refinements. Despite precautions to prevent escape with gaskets, neonates hatching from eggs laid on cage parts can escape. Tape and putty are applied to potential escape routes, but these also fail to contain neonates. During egg harvest, adults can hitchhike on egg sheets out of the cages and also escape. Time spent cleaning and preparing one cage for use can last up to 45 minutes, and harvesting the eggs from one cage (and resealing the cage) can last between 20 and 30 minutes. We therefore harvest eggs twice per week rather than daily. The sliding-door access into the cage for cleaning and for inserting reservoirs of adult diet is too narrow for some workers. A new design is planned for 2012 that will significantly improve handling efficiency and allow for daily egg harvest.

2.4.3. *Adult diet*. Adults of the parental generation lived longer when given access to a solution of 10% honey in water than without any provisions (see **Table 1**). We therefore continued to provide honey-water to adults in subsequent generations. However, in view of potential scaling-up to mass production and the need for cost reduction, we tested whether honey-water imparts a significant benefit to female fecundity and egg viability compared with less costly alternatives. Water was thought to be sufficient as an adult diet for maximum egg production by EGVM [(4)], but our moths were often more fecund than reported by Torres-Vila et al. [(4)], so we compared egg output and viability of adults provided with 1) only water, 2) 10% honey-water, and 3) no provisions (a dry dental wick). Furthermore, to test if interactions exist between adult and larval diet, we tested the three adult diets on adults reared on GM and PBW diets. The study was made with all treatment combinations. One freshly emerged female was grouped with two males in a quart-sized zippered polyethylene bag attached to a vial containing adult diet or a dry wick, and eggs were examined after the female died.

Table 4: *Comparison of two larval diets on female EGVM pupa weight, egg production, egg hatch, and adult longevity, and effects of adult diet on female egg output, egg hatch, and longevity.*

Treatment	Eggs per Female	Longevity (days)	Hatch (Proportion)	Pupa Weight
Larval Diet				
GM	225.2±8.1a (n=67)	13.2±0.5a (n=73)	97.2±2.0a (n=67)	12.8±0.2a (n=154)
PBW	192.5±8.4b (n=61)	13.3±0.5a (n=71)	90.6±2.1b (n=61)	12.3±0.2a (n=156)
Adult Diet				
10% honey-water	297.7±10.1A (n=43)	20.3±0.6A (n=51)	97.2±2.5A (n=43)	n/a
Water	205.0±9.9B (n=44)	10.5±0.7B (n=43)	92.5±2.5A (n=44)	n/a
Nothing	124.0±10.2C (n=41)	8.6±0.7B (n=43)	92.4±2.5A (n=41)	n/a

Different letters of the same case in each column indicate statistically different means (ANOVA followed by Tukey-Kramer HSD test to separate means, at α = 0.05).

Larval diet and adult diet significantly affected adult female performance, but no significant interaction was detected between adult and larval diet. Females reared on PBW diet had lower egg production and egg hatch compared with those reared on GM diet (**Table 4**). However, in contrast to the earlier comparison of diets conducted on isolated larvae in Eppendorf tubes (**Table 2**), female pupal weights on PBW diets were not lower than those reared on GM diet. Adult diet significantly affected female egg productivity and longevity, with 10% honey-water increasing egg production by 31% compared with water, and by 58% compared with no provisions. We continue to use honey water in our rearing system, as its benefit outweighs its cost at our production level, and plan to compare it with the less costly carbohydrate, table sugar. (We continue to use PBW diet rather than GM diet for reasons discussed in the Diet Selection section.)

2.4.4. *Adult cohort cycling*. The period of maximum egg productivity generally governs how often adult cohorts are replaced. Based on daily egg production patterns by the parental generation (**Fig. 1**) we initially cycled out spent adults after about 14 days (four or more egg harvests, 3 or 4 days between harvests). After several generations we found that estimated egg numbers in each cage tended to peak during the first or second harvest and declined by the third harvest, at 10-12 days after adults were added to the cage. To better define the egg productivity period we examined whether the proportion of eggs laid by individual females declines dramatically after the first 4 or 8 days after eclosion. Newly emerged females were individually grouped with two males in cups and provided with 10% honey-water on dental wicks for 12 days, and egg production was recorded at 4-day intervals.

Total number of eggs per female (about 108) was lower than for the full lifetime of the female, and also may have been negatively affected by the cup cages used for the study: four females laid no eggs and 17 laid < 30 eggs. However, the study clearly showed that egg production dramatically declined after the first two 4-day periods (**Table 5**). If a scale-up in production becomes necessary, consideration should be given to the costs and benefits of cycling adults out of production after 7 or 8 days. We continue to cycle adults after 10-12 days because of the effort involved in cage cleaning and preparation. When a more efficient oviposition cage is incorporated into the rearing system, we may consider cycling adults on a weekly basis.

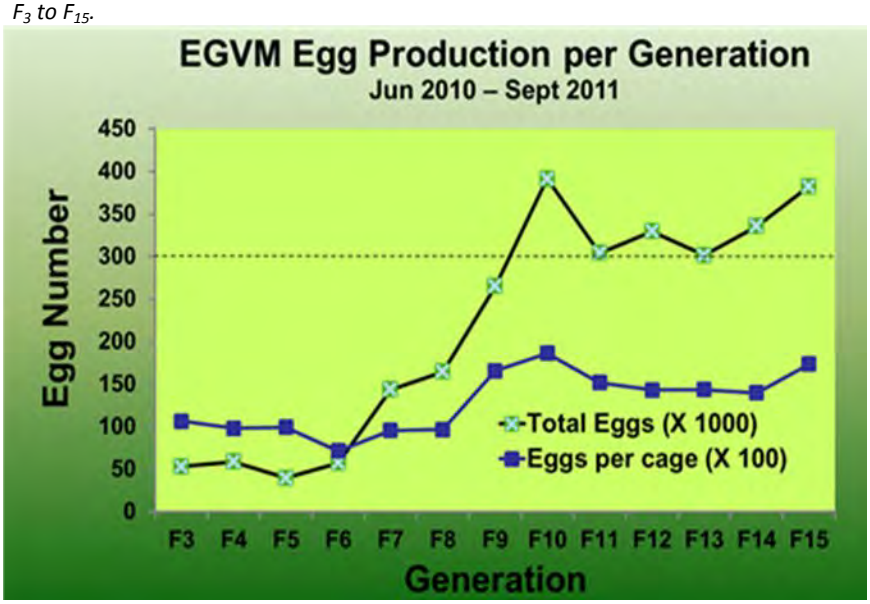
2.5. Colony productivity and quality control. Estimates of egg numbers produced per oviposition cage and per female are used to monitor colony health and productivity. Larval survival and pupa weight are also periodically monitored. Oviposition cages are currently comparable because each is populated by an equal number of adults and colony sex ratio is approximately 1:1. Initially, egg sheets harvested from cages were laid over a 30.5 x 45.7 cm marked grid with 16 equal blocks, and a subset of eggs was counted on six fixed blocks (37.5% of the total sheet surface area) that were randomly selected when the grid was made. By F_8 the number of eggs per cage increased to the point that too much time was devoted to egg counts, and the six blocks were each divided into four sub-blocks, of which two were counted (18.8% of total sheet surface area). The estimated number of eggs per sheet is calculated as the number of counted eggs divided by the proportion of surface area (0.19) they occupy. No attempt is made to estimate eggs laid off the wax paper on other parts of the cage. The number of eggs per female is calculated by dividing the estimated number of eggs per cage by the estimated number of females per cage.

Table 5: *Mean (±SE) egg production by individual females by 4, 8, and 12 days after eclosion.*

Oviposition Period	Total Eggs (n=52)	Eggs/Female	Proportion of Total Eggs (n=48)
Days 1-4	2581	49.6±8.7	0.44±0.05
Days 5-8	2164	41.6±6.6	0.40±0.04
Days 9-12	851	16.4±3.3	0.17±0.03

Productivity rose from about 10,000 eggs per cage before F_9 to about 15,000 eggs per cage in F_{15}, and from about 50,000 to about 400,000 per generation by September 2011 (**Fig. 6**). The increase was mainly due to higher numbers of cages cycled per generation, from five to 22. The average number of eggs per female in cages remained low through F_{15}, between 20 and 40 eggs, and increased thereafter due to a combination of new practices, including earlier replacement of spent adults and reduced adult density, but we will continue to research how to increase per-female productivity in cages, including better understanding effects of adult density, adult transfer from rearing boxes, and cage design.

Figure 6: *Estimated EGVM colony egg productivity per cage and per generation from F_3 to F_{15}.*



Acknowledgments

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References Cited

(1) Technical working Group for the European Grapevine Moth Program. 2011. Report. December 13-15, 2011, Sacramento, California. Available online at: http://cenapa.ucdavis.edu/newsletters/European_Grapevine_Moth42020.pdf. Accessed on 5/4/2012.

(2) Gilligan, T.M., M.E. Epstein, S.C. Passoa, J.A. Powell, O.C. Sage, and J.W. Brown. 2011. Discovery of *Lobesia botrana* ([Denis & Schiffermüller]) in California: An invasive species new to North America (Lepidoptera: Tortricidae). *Proceeding of the Entomological Society of Washington* 113: 14-30.

(3) Guennelon, G., F. D'Arcier, and J. Trincal. 1975. Description d'une production massive de l'eudémis de la vigne sur milieu artificiel (*Lobesia botrana* Schieff, Lepidoptera Tortricidae). *Annales de Zoologie Ecologie Animale* 7:295-309.

(4) Torres-Vila, L.M., J. Stockel, and M.C. Rodríguez Molina. 1996. Efecto de la indisponibilidad de agua sobre el potencial bíotico de la polilla del racimo *Lobesia botrana* Den. y Schiff. (Lepidoptera: Tortricidae). *Boletín de Sanidad Vegetal, Plagas* 22:443-449.

(5) Deseő, K.V., F. Marani, A. Brunelli, and A. Bertaccini. 1981. Observations on the biology and diseases of *Lobesia botrana* Den. And Schiff. (Lepidoptera, Tortricidae) in central-north Italy. *Acta Phytopathologica Academiae Scientarum Hungaricae* 16:405-431.

(6) Sinclair, E.R. 1974. A life system study of *Cryptophlebia ombrodelta* (Lower) (Lepidoptera: Tortricidae) in Southwest Queensland. *Ph.D. dissertation*, University of Queensland, Australia.

Gypsy Moth Colony Supports North American and European Research on Gypsy Moth Control and Insect Biology

Written by: Hannah Nadel

At any given time the European gypsy moth (Lymantria dispar dispar) (GM) colony at Otis Lab is comprised of about 63,000 egg masses, 15,000 larvae, and 30,000 pupae and adults (**Fig. 1**). It is the only mass-reared gypsy moth colony in existence with year-round availability of all life stages. The eggs are partitioned into 52 cohorts, one ready to hatch on any week of the year. The colony was started from individuals collected in New Jersey in the 1970s and by 2012 was reared on artificial diet through 66 generations. It is currently used to provide larvae for in-vivo production of GM baculovirus in large quantities for USFS and to support research funded by the Gypsy Moth Slow the Spread Foundation. However, over the decades the colony has also become the sole source of experimental insects for researchers who rely on it for ongoing work.

Figure 1: Larval rearing containers for gypsy moth colony at the Otis Laboratory.

The constant availability of all GM life stages, uniform rearing standards, and uniform quality of the insects have made the colony a valuable source of insects for researchers in the US, Canada, and Europe (Germany, Czech Republic, Turkey, Slovakia, Austria). Beside the option to receive live eggs, larvae, or pupae, researchers can facilitate their mark-recapture studies by requesting color-marked adults, which are produced with the addition of Calco Red dye to the larval diet. In trade for insects, researchers receiving moderate to large quantities have purchased GM rearing supplies.

In 2011, projects utilizing our GM were diverse in nature and scope although most were ultimately aimed toward improving GM control. The focus of studies can be roughly divided into five categories, each with two or more areas of study. The following list describes these areas in broad terms under each category and was constructed from researcher feedback provided in early 2012. It should be noted that some workers are conducting research on multiple areas of study while closely related studies are being addressed by multiple researchers. The list is by no means exhaustive.

1. **Host-plant-GM interactions:**
   a. Mechanism and efficacy of host-plant chemical defenses
   b. Protein chemistry and effects of various enzymes on the GM peritrophic membrane
   c. Feeding behavior of GM
   d. Influence of GM population and host plant on larval development
2. **Pheromones and mating disruption:**
   a. Evaluation of pheromone formulations
   b. Optimize mating disruption to control GM
3. **GM populations and ecology:**
   a. Population dynamics of GM
   b. Effect of climate change on southern spread of GM
   c. Effect of forest fragmentation on GM range expansion
4. **GM pathogens and host-pathogen interactions:**
   a. Biocontrol of GM with *Entomophaga mamaiga*
   b. Development of bioassays for presence of *E. mamaiga* in soil
   c. Physiology of microsporidian-GM interactions, mode of pathogenicity
   d. Transmission and spread of microsporidia
   e. Mode of action of Bt
   f. Pesticidal efficacy of Btk variants
   g. Distribution and metapopulation ecology of GM pathogens
   h. Natural enemies of pathogen spores
   i. Documentation of and mechanism of resistance to the baculovirus and *E. mamaiga*
   j. Changes in GM behavior induced by a GM virus gene
   k. Birds as GM virus vectors
   l. Roles of GM virus genes in binding of virions to midgut cells
   m. Potency of GM baculovirus recombinants
   n. Understanding initial host defense toward GM baculovirus
   o. Improving GM baculovirus strains
   p. Development of GM baculovirus in cell culture

q. Development of commercial in-vivo mass-production of GM baculovirus

5. **GM Parasitoids:**
   a. *Glyptapanteles liparidis* and *G. porthretiae* colony maintenance
   b. Physiological response of GM to parasitism
   c. Development of calyx cells in *G. liparidis*

Selection of North American and European research published in 2011 utilizing Otis colony gypsy moths:

(1) Blackburn, Michael B., Phyllis A. W. Martin, Daniel Kuhar, Robert R. Farrar Jr., Dawn E. Gundersen-Rindal. 2011. The occurrence of photorhabdus-like toxin complexes in *Bacillus thuringiensis*. *PLoS ONE.* 6(3): e18122. doi:10.1371/journal.pone.0018122.

(2) Duan, L.Q., I.S. Otvos, L.B. Xu, N. Conder, and Y. Wang. 2011. Comparison of the activities of three LdMNPV isolates in the laboratory against the Chinese strain of the Asian gypsy moth. *The Open Entomology Journal.* 5: 24-30.

(3) Goetz, D. and G. Hoch. 2011. Modeling horizontal transmission of microsporidia infecting gypsy moth, *Lymantria dispar* (L.), larvae. *Biological Control.* 56: 263-270.

(4) Hajek, A.E., Tobin, P.C. 2011. Introduced pathogens follow the invasion front of a spreading alien host. *J. Anim. Ecol.* 80: 1217-1226.

(5) Hoover, K., M. Grove, M. Gardner, D.P. Hughes, J. McNeil, J. Slavicek. 2011. A gene for an extended phenotype. *Science.* 333: 1401.

(6) Karowe, D.N. and J.K. Radi. 2011. Are the phytoestrogens genistein and daidzein anti-herbivore defenses? A test using the gypsy moth (Lymantria dispar). *J. Chemical Ecology.* 37: 830-837.

(7) Kereselidze, M., Pilarska, D., Hajek, A., Jensen, A.B., Linde, A. 2011. First record of *Entomophaga maimaiga* (Entomophthorales: Entomophthoraceae) in Georgia. *Biocontr. Sci. Technol.* 21: 1375-1380.

(8) Pecci, C.D. 2011. Oxidation of ingested phenolic compounds creates oxidative stress in the midgut tissues of *Lymantria dispar* caterpillars. University of Michigan Honor's Thesis (Bachelor's) (R. Barbehenn, Faculty Advisor). Accessed 17 August 2012: http://hdl.handle.net/2027.42/85311.

(9) Reilly, J.R., Hajek, A.E. 2011. Prey processing by avian predators increases virus transmission in the gypsy moth. *Oikos*. [DOI: 10.1111/j.1600-0706.2011.19752.x].

(10) Valaitis, Algimantas P. 2011. Histochemical study of lectin binding sites in fourth and fifth instar gypsy moth larval midgut epithelium. *In*: (eds) McManus, Katherine A; Gottschalk, Kurt W. 2010. *Proceedings. 21st U.S. Department of Agriculture Interagency Research Forum on Invasive Species*: 139.

(11) Valaitis, Algimantas P. 2011. Localization of *Bacillus thuringiensis* Cry1A toxin-binding molecules in gypsy moth larval gut sections using fluorescence microscopy. *Journal of Invertebrate Pathology*. 108: 69-75.

(12) Valaitis, Algimantas P., Podgwaite, John D. 2011. Comparative analysis of *Bacillus thuringiensis* toxin binding to gypsy moth, browntail moth, and douglas-fir tussock moth midgut tissue sections using fluorescence microscopy. *In:* (eds) McManus, Katherine A; Gottschalk, Kurt W. 2010. *Proceedings. 21st U.S. Department of Agriculture Interagency Research Forum on Invasive Species*: 140.

(13) Zúbrik, Milan, Gernot Hoch, Leellen F. Solter, Vincent D'amico, Michael L. Mcmanus, Jan Patočka, Daniela Pilarska , Július Novotný. 2009. Microsporidia and its potential for gypsy moth *Lymantria dispar* L. (Lep.: Lymantriidae) biological control. *In:* (eds) Pavol Hlaváč, Milan Kodrík: Disturbančné procesy pôsobenia na ekologickú stabilitu lesných ekosystémov a krajiny, *Proceedings of the Scientific Conference Held in Zvolen in 10. 9. 2009*. Technical University Zvolen, ISBN 978-80-228-2063-9. p. 49-59. Publ. 2011 (in Slovakian).

(14) Zúbrik, M., Kalmárová, G. 2011. Toxicity of water macerates of some plants for the larvae of gypsy moth (*Lymantria dispar* L.) (Lep.: Lymantridae). *Forestry Journal*. 57 (1): 42–47.

(15) Zúbrik, M., Kalmárová, G. 2011. Extraction of carbohydrates, lipids, polyphenols and tanstuffs from royal walnut *Juglans regia* L. and verification of their effect on larvae of gypsy moth *Lymantria dispar* L. (Lep,: Lymantriidae). *Acta Facultatis Forestalis Zvolen*, 53 (1): 9–19.

Improved Rearing Methods for Emerald Ash Borer

Project Lead: Dave Lance

Contributors: Ken Bloem (USDA, APHIS, CPHST)
Hannah Nadel, Victor Mastro, and Jason Hansen (Otis Laboratory)

Collaborator: Allen Cohen (North Carolina State University)

USDA-APHIS-PPQ has initiated an inoculative (classical) biological control program to attempt to slow the spread of emerald ash borer (EAB), *Agrilus planipennis*, and the resulting mortality of North America's ash trees (**Fig. 1**). Three species of hymenopteran parasitoids are being released at sites in most EAB-infested states. These insects must be reared in vivo, and rearing methods for the host EAB larvae are largely unrefined. This, in turn, makes rearing of the parasitoids inefficient and limits the capacity of the PPQ rearing facility in Brighton, MI.

Figure 1: *Emerald ash borer,* Agrilus planipennis.

In FY-2011, CPHST initiated a project to improve artificial diets and rearing methods for EAB and its parasitoids. The project will operate through a 2-year cooperative agreement that was established during the fourth quarter of FY-2011 with Allen Cohen at NC State University. The agreement will fund a post-doctoral position at NC State, though the incumbent will be stationed at the Otis lab. Jason Hansen has been selected to fill this position and will work primarily in conjunction with Hannah Nadel (Otis Lab) and Dr. Cohen.

Development of Emerald Ash Borer (*Agrilus planipennis*) Larval Diet

Project Lead: Jason Hansen

Collaborators: Allen Cohen (North Carolina State University)
Melody Keena (USDA Forest Service)

Contributors: Hannah Nadel, David Lance, Vic Mastro, and Juli Gould (Otis Laboratory)

Emerald ash borer (EAB) was first discovered in Michigan a decade ago and subsequent infestations have been detected in 17 other states in the eastern U.S. as well as southeastern Canada. In addition to the profound ecological consequences the loss of ash trees (*Fraxinus* spp.) will likely bring, replacement of ash trees in urban settings alone is expected to cost states billions of dollars. To meet this challenge, hymenopteran parasitoids are being mass reared in Brighton, Michigan and released in several states where EAB infestations have been found. The need for a reliable source of late instar larvae in quantities sufficient to mass rear the introduced larval parasitoids (i.e. *Spathius agrilli* (Hymenoptera: Braconidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae)), has prompted research into developing an artificial diet that fully meets the dietary requirements of EAB, thus eliminating the time consuming and laborious larval collecting methods currently employed.

Figure 1: *Larva of emerald ash borer,* Agrilus planipennis*, feeding on artificial diet in a petri dish.*

Building on successful diets for woodborers with similar life histories, and the success of Melody Keena (USDA-Forest Service) and Julie Gould (USDA-APHIS), new diet formulations are being assessed that are expected to improve larval survival. Key changes that have been tested are protein sources, the addition of host-specific sterols, differences in diet texture, antimicrobials, and varying amounts of host plant material. Growth chamber humidity, egg placement on diet and a clean work environment are also important factors. Our objective is to achieve 80% or more survival of EAB larvae to 4th instar (**Fig. 1**), the stage preferred for oviposition by EAB larval parasitoids. Work is ongoing to improve egg hatch and determine optimal diet formulation.

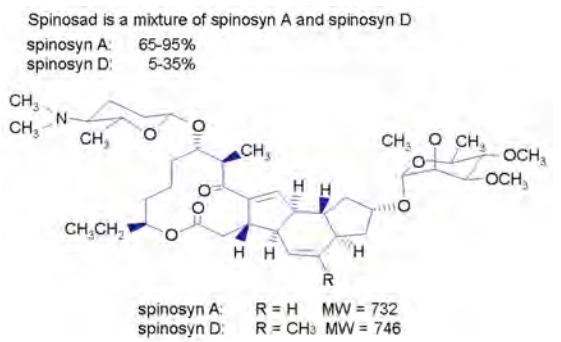
Control of Emerald Ash Borer with Aerial Applications of Spinosad in Michigan Woodlots

Project Lead: Phil Lewis

Collaborators: Dave Smitley and Terry Davis (MSU)

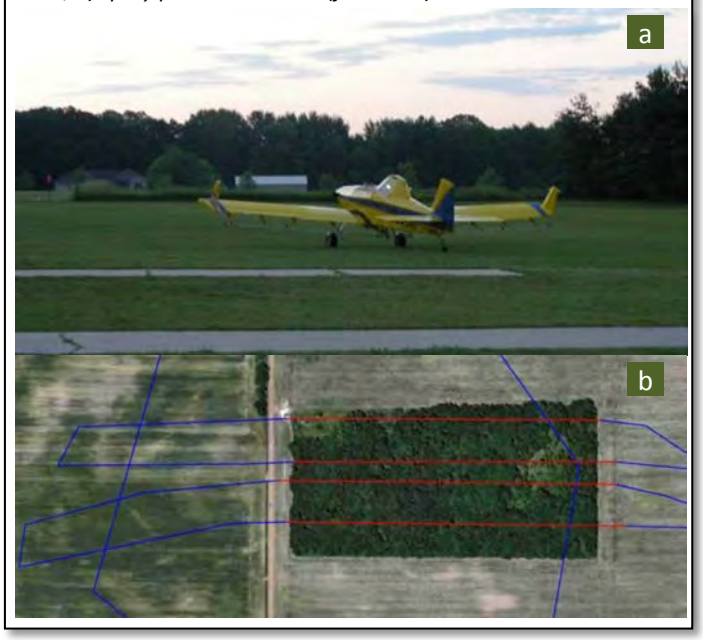
Spinosad is an aerobic fermentation product of the soil microbe, Saccharopolyspora spinosa *(**Fig. 1**) and is currently labeled for used on a wide variety of crops and on stored grain. The manufacturer, Dow AgroScience, considers it safe for forest use and supports the concept of using aerial applications of spinosad for control of emerald ash borer (EAB). Due to its low toxicity and low impact on the environment, EPA has registered spinosad as a reduced-risk pesticide.*

Figure 1: *Chemical structure of spinosad*



Suppression of EAB was tested using isolated woodlots in an intense farming area in Clinton County, Michigan. This area was recently infested with emerald ash borer, but woodpecker attacks and dieback were scarce in most of the test blocks. Isolated woodlots were selected for this study if they were surrounded by farm fields, contained enough ash trees to sample, and if some signs of EAB infestation were found. Ten suitable ash woodlots were chosen to be used as spray blocks and ten similarly infested woodlots were selected as unsprayed control plots.

Figure 2: *Application of spinosad over woodlots: a) Air Tractor 502B, b) spray path over site #3 (from GPS).*



Applications. Due to the cool spring and delayed emerald ash borer emergence, the applications were postponed 2 weeks. The first application was applied in late June between 5:50 and 7:10 am in excellent weather conditions (**Fig. 2**). There was no breeze, the humidity was high and the skies were clear and sunny. The applications were made with Micronair AU5000 nozzles on an Air Tractor 502B set at 55 degree pitch. The aircraft was flown at 140 MPH and over the trees high enough to give a 125 foot swath. A second application was made in early July between 10 and 12 am. The weather conditions were overcast with high humidity until a slight clearing of the cloud cover at 9:30am. There was occasional light drizzle during application, but not enough to cause drip off the leaves.

Tarp sampling. Two tarps were placed under 4 trees in each of 6 treated and 6 untreated woodlots between 18-23 June. On 25 June, the tarps were swept to remove leaves and other debris. The contents of the tarps were emptied into plastic bags and taken back to the lab to assay for emerald ash borer adults on 29 June. Tarp collections were again made between 6 and 7 July after which the tarps were removed from the woodlots.

A total of 54 EAB adults (range 1 to 20) were collected from sprayed plots after the 1st application, but only 9 (range 0 to 4) were observed on tarps after the July application. A total of two insects were observed on tarps placed within control plots for both applications.

Shotgun sampling for residue and bioassay. Leaf samples from the upper canopy of 3 trees in each of the 6 sprayed and unsprayed sites listed in **Table 1** were taken with a 12–gauge shotgun on 29 June (3 days after treatment) for residue analysis at the USDA/ APHIS facility at Otis. Three compound leaves from each

Table 1: *Foliage bioassay data, showing mean percent EAB mortality for adults placed on foliage samples collected from 6 treated and 6 control woodlots three days after aerial application of spinosad.*

		Mean Percent Mortality (± SD)		
Treatment	**n**	**24 h exposure**	**4 day exposure**	**6 day exposure**
Control Plots	48	5.5 ± 6.3	11.0 ± 11.1	30.8 ± 15.9
Sprayed Plots	48	43.5 ± 29.2	72.2 ± 36.9	84.0 ± 24.5

sample were placed in a floral pick with water before they were fixed in an arena consisting of two quart sized food containers held together with a nylon stretch-band. Arenas were set-up on 30 June in a lab at Michigan State University with approximately 8 adults in each arena. EAB adult mortality was determined on 1 July (24 h exposure to leaf samples), 4 July (4 d exposure) and 6 July (6 d exposure).

Canopy thinning and emergence hole sampling. Between 27 July and 11 September, 2009 a team of 3-4 people visited each site to identify and tag 30 ash trees in each plot, measuring their DBH, surveying them for emergence holes, and providing a canopy thinning rating (**Table 2**). Ash trees were identified first by their bark and then verified by their canopies of opposite-branching compound leaves. Flat, numbered aluminum tags were nailed to the trunk about 6 feet from the ground with aluminum nails, facing the direction of the following team members.

Percent dieback ratings were produced by taking into consideration both the size of the individual leaves of the canopy and the fullness of the canopy as a whole. The bottom 20 feet on the tag side of each tree trunk was videotaped. The taping was done with a tripod mounted digital video camera set up 6 paces from the tree to be sampled. Video was focused on the inner 75% of the tree's visible trunk surface and was taken in increments as it moved up the tree, pausing at the top of every frame for the focus to adjust for a clear view of the bark. The same trees were assessed in this way for three years.

The videos taken in the woodlots were viewed at later dates on a classroom projection screen by teams of two in order to count the number of EAB emergence holes. One site was done per sitting, taking approximately one hour to view 30 trees. Emergence holes were identified by the presence of a definitively D-shaped hole. Both team members reviewed and agreed upon each

Table 2: *Mean percent canopy thinning and emergence holes at the 20 study sites (30 trees per site, a total of 600 trees). At 15 sites emergence holes were counted from the ground to a height of 5 feet.*

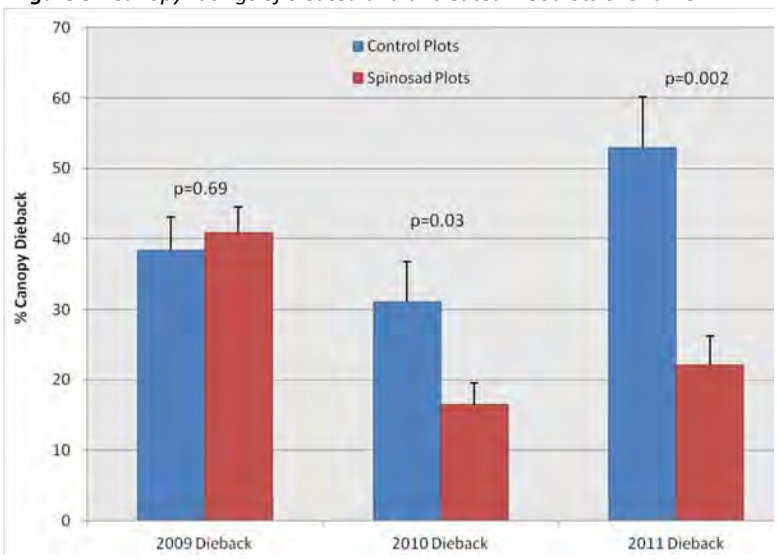
	Mean Percent Thinning & Emergence Holes (± SD)		
Treatment	**Canopy thinning**	**Emergence holes to 20' on video**	**Emergence holes visual to 5'**
Control Plots	38.3 ± 14.6	12.0 ± 5.4	4.0 ± 2.5
Sprayed Plots	40.9 ± 11.6	7.6 ± 5.3	1.7 ± 2.1

emergence hole before it was entered in the final data. The location on the trunk of the emergence holes was recorded in order to assist the samplers in identifying old from new holes in later observations.

The initial sampling conducted in 2009 was for the purpose of establishing the background population of EAB in the control and treated woodlots, which were similar (**Fig. 3**).

Overall, our data suggests that two applications of spinosad to woodlots in June or early July for two consecutive years will slow population growth. The 2010 dieback ratings reflect the impact of the first treatment in 2009. Additional data to be collected in late 2011 will reflect three years of spinosad application. At this point it appears that two annual applications of spinosad sprays to woodlots delay population growth of EAB by at least 3 years, in comparison with control woodlots. From this data we anticipate that annual aerial sprays will greatly reduce the natural spread of EAB (by flight) out of outlier infestations, and therefore could be a useful tool for local governments that would like to delay the spread of EAB in the first few years after it is discovered.

Figure 3: *Canopy ratings of treated and untreated woodlots over time.*



Control of Emerald Ash Borer Using Soil and Trunk Injection Methods

Project Lead: Phil Lewis
Collaborators: Deb McCullough (MSU)
Therese Poland (USFS)

Long-term evaluation of small ash trees. Small trees at 7 Lakes State Park have been treated annually from 2006-2008 with a trunk spray of either imidacloprid (Macho 2F) combined with the Pentra-Bark surfactant, a trunk spray of dinotefuron and the Pentra-Bark surfactant (Safari 20% WG), trunk injection with imidacloprid (10% Imicide in 3 ml Mauget capsules) or left untreated as Controls. Evaluation to date has consisted of collecting foliage samples for residue analysis (at 3-4 wk intervals post-treatment), adult EAB bioassays (coinciding with June and July residue sampling) and determining EAB exit hole densities in autumn. Because of their small size, residue levels in the foliage of these trees have been consistently at least 2-fold higher than residues in larger trees. These data, especially in combination with data from other sites and studies, enhances our ability to assess relationships between foliar residues and bioassay results, and to relate both to the density of emerging adults. In fall 2008, at least some of the untreated Control trees and a few of the treated trees were declining or dead. Our goal is to continue to treat and evaluate these trees as EAB density peaks and declines in this site. Results from long-term studies such as this are critically needed to determine whether these insecticides can adequately protect ash trees from EAB or if treatment simply delays tree mortality.

EAB colonization of trees treated once, twice or annually during a three-year period. This project began in 2008 and was completed in autumn/winter of 2010. Twelve blocks of trees were established in 2008, with each block consisting of 23 trees. Within each block, 5 trees were treated with one of four products/rates in 2008 and 3 trees remained untreated for controls. During the fall, one tree per treatment in each block were felled and intensively sampled to assess EAB larval density. This process was continued through 2010.

This design assesses how insecticide residues and product efficacy vary among trees treated annually for one, two or three years, trees treated with a single treatment only in Year 1, but not in Year 2 and trees treated in Year 1 but not in Years 2 or 3. The ability of emamectin benzoate to provide high levels of EAB control for three years will be determined for trees treated with low and high rates of the product. We can also address anecdotal observations that suggest EAB control in trees treated with neonicotinoid insecticides improves after two or more consecutive years of treatment.

Lethal trap trees. Localized outlier sites of EAB that are geographically distinct from major infestations continue to be discovered each year. A limited number of options are available for reducing the population growth of EAB infestations in newly identified EAB outlier sites. One idea that has been repeatedly discussed is the creation of “lethal trap trees” to attract ovipositing female beetles but ensure that any larvae on the trees die before completing emergence. A lethal trap tree would be created by injecting a tree with emamectin benzoate, waiting 2-3 weeks for the insecticide to move up the trunk, then girdling the tree by removing a band of outer bark, phloem and cambium from the circumference of the trunk. Girdled trees are highly attractive to adult beetles, especially in sites with relatively low densities of EAB. Female beetles preferentially oviposit on girdled trees, but if those trees are not felled and destroyed over the winter, the new cohort of larvae will emerge the following year. Issues related to uncertain funding for winter activity (e.g. when girdled trees must be destroyed) have so far prevented most managers from using girdled trees as “sinks” to absorb EAB oviposition. Determining whether lethal trap trees are effective could provide managers with a new, efficient option for slowing EAB population growth and the progression of ash mortality in outlier sites.

Project results summary. We quantified insecticide residues in composite foliage samples collected from 8 locations in each tree in mid-June, July and August 2008 using ELISA. For the trees treated in both 2007 and 2008, mean emamectin benzoate residues ranged from 5 to 8 ppm and dinotefuran residues ranged from roughly 2 to 3 ppm. Imidacloprid residues in trees treated with trunk sprays or with the Mauget capsules generally increased over the summer and were highly variable. Average imidacloprid residues in all months were < 2.5 ppm.

For the trees treated only in 2007, emamectin benzoate residues were detectable but averaged less than 1 ppm throughout the summer in 2008. Dinotefuran residues were highly variable but averaged approximately 2 ppm in June and July before sharply declining in August. Imidacloprid residues were detectable but generally averaged less than 1 ppm. Residues were consistently slightly higher on the trees treated with the 2007 trunk spray if the Pentra-Bark surfactant was added, but preliminary analyses indicated differences were not statistically significant.

Bioassays were conducted in mid-June in 2008 to assess survival of EAB adults caged with leaves from each study tree. Two leaves were collected from opposite sides of each tree and three beetles were placed on each leaf for four days.

For trees treated in both 2007 and 2008, mortality of beetles caged with leaves from the emamectin benzoate trees was > 90% in all three bioassays. For trees treated in both 2007 and 2008 with trunk sprays of dinotefuran or imidacloprid, or with the Mauget capsules, average beetle mortality ranged from 50-70%, while only 10-15% of the beetles on control trees died.

For trees treated only in 2007, on average at least 90% of the beetles that fed on leaves from the emamectin benzoate died. Beetle mortality on trees treated only in 2007 with dinotefuron or either of the imidacloprid products did not differ significantly from the control trees. The addition of the Pentra-Bark surfactant did not appear to influence EAB mortality on trees treated with trunk sprays in 2007 only.

Larval density was assessed by felling and debarking areas on the trunk and canopy of trees during the 2008-2009 winter. Number and instar of larvae were recorded and standardized by m^2 of phloem exposed on each tree.

For trees treated in both 2007 and 2008, larval density on untreated control trees averaged approximately 90 larvae per m^2. Trees treated with emamectin benzoate in both 2007 and 2008 had less than 1 larva per m^2, equivalent to >99% control when compared to larval density on control trees. Larval densities on trees treated with trunk sprays of dinotefuran + Pentra-Bark or imidacloprid + Pentra-Bark in both 2007 and 2008 were 65-70% lower than on control trees, a difference which was significant. Larval density on trees treated with the Mauget capsules or trunk sprays without Pentra-Bark were intermediate.

For trees treated only in 2007, larval density on untreated control trees averaged approximately 58 larvae per m^2. The emamectin benzoate trees had less than 1 larva per m^2 or > 99% control. Larval density on trees treated only in 2007 with any of the neonicotinoid products, however, did not differ from untreated controls.

The nearly complete EAB control provided by the emamectin benzoate, even two years post-treatment, was striking. These results show that trees could be treated at two or perhaps even at three year intervals, which would dramatically reduce costs and logistical problems associated with annual treatments. The neonicotinoid insecticides, particularly the trunk sprays of dinotefuran+ Pentra-Bark and imidacloprid+ Pentra-Bark did reduce EAB larval density. The level of control was not nearly as high as that provided by the emamectin benzoate, however, and these products provided virtually no reduction in EAB larval density if they were not re-applied the second year.

Control Activities and Long-Term Monitoring for the West Virginia EAB Infestation

Project Lead: Phil Lewis

Collaborators: Rick Turcotte (USFS), Rachel Braud (APHIS)

*Emerald ash borer (EAB) was discovered in late 2007 at a recreational area near Oak Hill in Fayette Co., West Virginia. A survey conducted within a ½-mile radius of the original trap tree identified over 300 ash trees, the majority of which showed no signs of EAB infestation (**Fig. 1**). An integrated control and monitoring study was initiated in 2008 due to the following favorable factors: limited ash resource over a large area; isolated and relatively light EAB population; infested ash trees were within a confined area.*

Ash trees were either cut down and disposed of (149 trees, mostly 3" DBH or less) or treated once by trunk injection in 2008 with emamectin benzoate (Tree-äge,

Arborjet) at varying dosages depending on tree diameter.

Treatment of trees. It took two applicators a little over two weeks to complete the tree treatments. **Fig. 2** indicates the amount of time needed to treat trees of varying size; dosage was doubled between the first three tree size classes. Time to treat was not significantly different between any of the size groupings, although it trended higher with increasing dosage. Injection time trended faster as tree size increased, probably due to the ability of larger diameter trees to more easily incorporate material into their vascular system.

Baseline monitoring. Foliar samples from 31 of the treated trees (~20%) were collected and analyzed for pesticide residue from 2008 to 2010. The ELISA analysis being employed to determine emamectin content in leaf tissue was confirmed as accurate when compared with an LC/MS analytical method used by the manufacturer (Syngenta).

Emamectin residue from the 2008 samples was present in significant quantities in all foliage samples, increasing by dosage applied but not differing between sample periods. The average amount of residue found in the foliage ranged between 5 and 8 ppm. **Fig. 3** shows that 2009 average residue was 4-5% of the previous year, dropping further in 2010 to 2-3% of the 2008 values.

Sample trees were also assessed for D-shaped exit holes, woodpecker feeding and general tree health by rating the tree canopy crown. This was conducted twice during each summer over the last four growing seasons. In 2008, about thirty percent of the trees showed evidence of canopy dieback from the August observations. There were very few woodpecker attacks on these trees, and only 6 of the 31 trees had 2-7 putative EAB exit holes, evidence of a light infestation. Additional tree observations during June and August of 2009 evidenced that the trees remain healthy with stable canopy

Figure 1: *Location of ash trees (white circles) within the West Virginia EAB infestation, Ace Adventure Campground. Green cross in circle center is the location of the original EAB positive trap tree.*

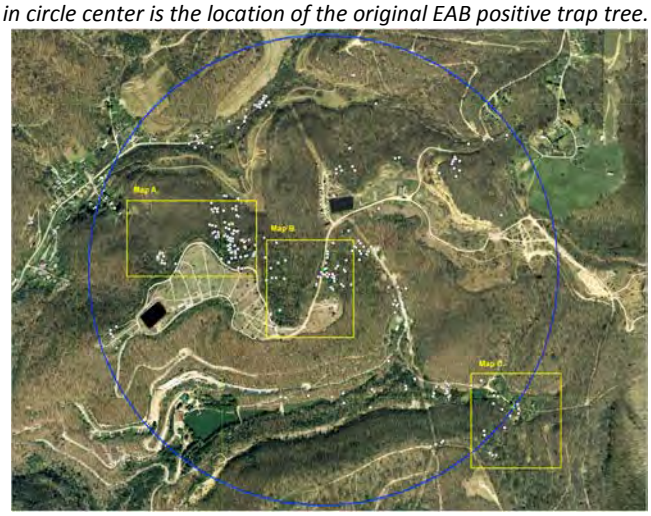


Figure 2: *Average time to treat 163 ash trees, grouped by DBH class and treatment rate. The 0.1 g rate equals 2.5 mL of undiluted product.*

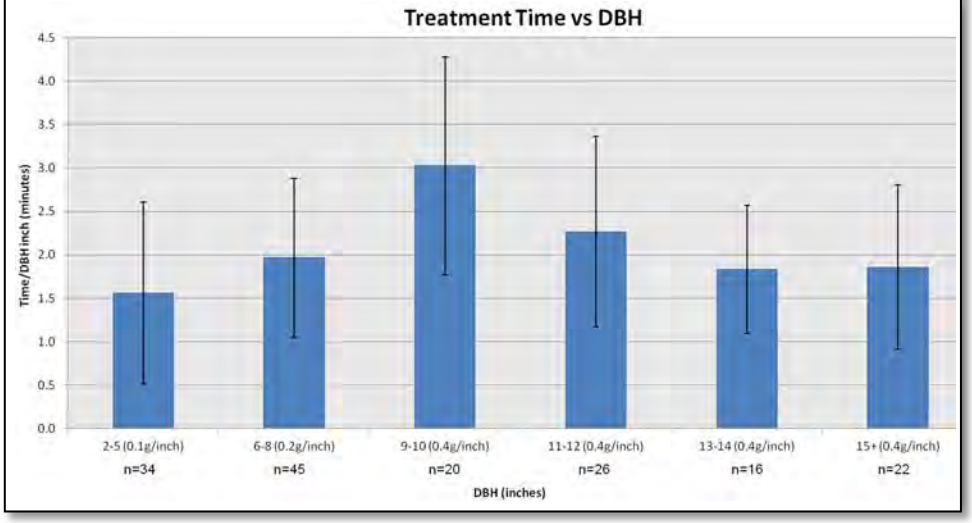
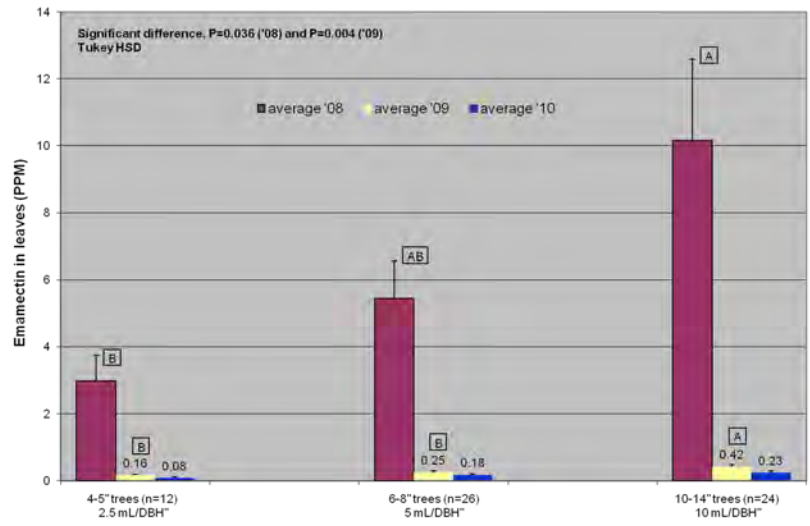
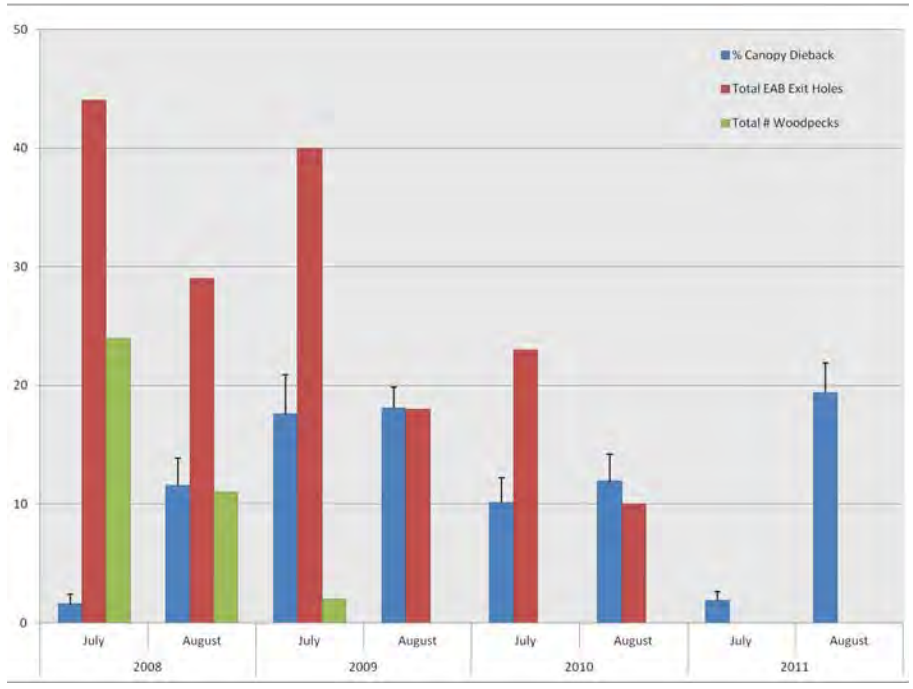


Figure 3: *Pesticide residue data from foliage of treated trees.*



ratings, a 33 to 55% decline in observed EAB exit holes and 90 to 100% decrease in the number of woodpecker attacks. The trends continued through the current year assessment, with no woodpecker attacks and no visible exit holes for 2011 (**Fig. 4**).

Figure 4: *Tree health and infestation level ratings post application.*



Trapping. A trapping grid was set up to monitor the general EAB population in the area. A more intensive trapping grid was targeted for those areas around the original infestation as well as for areas surrounding EAB infested trees that were first identified in the early spring of 2009 (red dots North of Ace, **Fig. 5**). All traps were EAB program panel traps green in color, initially baited only with Phoebe oil, but from 2009 onward the traps were also baited with Manuka oil. Ninety-four trapping sites were established in 2008 with an additional 111 sites in 2009. In 2010 a total of 274 traps were placed for an increase of 65 traps from the previous year. In 2011 only the Ace management area was trapped and 40 traps were set out to monitor EAB adults.

Traps were checked once in July and again in August before being taken down for the season. Only 2 adult beetles were captured on two of the traps placed around the original infestation site in 2008 (purple triangles, **Fig. 5**). In 2009 a total of 95 insects were captured on 27 traps, half of which captured only one insect each. Five of the traps had between 13-18 beetles (red triangles, **Fig. 5**). For 2010, there were 36 positive traps, many again had only single or small numbers of EAB, but 7 traps ranged between 12-42 beetles. For the limited trapping that was done this year within Ace, 12 of 40 traps caught 27 total beetles.

It is of interest to note that no insects were captured in either 2008 or in 2009 within the original ½ mile radius management area around the Ace Adventure Campground (inner red circle, **Fig. 5**). In 2010 there was one trap within Ace that caught 29 EAB adults, and this trap was located within the management area on the eastern edge. The ash tree the trap was placed in had not been identified prior to the 2008 treatments, but subsequently was used as a trap tree. It is interesting to consider that the trap tree was noted to be dead in 2010, and that within a few hundred meters of this tree several of the treated trees remain healthy and have good canopy cover. This information along with the 27 beetles caught on traps within the Ace management area demonstrates that strong insect pressure is present on the ash trees within this area.

Assessments. Twelve of the study trees were felled and examined in the fall of 2011 for a more complete assessment of treatment impact and EAB infestation levels. Four trees from each of the three application rates were selected, felled and debarked to characterize the EAB population within the treated trees. Four control trees from North of Ace that had obvious signs of EAB infestation were used as positive controls by which to compare.

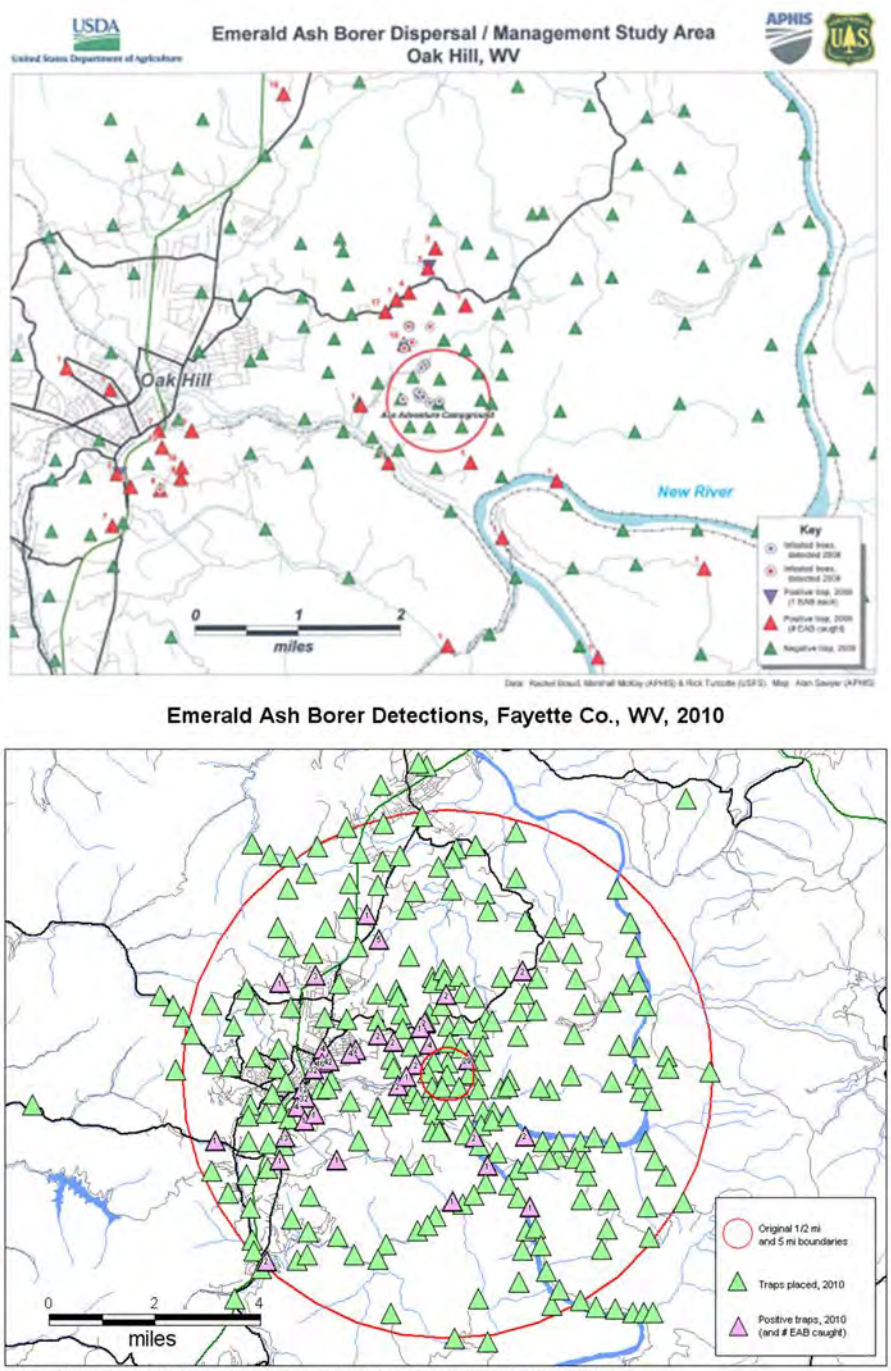
A visual check for exit holes was made of the entire trunk, which was then debarked up to 10 m, with alternate meter sections debarked beyond that. Visual checks were made on all branches > 2" in diameter for bark cracking and exit holes. For the control trees the infestation was so heavy that only a visual check of the trunk was performed for EAB exits and woodpecker attacks, along with a visual check on branches >2" in diameter. For the treated trees, 129 m^2 of log area were processed. No exit holes were found and only 7 woodpecks (<0.1/m^2).

From all of the bark scraping, only 2 galleries (one a year old) were noted on one of the trees and no live

EAB larvae were found. In contrast, control trees had 36 m^2 of log area processed and 392 EAB exit holes were identified ($11/m^2$) and there were 2,232 woodpecks ($62/m^2$).

The results of this study demonstrate that there are situations where EAB populations can be managed. An integrated approach of phloem reduction (removal of small diameter trees and felling and surface treatments of infested trees) and insecticide treatment can provide multi-year protection of the ash resource from EAB attack. Although the ultimate outcome of EAB on ash may not change, integrated approaches using currently available tools can provide land managers with options to remove and retain trees as desired. Thinning, timber stand improvement and crop tree techniques used in concert with an integrated management approach can be used to control the mortality trajectory of EAB, manage canopy disturbance and tree fall.

Figure 5: *Trapping results and infested trees found near Ace Adventure, 2009-2010.*



Improving Detection Tools for Emerald Ash Borer, 2011: Comparison of Prism and Multi-Funnel Traps at High and Low Population Density Sites

Project Lead: Joe Francese

Collaborators: Mike Rietz and Ivich Fraser (USDA, APHIS, CPHST, Brighton MI)
Dave Lance, Damon Crook, and Vic Mastro (Otis Laboratory)

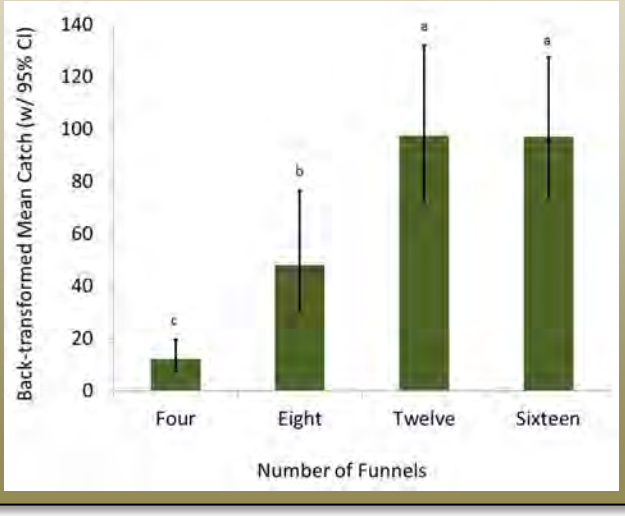
Efficacy of multi-funnel traps for capturing emerald ash borer: effect of color, size and trap coating. Field assays were conducted in southeastern Michigan in 2009 and 2010 to ascertain the feasibility of using non-sticky traps as survey and detection tools for emerald ash borer (EAB). In subsequent color comparison assays, green and purple painted multi-funnel (Lindgren) traps (and later, plastic versions of these colors) performed as well or better than prism traps. Multi-funnel traps coated with spray-on adhesive caught more beetles than untreated traps. The increased catch, however, occurred in the traps' collection cups, and not on the trap surface. In a separate assay, there was no significant difference detected between glue coated traps and Rain-X (normally a glass treatment) coated traps, but both caught significantly more *A. planipennis* adults than untreated traps[1].

The standard size multi-funnel trap we have used since 2009 is a 12-unit model. Miller and Crowe[2] found that funnel trap length (number of funnels) played a role in capturing some species of bark- and wood-boring beetles. We tested different sizes of traps (number of funnels) to determine if multi-funnel trap size plays a role in capture of EAB. We tested green and purple versions of 4, 8, 12 and 16-unit traps (**Fig. 1**) coated with Rain-X. There was no significant interaction between color and number of funnels per trap, so green and purple traps were combined for statistical analysis. Twelve-unit funnel traps were more effective than other traps as they caught more beetles than the smaller traps, and were not as unwieldy as the 16-unit traps (**Fig. 2**). Sex ratio was not affected by number of funnels per trap.

Flu-on has been shown to increase captures of cerambycids in baited intercept panel traps over Rain-X treated and control traps[3]. While Flu-on is clear when applied to a surface, it dries white. Concerned that this could change the attractiveness of the trap to EAB, we added pigments to a Flu-on base to change the color of the liquid and the dried residue. The pigments had previously been used to create the green and purple funnel trap plastic color matches (**Fig. 3**). The addition of the green pigment to Flu-on was unnecessary as

Figure 1: *Funnel traps containing 4, 8, 12, and 16 units.*

Figure 2: *Mean (95% CI) number of* A. planipennis *adults captured in funnel traps in a color comparison field assay in Clinton and Eaton Counties, Michigan in 2011 (n = 30).*



non-tinted (white) flu-on applied to green plastic funnel traps produced the highest catch of all trap treatments (**Fig. 4**). There was no significant difference among Rain-X and Flu-on treated purple multi-funnel traps and green-tinted Flu-on treated traps. Treating traps was necessary as uncoated traps were out-caught by Flu-on and Rain-X coated traps, regardless of color.

New prism trap colors for improving survey of emerald ash borer. The current emerald ash borer survey trap used in the US is a prism trap constructed from a stock purple corrugated plastic. Recent electro-retinogram assays demonstrated that male and female EAB are sensitive to light in the UV, violet and green (420-430, 460 nm and 530-560, respectively) ranges of the visible spectrum, while mated females are also sensitive to light in the red (640-670 nm) range[(4)]. Trapping studies have shown that green traps painted in the mid-range (22%-67%) of reflectance (brightness), and purple traps painted with a color originally shown to be attractive to buprestids, were most attractive to EAB[(5)]. Several plastics have been produced from these colors, and our goal was to determine if these plastics can improve on and serve as a new alternative to plastics already in use for EAB survey. In 2010, six colors were tested at about 5-8 m high in the lower canopy of heavily infested ash woodlots (**Table 1**). In 2011, treatments 1, 3, 4, and 6 were tested with two new colors, 7 and 8.

New purple and green plastic traps showed promise as alternatives to the standard purple corrugated plastic, but there was greater degree of variability in catch on green traps than on purple traps in 2010 and among all traps in 2011 (**Fig. 5**). Several of the purple card-stock traps fell apart during the field season so these may need work if they are to be an alternative to the current corrugated plastic.

Figure 3: *Tinted and untinted Fluon.*

Comparison of prism and multi-funnel traps at high and low population density sites. Results from studies performed in heavily infested sites may not always match with what is found along the edges of the infestation. In outlier infestations, purple prism traps while yielding lower catch than green traps in Michigan,

Figure 4: *Mean (95% CI) number of* A. planipennis *adults captured in funnel traps in a trap coating comparison field assay conducted in Clinton and Eaton Counties, Michigan in 2011 (n = 15).*

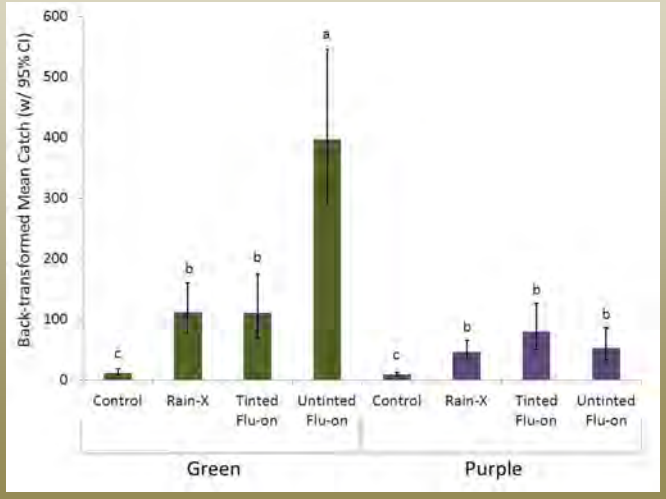


Figure 5: *Mean (95% CI) number of* A. planipennis *adults captured in prism traps in a color comparison field assay conducted in (a) Shiawassee County, Michigan in 2010 (n = 12), and (b) Clinton and Eaton Counties, Michigan in 2011 (n = 10).*

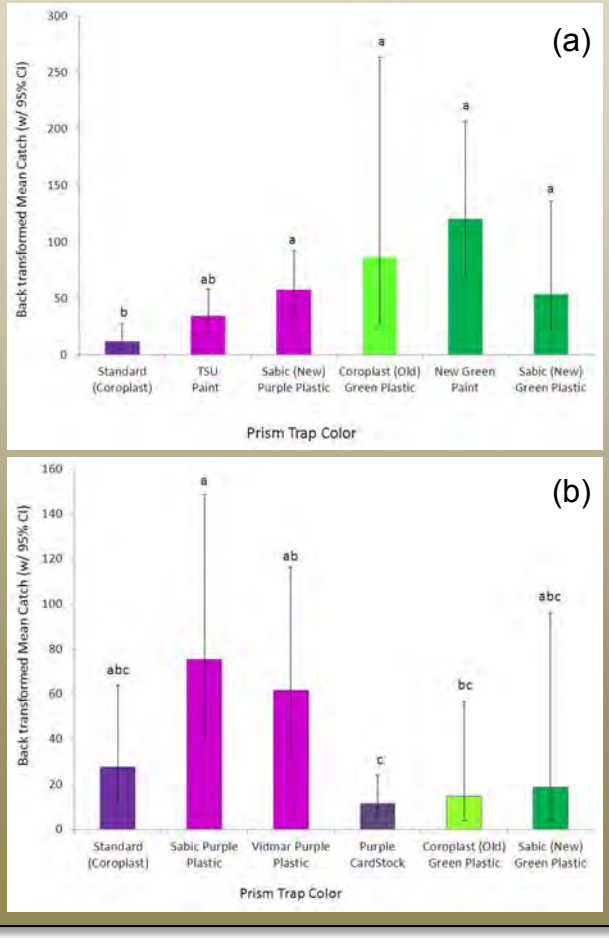


Table 1: *Colors and their descriptions, and the years they were tested.*

Trt	Test Year	Color	Description
1	2010, 2011	Coroplast Purple plastic	the standard color used for EAB survey in the US since 2008
2	2010	TSU Purple paint	a color found to be attractive to at least 31 species of buprestids including some Agrilus spp.[6]; painted on translucent prism traps
3	2010, 2011	Sabic Purple plastic	a newly manufactured plastic based on the TSU purple paint
4	2010, 2011	Coroplast (light) Green plastic	manufactured from a paint described as being attractive to EAB[4]; 540 nm, 67% reflectance; sometimes referred to as light green
5	2010	New Green paint	found to also be attractive to EAB[5]; 530 nm, 49% reflectance; sometimes referred to as medium or dark green
6	2010, 2011	Sabic Green plastic	a newly manufactured plastic based on the New Green paint
7	2011	Vidmar Purple plastic	a purple plastic based on TSU purple, manufactured by a different company than Sabic
8	2011	Purple Card-stock	a wax-coated card-stock prism designed to be more "user-friendly"; folds to a small size and is lighter than corrugated plastic

were found to have a higher detection rate (catch of at least one beetle)[7]. The detection tools/outlier study this year was conducted on public land in 9 states along or near the leading edges of the current emerald ash borer infestation in: Indiana, Kentucky, Maryland, Minnesota, Missouri, New York, Pennsylvania, West Virginia and Wisconsin.

Four trap designs were tested:

- Standard Purple prism
- Sabic Purple prism
- Sabic Green prism
- Green (12-unit) multi-funnel treated with Rain-X

Prism traps were coated with Tangletrap. All four designs were baited with a Manuka oil (50mg/d) / cis-3-hexenol (50mg/d) lure. Traps were placed at least 30m apart from each other within replicates, and replicates were placed at least 800 m from each other within states. Ninety-nine replicates were placed among the 9 states. Of the 99 reps, 5 were lost to Mississippi River flooding in Missouri and 17 did not catch a single beetle (no detection). For the analyses conducted, the 77 replicates that recorded at least one beetle were used. Marshall et al.[8] defined low and high density sites based on visual signs and symptoms on trees. They saw a natural break in trap catch between low (<87) and high (>273) density sites. We used these same numbers to define low (n=56) and high (n=7) density sites, but there were 14 sites that fell between these sites that we defined as medium density sites.

Trap catch was highest on Sabic Purple prism traps, and there was no significant difference between trap catch among the other trap types (**Fig. 6**).

The proportion of detection was highest on Sabic Purple prism traps among total sites (**Fig. 7**). It was also highest at low density sites (**Fig. 8**). There were no significant differences among any of the other trap types. In replicates where only a single trap recorded a detection and the other three trap types did not, the Sabic purple prism, Standard purple prism, Sabic green prism and green multi-funnel were responsible for 10, 2, 2 and 1 detections respectively.

Figure 6: *Mean (95% CI) number of* A. planipennis *adults captured in trap types traps in a detection tools comparison field assay conducted in nine states in 2011 (n = 77).*

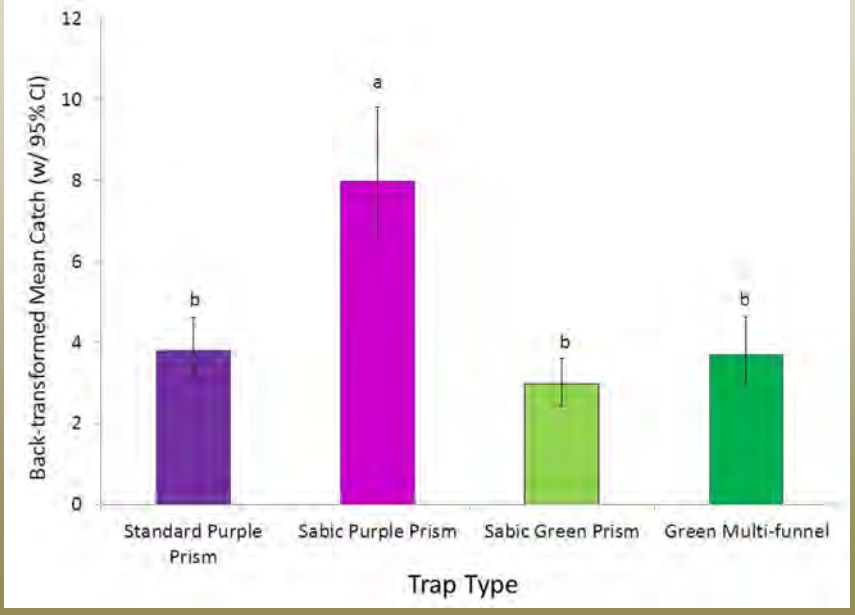


Figure 7: *Number of traps with and without EAB detections in 77 sites with at least one EAB detection. Numbers above bars represent the proportion of traps with detections. Different letters represent significant differences between trap types (χ^2 = 14.9, p = 0.002).*

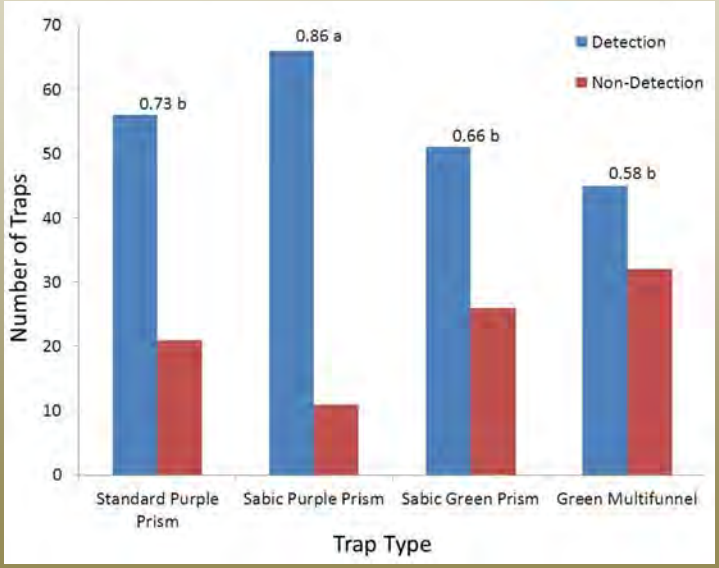
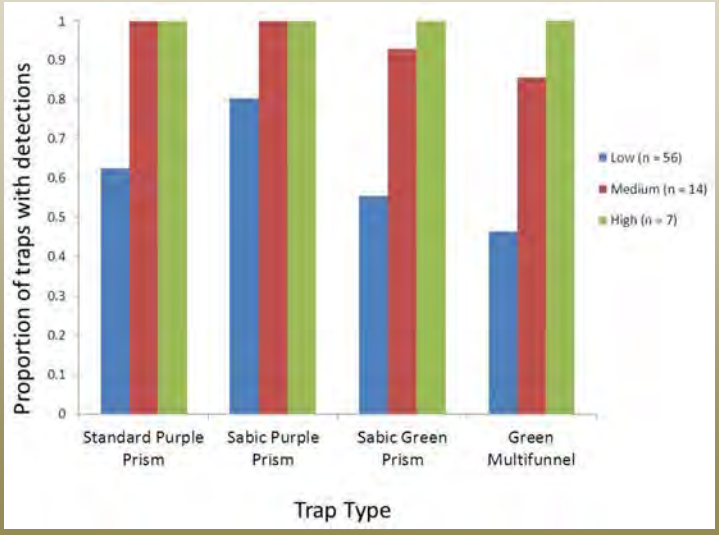


Figure 8: *Proportion of traps with detections at sites in a trap comparison study at three EAB population densities.*



References

(1) Francese, J.A., I. Fraser, D.R. Lance and V.C. Mastro. 2011. Efficacy of multi-funnel traps for capturing emerald ash borer (Coleoptera: Buprestidae): effect of color, glue, and other trap coatings J. Econ. Entomol. 104(3): 1235-1241.

(2) Miller, D.R. and C.M. Crowe. 2009. Length of multiple-funnel traps affects catches of some bark and wood boring beetles in a slash pine stand in northern Florida. Florida Entomol. 92 (3): 506-507.

(3) Graham, E. E., R. F. Mitchell, P. F. Reagel, J. D. Barbour, J. G. Millar and L. M. Hanks. 2010. Treating panel traps with a flouropolymer enhances their efficiency in capturing cerambycid beetles. J. Econ. Entomol. 103 (3): 641-647.

(4) Crook, D. J., J. A. Francese, K. E. Zylstra, I. Fraser, A. J. Sawyer, D. W. Bartels, D. R. Lance, and V. C. Mastro. 2009. Laboratory and field response of the emerald ash borer, Agrilus planipennis (Coleoptera: Buprestidae) to selected wavelength regions of the visible spectrum. J. Econ. Entomol. 102: 2160 - 2169.

(5) Francese, J. A., D. J. Crook, I. Fraser, D. R. Lance, A. J. Sawyer, and V. C. Mastro. 2010 . Optimization of trap color for the emerald ash borer, Agrilus planipennis (Coleoptera: Buprestidae). J. Econ. Entomol. 103: 1235 - 1241.

(6) Oliver J. B., N. Youssef, D. Fare, M. Halcomb, S. Scholl, W. Klingeman, and P. Flanagan. 2002. Monitoring buprestid borers in production nursery areas. pp. 17- 23. In G. Haun (ed.), Proceedings of the 29th Annual Meeting of the Tennessee Entomological Society. October 2002. Nashville, TN. 17: 12-13.

(7) Marshall, J. M., A.J. Storer, I. Fraser and V.C. Mastro. 2010. Efficacy of trap and lure types for detection of Agrilus planipennis (Coleoptera: Buprestidae) at low density. J. of Appl. Entomol., 134: 296-302.

(8) Marshall, J. M., A. J. Storer, I. Fraser, J. A. Beachy and V. C. Mastro. 2009. Effectiveness of different trap types for the detection of emerald ash borer (Coleoptera: Buprestidae). Environ. Entomol. 38: 1226 – 1234.

Baiting Emerald Ash Borer Dark Green Funnel Traps With Semiochemical Lures

Project Lead: Damon Crook

Contributors: Vic Mastro (Otis Laboratory)
Peter Silk, Kristal Ryall, & Taylor Scarr (Natural Resources Canada)
Allard Cossé & Ashot Khrimian (USDA, ARS)

In 2010 the standard survey trap used nation-wide by the USDA-APHIS-PPQ-EAB Cooperative Program was a glued, purple, prism trap, baited with a semiochemical lure (manuka oil, 50mg/d), hung at 6m in the subcanopy of an ash tree. A more user-friendly 'non glued' trap would be desirable because tens of thousands of glued traps are currently deployed and discarded at the end of each year. For program use, lure release devices should ideally last 3 months.

Trap color and design. Recent electroretinographic assays and trapping work have shown that light green traps catch significantly more emerald ash borer adults (especially males) than traps of other colors[(1)]. Light green traps typically catch more adults only when deployed high in the tree canopy. Thus, trap placement, as well as color and lure combination, must be considered when evaluating traps for a monitoring program. Francese et al.[(2)] recently improved the attractancy of light green (540nm) prism traps by adjusting green reflectance to 49% (i.e. creating a darker green). When this dark green color was incorporated into funnel traps and field tested alongside standard purple prism traps in an unbaited study, dark green funnel traps caught significantly more beetles when hung at 5~8m[(3)]. Funnel trap catch was improved further by the addition of Rain-X. No lures have yet been tested on the new dark green funnel traps.

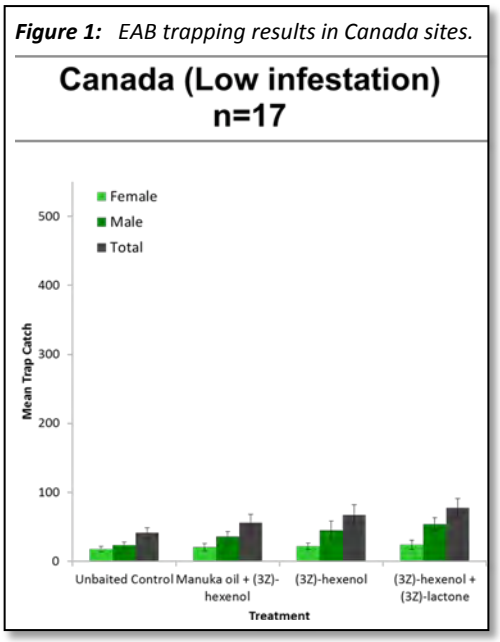
EAB lures. Specific host volatiles act as kairomones in the chemical ecology of *A. planipennis* and have proved useful as detection tools (reviewed by Crook & Mastro[(4)]). Two types of host volatiles have been demonstrated to be attractive to *A. planipennis*, bark sesquiterpenes (found in Manuka and Phoebe oil) and leaf volatiles (particularly (3Z)-hexenol). The first putative long-range pheromone for *A. planipennis* was identified as (3Z)-dodecen-12-olide [(3Z)-lactone][(5)] although no behavioral activity was reported.

The (3Z)-lactone significantly increased male trap catch when combined with the green leaf volatile, (3Z)-hexenol, in light green prism traps deployed in the canopy[(6)]. Captures of males with the (3Z)-lactone + (3Z)-hexenol were at least 50-100% greater compared to the (3Z)-hexenol. It appears that two cue modalities are required by *A. planipennis* in the host & mate-finding process: a visual cue (green) and a two-component olfactory cue: a foliage volatile (kairomone), (3Z)-hexenol, and the sex pheromone, (3Z)-lactone. By fine-tuning each of these three components it should be possible to improve trap effectivity even further.

Methods. Field tests on dark green funnel traps were carried out along the edges of infested white and green ash wood lots in Michigan, USA (n=15), as well as Ontario, Canada (n=17). USA field sites contained ash trees with moderate to severe levels of decline. Canadian field sites contained ash trees with low levels of decline. Each test consisted of four lure treatments:

1. unbaited control
2. Manuka oil (50mg/d) + (3Z)-hexenol (50mg/d)
3. (3Z)-hexenol (50mg/d)
4. (3Z)-hexenol (50mg/d) + (3Z)-lactone (0.5 mg septa loading)

Figure 1: *EAB trapping results in Canada sites.*



Rain-X coated dark green funnel traps were set within 2 m of tree stands, spaced 20-30 m apart in a randomized complete block design. Traps were checked thru June and July 2011 every week in the USA and every 2 weeks in Canada. All *A. planipennis* were collected, sexed and summed for the entire field season. Catches of males, females and total catch (males plus females) were analyzed separately for each experiment. Data from all experiments were transformed by log (x + 0.5) before being analyzed by randomized complete block design ANOVA. Tukey's honestly significant difference (HSD) test (α = 0.05) was used to compare differences in catch for each sex between treatments.

Results and conclusion for 2011. At the low infested sites in Canada (**Fig. 1**) and the high level infested sites in USA (**Fig. 2**), highest male catch was seen on green funnel traps baited with (3Z)-hexenol + (3Z)-lactone. No significant differences were seen in trap catch for males, females or total (male + female) in either study.

Our results show that in low and medium/high infestation areas dark green funnel traps (coated with Rain-X) caught high numbers of *A. planipennis* with or without the presence of manuka, (3Z)-hexenol or (3Z)-lactone lures. At the release rate used (3Z)-Hexenol did appear to help increase male trap catch but the differences were not significant. At the release rate used, the (3Z)-lactone lure improved male catch slightly (not significantly) in both regions. Further testing of the (3Z)-lactone and (3Z)-hexenol at difference release rates on these traps is ongoing, along with further trap placement studies. It is hoped that this will optimize trap catch and further improve the detection rate of the current monitoring program effort for this insect.

References

(1) Crook, D. J., Francese, J. A., Zylstra, K. E., Fraser, I., Sawyer, A. J., Bartels, D. W., Lance, D. R., and Mastro, V. C. 2009. Laboratory and field response of the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae), to selected regions of the electromagnetic spectrum. *J. Econ. Entomol*. 102: 2160–2169.

(2) Francese, J. A., Crook, D. J., Fraser, I., Lance, D. R., Sawyer, A. J. and Mastro, V. C. 2010. Optimization of trap color for emerald ash borer (Coleoptera: Buprestidae). *J. Econ. Entomol.* 103(4): 1235-1241.

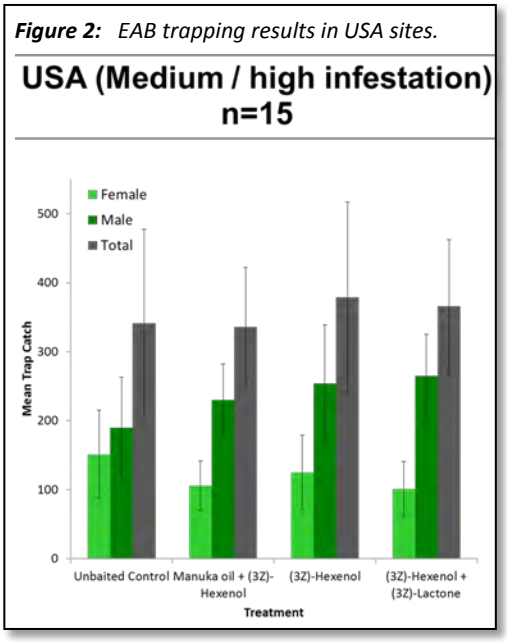
(3) Francese, J. A., Fraser, I., Lance, D. R. and Mastro, V. C. 2011. Efficacy of multifunnel traps for capturing emerald ash borer (Coleoptera: Buprestidae): Effect of color, glue and other coatings. *J. Econ. Entomol*. 104 (3): 901-908.

(4) Crook, D. J. and Mastro, V. C. 2010. Chemical ecology of the emerald ash borer *Agrilus planipennis*. Review article. *J. Chem. Ecol.* 36: 101–112.

(5) Bartelt, R. J., Cossé, A. A., Zilkowski, B. W., and Fraser, I. 2007. Antennally active macrolide from the emerald ash borer, *Agrilus planipennis* emitted predominantly by females. *J. Chem. Ecol*. 33: 1299–1302.

(6) Silk, P. J., Ryall, K., Mayo, P., Lemay, M. A., Grant, G., Crook, D. J., Cossé, A., Fraser, I., Sweeney, J. D., Lyons, D. B., Pitt, D., Scarr, T. and Magee, D. 2011. Evidence for a Volatile Pheromone in Agrilus planipennis Fairmaire (Coleoptera: Buprestidae) that Increases Attraction to a Host Foliar Volatile. *Environ. Entomol.* 40(4): 904-916.

Figure 2: *EAB trapping results in USA sites.*



Dorsal and lateral views of the emerald ash borer, Agrilus planipennis.

Emerald Ash Borer: Alternative Control Strategies

Project Lead: Juli Gould

Collaborators: Dave Williams (Otis Laboratory)
Leah Bauer (US Forest Service)
Jian Duan and John Vandenberg (USDA, ARS)
Jon Lelito (USDA, APHIS, PPQ, Brighton, MI)

Cooperators: Yang Zhang-qi and Wang Xiao-yi (Chinese Academy of Forestry)

Release and evaluation of establishment and impact. Three parasitoid species (*Spathius agrili, Tetrastichus planipennisi,* and *Oobius agrili*) have now been released in 12 of the 15 states infested with EAB, with the addition of four new states and 23 new sites in 2011. It can often take years after parasitoids are released to recover them in the field; however we have already recovered parasitoids in the five states where releases have been occurring for the longest time (MI, OH, IN, IL, MD). *Spathius agrili* has overwintered at 9 sites and is considered established (recovered at least one year after the last release) at six of these sites (**Fig. 1**). *Tetrastichus planipennisi* has established at 6 of the 10 sites where it has overwintered (**Fig. 2**). Options for samp-ling *Oobius agrili* are more limited, fewer recovery samples have been taken, yet we have determined estab-lishment at 7 sites and overwintering at two more sites (**Fig. 3**).

Prior to 2011, sampling for parasitoid recovery involved cutting trees and removing the bark. This method is destructive and labor intensive, but it was effective. USDA Forest Service developed yellow pan traps for sampling the two larval parasitoids, based on success in 2010. The benefits of this method are that it is less destructive, less labor intensive, and can cover a wider area because more traps can be deployed. I put together yellow pan trap kits and sent them to cooperators, who deployed 15 traps at each of 18 sites in four states (**Fig. 4**). The traps were very attractive to

Figure 2: Tetrastichus planipennisi *overwintered at sites represented by blue dots and established (was found one or more years after the last release) at sites with green dots. No parasitoids were recovered at sites with red dots.*

Figure 1: Spathius agrili *overwintered at sites represented by blue dots and established (was found one or more years after the last release) at sites with green dots. No parasitoids were recovered at sites marked with red dots.*

Figure 3: Oobius agrili *overwintered at sites represented by blue dots and established (was found one or more years after the last release) at sites with green dots. No parasitoids were recovered at sites marked with red dots.*

parasitic hymenoptera, and we recovered 22 *Spathius*, however only 2 of them were *S. agrili*. These two specimens, though, represented evidence of establishment in Illinois and Michigan. No *Tetrastichus* were recovered. These results, combined with evidence from past studies, suggest that we do not have one single method that recovers parasitoids at all sites and that a combination of yellow pan traps, sentinel logs, and bark peeling may be necessary to document establishment.

Figure 4: *Yellow pan trap on ash tree.*

Because determining EAB and parasitoid density is too labor intensive and costly to be done at more than just a few sites, I developed indirect measures of ash health that could quickly be assessed at sites where parasitoids had and had not been released. Unfortunately, parasitoids were unable to catch up to EAB at most sites and most of the monitored trees died. Methods to document parasitism and survival of ash trees as they grow to a size where they are susceptible to EAB are being developed. Interestingly, at two sites in MI where parasitoids were released when EAB was at very low density, the trees are still very healthy, but frustratingly we have not been able to recover parasitoids, possibly because there are few EAB to sample. We will continue to monitor these sites.

Life-table studies of EAB in its native range. Studies of the contribution of natural enemies and tree resistance to mortality of EAB in its native range are underway in collaboration with ARS and foreign cooperators. We now have sampled EAB populations in Liaoning Province, China on the native *Fraxinus mandshurica* and in Tianjin City on the American *F. velutina*. ARS has also studied EAB populations in Russia on both the native *F. rhynchophylla* and the American *F. pennsylvanica*. It was necessary to girdle the Asian species in order to attract EAB for sampling.

The stage specific mortality for EAB in two seasons at the two sites is shown in **Tables 1 & 2**. In Liaoning Province, the most prominent mortality factors were mortality from egg parasitoids and callusing, where the tree mounted a defense and killed the developing larva.

Table 1: *Mortality of EAB collected from 10 girdled F. manchurica in Liaoning Province, China.*

Site	Season	Stage	Fate	Percentage
Liaoning	Fall	Egg	Alive	21%
			Dead	9%
			Parasitized	32%
			Predated	18%
			Unhatched	19%
		Instar 1-2	Alive	56%
			Dead/Fungus	3%
			callous	39%
			Tetrastichus	2%
		Instar 3-4	Alive	70%
			Dead/Fungus	24%
			Tetrastichus	6%
		J-larvae	Alive	67%
			Dead	33%
Liaoning	Summer	Egg	Alive	43%
			Dead	1%
			Parasitized	32%
			Predated	10%
			Unhatched	13%
		1~2	Alive	39%
			Dead/Fungus	3%
			callous	58%
		3~4	Alive	87%
			Tetrastichus	11%
			Woodpecker	2%

Table 2: *Mortality of EAB collected from F. velutina in Tianjin City, China.*

Site	Season	Stage	Fate	Percentage
Tianjin	Fall	Egg	Alive	75%
			Dead	13%
			Parasitized ?	1%
			Predated	11%
		1~2	Alive	97%
			Dead/Fungus	0%
			callous	2%
			Spathius	0%
		3~4	Alive	31%
			Dead/Fungus	2%
			callous	0%
			Predated	8%
			Spathius	59%
			Unknown	1%
		JL	Alive	99%
			Dead/Fungus	1%
Tianjin	Spring	3~4	Alive	36%
			Dead/Fungus	16%
			Predated	23%
			Spathius	25%
		Pupa	Alive	93%
			Dead/Fungus	3%
			Predated	4%

T. planipennisi was present, but parasitism levels were low. Tree defenses killed very few EAB in the native *F. velutina* in Tianjin City, and parasitism by *S. agrili* was the most common mortality factor. Egg parasitoids were not a factor in Tianjin. The impact of parasitoids on EAB in non-native ash was similar in Russia. Seventy percent of EAB were killed by the tree when they were located in *F. rhynchophylla*, and only 20% were parasitized.

These percentages were reversed when EAB attacked *F. pensylvannica*, with 65% killed by a *Spathius* species and only 20% killed by the tree. The high levels of parasitism in Asia in ash trees native to the United States suggest that these parasitoids might contribute to EAB control in this country.

Use of girdled trees to enhance establishment and recovery. Release of parasitoids at sites with low to moderate EAB density has several advantages foremost of which is that parasitoids will have time to establish and build in number before the trees succumb to EAB and the host population crashes. However, parasitoids must find EAB for parasitization, and program personnel must find the parasitized EAB to determine establishment and impact. EAB adults are attracted to girdled trees for oviposition, and we (CPHST and the Forest Service) tested whether girdled trees attract EAB and enhance the likelihood of parasitoid establishment.

Three treatments were tested: Parasitoid release on girdled trees, release on non-girdled trees, and a no release control. We had five replicates of each treatment, with the density of EAB ranging from low to moderate. Trees were girdled in May 2010 and larval parasitoids were released in the late summer of 2010 and in 2011. *Oobius* was released in the summer of 2011. Half of the trees were sampled for parasitoid recovery in 2010. Unfortunately, few released parasitoids were recovered, but parasitoid releases had not yet been completed. Sampling will continue in the spring of 2013.

Application of sugar water to enhance the probability of parasitoid establishment. One reason that released parasitoids might disperse from a release site is a lack of a source of sugar for food. Parasitism has been shown to be enhanced for several species (including a *Spathius* that attacks bark beetles) by spraying sugar water prior to release. We selected four sites in Ulster Co. NY and four sites in Cattaraugus Co. NY for our study. Ash trees were girdled in May and larval parasitoids were released in August/September. At four of the eight sites, sugar water was sprayed on the tree trunks and foliage prior to release (**Fig. 5**). Larval parasitoids were released again in the spring of 2012 and *Oobius agrili* was released in the summer of 2012. Sampling to determine establishment Is ongoing, with recovery of *S. agrili* in yellow pan traps in our first sample.

Impact of woodpeckers on parasitized EAB. Woodpeckers can consume a large number of overwintering larvae, and I wanted to determine if this predation adversely affected the likelihood of establishing larval parasitoids, or if it could affect future estimates of parasitoid impact. I have seen a woodpecker attack where the *S. agrili* cocoons seemed uneaten, but it was a sample size of one. In Ulster Co. NY, five sites that were heavily infested with EAB (and presumably had naturally occurring larvae in the trunks) were selected. Two cages were put on six trees at each site (**Fig. 6**). *S. agrili* was released into both cages on two trees per site, *T. planipennisi* was also released

Figure 5: *Application of sugar water onto tree trunks and foliage.*

Figure 6: *Cages on tree trunks in woodpecker study.*

into cages on two trees, and the remaining two trees served as controls. In late August, the cages were removed, and one of the cage sites was covered with window screening to deter predation by woodpeckers, while the trunk that had been covered by the other cage was exposed to woodpeckers. The screening effectively excluded woodpeckers and woodpecker predation was high. Unfortunately, although cage releases have been successful, we did not find any parasitized EAB.

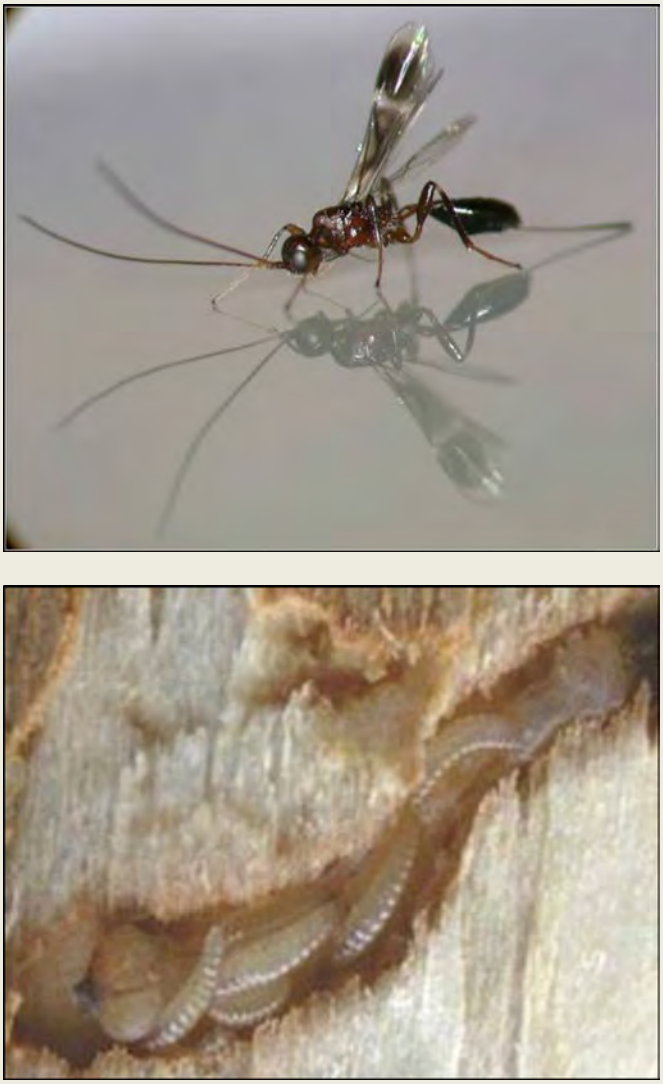
Oviposition and progeny development for parasitoids "released" in different climates. Program releases of parasitoids are often made well into the fall as long as temperatures remain sufficiently warm for adult oviposition. It is not clear, however, if parasitoids will oviposit under these short day conditions and if they do, if their progeny will develop to a diapausing stage before the onset of freezing temperatures. I programmed environmental growth chambers to simulate temperature, daylength, and humidity profiles for Baltimore, MD; Detroit, MI; and Duluth, MN. We "released" parasitoids whose parents experienced either warm/long-day or warm/short day conditions. Starting August 15th (simulated), parasitoids were "released" weekly until daytime high temperatures reached 8°C (a common developmental threshold). Percentage parasitism and development of progeny (**Fig. 7**) was be determined for all weekly releases to inform the program as to when to cease releases in the different climate zones. Parasitoid releases after the first of September resulted in few overwintering offspring in both Baltimore and Detroit, indicating that *S. agrili* should not be released after September 1. In Addition, as we evaluate establishment of parasitoids released by the EAB Biocontrol Program, this information will be important because many releases were made in September and October.

Host specificity, instar preference and biology of *Spathius galinae*. I am working with Jian Duan (USDA-ARS) to collect the data necessary to submit an application for a permit to release the Spathius species from Russia. We have tested ALB (no attack) and we are in the process of testing the gold spotted oak borer. Dr. Duan has tested additional species. We hope to complete the testing and start writing the application for release in the spring of 2013.

Proposed Research FY 2012. All of the studies described above will be continued in FY 2012, mostly with data collection to determine the effects of the various treatments. Two additional activities are planned.

Determining the impact of EAB parasitoids on EAB and ash populations. To date, there is no conclusive evidence that parasitoid releases can save large ash trees from dying from EAB damage. However, at sites where parasitoids have established, saplings will eventually grow to a size that is susceptible to attack by EAB. I am collaborating with colleagues to develop a method to monitor EAB density and mortality from parasitoids in the next generation of ash trees.

Figure 7: *An adult* S. agrili *female (top), and an emerald ash borer larva* parasitized by several S. agrili larvae (bottom).



Monitoring Tools for the Detection of *Spathius agrili*, a Parasitoid of Emerald Ash Borer

Project Lead: Miriam Cooperband

Contributors: Ashley Hartness & Juli Gould (USDA, APHIS, PPQ, CPHST, Otis Laboratory)
Allard Cossé (USDA, ARS, Peoria, IL)
Jon Lelito & Mike Rietz (USDA, APHIS, PPQ, Brighton, MI)

The parasitic wasp Spathius agrili *has been released in the United States as a biological control agent against emerald ash borer. To determine whether this species has established, monitoring tools are needed. A pheromone developed by ARS scientist Dr. Allard Cossé was used in studies to test 1) preferences for landing surfaces of different colors in the wind tunnel; 2) trap designs in the wind tunnel and field; and 3) test for attraction to pheromone by wasps of different mating status in the wind tunnel. It was found that yellow, white, and green colors were preferred over red, black, and purple. In our wind tunnel bioassay, the pheromone worked best for females that had not mated. Field trapping studies at four locations where they had been released at least one year before used different approaches (pheromone-color-sticky, pheromone-suction, and color-soap-pan traps), and found just 2 wasps in the color-soap-pan traps.*

Since its introduction into the United States, the emerald ash borer (EAB), *Agrilis planipennis*, has killed millions of ash trees in as many as fourteen states. There is an urgent need for intervention to control this species. The most sustainable approach to controlling an invasive species that has become so widespread is biological control. *Spathius agrili*, a braconid parasitoid of EAB from China, has been targeted as a suitable biological control agent, and that species has been released at a number of field sites in and around Michigan. It is necessary to evaluate whether or not the species has been able to overwinter and establish. Therefore, monitoring tools are needed for the detection of *S. agrili*.

Insects. Rearing of *S. agrili* was conducted at the Brighton laboratory under the supervision of Dr. Jon Lelito. Male and female *S. agrili* were sorted into separate containers prior to mating and sent to the Otis Laboratory where behavioral bioassays were conducted in the wind tunnel. Males and females were stored in separate environmental chambers at 20-25°C, 75% RH with 16L:8D light regime. Upon arrival at the Otis Laboratory, they were approximately 5 d old.

Figure 1: *Wind tunnel with color choice test.*

Wind tunnel bioassays. A push-pull wind tunnel (120 cm x 91 cm x 75 cm) was constructed for use inside a walk-in environmental chamber (**Fig. 1**). Air was cleaned of odors as it passed through carbon filters upon entering and leaving the wind tunnel. Paper discs (5 cm diam.) with holes punched in them were used as landing targets. Pheromone lures were obtained from ARS scientist Allard Cossé. The lure was placed in the center of the paper disc, and the holes around it allowed the air to flow around the lure and the plume to be carried downwind to the release stage. Insects were placed individually on the release stage and allowed 3 min to respond. Insects that flew upwind in the plume were considered to have responded, whereas insects that remained on the stage, or flew to the ceiling, wall, or floor of the wind tunnel were considered as non-responders.

Color. Six colors were selected to test in the landing preference study (white, yellow, red, green, purple, and black). Colors were scanned for reflectance and wavelength, and attempts were made to match the green and purple colors to those used in the EAB green and purple prism traps (**Fig. 2**). Paper hexagonal discs (5 cm diam.) were printed with combinations of two color

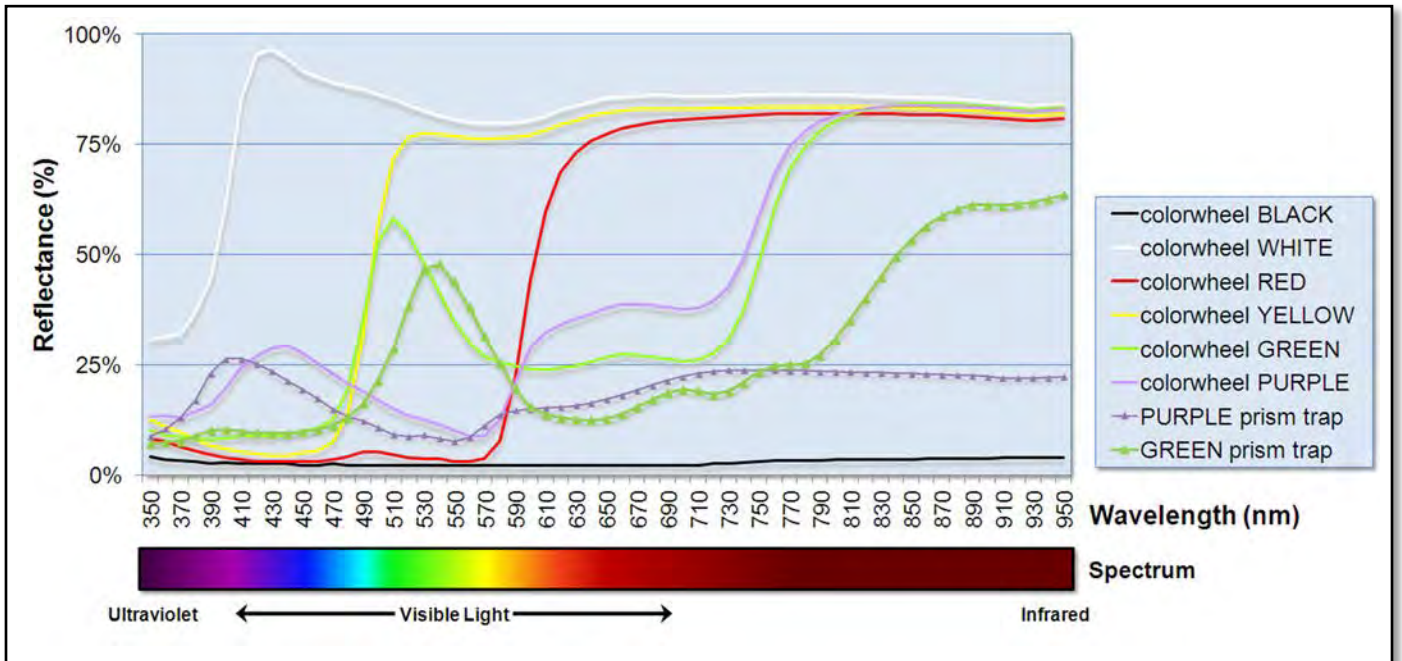
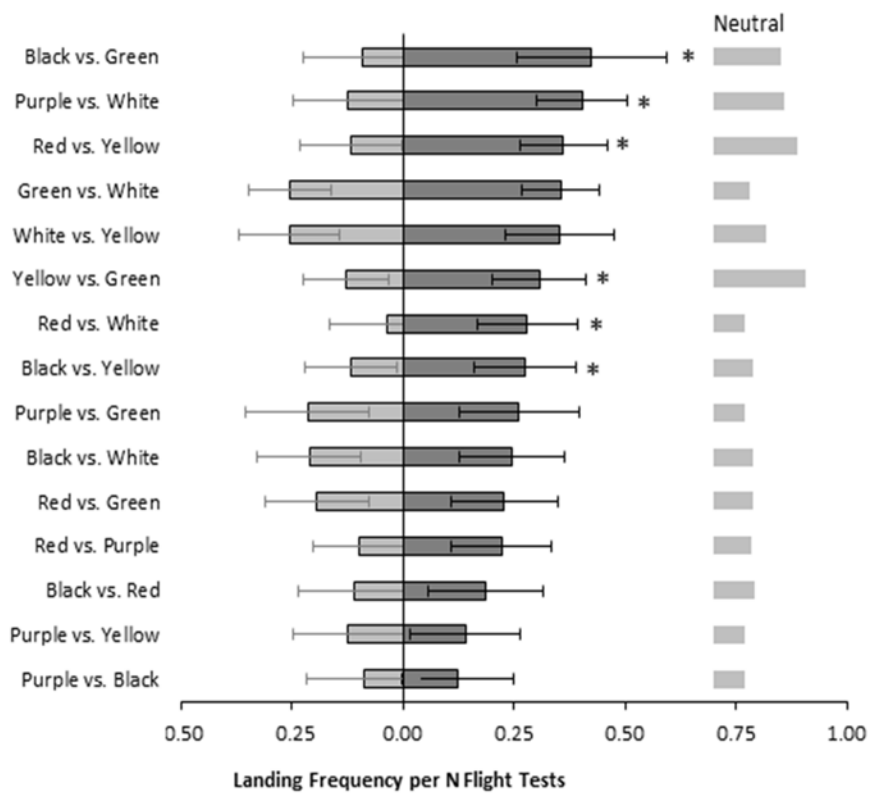


Figure 2: *Spectra of colors used in color choice bioassays.*

choices on the two halves of each disc. Fifteen holes were punched in the discs, a pheromone lure was placed in the center hole, and the disc was suspended in the upwind end of the wind tunnel. Virgin female wasps were individually released downwind and the color on which they landed was recorded. If they landed on the back of the disc, on the clip holding the disc, or on the rubber septum, the color choice was regarded as neutral.

Figure 3: *Landing choices made by* Spathius agrili. *Asterisk shows significance.*



Color preferences. The percent of virgin females that flew upwind to the lures ranged from 61.4% to 97.7%. Of those that flew upwind, landing frequency ranged from 39.6% to 81.5%. The most upwind flight and highest landing frequency occurred with green, yellow, and white targets, and the lowest with red, black, or purple targets (**Fig. 3**). Wasps that flew upwind and landed on a neutral part of the target, such as the septum, the mid-line, on the clip holding the target, or the back of the target, were excluded from the statistical analysis. There was no significant difference between white and green, or white and yellow. Although more wasps landed on the green when offered next to yellow, that combination resulted in more neutral choices than other color combinations. Green and yellow were chosen more than black; yellow and white were chosen more than red; and white was chosen more than purple. Based on these data, white,

yellow, or green traps would be expected to perform better than red, black or purple traps. Statistical analysis was conducted on choice to test the hypothesis that both colors were landed on at equal frequencies, using the Chi Square goodness-of-fit test at α=0.05.

Mating. To evaluate whether mating status had an effect on attraction to the pheromone lure, females were either virgin or mated. To ensure mating took place, male and female wasps were paired and placed in an Eppendorf tube and watched until mating was observed to take place.

Wasps were tested in the wind tunnel first as virgins to ensure they were responsive to the pheromone. After that, half of the wasps were mated, and then all wasps were tested again in the wind tunnel. The results found that although virgin females were strongly attracted to the pheromone lure, females were no longer attracted to the lure after mating (**Table 1**). Wasps were re-tested 11 d after mating, and attraction to the pheromone was not recovered.

Table 1: *Number of mated and virgin* S. agrili *females that flew upwind and landed on the pheromone target.*

	Upwind Flights	Landings on Target	N
Virgin	119 a	70 a	170
Mated	7 b	1 b	234

Totals in the same column followed by different letters are significantly different (Student's T test, P<0.05)

Effects of oviposition on attraction to pheromone. Another study tested the effect of oviposition on attraction to the pheromone, and whether females will regain attraction to the pheromone after oviposition. Females were split into two groups, those that would be mated and those that would remain virgins. They were all tested to verify that they would fly to the pheromone. Then half of them were mated as described above. They were all tested again in the wind tunnel to evaluate changes in attraction to the pheromone. Then each female was placed individually in a jar and provided with a stick of ash containing two EAB late instar larvae.

The EAB larvae had been carefully extracted by peeling naturally infested ash sticks, and uninfested ash sticks were prepared for the infestation of two EAB larvae each. Two larval chambers were created on uninfested sticks. Each chamber was created by peeling back 3 cm of bark on the uninfested sticks, carving out a channel in the exposed wood, placing an EAB larva in the channel, covering it back up with the bark, and holding down the bark with a rubber band. The odors and chewing sounds of the EAB larvae in the ash sticks stimulated *S. agrili* females to oviposit.

EAB larvae were examined daily for *S. agrili* eggs, and any larvae that were dead or parasitized were replaced with fresh larvae. Every day the *S. agrili* females were tested in the wind tunnel to evaluate flight response to the pheromone. Survival, frequency of oviposition, and total eggs oviposited were not affected by mating status, however upwind flights and target landings were significantly lower for mated females (**Table 2**).

Field testing of traps. To evaluate trap design, and determine if *S. agrili* had established, three trap designs (**Fig. 4**) were evaluated at four field sites in MI and OH, where *S. agrili* had been released in previous seasons by Dr. Juli Gould. The first trap was a yellow pan trap filled with propylene glycol and a drop of odorless detergent. The only attractant for this trap was the yellow color of the plastic bowls (Yellow Sunshine, Festive Occasion plastic party bowls and plates). No pheromone was used for this trap. The second trap design was the same color plastic plates (Yellow Sunshine, Festive Occasion) hung vertically, coated with Tanglefoot glue, with a pheromone lure in the middle, and 15 holes punched into them for air flow past the lure (similar to the paper discs used in the wind tunnel). The third trap design resembled a miniature version of a counterflow geometry trap used for mosquitoes. Eight D cell batteries powered a 12V CPU fan which sucked air into the trap through a yellow tube, and blew the air out of the trap above the tube. The pheromone lure was attached to the yellow tube just above the opening.

Traps were deployed for 4 weeks starting the second week of August, 2011 and running until the second week of September, 2011. A grid was drawn at each of the four field sites (three in Michigan and one in Ohio) containing ash trees. Each grid was 400 m x 400 m and centered around the trees where *S. agrili* had been released in previous seasons. Grids consisted of a

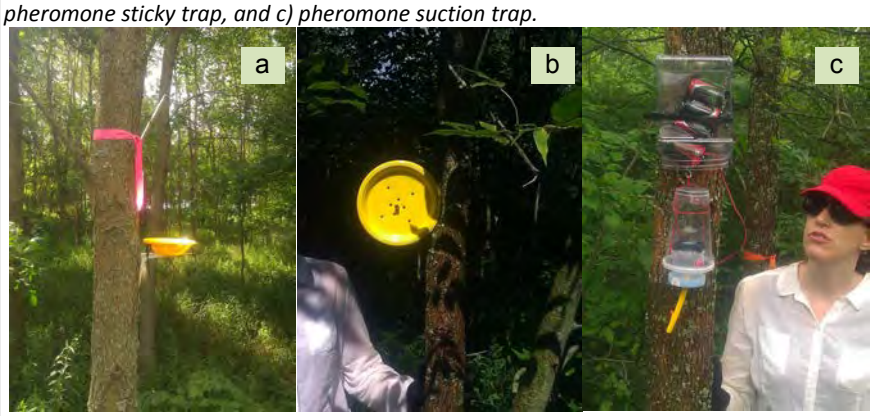
Table 2: *Means for virgin and mated female* Spathius agrili *that were provided with unlimited host material for 55 d.*

	Survival (d)	N Test Days	Days Eggs Present	Total No. Eggs	Frequency of Upwind Flights	Frequency of Target Landings
Virgin	41.4 a	16.6 a	5.2 a	22.4 a	0.4082 a	0.2063 a
Mated	38.4 a	15.8 a	3.8 a	21.0 a	0.0118 b	0.0078 b

Totals in the same column followed by different letters are significantly different (Student's T test, P<0.05)

square of 64 cells (8 cells x 8 cells). Each cell was 50 m x 50 m, and up to one trap was placed near the center of each cell. One of each trap type was placed in three of the four center cells (leaving one cell without a trap), two of each trap type was placed in the next ring out of 12 cells, four of each trap type was placed in the third ring out of 20 cells, and 8 of each trap type was placed in the last ring of 28 cells. Some cells did not contain ash trees, so those were excluded. Traps were monitored weekly, and pan and sticky traps were changed every two weeks. The batteries and pheromone lures were replaced after two weeks. Only two *S. agrili* were captured at the four study sites, both in the yellow pan traps. This confirms that *S. agrili* established at those sites, but such a small number does not allow for any comparison of traps in the field. More research is needed in areas where populations of *S. agrili* are more reliable.

Figure 4: *Three traps tested for S. agrili: a) yellow pan soap trap, b) yellow plate pheromone sticky trap, and c) pheromone suction trap.*



Classical Biological Control of Emerald Ash Borer

Project Lead: Dave Williams

Cooperators: Hai-Poong Lee (Dongguk University, Seoul, South Korea)
Galina Yurchenko (ar East Forestry Institute, Khabarovsk, Russia)

Our general objective in this work is to discover new species or biotypes of EAB natural enemies in South Korea and Russia and provide them as biological control agents for the Emerald Ash Borer Emergency Program. We are carrying it out in cooperation with Professor Hai-Poong Lee of Dongguk University, Seoul, and Dr. Galina Yurchenko of the Far East Forestry Institute, Khabarovsk. One of our greatest achievements in the past year or two has been the discovery and description of a braconid larval parasitoid, *Spathius galinae*, which shows great promise as a biological control agent (**Fig. 1**). Unfortunately, progress on this project was hampered in 2011 by an agency limitation on foreign travel that resulted in my being unable to visit our field sites. Because I was unable to participate in the tedious and painstaking collection process, I ultimately received fewer *Spathius* than desirable to start a culture.

Figure 1: *Females of* Spathius galinae *imported from South Korea.*

Improved Rearing Technology for Asian Longhorned Beetle, *Anoplophora glabripennis*

Project Lead: David Lance

Contributor: Barb Holske

The Asian longhorned beetle (ALB) program has been funding work to maintain colonies of ALB within the Otis Containment Facility (**Fig. 1**), develop improved rearing methods, and provide specimens for experimental and outreach work. Various studies associated with this project in the past have allowed us to develop a reliable artificial diet for rearing the insects from neonate to pupa, and develop an artificial substrate of cheesecloth on cardboard to replace logs as an oviposition site. Finding an artificial alternative to twigs as an adult food remains as a developmental goal, along with reducing time and labor required for rearing this insect. Basic overall goals of the project are:

- Develop diets and handling methods to allow for stable, cost-effective rearing of Asian longhorned beetle (ALB).
- Maintain colonies of ALB to provide a year-around source of test organisms for a variety of scientific studies.
- Provide ALB specimens for outreach and education programs.
- Develop information on basic biology and bionomics of ALB.

Strains in culture (FY 2011):

- Otis lab colony (mixed strain derived primarily from collections in NY and IL).
- Chinese strain (collected by B. Wang).
- Worcester strain.

Note: introduction of an Ohio strain is planned; the mixed strain will eventually be phased out.

Figure 1: *Asian longhorned beetle,* Anoplophora glabripennis.

Work in FY 2011:

- Maintained strains listed above. At the end of FY-11, there were approximately 120 adults and 1200 larvae and pupae in culture.
- Provided several hundred experimental insects for work in the following areas:
  - Visual and chemical ecology assays
  - Insecticide efficacy testing
  - Detector-dog studies (actually frass rather than insects)
  - Host-range testing (for pre-release evaluation of parasitoids)
  - Molecular (DNA) analyses
- Provided at least 200 each of larvae and adults for education and outreach displays.
- Continued study to characterize larval stadia (B. Wang).
- Updated ALB rearing SOP.
- Issue in FY-2011: There was a problem with low hatch this past spring. Like rearing problems sometimes do, it seemed to resolve itself without the cause ever being identified.

Evaluation of the Host Range and Preference of the Asian Longhorned Beetle

Project Lead: Baode Wang
Contributor: Vic Mastro (Otis Laboratory)
Cooperators: Ruitong Gao and Chuang Song (Chinese Academy of Forestry)
Yan Wang, Xiaoming Yang, and Yanfang Jin (Baiyin Municipal Forestry Bureau)

The purpose of the study has been to evaluate the host range, preference for feeding and egg-deposition and suitability for completing life cycle of tree species by the Asian longhorned beetle (ALB), Anoplophora glabripennis *(Motschulsky).*

Brief summary. This is a summary of our studies on host range of the Asian longhorned beetle (ALB), *Anoplophora glabripennis* in supporting the current USDA APHIS ALB eradication program. Studies prior to 2011 have produced useful information and the results were provided to the ALB national program for determining which tree species to be included in the surveys and treatment activities against the beetle. We confirmed the status of different *Acer* (maple), *Ulmus* (elm), *Salix* (willow), and *Populus* (poplar) species as ALB host and established the order of their preference by the beetle for feeding and egg-deposition. We also have found a few additional tree species that can be used by ALB as host. These include Russian olive (*Elaeagnus angustifolia*), goldenrain tree (*Koelreuteria paniculata*), grey alder (*Alnus incana*), green ash (*Fraxinus pennsylvanica*), Chinese ash (*Fraxinus chinensis*), and Japanese pagoda tree (*Styphnolobium japonicum*). Some of these trees were only attacked in certain situations.

Figure 1: *(a) field site in Baiyin, Gansu province, China, and (b) adult ALB emerged from a Japanese pagoda tree.*

In 2011, we conducted the following studies:

- Continued the evaluation of ALB host preference and suitability at two sites (Gansu and Beijing, China)
- Conducted small field plot preference testing of tree species planted in net-houses
- Surveyed different trees species planted in urban and rural areas in China to determine the preferences of these trees by ALB adults

Evaluation of ALB host preference and suitability at two sites (Gansu and Beijing, China). This study was conducted at two sites in "common garden" settings. One site was in the suburbs of Beijing with primarily North American tree species, and the other site is in Baiyin, Gansu province with primarily native trees in China.

Site 1: Baiyin, Gansu Province

Approach. This site is located in northwestern China with primarily native trees. In 2011, we evaluated:

1) Whether ALB can complete development on the Japanese pagoda tree,
2) Whether ALB can complete development in some 20 trees that had active sites in fall 2010,
3) Determine the suitability of Russian olive as ALB host.

In the summer of 2011, all live trees in 28 species were checked for the number of ALB adults on trees, egg sites, active egg sites and exit holes from July 12 to September 24, approximately once every week.

Results. No adult beetles were found during the time. The natural ALB population level has been very low at this site since 2008. Although some of the tree species (e.g., maple, willow and birch) at this site have been known as ALB preferred hosts, new egg sites were only found on the following three species: *Populus alba* var. *pyramidalis*, *P. tomentosa,* and *Ulmus pumila.*

Figure 2: Testing trees (a) planted inside a net-screen house, and (b) an exit hole on a tree of Aesculus hippocastanum.

One significant finding at this site in 2011 was that one female ALB adult emerged from a Japanese pagoda tree (**Fig. 1**). This tree had been caged with a pair (a male and a female) of beetles in 2008 and found to have an active egg site. This is the first record of *S. japonicum* as ALB physiological host. We had not seen much feeding or any other active egg sites or exit holes on other individual trees of the same species in any situation prior to this finding. It took about three years for the beetle to complete development on this tree.

A total of 15 Russian olive trees were caged with beetles. However, only 243 egg sites were found on sections of Russian olive trees in nine cages. No active sites were found by mid-September. We have found that this species is preferred for feeding by ALB adults and found a number of egg sites on them when ALB population level are high. We also found two exit holes on this species in 2000, and 2001, but have not seen any since.

Site 2: Beijing (Primarily Trees from North America)

In previous studies, we have compared different trees for their acceptance and suitability as ALB host. Trees at this site are primarily 20 tree species from the US, including maples (*Acer* spp.), and ash (*Fraxinus* spp.). No beetles were marked or caged with trees in both the choice and non-choice plots for trees planted prior to 2011. We simply checked all trees for signs of infestations once every week from mid-June to late September. We recorded new oviposition sites, larval activity (e.g., frass) and adult emergence (exit holes). No new exit holes were found on any of the host trees such as the Norway maple (*Acer platanoides*), and painted maple (*A. mono*) probably due to the low natural ALB population in 2010 and 2011.

Small plot tests of tree species were conducted in net-houses using transplanted trees to determine ALB preferences. In 2011, we added 5 tree species in this net-house host preference study that was initiated in 2010 (**Fig. 2**). Initially, we started with seven tree species including black walnut (*Juglans nigra*), paper birch (*Betula papyrifera*), Chinese goldenrain tree (*Koelreuteria bipinnata*), little-leaf linden (*Tilia cordata*), horse chestnut (*Aesculus hippocastanum*), the European hazelnut (*Corylus avellana*), and yellow poplar (*Liriodendron tulipifera*). The five species added in 2011 were: Norway maple, sugar maple (*A. saccharum*), painted maple, grey alder, and a poplar (*Populus* sp.).

One pair (1 female and 1 male) of newly collected adult beetles were released into the net house at 36 release points in early, mid and late July, for a total of three times at 7-10 d intervals. All beetles were marked to be distinguished individually.

The number of feeding sites was recorded. The area of leaves (include number of veins and petioles), twigs or the stem (trunk) consumed were also measured.

Results. When the 12 species were planted together, ALB adults stayed more frequently on horse chestnut, painted maple, Norway maple and little-leaf linden than they did on others such as gray alder and yellow poplar. The top 4 tree species ranked by feeding areas were painted maple, Norway maple, sugar maple, and little-leaf linden. The greatest numbers of egg sites were on horse chestnut, and Chinese golden-rain tree, while poplar, and painted maple had intermediate number of

egg sites. No egg sites were found on black walnut and yellow poplar trees. A few active egg sites were found on Chinese golden-rain tree, little-leaf linden, and painted maple. However, none were found on horse chestnut. In a previous test, ALB adults emerged from horse chestnut trees.

Beetles were caged with a section of a tree on July 17 for selected trees in 11 of the 12 species. Egg sites were checked and dissected on August 9. It was found that a few egg sites had some type of tree secretion (such as resin or foam), for example: 69% on poplar, 44.3% on Norway maple, 8.2% on horse chestnut, and 2.8% painted maple.

The order based on the percentage of viable eggs (normal eggs and newly hatched larvae) on trees: 1) box elder, and Norway maple (both >80%); 2) poplar, horse chestnut, grey alder (all >70%), 3) Chinese golden rain tree, painted maple (both >50%), and 4) paper birch, London planetree, and littleleaf linden (all <50%).

Surveys of urban and rural tree species to determine the preferences of these trees by ALB adults. In addition to the site testing reported above, we also conducted surveys in several locations in China to evaluate preference of tree species by ALB. We especially evaluated species whose status as the ALB host had not been determined at the time, or species that reportedly could be used as trap trees.

We did not find any ALB exit holes on black locust (*Robinia pseudoacacia*), although we found some egg sites and adult feeding sites. No ALB exit holes were found on hackberry (*Celtis* sp.), or rose- of-Sharon (*Hibiscus syriacus*).

To further evaluate whether *Acer mono* and *A. negundo* can be used as trap trees in the US ALB eradication program as suggested by some researchers, we visually surveyed the numbers of ALB adults on *Acer mono*, *Acer negundo*, and *Salix babylonica* growing in close proximities (**Fig. 3**). Surveys were conducted in between mid-June to late July during the ALB flight season. Willow (*Salix babylonica*) were planted in a row along a river bank. The approximate distances are: 10-15 m (willow trees to the mixture of 2 rows of *A. mono* and *A. negundo* in Zhengzhou), and 10m (between willow and *A. mono* in Tianjin).

Figure 3: *Survey for ALB infestation (a) at site where painted maple (*Acer mono*), box elder (*A. negundo*), and willow (*Salix babylonica*) planted adjacent to each other, and (b) exit holes and frass.*

Many more beetles were found on *S. babylonica* trees than on *A. mono* or *A. negundo*. It should be noted that both the later species were smaller in size in these surveys (**Table 1**). The results of this survey indicate that the efficacy of using *A. mono* or *A. negundo* as trap trees in areas where large host trees (in this case, *S. babylonica*) exist would be doubtful.

In addition, we also evaluated whether the Chinese chestnut, *Castanea mollissima* is an ALB host. There were anecdotal reports that ALB adults feed on Chinese chestnut trees. We caged pairs of virgin adults (males and females) with a section of a tree trunk, twigs and leaves) of live a Chinese chestnut tree. Although adults survived quite a few days (9.3±4.38 for the male, and 10±5.8 for the female) on the tree, they did not make any egg pits, nor there were any viable eggs found when females were dissected after they were dead.

Table 1: *The number of ALB adults on trees.*

Location	Tree species	# Trees	DBH (cm)	#Adults/tree
Zhengzhou 1	*Salix babylonica*	60	20-27	0.12
	Acer mono	30	10-15	0.03
	Acer negundo	10	10-15	0.00
Tianjin	*Salix babylonica*	30	10-15	1.19
	Acer mono	26	10-15	0.50
Zhengzhou 2	*Salix babylonica*	30	21-28	0.83
	Acer mono	30	10-16	0.17

Efficacy Evaluation of Insecticide Treatments Against *Anoplophora* spp.

Project Lead: Baode Wang
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Cooperators: Ruitong Gao (Chinese Academy of Forestry)
Zhichun Xu (Beijing Forestry University)
Xinsheng Cheng, Fangxiao Wang, and Jiabao Hong (University of Science and Technology of China)

The efficacy evaluation of insecticide treatments against the Asian longhorned beetle (ALB), Anoplophoa glabripennis is to develop an effective chemical control strategy to be integrated into the current USDA APHIS ALB eradication program.

Efficacy evaluation of nursery tree treatments with insecticides against Anoplophora spp. The objective is to determine feasibility and efficacy of neonicotinyl systemic insecticide treatments against ALB and CLB feeding on host tree species in commercial nurseries.

Materials and methods. The treatments tested were Critrion 75 WP (A.I. imidacloprid, soil basal injection =**A**), Arena 50 WDG (A.I. clothianidin, soil injection =**B**), Flagship 25 WG (A.I. thiamethoxam, soil injection =**C**), Safari 20 SG (A.I. dinotefuran, soil injection =**D**), Safari 20 SG (A.I. dinotefuran, bark spray = **E**), and the untreated trees as the control (**CK**).

Site 1: The first primary site is located in Quanjiao, Anhui province, China. Red maple (*Acer rubrum* L) trees infested by both CLB (primarily) and ALB were selected since no suitable sites and trees were found infested primarily by ALB. Two plots of all red maple were established and a Randomized Block Design was used in 2010 (6 blocks, each with 5 trees). The first plot were smaller trees (average DBH =1.7 cm), while the second plot had larger trees (average DBH =3.1 cm). Only treatment trees in the "smaller tree plot" were left in 2011. Half of these trees were treated with the same insecticide and dose a second time in mid-April 2011. Pairs of male and female beetles were caged with treated trees in June 2011 (**Fig.1**). These trees were checked weekly for the numbers of dead beetles and egg sites as well as active egg sites.

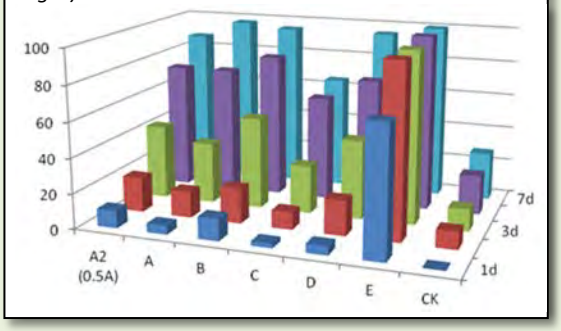
Site 2: A second primary site had to be selected by our cooperator because larger trees at site 1 were sold by the farmer in 2010. The second site is located in Hebi, Henan province. The tree species at this site was painted maple, *Acer mono*. Tree size was similar to trees at Site 1. Both the mortality of caged beetles and the number of dead beetles under treatment trees were checked and recorded during the ALB flight season. Soil applications were conducted on June 15. Trunk sprays (from tree base to 1.5 m high) were conducted on July 6. Adult peak emergence was estimated to be in early July. There were 30 trees in each treatment. Ten trees from each treatment were selected to cage beetles on. One pair (1 male and 1 female) of ALB adults were caged (1 m long and 22 cm diameter) with tree trunks. The bottom of the cage was 20-70 cm from ground. Live twigs and leaves were included in the cages. The mortality of ALB adults was checked 1, 2, 3, 5, and 7 days after they were caged (**Fig. 2**). The same method was also used for a second cage on the same tree but with twigs and leaves freshly cut from the same tree only. The number of feeding sites and areas consumed by the beetle were measured after the beetles were dead.

Figure 1: *Beetles were caged with red maple trees at site 1 in Quanjiao, Anhui province, China.*

Figure 2: *Caging beetles on painted maple trees at site 2 in Hebi, Henan province, China.*

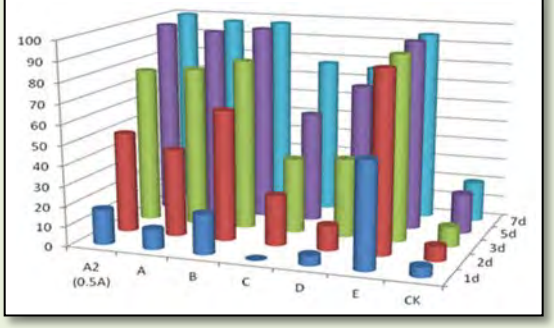
Results. For Site 1: Mortality of ALB adults in cages rank from high to low in 2011: A (imidacloprid), B (clothianidin) and C=D=E= CK for trees treated only in 2010, and A, B, D, E, C, CK for trees treated both in 2010 and 2011. Adult mortality was still the highest for CLB adults caged with trees either treated with imidacloprid only in 2010 or in 2010 and 2011. The mortality of CLB adults caged with trees treated with other insecticides was also higher than CLB adults caged with the untreated trees. No active egg sites were found on trees in treatment A when trees treated only in 2010, and in treatment A, B, C, D when trees treated both in 2010 and 2011. In both cases, the number of active egg sites was the highest on untreated trees. The results indicate the great protection of trees after one or two time treatment with imidacloprid for small trees.

Figure 3: Cumulative mortality of adult pairs caged on a section of tree trunk with live twigs and leaves (20 cages)



For Site 2. Mortality was higher in all insecticide treatments than in the control (**Figs. 3 and 4**). Relatively, lower mortality in treatment C and D, especially when the beetles were provided with only the cut twigs and leaves from the same tree they were caged with (**Fig. 4**).

Figure 4: Cumulative mortality of adult pairs caged on a section of tree trunk and provided with cut twigs and leaves from the same tree (20 cages)



All insecticide treatments significantly reduced feeding by ALB adults and the number of egg sites made by females, especially in the 20% dinotefuran trunk spray, 50% clothinidin and 75% imidacloprid soil drench.

No exit holes were found on trees in treatment A, but a few exit holes were found on untreated trees.

Efficacy comparison of different treatments (insecticide, formulation, and dosage) for ALB.

A new test (TEST 1) was established in 2011 to compare the efficacies of new insecticide formulations. The test was conducted in Ningxia Autonomous Region, China, in a mixture of several different poplar species. The insecticide application was carried out on July 23, 2011. The treatments are list in **Table 1**.

Because of the low wild ALB population in this plot, only a few dead wild adult beetles were found under treated trees. Therefore, our emphasis was caging beetles on treated trees and checking their mortality. Three pairs of beetles (3 males and 3 females) were caged with a section of trunk of each individual treated tree. The status of these beetles was checked once every day for 7 days after they were caged on the treated tree.

The procedure was repeated three times from July 24 (1st check on July 25) to August 6 (1st check on August 7 and until August 14). At the end of August, the caged section of each tree was checked for the total number of ALB egg sites.

The results (**Fig. 5**) indicate higher mortality for caged adults in all insecticide treatments than adults caged with the untreated trees. On the beta-cypermethrin treated tree, mortality was higher than 95% at the first check (1 day after the beetles were caged). Beetles in all but imicide HP treatment and the control had mortality close to or higher than 80% 4 days after the beetles were caged. The number of egg sites made by caged beetles did not differ significantly although the numbers ranged from 2.0 egg sites per tree (caged area) in the Rainbow CVG696 treatment to 5.9 in the untreated control.

Table 1: New insecticide treatments in 2011.

Treatment abbr.	Insecticide and Formulation	Delivery	Dose	No. Trees
HP	Imicide HP (10% imidacloprid)	Trunk injection	4 ml per 5cm DBH	10
CVG696	Rainbow CVG696 (10% imidacloprid)	Trunk injection	4 ml per 5cm DBH	10
Ima	Imajet (10% imidacloprid)	Trunk injection	4 ml per 5cm DBH	10
Dino	Rainbow DCPW (22.5% dinotefuran)	Trunk injection	4 ml per 5cm DBH	10
Cyper	3% beta-cypermethrin capsules suspension	Cover spray	500 x	10
Thia	2% thiacloprid micro-capsule granular	Cover spray	2000 x	10
CK	CK			10

TEST 2 was conducted in central China Kaifeng, Henan province by Chinese Academy of Forestry. This was a continuation of the study started in 2010. Six treatments were used:

1) TreeAzin (azadirachtin) applied through Tree IV,

2) Transtect 70 WSP (dinotefuran) applied through both bark spray,

3) Transtect 70 WSP (dinotefuran) applied through soil drench,

4) Imicide (imidacloprid) capsules though tree trunk injection,

5) Acelepyrn (chlorantraniliprole) through soil drench, and

6) the untreated trees as the control.

In 2010, The insecticides were applied 2 weeks (trunk application) to 2 months (soil application) before the estimated ALB adult emergence.

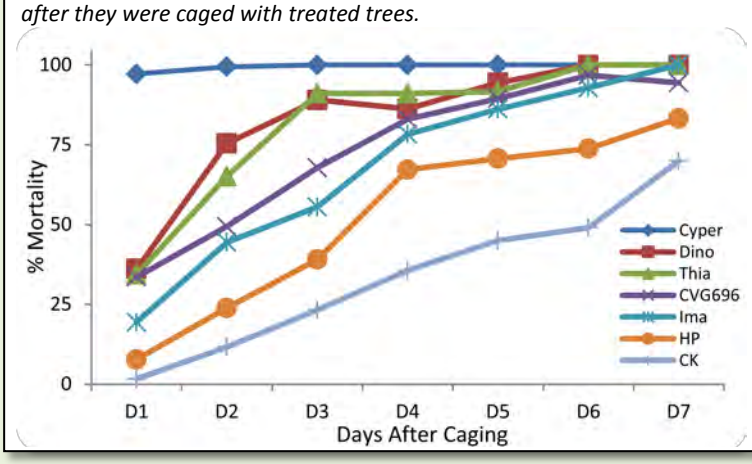
Evaluations of efficacy in 2011 were based on comparison of adult mortality, both caged adults and the number of dead free living adults under treated trees. Twig samples were taken from 10 trees for each insecticide treatment for residue analyses.

Results. Similar to the results in 2010, the number of dead beetles under trees treated with both Transtect 70 WSP (dinotefuran) soil drench and Imicide (imidacloprid) trunk injection were much greater than that was found under other treated trees as well as the control (**Table 2**). By day 20, the greatest mortality was found for beetles caged with trees treated with Transtect 70 WSP (dinotefuran) soil drench and Imicide (imidacloprid) trunk injection. Some beetles caged with trees treated with imidacloprid or dinotefuran fed initially, and then became too sick to feed. They either remained in a moribund state for a time and died or were able to recover late because they were in cages.

Table 2: *Mortality of free moving beetles on and beneath treatment trees in 2011 (total number in 40 days; soil basal drench was in late May, 2010 and all other application was in early July, 2010).*

Treatment	No. Trees	% Mortality	No. Dead Adults/Tree
CK	30	3.3	0.4
TreeAzin, tree IV	27	10.4	0.3
Acelepyrn, soil drench	30	14.9	0.5
Transtect 70 WSP, soil drench	30	**50.5**	1.6
Transtect 70 WSP, bark spray	30	13.1	0.3
Imicide, capsule	30	**66.7**	2.7

Figure 5: *Mortality of caged beetles in different treatments 1 to 7 days after they were caged with treated trees.*



Assess the efficacy of imidacloprid treatment in natural situations. Thirty uninfested willow trees (average DBH = 7 cm) were planted in March 2008 in a plot among heavily infested willow trees (average DBH = 13 cm). The natural ALB population was estimated to be not less than an average of two adult beetles per tree during the flight period.

Twenty of the planted 30 trees were randomly selected and trunk injected with imidacloprid (10%, 4 ml Imicide capsule, 1 capsule / 5 cm) in June 2008.

However, by June 2009 only 13 trees remained and were retreated. Only 6 of the 10 untreated trees were left by June 2009, and 2 more were dead as a result of ALB infestation by June 2010. All trees were not treated with insecticide in 2010. Tree size, the number of live adult ALB on trees, dead ALB adults under each tree and the number of exit holes were recorded from early June to mid-August in 2008, 2009, and 2010. Additionally, the number of egg sites and active egg sites were recorded for willow trees that were transplanted in March 2008.

Results. Generally, the ALB population level was low in summer 2011 in this area. No exit holes were found from 2008 to 2011 on any of treated, previously uninfested, willow trees after two years treatment, whereas exit holes were found on both the untreated surrounding trees and untreated transplanted trees in 2010. We also found ALB exit holes on the untreated surrounding trees in 2011. There were more active egg sites on the untreated trees than that on the treated trees. The results indicate that prophylactic treatment of uninfested trees using imidacloprid may prevent the trees from being infestated.

Development of Equipment and Treatment Options for Combating the Asian Longhorned Beetle

Project Lead: Phil Lewis

APHIS is actively conducting a multi-million dollar program to eradicate the Asian longhorned beetle (Anoplophora glabripennis) (ALB) in New York, New Jersey, Ohio, and Massachusetts. Successful eradication of this insect has been declared in portions of New Jersey and in Illinois and additional locations are being slated for eradication status in the coming years. This program is dependent on visual techniques for survey and delimitation, infested tree removal for population reduction, and treatment of surrounding uninfested trees to prevent population expansion and growth.

The systemic insecticide imidacloprid was chosen as the primary active ingredient for treatment based on extensive testing using field populations of ALB in China. In the U.S. eradication program, treatment of trees up to ¼ mile of known infestation sites has been accomplished using either direct tree injection (initiated in 2000) and/or soil injection (initiated in 2002) of imidacloprid. A minimum of three years of treatment and an area-wide approach is necessary to ensure effectiveness. Treatments are applied between April and early June, prior to adult emergence and have been shown to have a 99.9% success rate. Treatment failures have been attributed to such factors as improper application and poor health/condition of the tree. Research into the chemical treatment process is used to further understand and maximize the impact of chemical applications for control of ALB populations in order to improve Program performance, lower costs and increase the chance of success. This research unit has helped the ALB Program determine the optimal timing of treatments, verify their efficacy and investigate how systemic insecticide applications can be applied to situations unique to the urban and forest environments where treatments occur.

Figure 1: *Approved tree-injection equipment: a) EcoJect Canisters, b) Arborjet's VIPER gun, c) Mauget capsules, and d) Davey Injector*

Trunk injection methodology and equipment testing. There are a number of competing technologies and methods within the arboricultural community that have been tested for suitability for use within the ALB Eradication Program (**Fig. 1**). In order to facilitate a fair and orderly assessment of these technologies, a trial system was implemented where companies demonstrated their products, subjecting them to rigorous use and evaluation. The Program has treated over 400,000 trees over the last decade in a number of cities and municipalities, so it is important that treatments be confirmed as effective, are as non-injurious to trees as possible, and are acceptable to our stakeholders. **Table 1** lists the equipment and/or formulation tested and the result of the testing.

Soil injection methodology and equipment development. Through collaborative efforts with Davey Tree, one of the treatment contractors, research studies were implemented to modify and improve on standard arboricultural treatments. As a result, soil injection methods have evolved from using large trucks that delivered gallons of chemical mixture within the dripline of infested trees, to the use of portable pump-up spray containers that deliver a concentrated chemical solution at the base of the tree (**Fig. 2**). The smaller, portable equipment combined with a subsequent reduction in the amount of water needed to treat individual trees has resulted in lower program costs, allowing for more trees to be treated each year. Insecticide residue levels from foliage of trees treated with the new methodology were magnitudes higher as compared to the previous method.

Table 1: *Trunk Injection Devices and Formulations Approved for use in the Asian Longhorned Beetle Eradication Program.*

Injection Device / System	Company	Year Tested	Pass / Fail
Mauget Micro-Injection System	JJ Mauget Co.	1997	**PASS**
Arborjet VIPER with USDA Tips	ArborJet, Inc.	2003, 2004	**PASS**
Arborjet Hand-held Injection System	ArborJet, Inc.	2004	FAIL
BioJect System	BioForest Technologies, Inc	2004	FAIL
ChemJet Tree Injectors	Chemjet Trading, Pty. Ltd.	2004	FAIL
Davey Beetle Buster Tree Injector	Davey Tree Expert Co.	2004	FAIL
Bayer Tree Injector	Bayer CropScience	2004	FAIL
Sidewinder Tree Injector	Sidewinder, Pty. Ltd.	2004	FAIL
Davey Beetle Buster Tree Injector	Davey Tree Expert Co.	2005	**PASS**
EcoJect System	BioForest Technologies, Inc	2005	FAIL
Bartlett Tree Injector	Barlett Tree Research Lab	2005	FAIL
Mauget Liquid Loadable System	JJ Mauget Co.	2006	FAIL
M3 capsules (revised Barlett system)	Rainbow Treecare Scientific	2007	FAIL
EcoJect System	BioForest Technologies, Inc	2007	**PASS**
Tree Tech microinjection systems	Tree Tech, Inc	2008	FAIL
Imidacloprid Formulation			
Imicide HP (110 g/l, 10% formulation)	JJ Mauget Co.	2003 to 2005	**PASS**
Confidor 200 SL / Merit Tree Injection Insecticide (200g/l, 17% formulation)	Bayer CropScience	2005, 2008, 2010	FAIL
EcoPrid Tree Injection Solution (50 g/l) [use contingent on available US Label]	Bayer CropScience & BioForest Technologies, Inc	2005	**PASS**
IMA-jet (50 g/l, 5% formulation)	ArborJet, Inc.	2005	**PASS**
Pointer Insecticide (50 g/l)	ArborSystems, Inc.	2005	**PASS**

The application method now in use by the ALB program is termed basal soil injection and it is the primary method of treatment, costing 1/3 that of trunk applications. Imidacloprid is applied at the maximum labeled rate 6 to 8 inches below the soil and within 12 inches of the base of the tree. The pump-up sprayers are attached to hand carts allowing the applicators to maneuver easily through the congested city environment. For soil treatments, the applications are completed in time so that residue levels are sufficient at peak ALB emergence.

Chemical residue determination. HPLC analytical methods that detect imidacloprid residues in tree tissues are expensive ($75-$300 per sample), and the average wait time between sampling and receiving results can be lengthy, hampering the ability to assess the efficacy of various treatment methods. We developed and verified an efficient leaf extraction method, and analyze pesticide residue with a commercially available immunological assay (ELISA) in-house.

Use of this assay system has the following advantages: a per sample cost of around $10, detection in the parts per billion range (versus parts per million with HPLC), and rapid quantification of imidacloprid residues.

Analytical results from our laboratory achieved similar results when compared with HPLC analyses conducted by APHIS's Gulfport lab, by a private lab hired by Suffolk county, New York and by chemists working for Syngenta.

While it takes one to two weeks to reach effective residue levels in plant tissues via trunk injection, two to three months are necessary when trees are treated by soil application. The typical residue level obtained in foliage from treated trees ranges from 5 to 30 ppm for soil injection and 20 to 60 ppm with trunk injection.

Figure 2: *Soil injection methods*

Effectively Treating Trees in the Urban Forested Environment to Combat the Asian Longhorned Beetle

Project Lead: Phil Lewis

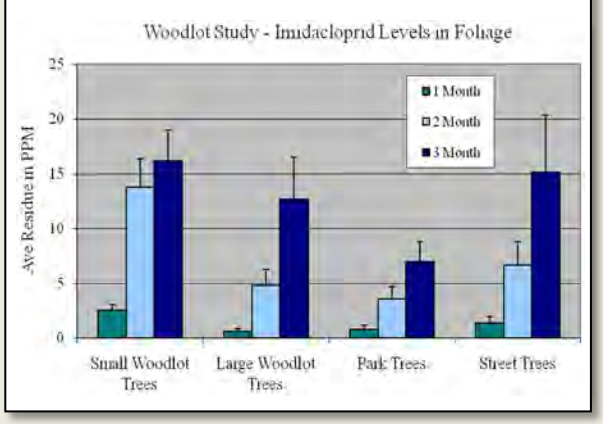
The majority of pesticide applications used in the eradication effort against the Asian Longhorn Beetle (ALB) are soil injections applied in the spring and early summer. Tens of thousands of trees are treated each year in a wide variety of urban and forested landscapes in New York, New Jersey and Massachusetts. Most tree treatments fall within expected parameters, but there are always some for which treatment efficacy is in question. One of these situations is the soil treatment of small diameter trees that are growing in close proximity to a large diameter tree that is not a host for ALB (and therefore, not being treated). In this scenario, the concern is that the roots of the non-host tree will take up a significant portion of the treatment chemical leaving the host tree inadequately protected. If that is the case, then a direct trunk injection application would be warranted, although this is not ideal due to the higher treatment cost of trunk injections and logistical concerns (switching between application types). Information from studies such as these assist us in developing and understanding the dynamics of uptake and resultant residues of insecticides injected in and around trees that are a part of the ALB eradication program.

Figure 1: *Basal soil application.*

Methods and results. Basal soil applications were made on trees in an open environment (park setting and street-side trees) and on trees growing in a woodland environment. Twenty understory maple trees (ave. 4.5" DBH) were selected to create intense root competition for the imidacloprid treatment as they were growing within an extensive undergrowth of weeds and ivy and were in close proximity to large untreated trees (10 to 24" DBH). Trees were given 1 cup of a maximum labeled rate solution per inch of tree diameter, delivered as a basal injection. All applications were made with a modified pump-up sprayer at 30 psi with a 6" soil injection probe placed within 6" of the base of the tree (**Fig. 1**).

Composite foliage samples were collected each month for three months post-treatment and the amount of imidacloprid residue was assessed using an ELISA assay. After 3 months, maple trees under intense root competition had comparable residue levels to trees growing in similar and in more open environments (**Fig. 2**). Although some of the large non-host trees were as close as 6" to the small diameter trees, tree diameter was the only significant factor in differences between residue levels (residue decreases with increasing tree DBH; ANOVA P< 0.02).

Figure 2: *Foliage residue level comparisons.*



The results of this study demonstrated that the basal soil injection method can be effectively used to treat trees in woodland habitats or other situations where intense root competition from neighboring, non-treated plants is encountered. All trees treated in this study had imidacloprid residue values well above the target level of 1.9 ppm (ALB LC50) at 2 and 3 months post-injection. The proximity of neighboring trees did not influence the amount of residue detected in foliage. Instead it appears that the only factor influencing insecticide residue was increasing tree diameter, which yields lower levels of pesticide residue. This is a result of the near exponential increase in tree mass as diameter increases, versus a small incremental increase in the amount of chemical applied as tree diameter increases.

Evaluation of Diverse Trap Designs in a Newly Discovered Asian Longhorned Beetle Population in Bethel, OH

Project Lead: Dave Lance and Joe Francese

Collaborators: Mike Rietz (USDA, APHIS, CPHST, Brighton MI)
Damon Crook and Vic Mastro (Otis Laboratory)

Asian longhorned beetle (ALB) was recently discovered in Bethel, OH. The goal of this study was to test possible candidate traps for ALB in an (at the time) unmanaged area.

Traps were tested with and without a lure that was developed and is currently being field tested by cooperators at Penn State University and USDA ARS. The lure consisted of a pheromone [4-(*n*-heptyloxy)butan-1-ol and 4-(*n*-heptyloxy) butanal] and several host volatiles [*cis*-3-hexenol, (-)-linalool and *trans*-caryophyllene] (Zhang et al 2002; Nehme et al. 2009). Five trap designs were tested (**Fig. 1**).

1. Intercept Panel (Black)
2. Black Multi-funnel
3. Green Multi-funnel
4. Screen Sleeve
5. Modified Plum Curculio

Of the trap designs we tested, the intercept panel is the most widely used in cerambycid trapping studies, and is usually the standard for ALB. We also tested a sleeve trap design and a modified plum curculio trap both of which have shown promise as a trap for ALB. Two other traps that we tested were versions of the Lindgren funnel (multi-funnel) trap, a black and a green model. Green is a color that has shown promise in early behavioral and trapping studies (B. Wang, unpublished data; J. Lund, unpublished data) as well as preliminary electro-retinogram assays. The green trap is the color used for emerald ash borer. Multi-funnel and intercept panel traps were hung at ~5-8 m in the canopy of maples and boxelders (*Acer* spp.) at sites that had been shown to have positive ALB finds. Screen sleeve and modified plum curculio traps were attached to the trunks of *Acer* spp. A pair of baited and unbaited versions of each trap type were in adjacent trees, and pairs were at least 10-15 m apart from one another (**Fig. 2**). Each replicate consisted of two traps of each type, one with and one without a lure (n=10).

Figure 1: *Five trap designs tested, a) intercept panel (black), b) black multi-funnel, c) green multi-funnel, d) screen sleeve, and e) modified plum curculio.*

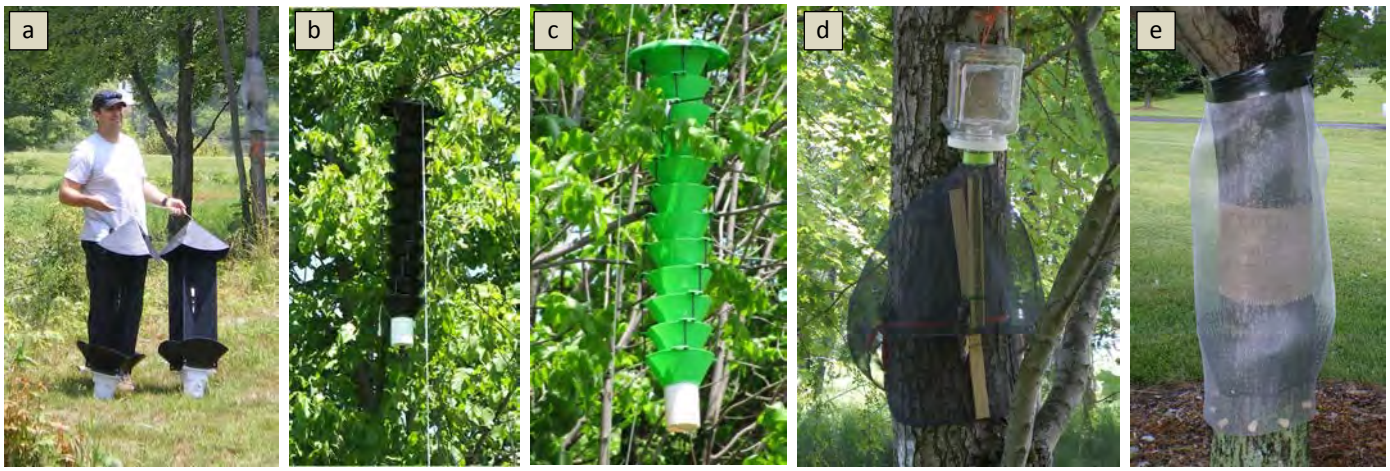
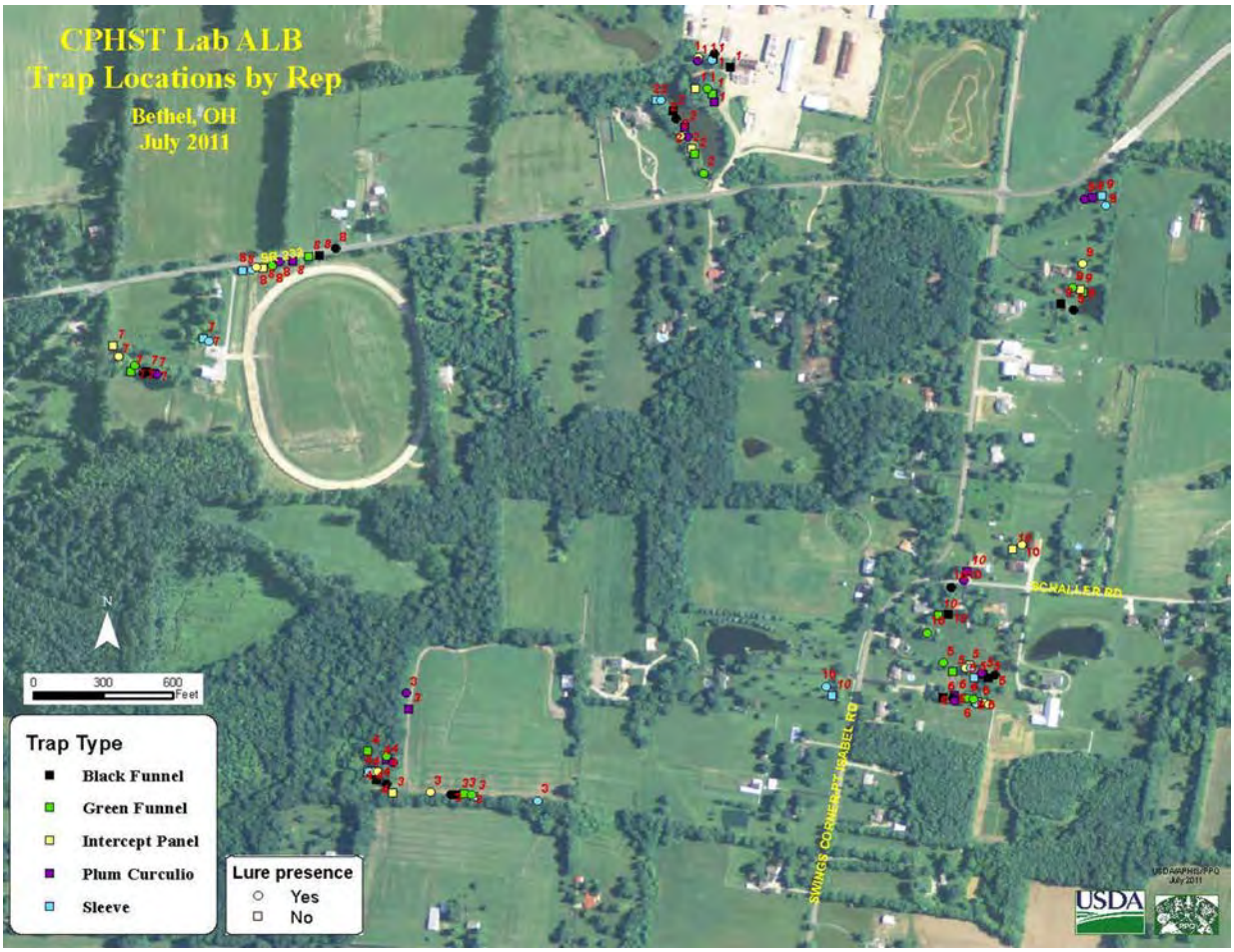
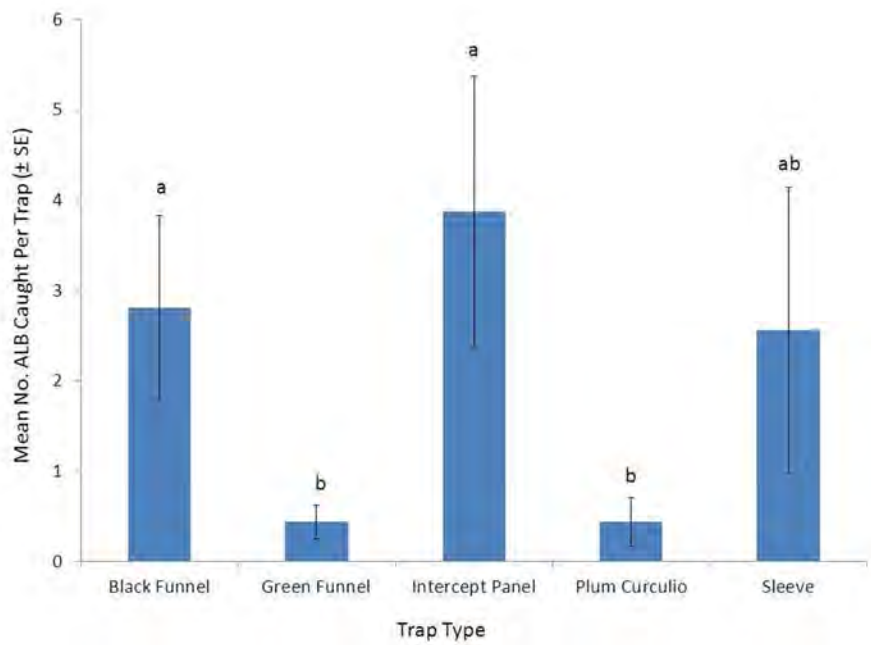


Figure 2: *Locations of different trap designs in a field study conducted in Bethel, OH in 2011.*



Replicates 2 and 7 were negative for catch, and were removed from the analysis. There was no significant trap type x lure interaction, so the two factors were each analyzed separately. There was no significant difference between lure baited (1.95 ± 0.70 beetles / trap) and unbaited (2.10 ± 0.72 beetles / trap) traps. The overall effect for trap type was significant with black multi-funnel and intercept panel traps catching more beetles than green funnels or modified plum curculio traps (**Fig. 3**). No other differences were found among the other trap types.

Figure 3: *Mean (± SE) number of A. glabripennis adults captured in a trap design comparison field assay conducted in Bethel, OH in 2011 (n = 16).*



Phenology and Flight Periodicity of *Sirex noctilio* (Hymenoptera: Siricidae) in Central New York

Project Lead: Scott Myers

Contributors: Kelley Zylstra and Joe Francese (Otis Laboratory)
Daniel Borchert (CPHST, Raleigh, NC)

Field and laboratory studies were performed to determine phenology of flight activity and the thermal requirements for adult emergence of Sirex noctilio*. To measure time from egg to adult under laboratory conditions* Pinus syvelstris *was infested with lab reared adults and monitored for adult emergence. Similarly, bolts of* P. sylvestris *cut from trees infested in the field by wild populations of* S. noctilio *were brought indoors for rearing at constant temperature. Adult emergence from laboratory infested bolts was strongly male-biased, while field collected material produced a 2.7:1 male-biased sex ratio. Mean degree-days to emergence was 1477.0 ± 13.4 (males) in laboratory infested bolts; and 1455.2 ± 11.2 (males) and 1577.8 ± 19.5 (females) in field collected material. Given the similar emergence times and the fact that winter collected material typically contained mid to late instar larvae rather than eggs, these data suggest a lag in development in field collected material that is likely the result of time required to break diapause. Field trapping studies were conducted concurrently over four years in central New York to compare flight activity with rearing data. Trap captures showed first flight activity and peak catch occurred at 709 and 1145 degree-days, respectively. The resulting degree-day model predicts early flight activity in early to mid-April for pine stands in southeastern United States, early to mid-May in the Mid Atlantic region, and late June to early July in the Northeast.*

Introduction. When exotic insects become established in a new environment, survey tools are useful to determine the extent of establishment and to monitor their movement and colonization of new areas. One of the most valuable tools for detecting and monitoring invasive populations is reliable information on flight activity. This is particularly important for large scale survey efforts that can be costly in terms of resources and time invested. Phenology models of flight activity can help focus trapping efforts to reduce labor in trap collections, minimize time when traps are in the field and make the most of pheromones and other attractants when they are employed. *Sirex noctilio* is a serious pest of pines in commercially grown pine stands in many parts of world where it has been introduced. *Sirex noctilio* was first reported in North America in central New York in 2004, and has since been found in CT, MI, PA and VT in the United States, as well as Ontario, Canada. Barring large scale control measures, it's likely that *S. noctilio* will continue to expand its range in North American through natural migration, as much of the eastern U.S. contains suitable host trees.

This project was undertaken to determine the timing of flight activity in central New York where *S. nocitilio* is established through trapping efforts and to compare that with emergence data collected under controlled conditions in the laboratory. The overall goal was to develop a degree-day (DD) model to predict larval development and adult emergence in Scots pine that could serve survey and detection efforts monitoring *S. noctilio* populations as it moves to other parts of the U.S. and Canada.

Figure 1: *Lidgren funnel traps used to capture adult female* Sirex noctilio *in the field.*

Infestation of *P. sylvestris* bolts. To evaluate developmental time for *S. noctilio* from egg to adult, bolts of *P. sylvestris* were infested using *S. noctilio* adults reared from pine bolts collected from the field during the winter. Upon emergence, adult females were collected and placed in screen-topped, clear plastic containers with three males for 12-24 h. After a female was observed mating with one of the males, she was removed and placed in a plexiglass cage with six 1 m x 7-10 cm diam *P. sylvestris* bolts for oviposition. Following oviposition infested bolts were held at 22.0 ±

1.0 °C until adult emergence. Date of adult emer-gence and sex of each individual were recorded.

Rearing of *Sirex noctilio* in field-girdled *P. sylvestris* trees. Thirty field sites were established from 2008-2010 (10/yr) in forested stands of red and Scots pine in central New York to collect *S. noctilio* infested trees (**Fig. 1**). Field sites were set up as a single group of three traps at each forest stand location spaced a minimum of 100 m apart. Sites where no *S. noctilio* adults were collected were dropped from the experiment, as no trees were harvested. Four *P. sylvestris* trees were chemically girdled using the herbicide Banvel® to attract female *S. noctilio*. The girdled trees were felled from Oct. through Dec. each year and cut into bolts measuring ~70 cm. Bolts showing signs of *S. noctilio* attack (i.e. sap and resin beads) were selected for rearing adults. The ends of each bolt were painted with AnchorSeal. Bolts were placed into plastic barrels with widow screen tops and held at 23.0 ± 1.0°C to allow adults to emerge from the logs. Barrels were checked daily during the work week and occasionally on weekends. The emergence date and sex of each individual was recorded. A subset of 50 bolts were dissected 60 d after the last adult emerged to collect any larvae remaining in the wood. These larvae were used to evaluate the success of larvae to develop to adults under these conditions and determine what proportion of *S. noctilio* larvae may require a longer period of development. The number and life stage of *S. noctilio* found in the wood were recorded.

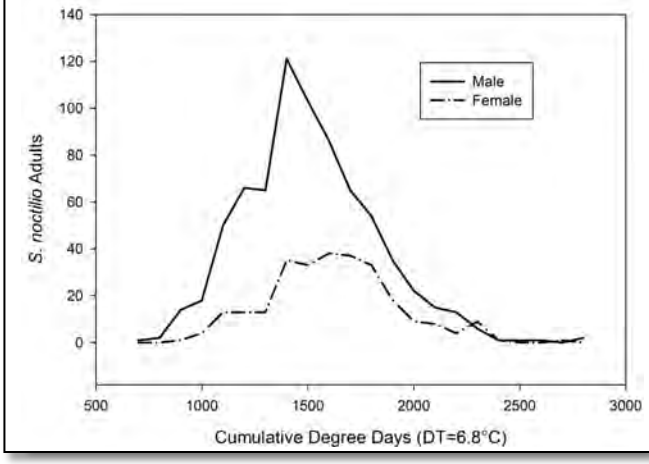
Field Trap Captures. To compare adult emergence time in the laboratory with flight periods in the field, trap catch data were collected from sites across 11 counties in New York from 2007-2010. At each location data loggers were used to record daily temperatures at 1 hr intervals. An additional 46 locations across central New York from related projects on *S. noctilio* designed to develop trapping and survey techniques were also included. Trap trees were chemically girdled using herbicide as above, or Garlon 3A, or mechanically girdled to attract females. Lindgren funnel traps (12-funnel) were hung at ~6 m from *P. resinosa* and *P. sylvestris* trees in areas thought to be infested. Trap collections were made weekly from late June through September.

Data Analysis. The single sine method for degree-day (DD) calculations[1] was used to calculate DD for adult emergence in the lab and flight in the field (from 1 January) using the base developmental threshold of 6.8 °C from Madden[2]. An upper development temperature of 35.0 °C. Minimum and maximum daily temp-eratures were derived from data loggers when they were associated with trapping sites. When the trapping locations did not have data loggers, daily temperature data were used from the nearest NOAA data station. T-tests were used to compare mean DD for adult emergence between males and females in the laboratory, and between laboratory reared and field collected adult females. Probit regression models were used to fit the cumulative proportion emergence data from laboratory reared and field collected *S. noctilio*. First flight and peak flight predictions were based on 5% and 50% emergence estimates, respectively, from the probit model. Means are reported ± standard error, and median emergence estimates from probit regression models with 95% confidence intervals.

Using the degree-day modeling function in the North Carolina State University-APHIS Plant Pest Forecasting (NAPPFAST) system[3], DD models were created using 6.8°C as the base developmental temperature and the model was initiated to start accumulations on January 1 of each year.

The NAPPFAST system produced geotiff raster maps with 10 km^2 resolution that use multiple years of data (10, 20, or 30) to display the frequency of which an event occurs during the time period of interest. Frequency maps were set to display the area and the time for DD accumulation to initial and peak emergence, respectively. The map was reclassified into to a single class for all occurrences more frequent than three years. Map generation and reclassification for subsequent time periods were performed to illustrate where and when and initial and peak emergence of *S. noctilio* would occur in the US.

Figure 2: *Emergence of* S. noctilio *adults from winter harvested* P. sylvestris *collected in NY from 2008-2010 reared in the laboratory at 22.0 ± 1.0 °C.*



Results & Discussion. A total of 142 adult males and two females emerged from two groups of lab infested 1 m bolts. The first adult emerged after 71 d and the last was collected at 115 d. Mean DD to emergence was 1477.0 ± 13.4. The skewed sex ratio indicates that while the females were observed to copulate with males before they were introduced to the pine bolts they may have been less successful in mating, and as such, unfertilized females would have only been able to oviposit male eggs. Alternatively, females may be reluctant to oviposit fertilized eggs in an artificial environment or consider cut bolts a poor host and consequently deposited predominately male eggs.

Field collected *P. sylvestris* over three years produced 741 adult males and 272 females (2.72:1, M:F). Males emerged as early as 43 d after they were introduced to 23°C. The longest emergence was a female at 190 d. Despite different starting points (eggs vs larva), mean DD to emergence was similar to that of the laboratory infested bolts with no significant difference found between males from each group (P=0.83). A significant difference in mean degree days to emergence was, however, observed between male (1455.2 ± 11.2) and female (1577.8 ± 19.5) adults reared in the lab (P<0.01) (**Fig. 2**). Median emergence time as estimated from probit regression models was 1445 and 1559 for males and females, respectively.

Dissection of 50 *P. sylvestris* bolts found a higher than expected number dead adults (102) along with 51 dead larvae inside wood that had produced 527 live adults. Similar data (unpublished) from *S. noctilio* infested *P. sylvestris* bolts that were held vertically in individual emergence barrels with little or no contact with the sides of the barrels found no live larvae or dead adults remaining in the logs. These results suggest adults may have trouble emerging from logs when they are blocked by other logs or the sides of barrels when barrels contain multiple logs. Six live larvae were also found (0.8%), indicating a relatively small portion extends diapause into a second year. Reports from Australia and South America in the literature suggest that as much as 25% of *S. noctilio* larvae may extend diapause and emerge as adults two or three years post oviposition.

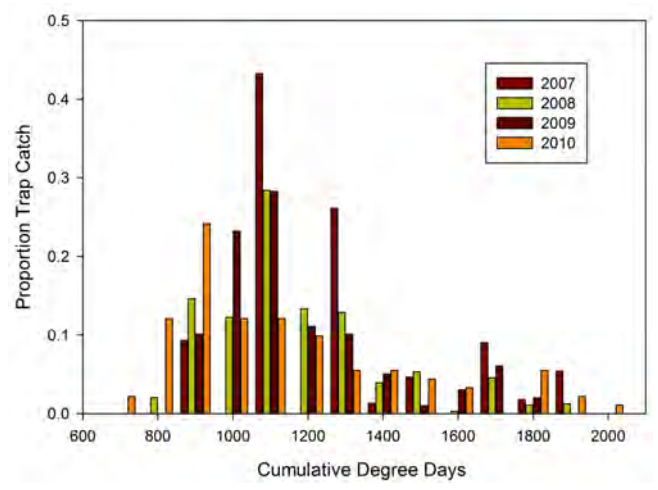
In the field, the number of *S. noctilio* females captured in traps varied considerably from year to year, however, overall emergence patterns were similar (**Fig. 3**). A total of 1067 females were collected. The earliest emergence was observed at approximately 700 DD, while mean DD catch was 1158.3 ± 7.3 for the combined data from all four years.

In comparing rates of development under constant temperature in the laboratory we found that adults emerged from winter field collected material at a similar DD as they did in the bolts infested with adult females in the laboratory. We know from prior dissections of field collected host trees that *S. noctilio* larvae are mid- to late-instar during the winter months. This result suggests that *S. noctilio* larvae in field collected material are in winter diapause, and delay in resuming development after they were brought indoors and acclimated to 23 °C. Larvae developing in lab infested bolts do not require a diapause and thus are able to develop to adult without delay.

Similarly, in the field, cumulative mean DD trap catch for females was significantly less than DD accumulation to emergence for field collected material that was brought into the laboratory and held under constant temperature. Mean emergence was 409 DD higher in field collected material reared in lab compared to trap captures. Over-wintering larvae in the field are presumably able to break diapause and resume development as field temperatures become favorable without the delay in development that is experienced by larvae in winter collected bolts.

Madden[(2)] predicts median emergence at close to 1700 DD based on lab infested *P. radiata*. This is ~15% higher than our observations with *P. sylvestris* bolts, and may indicate differences in developmental rate between the different tree species used and/or the

Figure 3: *Proportional distribution of* S. noctilio *adult females captured in Lindgren funnel traps from four years of field surveys in western New York state. (2007: n=387, 2008: n=507; 2009: n=100; 2010: n=73).*



overall suitability of the bolts for growth of *A. areolatum*.

Regression models of probit transformed cumulative trap catch data predicted 5% trap (early emergence) occurring at 709 DD and 50% catch at 1145 DD. This coincides with late June to early July in northern PA and central NY, where *S. noctilio* is currently known to occur. If this relationship holds true in warmer parts of the U.S., some of the pine growing regions from eastern TX to northern FL and southern portions of GA and SC would experience first emergence of adults as early as mid-April and peak flight activity during the first half of May (**Fig. 4**).

Our results show that consistent annual patterns of emergence, development times and DD relationships may change as *S. noctilio* moves southward and encounters new host conifers. The degree day relationship provided here provides a starting point to guide survey efforts in eastern U.S. and Canada. Accurate timing of sampling is especially important with *S. noctilio*, as current trapping methods are inefficient and may not readily detect low level populations in areas where it is newly established.

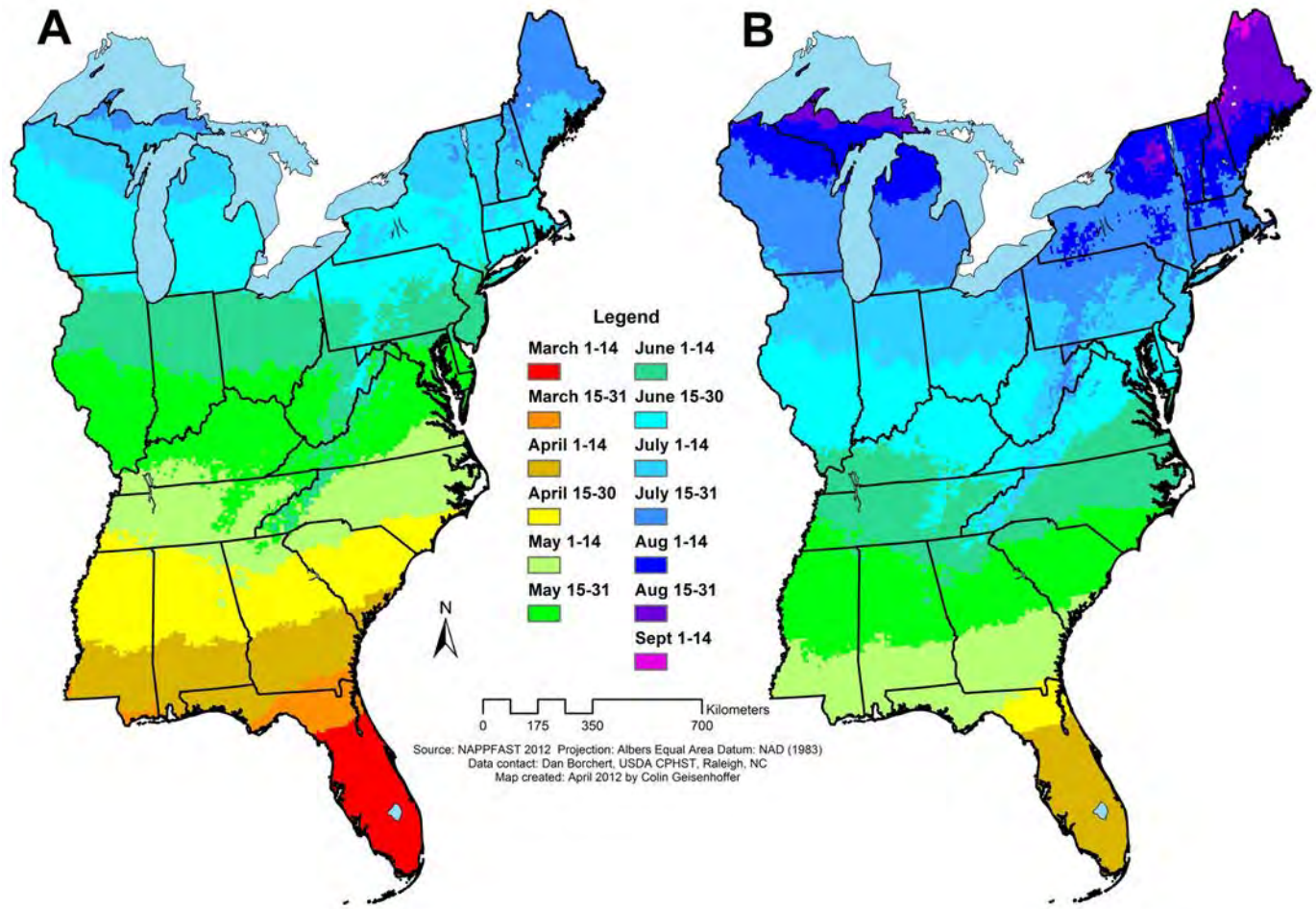
References

(1) Baskerville, G.L., and P. Emin. 1969. Rapid estimation of heat accumulation from maximum and minimum temperatures. *Ecology.* 50: 514-517.

(2) Madden, J.L. 1981. Egg and larval development in the woodwasp *Sirex noctilio* F. *Aust. J. Zool.* 29: 493-506.

(3) Borchert, D. M., and R. D. Magarey. 2007. A guide to the use of NAPPFAST. (www.nappfast.org). Date accessed 4/30/2012.

Figure 4: *Degree-day model predictions of first emergence (A; 700 DD) and peak adult flight (B; 1145 DD) for Sirex noctilio in the eastern United States. Degree-day calculations were based on a developmental threshold of 6.8 °C and upper threshold of 35 °C. Probit regression of cumulative adult female trap captures in central New York from 2007-2010 was used to estimate early and peak emergence periods using 5 and 50% cumulative emergence estimates, respectively.*



Discovery of an Aggregation Pheromone for *Sirex noctilio*, and its Application

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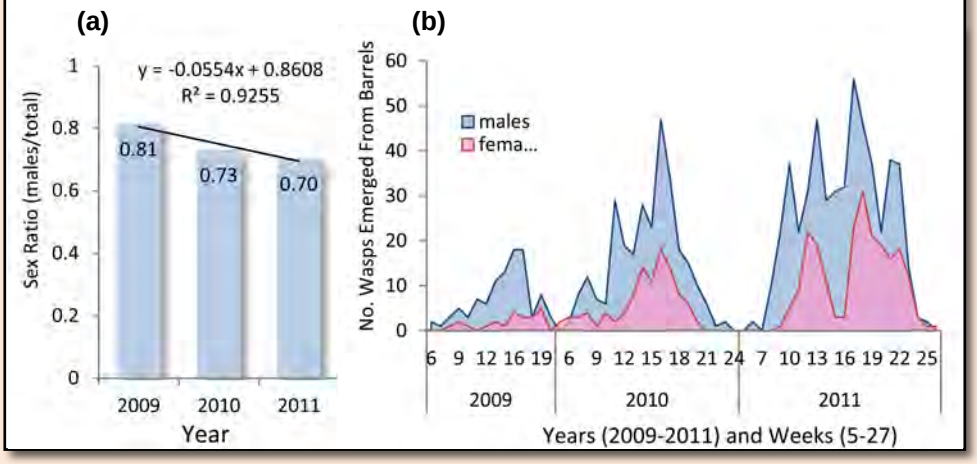
A 3-component male-produced sex pheromone was discovered for the European woodwasp, Sirex noctilio*, using a combination of techniques including aerations, GC-MS, GC-EAD, Y-tube olfactometer and wind tunnel behavioral bioassays. Females did not produce the main component, nor did males younger than 2 d old. The major component and two minor components were identified, and the ratio of the three compounds was determined to produce an attractive synthetic blend. The pheromone, consisting of a ratio of 100:1:1 parts (Z)-3-Decen-1-ol, (Z)-4-Decen-1-ol, and (E,E)-2,4-Decadienal, was found to be attractive to both males and females in a laboratory wind tunnel bioassay. Multiple Y-tube tests revealed that the addition of other suspected compounds reduced attraction, as did omission of the compounds in the blend, or blending the compounds at a different ratio. Prior to positive identification of the two minor components in the laboratory, a 2010 field trapping study in South Africa found that, although the main component in the absence of the correct minor components did not out-compete the kairomone lure, a significant dose-response to the major pheromone component alone existed. Since the discovery of the two minor pheromone components in 2011, a) a preliminary field trapping study was conducted near Syracuse, NY to test the pheromone at 3 concentrations against the kairomone lure, b) trap designs were evaluated in a large field wind tunnel in Ithaca, NY, and c) a large field trapping study was initiated in* Sirex*-infested South African pine plantations.*

Background. Early in the 20th century, *Sirex noctilio* was introduced into New Zealand, and became a serious pest of *Pinus radiata* plantations following a period of drought in the 1940's[1]. Since then, much of its basic biology is still poorly understood. Although there has been research on host plant volatiles, few studies have focused on how they find their mates.

Aside from the use of girdled trap trees, lures containing host plant volatiles have been the next best tool available to date for detection and delineation trapping. However, those lures only target ovipositing females. In new infestations, females usually make up much less than half of the population (**Fig. 1**) due to the haplo-diploid sex determination in Hymenoptera. That is, when mates are scarce, diploid females deposit unfertilized eggs which develop into haploid males. As populations become more established, mates become more abundant, and the sex ratio adusts itself to be more female. Thus, in early invasions, females have been outnumbered by

Figure 1: *Declining annual sex ratios (a), and number of males and females that emerged weekly from 25 staggered barrels of logs (b).*



males by as much as 14:1[2], and in extreme instances, 100:1 ratios of males:females have been reported from individual trees[3]. A lure and trap combination that targets males or both sexes could potentially tap into this untargeted population and may provide a more sensitive tool for early detection and delineation purposes.

This report describes work conducted in FY 2011, and progress in the discovery of the sex pheromone for *S. noctilio*, as well as progress in improving trapping technology.

Insects. In the fall, girdled and infested trees were felled, cut, and placed into barrels which were brought indoors in five batches over five months in order to stagger emergence over a long period of time. The 2011 emergence pattern from staggered log barrels in the quarantine at Otis, and the sex ratio of emerging wasps over time, are shown in **Fig. 1**. Wasps were only viable for use in experiments for 7 or 8 days. To maximize the use of a limited number of wasps, young wasps were used in behavioral bioassays, males over 2 d old were used in aerations, and the oldest wasps were usually used in electrophysiology after they were first used in behavioral bioassays and/or aerations.

Volatiles and bioassays. The air delivery system, volatile collection chambers, Y-tube olfactometer, and wind tunnel bioassay at the Otis Laboratory are depicted in **Fig. 2**. Males, 2-7 d old, were aerated and odors were collected on SPME fibers, as well as charcoal and Porapak traps that were subsequently eluted in solvent.

Gas chromatography coupled with electro-antennographic de-tection (GC-EAD) was conducted using four male antennae in parallel to search for minor pheromone components. Gas chromatography coupled with a mass spectrometer (GC-MS), was used to preliminarily identify peaks of interest.

Table 1: *Suspected minor components tested for male antennal responses (check boxes). Those eliciting the strongest EAD responses (two checks) were tested for attraction in the Y-tube.*

Compounds	Response
α-Pinene	☑
β-Pinene	☑
(+)-α-Longipinene	☐
Nonanal	☑☑
Verbenol	☑
(1S)-(-)-Verbenone	☐
Myrtenol	☐
(E)-3-Decenol	☑☑
(Z)-5-Decenol	☑☑
(Z)-3-Decenol *	☑☑
(Z)-4-Decenol	☑☑
9-Decenol	☐
(E,E)-2,4-Decadienal	☑☑
Nonanoic Acid	☐

** Major component*

Retention times, peak shapes, and Kovat's indeces were also used to match peaks producing antennal responses in the GC-EAD to peaks in the GC-MS. Once tentative identifications of active compounds were made, those synthetic compounds were purchased or synthesized and tested for GC-EAD antennal activity and matching retention times (**Table 1**). Synthetic minor compounds that were suspected to be in the male headspace, and also produced strong EAD responses, were then tested for male attraction in the Y-tube olfactometer in different blends, concentrations, and ratios.

However, females tested in the same Y-tube olfactometer appeared to have difficulty walking and flying, and lacked motivation to respond to natural male odors in the confined space. Consequently, experiments in 2011 focused initially on the preferences of males to synthetic odors using the Y-tube bioassay. It was later necessary to develop a bioassay in which females also could be tested for attraction to odors in the laboratory. After a synthetic blend was determined to attract males, it then was used to develop a behavioral bioassay that could test females for attraction as well as males.

A laminar flow wind tunnel (120 cm long x 91 cm high x 75 cm wide) was constructed for use inside a walk-in environmental chamber in the quarantine facility at the Otis Laboratory. A female or male *S. noctilio* released at the downwind end of the wind tunnel could fly to the source of an odor placed somewhere upwind. Two targets, made of 5-cm diam. black disks (cut from black intercept trap panels), were suspended at the upwind end of the tunnel, each one with a rubber septum attached above it (**Fig. 2**). One septum was loaded with the test odor(s) in hexane, and the other with an equivalent amount of hexane alone. The targets were alternated after every few replicates to avoid any directional bias in the bioassay.

Figure 2: *Air delivery system with a volatile collection chamber (top), Y-tube olfactometer (bottom left), and wind tunnel (bottom right).*

A second, much larger, wind tunnel (360 cm long x 180 cm high x 180 cm high) was constructed for field or greenhouse use in areas where *S. noctilio* occurs. It consisted of a 1" PVC pipe frame, with laminizers on both upwind and downwind ends, and a semi-transparent plastic sheet attached by Velcro® to form the ceiling and walls. At the upwind end, an expansion chamber was constructed of the same plastic material which connected a fan to the upwind laminizer.

Trap design. With the development of an attractive synthetic lure, behavior was examined of *S. noctilio* flying to the lure and entering different devices in the small wind tunnel at the Otis Laboratory with the goal of learning about their trap-approaching behavior, in order to design a more effective trap. Based on these observations, two new trap designs were developed at the Otis Laboratory and sent to cooperator Ann Hajek at Cornell, along with the commonly used black intercept panel trap, for testing in a large field wind tunnel in the summer of 2011. The wind tunnel was constructed at the Otis Laboratory and transported to Cornell University in Ithaca, NY. It was set up inside a greenhouse which was well-ventilated to allow clean outside air to enter the upwind end of the tunnel, and air exiting the tunnel was exhausted out of the greenhouse. The two new trap designs consisted of an upside-down cone-shaped trap, and a clear jar trap. Traps were baited with a 3-component pheromone lure and suspended at the upwind end of the wind tunnel. Two lure concentrations were tested: 1.0 mg and 0.1 mg of the main component, at 100:1:1 of (Z)-3-decenol, (Z)-4-decenol, and (E,E)-2,4-decadienal, respectively. Male and female *S. noctilio*, reared from infested wood and collected upon emergence, were kept individually in vials. Individuals were released in the downwind end of the wind tunnel and video recorded flying upwind towards a trap. The number of wasps that encountered and entered each of the traps was recorded.

Figure 3: *Setting up intercept traps in South Africa.*

Field studies. Three field studies were conducted in FY2011. The 2010 study was conducted from October to December in South Africa by cooperator Dr. Brett Hurley at University of Pretoria. A study in the summer of 2011 was based around the Syracuse CPHST laboratory and took place from June to August. The last study was initiated in October of 2011 in South Africa by cooperator Dr. Brett Hurley.

The cooperative agreement established in 2010 with Dr. Brett Hurley was a large replicated field trapping study to test the antennally active major component in areas heavily infested with *Sirex*. At the time of this study, the identity or importance of any minor components had not been discovered yet. Due to multiple overlapping GC peaks in the area of the second strong antennal response, efforts to identify a minor component were complicated. Concentrated headspace extracts were sent to Dr. Ashot Khrimian (chemist at USDA ARS) for further chemical analysis, and he found that one of the hidden peaks matching the retention time of the the strong antennal response contained nonanoic acid. It was suspected as a possible minor component. Since there were no adult *S. noctilio* available to conduct GC-EAD on nonanoic acid, it was decided that the field study in South Africa would test the major component alone and in a 100:5 blend with nonanoic acid, at three different concentrations, in the presence and absence of a kairomone lure (Alpha Scents) containing *S. noctilio* host plant odors, and with a positive and negative control. The study was conducted in pine plantations of 10-15 year old *Pinus patula* in South Africa, with three blocks and five replicates (**Fig. 3**).

The first 2011 field study was based around the Syracuse laboratory and tested three concentrations of the newly discovered 3-component blend (0.1 mg, 1.0 mg, and 10 mg) against the 8-component kairomone lure which was used successfully in the above-described 2010 South Africa field study. This study consisted of the four treatments at 10 field sites in Oneida, Wayne, and Oswego Counties (relatively small, mostly private properties, each with a stand of Scots pine, *Pinus sylvestris*). Each site had 2 replicates of 4 treatments, for a total of 80 traps. Traps were deployed from June 27 to July 1, 2011. Lures were replaced in the week of August 1, 2011.

The second 2011 study, initiated in South Africa, was going to utilize the best concentration determined from the Syracuse study and focus on questions of trap height and design. However, due to low trap catch in Syracuse for all four treatments, it was necessary to test

concentration in South Africa as well. Therefore, the South African study was designed to test the pheromone blend at two concentrations (1.0 mg and 0.1 mg), with a negative control (no pheromone), each with and without the kairomone, at two heights (3 m and 7 m), using the black intercept panel traps.

Additionally, based on results from the Hajek wind tunnel trap design study, the clear jar trap would be tested, at the 7 m height only, with and without the 1 mg pheromone lure, and with and without the kairomone lure. The study was to be conducted at four field sites consisting of large pine plantations (*Pinus patula*) heavily infested with *S. noctilio*. Each treatment was replicated five times per site. Due to the steep terrain at one site, and the extra time required to hang traps at the 7 m height, it was necessary to drop one of the sites from the planned study, so three sites were used for a total of 15 replicates per treatment. Traps were deployed from October 17 to 21, 2011, with a change of lures scheduled to take place after three weeks.

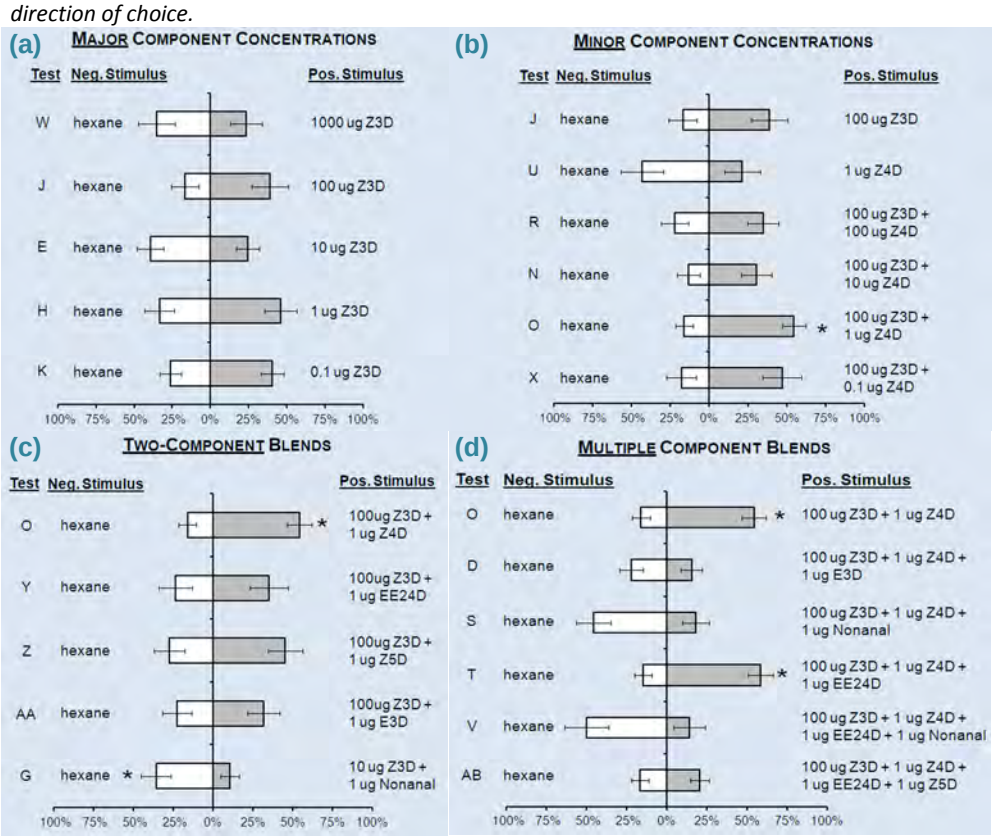
Male responses to synthetic blends in the Y-tube olfactometer. Experiments to determine which compounds, blends, concentrations, and ratios attract males were conducted in the Y-tube. Although the main component alone did not significantly differ from hexane in these tests at any of the concentrations tested (**Fig. 4a**), the concentration that produced the least negative response (100 ug) was selected for testing with potential minor components. That the main pheromone compound alone did not strongly attract at any concentration suggests it was missing at least one required component.

When offered alone, neither the main component (Z)-3-decenol, nor the minor component (Z)-4-decenol produced significant attraction (**Fig. 4b, tests J and U**). However, the 2-component blend of 100:1 ug (Z)-3-decenol and (Z)-4-decenol was chosen significantly more often than the hexane alternative (**Fig. 4b, test O**), and other ratios of the two compounds failed to attract males (**Fig. 4b, tests R, N, and X**). The 100:1 ratio of major to minor components was close to the ratio found in extracts of the natural male headspace odors. The sum of the attraction by each of the two compounds separately was less than the attraction of the 2-component blend, indicating that these two compounds act synergistically.

Other candidate minor components with strong antennal responses were also tested in 100:1 binary blends with (Z)-3-decenol, but those blends failed to produce attraction (**Fig. 4c, tests Y, Z, and AA**). When a 10:1 ug blend of (Z)-3-decenol and nonanal was tested, males selected the hexane significantly more, suggesting repellency (**Fig. 4c, test G**).

The addition of one or more minor components to the 2-component attractive blend of 100:1 (Z)-3-decenol and (Z)-4-decenol was also investigated (**Fig. 4d**). The addition of 1 ug of either (E)-3-decenol or nonanal to the attractive 2-component blend completely removed attraction. The addition of 1 ug of (E,E)-2,4-decadienal to the attractive 2-

Figure 4: *Key results of Y-tube olfactometer experiments, testing males when offered a choice between the synthetic component(s) in hexane and hexane alone. Bars show percent responding and direction of choice.*

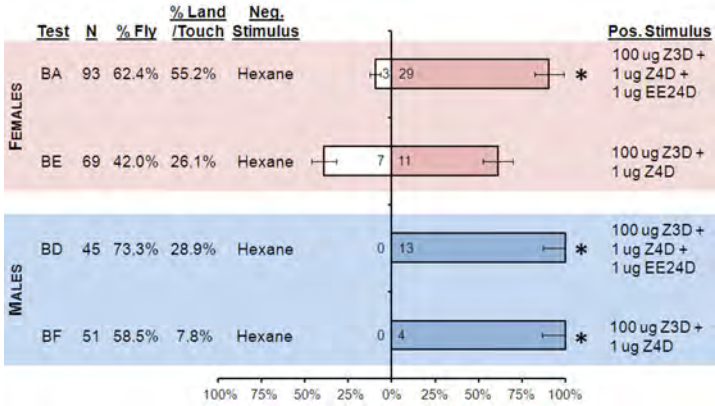


** asterisk indicates significant difference between sides; Z3D=(Z)-3-decenol; E3D=(E)-3-decenol; Z5D=(Z)-5-decenol; Z4D=(Z)-4-decenol; EE24D=(E,E)-2,4-decadienal*

component blend was significant, and produced slightly higher attraction and response rate than the attractive 2-component blend alone (**Fig. 4d, test T**). The addition of 1 ug of either nonanal or (Z)-5-decenol to this attractive 3-component blend completely removed attraction (**Fig. 4d, tests V and AB**).

These results establish the first discovery of a 2-component blend of 100:1 ug (Z)-3-decenol and (Z)-4-decenol, and a 3-component blend of 100:1:1 ug (Z)-3-decenol, (Z)-4-decenol and (E,E)-2,4-decadienal, both attractive to male *S. noctilio* in a Y-tube olfactometer. These results also demonstrated that nonanal, (E)-3-decenol, or (Z)-5-decenol, when added to the attractive blends, resulted in a loss of attraction.

Figure 5: *The 2- or 3-component blend in hexane was offered next to a negative stimulus (hexane alone) in the wind tunnel. Bars represent the percent of males or females that landed on or touched either target (actual numbers that chose each target are inside each bar).*



* asterisk indicates significant difference between sides; Z3D=(Z)-3-decenol; Z4D=(Z)-4-decenol; EE24D=(E,E)-2,4-decadienal.

Synthetic blends attractive to both sexes in the wind tunnel. The two- and three-compound blends found to be attractive to males in the Y-tube were subsequently tested in the small wind tunnel in the quarantine at the Otis Laboratory. The wind tunnel bioassay allowed for successful testing of both sexes to the new attractants.

Results found that for both sexes a higher percent of wasps flew upwind to the 3-component blend than to the 2-component blend (**Fig. 5**). Similarly, a higher percent of wasps landed on or touched the target containing the 3-component blend than the 2-component blend. All males that made a choice selected the positive stimulus over the hexane, regardless of whether the 2- or 3-component blend was offered. Females chose the 3-component blend significantly more than the hexane, however, they did not chose the 2-component blend more often than the hexane. Therefore, in order to find the correct target females required the 3-component blend. For both sexes, the proportion of wasps released that chose the 3-component blend was at least double that for the 2-component blend.

Laboratory observations and trap designs. Differences in behaviors occurred between males and females. Males were less likely to touch or land on a target than females. The target baited with the 3-component lure was intercepted by 28.9% and 55.2% of males and females, respectively (**Fig. 5**). A similar pattern was observed with the 2-component blend.

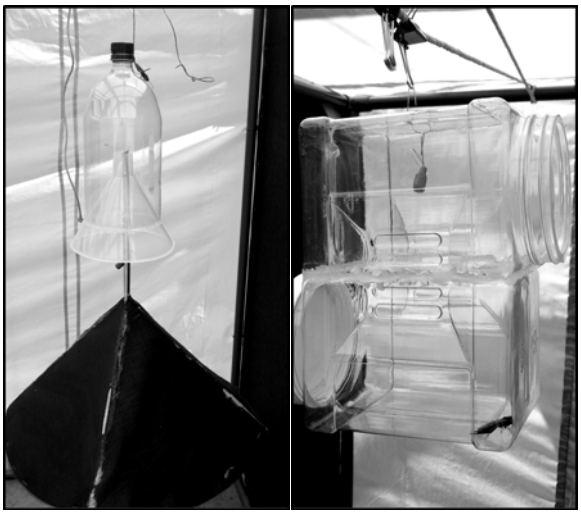


Figure 6: *Two novel trap designs, the "skirt" trap (left) and the "clear" trap (right). A female S. noctilio landed on top of the cone trap and inside the bottom of the jar trap.*

Based on these observations, it was hypothesized that males were less likely to land or come into contact with a solid surface than females. This behavioral difference is supported by the differences in their biology, in which females are required to land on trees to oviposit, and not males, and males are thought to fly in leks at the tops of trees to find mates.

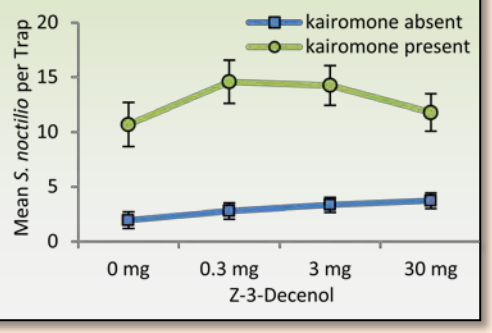
Two new traps were designed with the goal of increasing the male capture rate (**Fig. 6**). The first trap design aimed to exploit the strong phototactic responses of males. This trap had the top of an intercept trap as a "skirt" suspended beneath an inverted clear funnel leading into a clear bottle. This skirt trap required wasps to approach the lure at the top of the skirt, land on the skirt, and then switch to phototaxis to climb into the bottle above.

The second trap consisted of two clear plastic square jars on their sides, stacked on top of each other with the section between them cut away. The top jar was uncovered and had a large mouth (9.5 cm diam.), and contained the lure. This design exploited the fast flight of both sexes by allowing them to follow the odor plume into the trap and

then bump into the clear wall of the trap, and fall into the jar which could contain propylene glycol.

The two novel trap designs, and a standard black panel intercept trap, were tested by cooperator Dr. Ann Hajek and Dr. Mark Sarvary, who borrowed our large walk-in wind tunnel for use in a green house in Ithaca, NY. Wasps reared from infested Scots pine were individually released and recorded in the wind tunnel. Three trap types were tested, and two concentrations of the new 3-component pheromone lure, 1.0 mg or 0.1 mg. Of those that flew upwind, the number of wasps that were caught in each trap was recorded (**Table 3**).

Figure 7: *Mean number of* S. noctilio *caught per trap showing dose response of the main pheromone component, and an interaction between the kairomone and the pheromone. Nonanoic acid had no effect.*



The new skirt design did not work for either sex, but when baited with the 1.0 mg pheromone lure, the new clear trap caught more males than the standard intercept trap. When baited with the 1.0 mg lure, females were caught by both the clear trap and the intercept trap at similar frequencies. But with the 0.1 mg lure, the intercept trap caught more females than the clear trap. These results suggest that although both sexes are attracted to the pheromone, the visual trap silhouette is attractive for females but deterrent for males.

Field study results. In the field study conducted in South Africa from October to December, 2010, prior to the discovery of the minor pheromone components, 3409 females and 14 males were caught in 420 black panel intercept traps, with 14 treatments, over a period of five weeks. All traps, including unbaited control traps, caught wasps. The fact that unbaited intercept traps caught wasps supports the idea that there is a visual component to the trap that contributes to its success, at least for female *S. noctilio*. There was no significant effect of nonanoic acid, but the kairomone was significant, and there was a significant interaction between the kairomone and the major compound (**Fig. 7**). A significant dose response was found for the major compound in the absence of the kairomone. The kairomone alone clearly outperformed (Z)-3-decenol alone or in combination with nonanoic acid.

The field study in Syracuse, NY, from June 27 to September 13, 2011, aimed to evaluate three concentrations of the new 3-component pheromone. The kairomone lure successfully used in South Africa was used as a positive control. All four lure treatments were tested using the same black intercept traps that were successfully used in South Africa, however, resulting capture rates were low. A total of only 17 female *S. noctilio* were caught in NY: 14 in the kairomone traps, and 3 in the 0.1 mg traps, and captures were clustered in field locations. Thus, the Syracuse study was inconclusive.

Based on behaviors observed in these studies, and field observations in the early literature[2], this pheromone may serve as a lekking pheromone. If so, males swarming around the tops of trees, producing the pheromone, would attract other males and females seeking a mate. This report marks significant progress in characterizing the sex pheromone of *S. noctilio*, but many questions remain unanswered and more research is needed. Plans to continue field testing the 3-component pheromone blend are underway.

Table 3: *Of* S. noctilio *wasps that flew upwind in the wind tunnel (N), proportion caught by trap designs baited with the 3-component synthetic pheromone lure.*

Lure		Intercept	Clear	Skirt
0.1 mg	Male	0.04 (46)	0.02 (52)	0 (25)
1.0 mg		0.03 (33)	0.13 (32)	0 (23)
0.1 mg	Female	0.16 (37)	0.05 (42)	0 (39)
1.0 mg		0.21 (28)	0.20 (30)	0 (29)

References

(1) Coutts, M. P. 1965. Sirex noctilio and the physiology of Pinus radiata. Some studies of interactions between the insect, the fungus, and the tree in Tasmania. *Forestry and Timber Bureau, Australia Bulletin*. p 79.

(2) Hurley, B. P., Slippers, B., Croft, P. K., Hatting, H. J., Van Der Linde, M., Morris, A. R., Dyer, C., and Wingfield, M. J. 2008. Factors influencing parasitism of Sirex noctilio (Hymenoptera: Siricidae) by the nematode Deladenus siricidicola (Nematoda: Neotylenchidae) in summer rainfall areas of South Africa. *Biol. Control* 45:450-459.

(3) Morgan, D. and Stewart, N. C. 1966. The biology and behaviour of the woodwasp Sirex noctilio F. in New Zealand. *Tran. Roy. Soc. N. Z., Zool*. 7:195-204.

Biological Control of *Sirex* Woodwasp

Project Lead: Dave Williams

Contributors: Carrie Crook, John Molongoski, and Dyanna Louyakis (Otis Laboratory)
Evan Braswell (CPHST, Mission, TX)

Controlled releases in 2011. Field releases of the Australian "Kamona strain" of *Beddingia siricidicola* continued to be "controlled" this year. That is, we felled infested pine trees and inoculated them with the nematode in the fall (**Fig. 1**). They passed the winter in the field, and in the spring, we cut billet samples for rearing in screened barrels and chipped the remainder to prevent escape of the nematode into the environment.

Figure 1: Sampling an infested red pine treated with Australian Kamona nematodes.

Our primary objective this year was to compare the Kamona strain with the pre-existing "North American strain" of *B. siricidicola* (**Fig. 2**). We hypothesized differences in the rate of infection and in the sterilization of hosts. Sterilization is the ability of juvenile nematodes to invade the *Sirex* egg (**Fig. 3**), and it has been touted as an important feature in the effectiveness of the Kamona strain. Entering the host egg serves both to disperse juvenile nematodes and to reduce *Sirex* populations. By contrast, the North American strain has been reported to be non-sterilizing, rendering it less useful as a biological control agent.

Figure 2: The parasitic form of the Kamona strain, showing the long stylet at the mouth.

To investigate possible strain differences, we contrasted trees inoculated with Kamona nematodes in a gel suspension with equal numbers of controls treated with gel alone at five sites in red pine plantations in southern New York and northern Pennsylvania. We used 84 trees in all and a total of 356 *S. noctilio* emerged from them. Variability was quite high; for example, fewer than ten woodwasps emerged at two of the sites, whereas another had almost 80 % of the emergences. We did not recover any nematodes at two sites. At one site, we found no nematodes in the controls and 67% infection in the nematode treatments, whereas in another we found 12% infection in the controls and 22% in the treatments. The latter case suggests that both nematode strains were present and that their numbers were additive. Interestingly, the last case had a higher infection rate (11%) in the control than in the nematode treatment (5%). We provide no explanation for this. We also found no cases of sterilization at any of the sites, suggesting either that the Kamona strain did not establish or that it did but was unable to enter the host egg.

In addition to woodwasps, many parasitic wasps that are known *Sirex* parasitoids emerged in our billet barrels. Most prevalent were *Ibalia leucospoides* and *Rhyssa* species. *Ibalia* produced high rates of parasitism, ranging from 3-65% with an average of 34%, whereas *Rhyssa* had considerably lower rates, ranging from 0-11% with an average of 2%. Because of their relatively high parasitism rate, other researchers have speculated that *Ibalia* might be an important factor in the natural control of *Sirex*. We investigated this idea using our four years of field data on *Ibalia* emergence. We plotted the densities of *Ibalia* populations at individual sites in individual years against total "*Sirex*" density,

Figure 3: *A* Sirex *egg full of juvenile nematodes.*

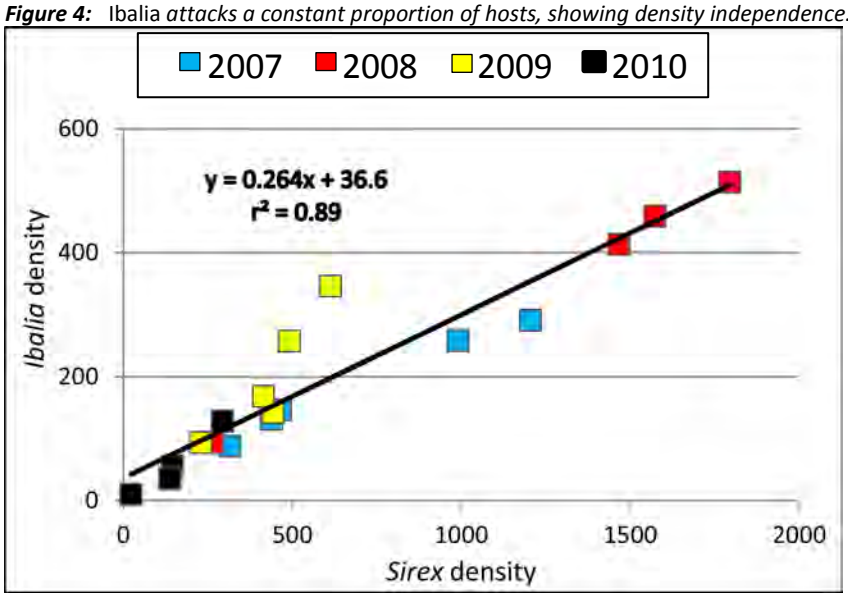
which included both *S. noctilio* and its parasitoids (**Fig. 4**). The highly significant linear relationship indicates that *Ibalia* attacks a constant proportion of the number of hosts regardless of their density. Thus, *I. leucospoides* acts as a density independent agent. It kills a sizeable population of *Sirex* larvae, which is useful, but it cannot exert real control in a density dependent fashion.

Laboratory Studies. Our first objective was to shorten the time needed to determine infection rates of the Kamona and New York nematodes by inoculating and rearing them in the lab. We collected billets from trees with *Sirex* signs in two state parks in Pennsylvania in October 2010. Back in the lab, we inoculated them with the Kamona strain, the North American strain, or a gel control and held them for emergence. Unfortunately, none of the emerging *Sirex* was infected with nematodes, probably because the billets desiccated rapidly in the lab. Combined parasitism by *Ibalia* and *Rhyssa* was high, 35-58%.

Our second objective was to investigate possible susceptibility of the native *Sirex nigricornis* to the Kamona strain. Experimental billets were provided by Forest Service cooperators in Louisiana. Billets from three pine trees, including loblolly and shortleaf pines, were exposed to *Sirex* oviposition and then shipped to Otis. We inoculated them with either the Kamona strain nematodes or a gel control. None of the emerging *Sirex* adults was infected with nematodes. As with the previous experiment, nematodes may have failed to establish because the billets desiccated. Alternatively, the Kamona nematodes may not have established because they cannot survive or reproduce on the native *Amylostereum chailletii* fungus, which is the associate of *S. nigricornis*.

Another objective was to develop molecular tools to identify the strains of *B. siricidicola*, This is of obvious importance to our field studies as reported above. John Molongoski and Dyanna Louyakis with advice from Evan Braswell have sequenced the CO1 region of the nematode. They identified differences in two base pairs between the Kamona and North American strains. We are in the process of identifying field samples.

Figure 4: Ibalia *attacks a constant proportion of hosts, showing density independence.*



Evaluation of Vacuum Steam Treatment for Whole Log Export

Project Lead: Ron Mack

Contributors: Zhangjing Chen and Marshall S. White (Virginia Tech University)

Available phytosanitary treatment for whole veneer logs destined for export are currently limited to fumigations using methyl bromide. Alternative whole log treatments are needed as we approach the phase out of methyl bromide. To this end, PPQ CPHST has partnered with Virginia Tech University to evaluate the prospect of treating whole logs with vacuum steam treatment (VST) as a phytosanitary measure.

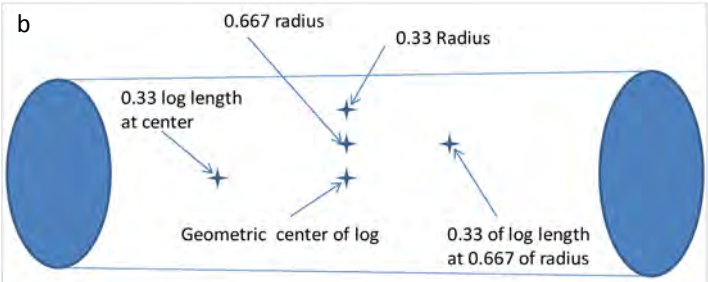
The objectives of the experiment include the following:

- Test the time necessary to attain a temperature of 56C/ 30 minutes throughout the entire profile of the log.
- Characterize energy consumption for each tested log.
- Evaluate any veneer damage or quality reduction due to treatment on four counts (end checks, heart checks, color change, veneer yield).

Methods. Veneer grade logs purchased from Danzer Veneer, Indiana were shipped to Brooks Forest Products Center at Virginia Tech in the fall of 2011 for VST processing (**Fig. 1a**). Hardwood logs representing the range of porosity classes were chosen for the study. These included pignut hickory (*Carya glabra*), black cherry (*Prunus serotina*), American black walnut (*Juglans nigra*), yellow poplar (Liriodendron tulipifera), and red oak (*Quercus rubra*). A total of three logs of each species were earmarked for treatment, with a fourth log set aside as an untreated control. All logs were trimmed to 8 ft. lengths, debarked, and end coated with Anchorseal (**Fig. 1b**). Commercial end savers were applied to restrict end checking (**Fig. 1c**). Logs were then stacked outside and maintained with a water sprinkler system to prevent surface checking and moisture loss prior to VST testing (**Fig. 1d**).

Figure 1: *Veneer grade logs a) being transported for VST processing, b) trimmed, debarked, and end-coated, c) applying commercial end savers, and d) maintained with sprinkler system.*

Figure 2: *Logs were a) affixed with thermocouples, b) locations of temperature measurements within the hardwood logs.*



Holes were drilled in each log at selected locations for inserting thermocouples, then backfilled with plumbers' putty (**Fig. 2a,b**). Individual logs were then inserted into a zippered, flexible bag specifically designed to withstand vacuum and temperature extremes (**Fig. 3a**). The remaining VST system components include the vacuum pump, steam generator (boiler), flow meter, and data acquisition hardware/software (**Fig. 3b**). Logs were processed to 56C for 30 minutes at 200mm Hg vacuum for a single cycle.

After visual inspection, both treated and control logs were shipped to Danzer Veneer, Indiana for veneer slicing and subsequent quality assay. Logs were processed into flitches and soaked in hot water baths for softening. Flitches were then sliced into veneer and conveyed to dryers (**Fig. 4**). After drying, veneers were hand pulled and stacked in order for quality inspection.

Results. Treatment times to reach target temperature were on the order of a full day (**Table 1**). Energy use was calculated using total steam applied based on flow meter readings, then referencing standard steam tables (**Table 1**). End checking due to treatment was more pronounced on pignut hickory and black walnut, and less of a factor on yellow poplar (**Fig. 5**). Walnut heartwood was judged slightly darker by Danzer Veneer grading staff, and slight darkening in color of sapwood of black cherry, yellow poplar, and pignut hickory was also noted (**Fig. 6**).

Overall, the grading staff at Danzer Veneer found that the vacuum steam treated logs in this study did not differ much from normal production logs in terms of overall veneer quality and yield. There are a number of factors that impact veneer color, such as time to processing after logging, time of year harvested, and holding temperature of logs. VST logs in this experiment were 2 months removed from logging at treatment time, and were not cooled below 55F with sprinklers for the period of transport to Virginia Tech. These factors alone could account for the slight darkening of sapwood veneer. Future work planned will consider eliminating variables that impact quality. If possible, logs of each species should come from a single tree, and log testing should begin soon after harvest.

Figure 3: *Logs were a) placed into a vacuum steam bag, and b) conditions were electronically controlled and monitored.*

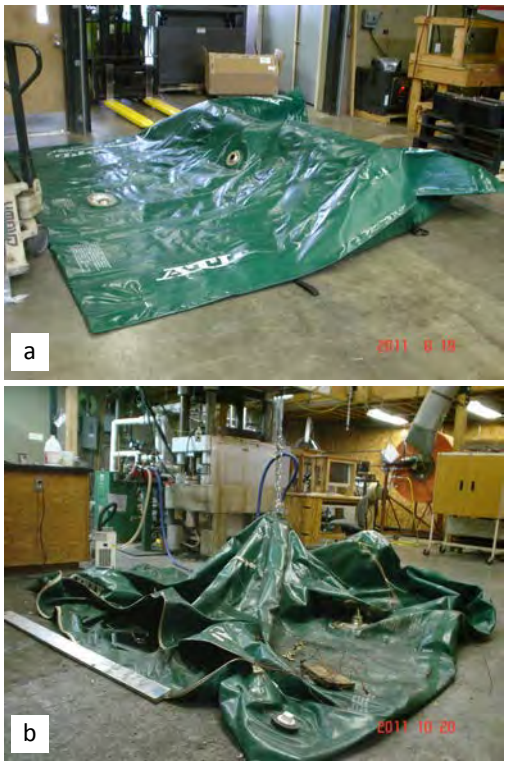


Figure 4: *Flitches were sliced into veneer and conveyed to dryers.*

Figure 5: *End checking due to treatment varied by wood type.*

Figure 6: *Minimal darkening of heartwood was noted as a result of treatment.*

Table 1: *Mean treatment time and energy used for hardwood logs treated with vacuum steam.*

	Avg. Log Diam. (inches)	Mean Treatment Time to 56C/30min (hrs)	Mean KWh Used
Pignut Hickory	22.75	25.27	148
Black Cherry	20.25	21.80	521
Black Walnut	22.38	23.64	148
Yellow Poplar	19.38	22.20	87
Red Oak	21.00	24.74	124

Development of a Cold Treatment for Peach Fruit Fly, *Bactrocera zonata*

Project Lead: Scott Myers

Cooperators: Guy. J. Hallman (USDA ARS, Weslaco, TX)

Gustavo Taret (FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria)

Initial experiments were conducted to compare cold tolerance of 3rd instar Bactrocera zonata *with* Ceratitis capitata*, and* Anastrepha ludens *using egg infested navel oranges under simulated in-transit cold treatment conditions. Results demonstrated that* A. ludens *is the most cold tolerant of the three species. Cold tolerance between* B. zonata *and* C. capitata *did not differ significantly. Confirmatory cold treatment tests were performed for* B. zonata *under conditions of 1.7°C for 18 d. A total of 36,820 second and third instar larvae were treated in fruit with no survivors. These results provide strong evidence that an 18 d cold treatment of 1.7°C or below will this treatment is adequate for use on citrus exports from Egypt in to the US.*

Introduction. The peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) poses a significant trade barrier for the export of fruit from Egypt into the United States. Originally native to south and southeast Asia, *B. zonata* is known to infest a variety of fruits including peach, mango, guava, papaya and citrus. There is currently no USDA-APHIS approved cold treatment for *B. zontata* that will allow for the import of fruit from countries where it is known to be established. Recent detections of *B. zonata* in California (2006) and Florida (2010) highlight the importance of establishing reliable treatment schedules for imported fruit to protect the fruit growing regions in the U.S.

Cold treatment can be an effective means to eliminate the risk of introduction for many tropical fruit flies as they are tolerated by many fresh fruit commodities and are often compatible with standard industry practices. In addition, they are capable of being implemented during overseas transit periods. One significant challenge to developing cold treatments is that producing the large data sets that are often required to validate a treatment can be expensive and time consuming. A shortcut is to develop experiments that directly compare multiple species of concern to determine their relative cold tolerance under treatment conditions. By this method, treatments that have been developed for species that are more cold tolerant can be applied to less tolerant species without the cost and rigor of developing a standalone treatment. Hallman et al.[1] utilized this approach to determine that the new invasive tephritid *Bactrocera invadens* Drew, Tsuruta & White, was more susceptible than *Ceratitis capitata* (Wiedemann) to cold phytosanitary treatment temperatures when both insects were treated in Petri dishes with diet.

The aim of this project was to develop a cold treatment schedule to be used for in-transit shipment of citrus from origins where *B. zonata* is known to occur. The objectives were to first compare the cold tolerance of 3rd instar *B. zonata* with that of two species with well-developed cold treatment schedules, *C. capitata* and *A. ludens* side-by-side using adult infested navel oranges at 1.7°C; and if the results of the previous objective were insufficient, conduct confirmatory treatments with greater than 30,000 *B. zonata* larvae in infested fruit to validate the efficacy of the treatment with a high degree of confidence.

Methods. All experiments were conducted at the IAEA Insect Pest Control Laboratory in Seibersdorf, Austria from June 2011 through January, 2012. Fly colonies used were *A. ludens* from a Mexican laboratory colony that originated from mango and was transferred to the FAO/IAEA Agriculture and Biotechnology Laboratory in Seibersdorf, Austria in 2009, *C. capitata* (Wiedemann) originated from laboratory strain pupae reared from unspecified wild-infested fruit in Argentina established in

Figure 1: *Fruit fly colonies at the IAEA Seibersdorf laboratory, adult room.*

Figure 2: *Fruit fly experimental setup showing preparation of oranges (top), and cages containing fruit and adults (bottom).*

2006, and *B. zonata* arrived from a colony originating from wild infested fruit collected in Mauritius in 2010. All test fruit fly species used in these experiments were reared under laboratory conditions described by Hallman et al.[1] (**Fig. 1**).

The fruit used for all experiments were navel oranges imported from Egypt. Preliminary infestations were conducted with each tephritid species to assay for insecticide residues that may have impacted larval survival. Larvae from all species developed normally to pupation in these trials. To achieve infestation, fruit was stored overnight to acclimate to room temperature (~21°C), then washed with a mild detergent and allowed to air dry. Fruit was then arranged in groups of 6 oranges in 7 × 40 cm plastic trays, and fine tipped forceps were used to pierce six holes into each fruit to a depth of approximately 2 cm (**Fig. 2, top**). Trays with fruit were placed into cages and exposed to adults of each species (**Fig. 2, bottom**). This arrangement allowed female flies to oviposit into the punctures (**Fig. 3**). Exposure times were 30-120 min depending on the age and number of adult flies available. The goal was to achieve an infestation rate of 30-50 larvae per fruit. Following the infestation period fruit was stored in temperature controlled environmental chambers at 26°C to allow eggs to hatch and larvae to develop to third-instar before fruit was treated. Prior work had determined that 3rd instar is the most cold-tolerant stage for each of these species (AL[2], BZ[3], CC[4]). Following the infestation period the fruit was held at 26°C for 11-12 d to allow the majority of larvae to develop to the 3rd instar. A subset of fruit from each infestation date was randomly selected as a control (untreated). These fruits were dissected and the number of live and dead larvae recorded.

Treatment groups were arranged into 50 × 38 × 12 cm (L × W × H) bins, and placed into a 1,965 L capacity environmental chamber (Thermotron Model SE2000-4, Holland, MI), customized with an internal glass door with four 18 cm access ports to allow access to the items in the chamber without opening the doors. The manufacturers stated temperature control of the chamber is ± 0.3°C, and stated uniformity is ± 0.7°C.

Treatments were added and removed from the chamber at the same time (11:00 am) each day to minimize any temperature fluctuation that would result from opening the interior doors more than once per day. One fruit from each replicate was monitored via thermocouple to measure the cooling rate of the fruit. An 8 channel type-T thermocouple data logger (GEC Instruments, Model S8TC, Gainesville, FL, USA) was used to monitor and record internal fruit temperature during the treatment period. Thermocouples were checked prior to the start of the experiment using an ice bath and found to read within 0.05°C. Internal temperature of the chamber was also recorded during the treatment period with the integrated data collecting software, temperature measurements were logged every 30 minutes. The temperature inside the chamber was set to establish a temperature as close to 1.7°C as possible without

Figure 3: *Oviposition of adult* B. zonata *into orange.*

dropping below it. This temperature allows the resulting treatment schedule to use a fruit pulp temperature of 1.7°C or below and align with existing cold treatment schedules at the 1.67°C (35.0°F). Treatment periods ranged from 8 to 20 d to develop a dose-mortality relationship for all three species in side by side comparisons.

At the conclusion of each treatment the fruit was removed from the chamber and held at 24º for about 24 h, then dissected and visually examined to evaluate the number larvae present and their status. Larvae that were not moving, but not obviously dead (possessing normal larval color) were held in small containers until either they were observed to move, pupariate, or turn a dark color and begin to decompose. Pupariated larvae were counted as survivors regardless of the normality of the puparium.

A probit model was used to evaluate dose (days cold treatment) mortality relationships and estimate regression parameters for each species to determine levels of control and confidence intervals[5]. Chi square tests were performed to make comparisons between species to determine significant differences in slope and intercept coefficients to evaluate relative cold tolerance at the treatment temperature. Probit 9 treatment efficacy and confidence limits for confirmatory trials with *B. zonata* were calculated from the equation described by Couey and Chew[6] (see below).

Results and discussion. Fruit required nearly 24 h to completely cool down to the operating temperature of 1.7-2.0°C. Each replicate was standardized by allowing the first 24h in the cold chamber to serve as a pre-treatment cooling period.

Probit model to compare slope and intercept across species showed significant effect of slope (df=1, χ^2=58.94, P<0.001), but none for intercept (df=1, χ^2=2.14, P=0.14). The species term was removed and model was re-analyzed with common intercept to compare slopes. Max likelihood estimates indicated *A. ludens* model significantly different from both *B. zonata* (df=1, χ^2=11.78, P<0.001) and *C. capitata* (df=1, χ^2=12.78, P<0.001). No differences in the models of C. capitata and *B. zonata* were detected (df=1, χ^2=0.07, P=0.79). Since lack of significant difference in cold tolerance between *C. capitata* and *B. zonata* did not provide clear evidence that *B. zonata* was less tolerant to cold treatment additional tests were conducted at the 18 d cold treatment, although it seems that the latter may be no more cold tolerant than the former (**Table 1**).

Table 1: *Regression parameter and dose (days cold storage) estimates for mortality from probit models for three tephritid fruit flies.*

Species	Intercept	Slope†	% Control Level (95% Fiducial Limits) 95.0	99.9	99.99682
Bactrocera zonata	-0.320 (-0.803, 0.163)	0.258 A (0.166, 0.349)	6.23 (1.80, 7.95)	14.55 (12.86, 18.86)	19.79 (16.55, 28.97)
Ceratitus capitata	"	0.261 A (0.204, 0.319)	7.84 (6.90, 8.45)	12.63 (11.74, 14.18)	15.64 (14.11, 18.45)
Anastrepha ludens	"	0.178 B (0.075, 0.281)	12.56 (9.40, 15.38)	18.25 (15.42, 23.73)	21.83 (18.31, 29.88)

† *Means followed by the same letter indicate no significant difference (P<0.05)*

In the 18 d confirmatory tests a total of 36,820 *B. zonata* larvae were treated in infested navel oranges with no survivors (**Table 2**). Using equation 1 adopted from Couey and Chew[6]:

$$P_c = (1\text{-}C)^{1/n}$$

Where P_C is the probability of control, C is the one-sided confidence interval, and *n* is the number of insects treated with no survivors. Calculating the probability of control for which we have a 95% confidence interval using the number of insects tested we solve for P_C to get 0.999919, or 81 survivors per million.

Control mortality was minimal, mean of 1.67% after 24 h across all replicates (29 of 1786 larvae). Summary statistics for fruit pulp temperature are reported in **Table 2**. Treatment temperature statistics for each replicate were calculated from 30 minute readings using the mean across thermocouples in fruit that had been in the chamber for longer than 24h. This was done to exclude data from the thermocouple channels used to measure the cooling rate when new fruit was added to the chamber.

Treatment temperatures from 29 September increased above 2.5°C for approximately 6 h (max of 5.98°C) as a result of technical problems with the cold chamber (**Table 2**). No survivors were observed from any of the infested fruit that were in the chamber during that period (Replicates 8-13), providing evidence that this treatment is adequately conservative to remain effective even with a short period of temperature increase. In summary, these experiments provide strong evidence that a cold storage treatment of temperatures not to exceed 1.7°C, will safely mitigate the risk of introducing *B. zonata* into

the United States on oranges or other citrus varieties that may be a host.

References

(1) Hallman, G.J., S.W. Myers, A.J. Jessup and A. Islam. 2011. Comparison of in vitro heat and cold tolerances of the new invasive species Bactrocera invadens (Diptera: Tephritidae) with three known tephritids. J. Econ. Entomol. 104: 21-25.

(2) Baker, A. C., W. E. Stone, C. C. Plummer, and M. McPhail. 1944. A review of studies on the Mexican fruit fly and related species. U.S. Dep. Agric. Misc. Publ. No. 531.

(3) Mohamed, S.M.A and M.F. El-Wakkad. 2009. Cold storage as a disinfestation treatment against peach fruit fly, Bactrocera zonata (Saunders), (Diptera: Tephritidae) on Valencia orange. Egyptian J. Applied Sci. 24: 290-301.

(4) Gazit Y., Rossler Y., Wang S., Tang J. and Lurie S. 2004. Thermal death kinetics of egg and third instar Mediterranean fruit fly (Diptera: Tephritidae). J. Econ Entomol. 97: 1540-1546.

(5) SAS Institute. 2011. SAS Users Manual, Version 9.3. SAS Institute, Cary, NC.

(6) Couey, M.H., and V. Chew. 1986. Confidence limits and sample size in quarantine research. J. Econ. Entomol. 79: 887-890.

Table 2: *Fruit, larvae and temperature data from confirmatory treatment trials of* B. zonata *in naval oranges.*

			B. zonata					Treatment Temperature (°C)					
Treatment dates	Rep	# Fruit	N	Live	Dead	2nd Instar	3rd Instar	Mean	SEM	Var	Range	Min	Max
7/30 - 8/17	1	38	2660	0	2660	1398	1262	1.810	0.004	0.016	2.173	1.660	3.833
8/1 - 8/19	2	37	2135	0	2135	575	1560	1.817	0.004	0.012	2.173	1.660	3.833
8/4 - 8/22	3	29	2329	0	2329	809	1520	1.819	0.002	0.003	0.544	1.667	2.211
8/9 - 8/27	4	29	2279	0	2279	584	1695	1.823	0.004	0.015	1.172	1.661	2.832
8/11 - 8/29	5	29	2170	0	2170	367	1803	1.826	0.005	0.023	1.525	1.661	3.186
8/13 - 8/31	6	29	1455	0	1455	181	1274	1.837	0.005	0.026	1.535	1.650	3.186
8/18 - 9/5	7	34	766	0	766	123	643	1.913	0.011	0.098	2.525	1.613	4.138
9/15 - 10/3	8	34	1400	0	1400	134	1266	1.728	0.011	0.102	4.430	1.546	5.976
9/16 - 10/4	9	22	1327	0	1327	86	1241	1.724	0.011	0.102	4.430	1.546	5.976
9/17 - 10/5	10	33	1279	0	1279	254	1025	1.737	0.011	0.114	4.430	1.546	5.976
9/22 - 10/10	11	34	1408	0	1408	90	1318	1.750	0.012	0.116	4.430	1.546	5.976
9/23 - 10/11	12	33	1463	0	1463	74	1389	1.756	0.012	0.117	4.430	1.546	5.976
9/24 - 10/12	13	34	2063	0	2063	131	1932	1.763	0.012	0.117	4.430	1.546	5.976
11/19 - 12/7	14	31	196	0	196	17	179	1.680	0.000	0.000	0.255	1.562	1.817
"	15	32	338	0	338	94	244	"	"	"	"	"	"
"	16	32	278	0	278	35	243	"	"	"	"	"	"
"	17	32	224	0	224	25	199	"	"	"	"	"	"
"	18	32	244	0	244	18	226	"	"	"	"	"	"
"	19	32	101	0	101	9	92	"	"	"	"	"	"
11/20 - 12/8	20	34	206	0	206	5	201	1.683	0.001	0.000	0.346	1.465	1.811
"	21	34	256	0	256	55	201	"	"	"	"	"	"
"	22	33	72	0	72	5	67	"	"	"	"	"	"
"	23	33	42	0	42	4	38	"	"	"	"	"	"
"	24	33	86	0	86	12	74	"	"	"	"	"	"
"	25	34	38	0	38	4	34	"	"	"	"	"	"
12/29 -1/16	26	34	2195	0	2195	41	2154	1.850	0.001	0.001	0.196	1.813	2.009
"	27	34	1508	0	1508	96	1412	"	"	"	"	"	"
"	28	34	1552	0	1552	279	1273	"	"	"	"	"	"
"	29	34	413	0	413	13	400	"	"	"	"	"	"
"	30	33	871	0	871	46	825	"	"	"	"	"	"
12/30 - 1/17	31	34	941	0	941	48	893	1.847	0.001	0.001	0.201	1.693	1.894
"	32	34	823	0	823	96	727	"	"	"	"	"	"
"	33	34	1196	0	1196	7	1189	"	"	"	"	"	"
"	34	29	680	0	680	64	616	"	"	"	"	"	"
"	35	33	384	0	384	5	379	"	"	"	"	"	"
"	36	34	968	0	968	32	936	"	"	"	"	"	"
"	37	34	474	0	474	2	472	"	"	"	"	"	"
		Totals	**36,820**	**0**	**36,820**	**5,818**	**31,002**						

Development of Survey Systems for Exotic Wood Borers A Farm Bill Funded Project – Year 4

Project Lead: Vic Mastro

This project has focused on the development of chemical and visual attractants that can be utilized for surveying for either the detection of introductions of exotic wood inhabiting pests or to monitor their populations if they are found to be introduced. This project has also focused on the improvement and/or development of traps that can be used in conjunction with attractants to optimize survey tools for exotic wood borers. Discoveries of populations of exotic wood borers, over the past 20 years, have highlighted the importance of these exotic insects to Northern American natural and urban forest systems. The pest potential of many of yet to be introduced exotic insects is unknown, but approach rates continue to be high. Once established, many of these have demonstrated a large potential to spread rapidly and damage North American urban and natural forests. Many have been shown, to move on firewood and in nursery stock but others are apparently are moving on logs and wood products as well in tree parts used by hobbyist.

The potential impacts on North American ash trees from emerald ash borer (EAB) were not recognized or prior to its discovery in Detroit, MI in 2002. At that time, there was little known about the insect and no effective survey tools were available. In a similar way, after the discovery of the Asian longhorned beetle (ALB) in NY City in 1996, no effective traps were available and there was no scientific information on how beetles locate hosts or mates. Several other wood inhabiting pests have also been discovered and survey tools remain undeveloped. The collective research goal of this multi organization effort is to develop effective survey tools for Cerambycids, Buprestids and Scolytids.

The objectives follow:

1. Develop generic and specific attractants for exotic wood borers.
   a. Attractants that will be investigated are chemical cues that wood borers utilize for either locating their hosts or for locating each other.
   b. Visual cues (color, reflectance, shape) will be characterized for species that may use these singularly or in combination with chemical cues for host or mate location.
2. Develop more efficient and effective trap designs for wood borers. Consideration will be given for the effectives of the trap design for capturing the target species, practicality for large scale surveys and manufacturing costs.
3. Develop synthesis information for chemical compounds which cannot be readily purchased.
4. Develop chemical emission rate information for chemical compounds and release devices suitable for baiting traps.
5. Develop trap placement information to optimize capture of target species.

Impacts

Wood inhabiting insects continue to be intercepted in international shipments containing wood packing material even after the adaption of the IPPC standard ISPM 15. Also, because of long latent periods before discovery, undiscovered populations of various woodborers may already reside here. Similar to the Asian longhorned beetle and the emerald ash borer, there are a large number of exotic species where little is known about their pest potential or survey techniques that can be used to determine when and where introductions occur or for monitoring control activities if they are found established.

At the inception of this project, trapping information was limited to a few species of cerambycids. This lack of knowledge about the beetle's behavior and effective ways to survey for them severely restricted the USDA and their cooperator's ability to detect populations that could possibly be controlled before they became too large. Although there was some efforts to find pheromones and attractants and describe the behavior for this group of insects, six years ago there were only a handful of insects with known attractants. Through the efforts of the cooperators in this project, attractants are now know for over 100 species of cerambycids and specific pheromones have been identified for several species. In addition, there have been improvements of trap designs and advances in the knowledge of where to place traps to maximize capture. The outputs of this

group of researchers has had a spinoff effect and has stimulated other groups, unfunded by this effort, to become engaged in developing knowledge about the behavior, biology and attractants and survey technology for the Cerambycids and, to a lesser extent, for the Buprestids and Scolytids.

Additional information has been developed about the impacts on trapping multiple target insects using individual or blends of chemical attractants. From these studies, we now know which blends can be used to survey, for which species optimally, and which compounds will inhibit the attraction of specific species. This information will allow the USDA to tailor surveys for individual or groups of target insects. Specific high risk species are being targeted for developing pheromone, host plant attractants and or an optimized combination of host plant volatiles and pheromone compounds. A pheromone, through these efforts, has already been described for a Monochamus species and high risk pests such as the citrus longhorned beetle are being targeted for pheromone identification.

In a similar way, little was known about the host or mate finding of the Buprestids prior to the introduction of EAB in 2002. Attraction to girdled host trees had been shown for the bronze birch borer and for the two lined chestnut borer, but no specific traps had been developed for any species. Also, no information had been developed on what specific cues beetles use to locate host trees or others of the same species. Through the efforts of this project, which has built upon knowledge generated by EAB research, we now have prototype traps for two or, possibly three, species of exotic Buprestids in the genus, *Agrilus*. One of these is *A. biguttatus*, a CAP target species. Through this FB funded research, however, we have a much better knowledge of the behavioral cues that this group of insects utilize to locate host and mates. This in-depth knowledge of these insect's vision has allowed the cooperator to design artificial surfaces (nano-manufactured decoys) that, when incorporated into trapping surfaces, should further enhance the survey potential for these species. Also, in a species specific project for the Gold-spotted oak borer, purple traps, developed for EAB, have been shown to trap GSOB for survey, but poorly. Recent electro-retinograms have revealed that the color spectrum that GSOB eyes react to is different than EAB. Traps with the spectrum of color that are in-line with results of the visual analysis are being prepared for testing. Host plant volatiles from oak bark and stumps, from freshly cut oak, are being collected for identification of attractive compounds. This spring, these compounds will be bio-assayed to determine if there is antennal or behavioral activity.

The cooperators on this project have isolated behaviorally active compounds from host and insects and formulated these and previously discovered compounds and screened these for attraction to various species by running field trials in North America, Europe, Asia, New Zealand, Australia and South America in a "shotgun" approach to determine which additional species are attracted to them singularly or in combinations. This screening, not only provides information on the "best blends" to use for trapping a species or a group of species, but also identifies those compounds that inhibit attraction of a species. The result is that blends can be tailored for the species that is targeted. Additional field studies will be undertaken to compare capture rates when traps are placed at various heights, in different host trees and at various locations to develop information that can be used in operational survey programs. Lure formulation development is also an ongoing part of this project with field and laboratory trials conducted to determine the best release devices, best release rates, and to maximize field life. Additional studies with trap design improvement will also be conducted to determine if improvements in traps can be made in terms of traps efficiency, ease of use, specimen retention, and/or preservation. Many of the aspects of survey tool development are intertwined and dependent on earlier developments. As the past three years of research has built on earlier findings, the following year will build on and expand what we currently know and further accelerate the developmental process.

I. Development of Chemical Attractants and Improved Trap Designs to Facilitate Detection of Exotic Cerambycidae

Project Lead: Vic Mastro
P.I.: Jocelyn Millar (Univ. of CA-Riverside)
Co-P.I.s: L. Hanks (Univ. Illinois), J. Allison (Louisiana state U.), S. Teale (SUNY, Syracuse), plus 26 collaborators (17 in the US)

The goal of this project is to develop and test selective and effective methods for detecting and monitoring exotic cerambycid species that are likely to be introduced into the United States by global commerce. Five separate objectives are include in the scope of this effort:

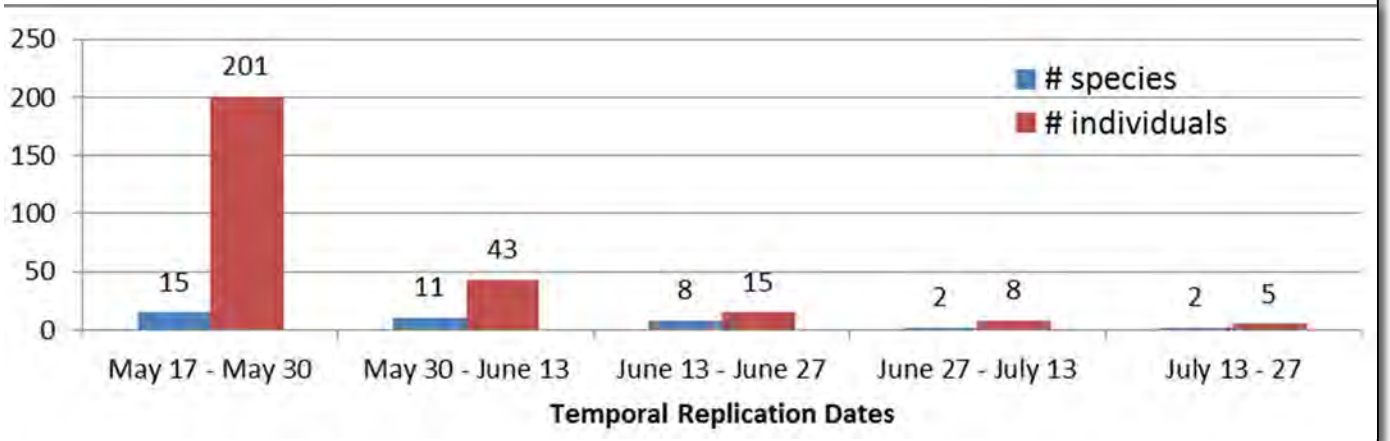
1. Synthesize and screen a library of known pheromones in areas having extensive trade with the U.S. (Asia, South America, Europe, Australia & New Zealand.
2. Target high risk species in China identified during pheromone screening studies in 2010.
3. Optimize release rates for various pheromone compounds.
4. Identify diagnostic morphological structures that are correlated with pheromone production or use.
5. Develop and optimize semiochemically baited traps for detection and monitoring invasive Cerambycidae with emphasis on the subfamilies Lamiinae and Cerambycinae.

A total of 14 candidate pheromone compounds were either synthesized or purchased and screened for activity in China. Effective attractants have been identified for *Monochamus alternatus* and *M. sutor* . Two separate publications cover this work. Also the studies produced some leads for attractants for *Anoplophora chinensis.*

Generic pheromone tests with some modifications were conducted in Guangxi, Yunnan, Fujian, and Heilongjiang Provinces. The base for these test consisted of 14 pheromone treatments, an ethanol blank, and an unbaited trap. In Yunnan 15 species were captured with two species, *M. alternatus* and *A. chinensis* captured in significant numbers (**Fig. 1**).

In Yunnan province results generally duplicated and confirmed results from 2010 field trials. More *Rhaphuma horsfieldi* and *Pharsalia subgemmata* were attracted to traps and captured than other species. These have both been targeted for pheromone identification in 2012. The effective height of trap placement was also explored in a separate experiment. Generally taps hung higher in the canopy captured more species and larger numbers of beetles (**Fig. 1**).

Figure 1: *Number of cerambycid species and individuals captured in traps in China over time.*



II. Generic Pheromone Blends for the Early Detection of Longhorned Beetles

Project Lead: Vic Mastro
Co-P.I.s: J. Sweeney & P. Silk, and 12 collaborators

The overall goal of this project, similar to other projects funded out of this FB initiative, is to develop a suite of semiochemical lures and traps for surveillance and early detection of exotic bark and wood boring beetles in North America. The specific objectives are to:

1. Field test various combinations of Cerambycid pheromones and host plant volatiles for attractiveness to longhorn beetles in China, Russia, Europe, and Canada.
2. Produce a list of species and sex (es) significantly attracted to each lure.
3. Determine optimal lure combinations for maximum number of longhorn species detected.
4. Determine effect of trap height on detection efficacy.
5. Compare detection efficacy of standard multiple funnel traps with alternate trap designs.

Although this project shares similarities with other projects under this umbrella it focus on some geographic areas not covered by the other cooperators and bring another perspective to traps design, placement and pheromone isolation/identification. These trials have been conducted in Russia, Poland, China, Canada and the U.S.

To date these studies have focused on the attraction of C6-ketols, C8-ketols and C6-diols, alone or in combination with each other or host plant volatile compounds (ethanol in hardwood forest and alpha pinene in conifer stands). These plant volatiles have also been tested separately. A total of 29 longhorned species (22 in the subfamily Cerambycinae) were significantly attracted to the traps baited with generic pheromones of the combination of pheromones and ethanol (**Table 1**).

- Eleven species were significantly attracted to the C6-ketols alone and an additional four species were significantly attracted to the combination of C6-ketols + ethanol.
- Six species were significantly attracted to the C8-ketols alone and an additional five species were significantly attracted to the combination of C8-ketols + ethanol.
- One species (*Plagionotus detritus*) was significantly attracted to the combination of C6-ketols + C8-ketols.
- Two species were significantly attracted to S*S*C6-diols and only one species was attracted to R*S*C6-diols (but the latter lure was tested in very few sites).

By itself ethanol is significantly attractive to 7 species of long-horned beetle and significant increased the attraction to 14 species when used with the ketols.

From the trap height experiments taps hung the mid-upper canopy captured and average of 80% of the species per site compared to 52% of the species captured when traps were hung at 2 meters. For all Lamiinae species high traps significantly out caught low traps. For the sub families Cerambycinae and Lepturinae impacts of trap height was mixed. Overall placing traps high in the canopy would be beneficial for capturing most species.

Multi-funnel traps function well for detecting beetles in the Cerambycidae and other families of beetles. A number of new records were recorded from the Canadian field trials including 5 new provincial records for Cerambycids and more than 100 new records for other beetle species in New Brunswick and the Maritimes.

Table 1: *Cerambycid species significantly attracted to racemic 3-hydroxyhexan-2-one (C6-ketols) or racemic 3-hydroxyoctan-2-one (C8-ketols), tested with- and without ethanol lures, in field trapping experiments conducted 2008-2011 in Canada, US, China, Russia, and Poland (P<0.05, Friedmans ANOVA,or Cochran's Q for proportion of positive traps). X = attractive by itself; (X) = binary combination is attractive but neither compound is attractive by itself; neg = significant negative effect on attraction when combined with attractive pheromone on trap.*

Cerambycinae	C6-ketols	C8-ketols	S*S* C6-diols	S*R* C6-diols	Ethanol*
Chlorophorus motschulskyi				X	
Neoclytus conjunctus	X			na	N
Neoclytus scutellaris	X		neg	na	X, +
Neoclytus mucronatus	X		neg	na	+
Neoclytus j. jouteli			X	na	+
Neoclytus a. acuminatus	Neg		X	na	X, +
Phymatodes aeneus	X			na	+
Phymatodes aereus	X			na	–
Phymatodes maculicollis	X			na	N
Phymatodes testaceus	(X)			na	(X)
Sarosesthes fulminans	X			na	+
Eburia quadrageminata	(X)			na	(X)
Elaphidon mucronatum	(X)			na	(X)
Anaglyptus colobotheoides	X				N
Plagionotus pulcher	X				+
Plagionotus detritus	(X)	(X)		na	N
Xylotrechus colonus	(X)			na	(X)
Xylotrechus cuneipennis		(X)			(X)
Molorchus minor		(X)		na	(X)
Microclytus compressicollis		X		na	+
Clytus marginicollis		X		na	–
Clytus planifrons		X		na	N
Curius dentatus				na	X
Lamiinae					
Psenocerus supernotatus				na	X
Hyperplatys maculata				na	X
Astylopsis macula		(X)		na	(X)
Liopinus misellus		(X)		na	(X)
Lepturinae					
Bellamira scalaris		X		na	N
Gaurotes cyanipennis		X			N
Evodinellus borealis			na	na	X
Macroleptura thoracica		X			N
Parandrinae					
Neandra brunnea	X			na	N
Prioninae					
Sphenostethus taslei	X			na	

** +, –, N = addition of ethanol had significant positive, negative or neutral effect on attraction, respectively.*

III. Development of Chemical Attractants for Monitoring *Monochamus alternatus* and *Anoplophora chinensis*

Project Lead: Vic Mastro
P.I.s: F. Zhang, Z. Liu and Q. Lin

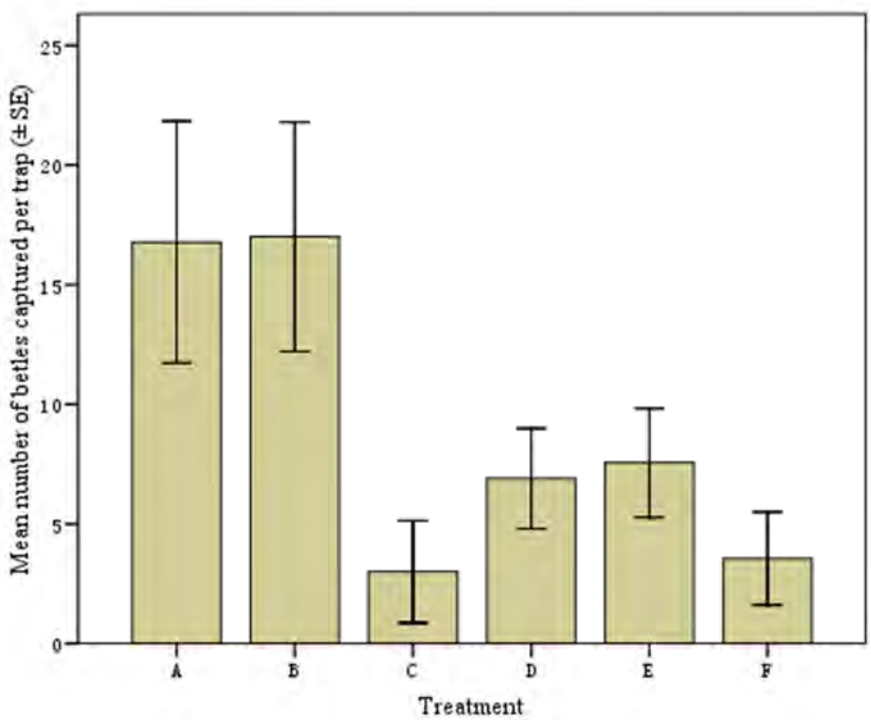
Focus of wood borer work in China has been on the Japanese pine sawyer, *Monochamus alternatus*, and the Citrus longhorned beetle, *Anoplophora chinensis*. The work on the Japanese pine sawyer (JPS) has results in the development of an attractant blend which is composed of a mixture of a male produced aggregation pheromone and two host tree volatiles. This combination performed better than other combinations tested (**Fig. 1**). The attractant combination is now the subject of a patent application and is described in a publication.

An additional field test was conducted to determine if the individual three components of the male produced pheromone compounds significantly effect trap captures. Modifying these components chemically did not enhance captures.

The citrus longhorned beetle (CLB), a conger of the Asian longhorned beetle, has the potential to be as destructive to southern areas of the U.S. as ALB is projected to be in Northern areas. This insect has been introduced and was the subject of one eradication program in Seattle Washington. To date, no attractant has been identified for this insect. Work has been conducted on identifying host plant volatiles and insect produced compounds using GC-EAB and Y-tube olfactometers. Based on these studies, field bioassays were conducted in 2011. A blend 50:50 of two compounds butanol and butanal induced the highest EAG responses in females. Male responses were equally reacted to only butanol on the 50:50 blend.

Y-tube bioassays where conducted on extracts of *Melia azedarae*, and *Casuarina equisetifolia,* hosts of the CLB. In the coming year, additional host plant volatiles will be evaluated in Y-tube and field trials.

Figure 1: *The number of Japanese pine sawyer adults captured in traps baited with six combinations of different compounds. Traps with no lures in it did not capture any beetles, and are not included in the figure.*



IV. Tree Injections and Other Methods to Improve Trapping of Buprestids, Cerambycids, and Scolytinae Borers

Project Lead: Vic Mastro
P.I.: J. Oliver (Univ. Tenn),
Co-P.I.s: A. Bray, and K. Addesso,
Collaborator: C. Ranger (ARS, Wooster, OH)

Similar to the overall goal of this project, this agreement goal is to develop improved attractants and trapping methods for a variety of cerambycid, buprestid and scolytid wood borers. The approach however is somewhat different and the specific objectives are:

1. Determine if the injection of trees with stress compounds can enhance the attraction to wood borers,
2. Characterize the emission profile of injected trees versus trees not injected,
3. Assess the electrophysiological response of beetles to volatiles from tissues of injected trees,
4. Evaluate a variety of traps for use on the target groups of insects and
5. Assess tree stress volatiles as general lures for the different groups of wood borers.

Major findings from the 2010 and 2011 and preliminary findings from 2012 work include the following:

- Trees injected with ethanol are highly attractive to Scolytinae but do not significantly impact the capture of Buprestids or Cerambycids.
- Ethanol injected trees were more attractive than commercial ethanol baits.
- When multiple species of trees are injected with ethanol specific complexes are attracted to each species.
- Tree bolts injected with ethanol can be stored up to 9 months frozen and remain attractive.
- Tree bolts hollowed and filled with ethanol are as or more attractive and remain attractive longer than injected bolts or commercial ethanol lures.
- Purple panel traps are effective for capturing many species of buprestids and were more effective that semi-transparent traps.
- Vane traps were more effective for capturing Scolytids than multi-funnel traps.
- The volatiles (ethanol, benzaldehyde and hexanal) which in previously studies were reported to increase buprestid attraction did not increase trap capture of any Buprestids or Cerambycids.
- Although purple panel traps capture Scolytids in large numbers they are difficult to see on the trap. This may be overcome through the use of clear cover panels.
- Of five new trap designs (non-sticky) tested , some of these may be more effective than standard multi-funnel traps.
- A color test of traps most effective for Scolytinae preliminarily found that grey and opaque traps captured the greater numbers of beetles.
- Volatile emissions from one species of tree with and without ethanol injection have been characterized.
- Elevated ethanol emission peak after 1 or 2 days and return to normal after 16 days.
- A scolytid , *X. germanus*, that utilizes the tree species as a host, antennae (GC-EAD) only responded to ethanol and not the other identified volatiles (alpha-pinene, (+)-camphene, (-)-B-pinene, (+)myrcene, P-cymene, limonene, and eucalyptol.
- Ethanol and monoterpene baits in field trials were no more attractive than ethanol alone. This was true for at least 10 other species trapped.
- Additional tree species emission pre and post ethanol injection will be characterized and GC-EAD will be used to test response of beetle associates.

Development of Detection Tools for Exotic Buprestid Beetles

Project Lead: Vic Mastro
P.I.: Thomas Baker
Collaborators: Miklos Toth, György Csóka, Gábor Vétek and Prof. Dr. Béla Pénzes (Hungary)

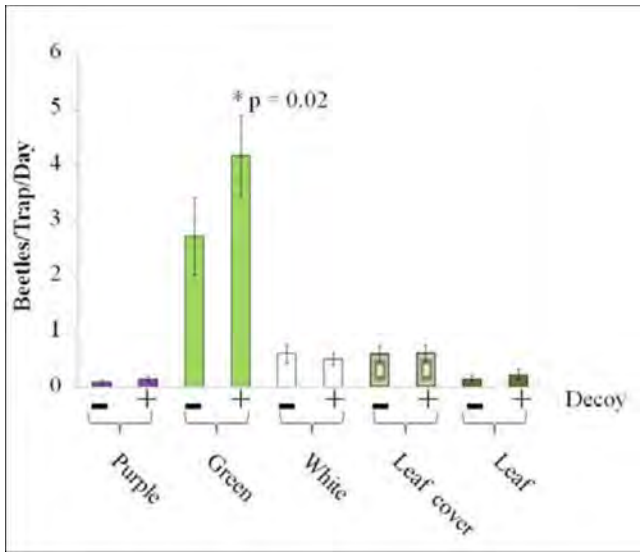
The goal of this project is to utilize information generated on the mate and host finding behaviors of buprestid beetles to develop traps and attractants for survey. This project has largely focused on developing survey tools for two European species of concern to the US and APHIS. Both of these species, *Agrilus biguttatus* and *A. sulcicollis*, feed on oak. *A. sulcicollis* has been found in North America but *A. biguttatus* has not. This work builds on earlier studies with the emerald ash borer *A. planipennis*, *A. subcinctus* and *A. cyanescens* that all have been shown to respond to cues of females resting on foliage. It was shown that EAB is responding iridescent color of the cuticle generated by cuticular hydrocarbons that causes interference of light having wavelength longer or shorter that the "green part" of the spectrum. Other species that do not have iridescent green color or no iridescent also appear to use visual cues for mate finding. This common template appears to apply for all Agrilus species studied so far. Contact pheromones have been identified for one Agrilus species and demonstrated for at least two others. This information may be useful in designing traps that would benefit from having the male retained on a surface for a longer period of time (ie toxicant based traps or traps employing an entomopathogen. No long range pheromones have been identified but host plant volatiles have been shown to be attractive.

Because this work is closely linked with another project "Nanofabrication of Visual Lures for the Emerald Ash Borer" results of both projects will be included here. In the first year of the project the behavior of three European beetles was studied and compared to that which had been described for EAB; in the next 2 years, traps were developed employing model (dead, pinned adults of both EAB and *A. biguttatus*). These traps utilized the background colors developed by the Otis laboratory (Joe Francese) which have been shown to be attractive by themselves and which are the basis for the standard EAB survey trap (**Fig. 1**)

Figure 1: *Green and purple traps deployed in a field trial in Hungary.*

In field trials trap variables were explored, including; trap color (green, purple and white versus natural leaves) with and without a decoy (pinned specimens of either EAB, *A. biguttatus*. or *A. sulicicollis,* with and without host plant volatiles. The Otis green color is the most attractive color when deployed on oak trees for all *Agrilus* spp. (**Fig. 2).**

Figure 2: *Trap captures of all Agrilus spp. comparing traps of different colors and visual appearances.*



Significantly more *Agrilus* adults were captured on green traps with decoys that normal green traps. Odors lures in combination with traps significantly increased the captures **(Fig. 3).**

The light emitting characteristics of the three specie *A. planipennis, A. cyanesus* and *A. subcinctus* have been compared using a light microscope, transmission electron microscopy and wavelength-scattering suing a white laser. All of these finding are included in a publication (in press).

In 2013 the 4th year of the project the project will focus on understanding the visual cues used for mate finding behavior of Buprestid adults. Indications so far are that Agrilus species use a common system that is not species specific. If so a template may be constructed that can be used for many or all species in this group. Also further studies will be undertaken to refine the trapping systems to include formulations of chemical attractants in the most effective proportions and rates based on our knowledge of the behaviors of these species.

The physical dimensions of the traps will be investigated to promote more beetle captures, and allowing deployment at different levels of the canopy. The project will also focus on developing and including nanofabricated decoys such that the visual lure-based traps will be fully artificial and amenable to mass production.

Working closely with the Otis laboratory we will focus on developing a non-sticky trap alternative that electrocutes or senses insects landing on the visual decoy.

A separate project was funded (FB) for 2001/12 for Nano- fabrication of decoys that would display all of the visual cues necessary to attract Agrilus species. In this period of time nanofrabication was used to deposit ¼ wave Bragg stacks of two polymers having different refractive indices. The layers of two different polymers having the desired complementary refractive indices were sequentially laid down at set durations in different solutions to form repeated layers of ca. 142 nm to produce a Bragg stack film having a reflectance optimum of ca. 550 nm (green).

A second process (nano-imprinting) was used to mold the decoy model. First a negative die was created using dead EAB adults using a film by evaporation method. The 10um thick negative die of the elytra is formed followed by two days of electroforming to for a 100 um thick negative die. After the die is formed it is used to stamp the nano –detailed sheets of ¼ Bragg stacked polymer sheets created by the first process. Stamping pressure is critical to retain the color. When done properly, the decoys resemble dead EAB adults and preliminary field trial results indicate that the attraction to the decoys is equal to dead pinned adults (**Fig. 4**).

If the process can be standardized these sturdy decoys can be incorporated into EAB or traps designed for the introduction of other exotic *Agrilus* species.

Figure 3: *Trap captures of green versus leaf traps (left) or purple plastic versus leaf traps (right) with and without beetle decoys, while baited with different odors.*

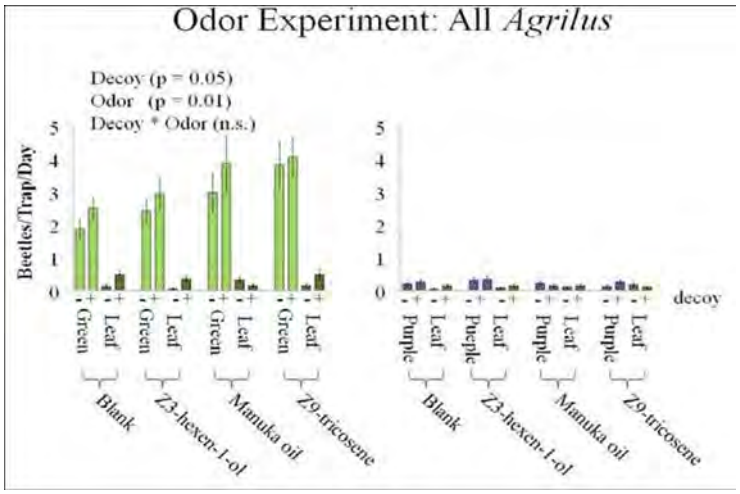


Figure 4: *A dead EAB with two different nano-frabicated decoys on either side.*

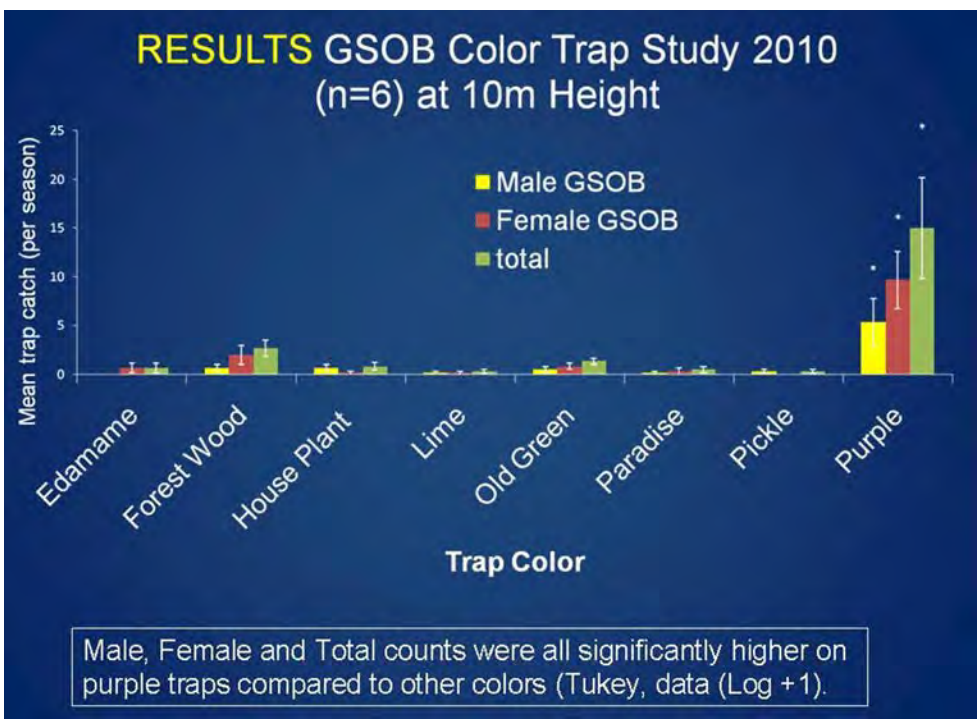
Enhancing Survey Techniques for the Exotic Goldspotted Oak Borer (GSOB) *Agrilus auroguttatus* (Coleoptera: Buprestidae) in Southern California Co.

Project Lead: Vic Mastro
P.I.s: Mary Louise Flint, Steven Seybold and Tom Coleman
Contributor: Damon Crook

This project unlike most others included under the overarching goal of developing attractant and traps for exotic wood boring pests focuses on a single pest species, the goldspotted oak borer, which is native to the US but recently has expanded its range into California where it is now causing significant mortality on oaks. The overall goal of this project is to improve the efficacy of the detection and survey techniques. An effective survey tool would facilitate survey to monitor the spread in California populations and survey in other at risk oak growing areas of the United States. This project first year of funding was in 2011 so it has been as long running as some of the other projects funded through this project. As was noted under the description of the T. Baker project on Buprestids, this species is suspected to rely on vision for the location of its host and its mates. In the first year of study this project, in filed test, investigated trap color. In field test the emerald ash borer traps (purple prism) was compared with the green prism traps developed by the Otis laboratory as an alternative to the purple prism, (also developed by the Otis Lab.). Two other variable were investigated: the attractant used as a bait (ethanol, manuka oil, phoebe oil, and (Z)-3-hexanol and trap height (1.5, 3.0, 4.5 and 6m).

Trap color had a significant impact on trap catch, (**Fig. 1**). The purple color used in the EAB program is clearly more attractive than other colors, including those developed to mimic the foliage of the oak species that they utilize. This is true for both female and male adult GSOBs.

Figure 1: *GSOB catch on unbaited panel traps where trap color was based on foliage scans from southern California oak foliage by D. Crook (USDA APHIS). Purple-colored panel traps were included as a control.*

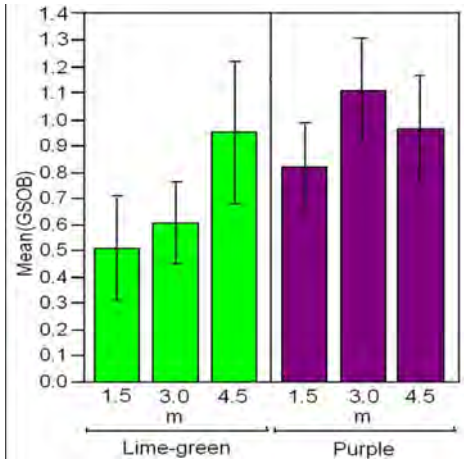


None of the semiochemicals tested in 2011 significantly enhanced the attractancy of the purple prism trap although ethanol appeared to have a negative effect. These treatments were also tested on the green prism traps. Again there were no significant differences and the trap catches were much lower than any of the purple prism traps baited with the same semiochemicals.

Trap height was investigated in separate field trials using the standard purple and a lime-green prism trap. Trap height, unlike EAB, does not appear to strongly effect GSOB captures (**Fig. 2**).

Volatiles were also collected from the bark of costal live oak and Engelmann oak, two of the species utilized by GSOB, to determine if the quantity or composition of volatiles change in stressed (girdled) and if any components are antennally active, GC-EAD analysis. Several compounds elicited strong responses from both male and female GSOB. Although all of the active compounds were not identified in 2011 a small field test did not demonstrate a significant difference between unbaited purple prism traps and those baited with either camphene, camphene-g-terpene or a "bark blend". Additional studies in 2012 also involve at characterizing the volatiles form oak foliage, bark and phloem of the various oak species and stumps of recently cut oaks, which are highly attractive.

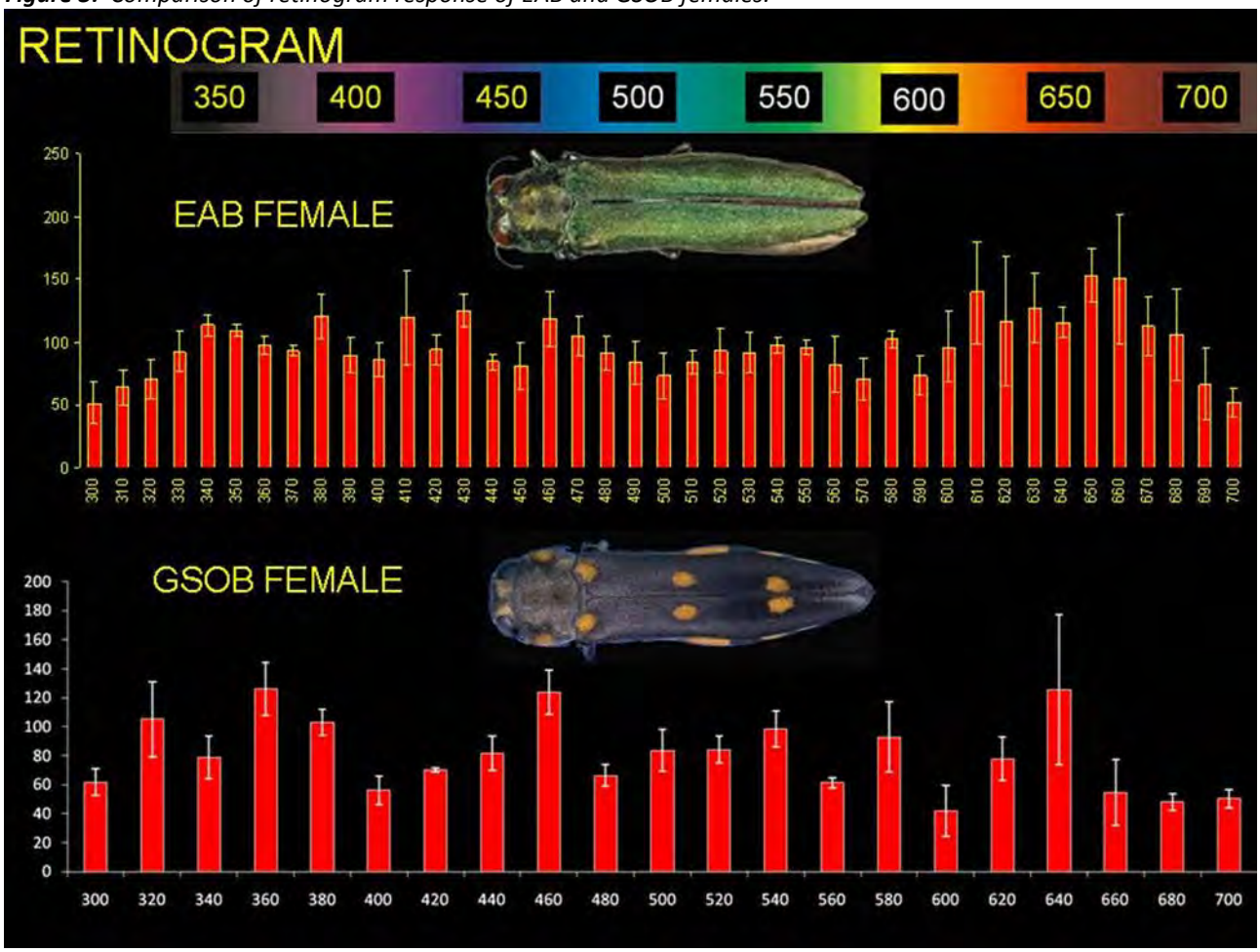
Figure 2: *GSOB catches on traps of two colors placed at various heights.*



The optoneural (retinogram) response of GSOB was evaluated to determine if trap performance could be improved by determining which light spectra GSOB is most sensitive to and designing traps with those colors. The retinal responses of male and female GSOB were tested in response to wavelengths ranging from the UV to red Results indicate that GSOB females were sensitive to the UV, purple, green, and red regions of the spectrum (**Fig. 3**). This is in agreement with the responses to purple traps relative to other colors of traps (**Fig. 1**).

These finding will be explored in field trial where custom colored traps will be compared to the standard purple prism.

Figure 3: *Comparison of retinogram response of EAB and GSOB females.*



Development of Attractants for the Juniper Bark Borer, *Semanotus bifasciatus* (Motschulsky) and Preliminary Behavioral Study on the Mulberry Longhorned Beetle, *Apriona germari* (Hope)

Project Leads: Baode Wang and Vic Mastro

Cooperators: Zhichun Xu and Lin Zhang (Beijing Forestry University, Beijing, China)

In 2011, our focus was on the mulberry longhorned beetle since we had already made progress on the juniper bark borer (JBB) in 2010. The goal of this project is to develop a lure based trapping technique for the mulberry longhorned beetle (MLB) survey with the following primary objectives:

1. Study MLB host feeding and acceptance behaviors.
2. Determine the compositions and relative contents of volatile compounds released from selected preferred host trees as well as extracts from these trees.
3. Field bioassay to determine the effectiveness of extracts and volatiles for trapping adult MLB.
4. Screening candidate volatiles to be used as lures for trapping JBB.

Summary. The results of the color-wrapped twig study indicate that MLB adults did not choose feeding objects based on color. Plant odor may play an important role in host feeding selection for MLB adults.

A total of 56 different volatiles were identified in four Moraceae tree species using GC-MS method, their relative contents were also determined, of which, 31 present in *Morus alba*, 30 in *Broussonetia papyrifera*, 29 in *Cudrania tricuspidata*, and 27 in *Ficus carica*. The 56 volatiles can be divided into four major categories: alcohols, aldehydes, alkenes, and esters. These host plants differed in their 'chemical fingerprints', characterized by specific types and content of volatiles. The major volatile components of *M. alba* were alcohols (25.06%) and esters (66.61%), accounting for 91.67% of the total. In *B. papyrifera*, alkenes (46.22%) and esters (39.59%) accounted for 87.81% of the total. For *C. tricuspidata*, alcohols (11.80%), aldehydes (22.92%), alkenes (36.15%) and acids (14.58%) accounted for 85.45% of the total. For *F. carica*, alkenes (33.25%), esters (33.36%) and phenols (18.06%), accounted for 84.77% of the total.

Among all four plants, the most common constituents were vinyl compounds. Compared with the other three species, *F. carica* had more ketones and fewer alkanes, while *C. tricuspidata* had fewer esters. Eleven of the volatiles were common to all four plants. These were: leaf alcohol, capryl alcohol, benzaldehyde, 1-nonanal, decanal, 1-octene, ocimene, leaf acetate, hexadecane, 2,6-di-tert-butyl-4-methylphenol and 2,4-di-tert-butylphenol. The total quantities of these 11 compounds accounted for 79.98% of their total volatiles in *M. alba*, 60.43% in *B. papyrifera*, 34.62% in *C. tricuspidata*, and 45.97% in *F. carica*.

Bioassays were conducted subsequently on the antennal responses of adult beetles to these volatiles to screen candidate compounds for further evaluation. Traps baited with two candidate volatiles caught a few beetles in an enclosed arena, but did not catch any MLB in field studies with candidate volatiles including crude tree extracts and commercially available compounds as lures. Further studies will be conducted.

Flight propensity test. A flight mill was built for this test. Flight mill testing indicates that female MLB possesses strong flight ability, and can fly longer in light conditions than in dark conditions. The average speed, flight duration and calculated flight distance, respectively, for MLB adult during the period was 5,274 ± 769 m/h, 2.31 ± 0.22 h, 12,371 ± 2,803 m under 24 h constant light, and 5,184 ± 2, 164 m/h, 1.31 ± 0.98 h, 7,990 ± 7,803 m under 24 h constant dark.

Field bioassay was conducted at a site in Shandong province with a cross-vane trap (**Fig. 1**). Several effective chemical compounds have been identified for trapping JBB adults. Trapping method will be optimized in 2012 and 2013.

Figure 1: *Juniper bark borer traps with lure at a site in Shandong province.*

Behavioral Study and Attractant Development for the *Citrus* Longhorned Beetle, *Anoplophora chinensis*

Project Leads: Baode Wang and Vic Mastro

Cooperators: Haijun Liu (Guangdong CIQ IQTC)
Huanhua Huang (Guangdong Academy of Forestry)

Contributors: Xuenan Hu, Li Lin, and Yujuan Gu (Guangdong CIQ IQTC)
Yonghuai Huang (Guangdong Academy of Forestry)

Project goals. The primary goal of this project is to develop chemical or physical lures for the citrus longhorned beetle (CLB, **Fig. 1**) though host preference testing, behavioral studies of adult beetles and through chemical analysis.

Summary. In 2011, our studies focused primarily in two areas: 1) host preference testing of adult beetles on different trees, and 2) bioassays of volatiles from host plants and insects. Among the beetles emerged, 17 were from *Citrus* reticulate, six were from *Salix* sp., and one was from *Melia azedarach*. Although these adults were smaller in size than that emerged from trees in natural conditions, their behaviors including host locating, feeding, mating and oviposition did not show significant differences.

In the net house, adult beetles were observed to be attracted to a 40W florescent light when placed within 3 m of the light sources. Beetles also tended to aggregate on trees beneath the light source. However, no beetles were caught in any of the traps baited with light sources of different wave-lengths in a field test. The behaviors of beetles on the following nine tree species were compared to determine their preferences: *Citrus reticulate*, *Salix* sp., *Melia azedarach*, *Lagerstroemia indica*, *Rosa* sp., *Ficus* sp., *Camellia oleifera*, *Eucalyptus* sp., and *Castanopsis hystrix*. The results indicate that of the nine species, *Citrus reticulate* was the most preferred species for the beetle. The results from the adult feeding studies of freshly cut twigs in individual cages indicate that adults preferred to feed on *Lagerstroemia indica*, and *Ficus* sp.

Six compounds and extracts from the bark and leaves of *Melia azedarach* were selected for the bioassay using GC-EAD. The result suggested that CLB adults responded to volatiles found in both the leaf and bark extracts, but did not respond to other synthetic compounds used as lures. Field evaluation of traps with five different lures did not show significant effects.

Figure 1: *Adult* Citrus *longhorned beetle on host plant (left) and in behavioral studies in a net house (right).*

Development of Chemical Attractants for Monitoring *Monochamus alternatus* and *Anoplophora chinensis*

Project Leads: Baode Wang and Vic Mastro

Cooperator: Feiping Zhang (Fujian Agriculture and Forestry University)

Contributors: Zhijie Liu, Jun Su, Liping Zheng, Long Chen, Guanghong Liang, Wei Xue Mingding Wang, Weiqi Zhang (Fujian Agriculture and Forestry University), Qiang Lin (Provincial Forestry Department of Fujian), Jiujiang Guo (National Forest Park of Fuzhou, Fujian), Shangqian Li (Yangmeiling Forestry Center of Xiapu, Fujian), Yu Zhang (Forestry Bureau of Lianjiang, Fujian), and Wenlin Huang (Forestry Bureau of Shanyuan, Fujian)

Goal. Develop efficient methods for attracting and monitoring the Japanese pine sawyer (JPS), *Monochamus alternatus* and the citrus longhorned beetle (CLB), *Anoplophora chinensis.*

Summary. The purposes of this project are to identfy highly efficient chemical lures, and develop trapping methods for attracting and monitoring two important word boring longhorned beetles, the Japanese pine sawyer (JPS), *Monochamus alternatus* and the citrus longhorned beetle (CLB), *Anoplophora chinensis*. Initially, volatiles from both adult beetles and host plants were extracted. The extracts and individual compounds that have been previously identified or their combinations were further screened and evaluated through laboratory and field bioassay. In addition, a pheromone found previously from JPS was reconstructed and optimized. Trapping devices and traps with efficient lures were evaluated in field studies.

We have made a great progress on JPS trapping in 2011-2012. Live JPS adults were caught from a forest park using panel traps (**Fig. 1**) baited with lures including a male produced pheromone, and two modified pheromones. Both males and fe-males had different re-sponses to the nine volatiles. Although the two modified compounds might not have any significant attraction to JPS, we have confirmed that three of the nine candidate compounds all played important and positive roles in JPS capture in traps by adding or subtracting individual compound from the lure tested in field.

For the *Citrus* longhorned beetle, the following studies have been conducted: 1) EAG responses of CLB to sixteen compounds including extracts from *Melia azedarach*, 2) The response of CLB adults to seven compounds using Y-Tube olfactometer, and 3) field bioassays to screen efficient CLB chemical attractants. Significant differences were found on tested compounds in EAG response in both sexes in the bioassay.

The result from a Y-tube bioassay indicated that odors from *Melia azedarach* attracts CLB adultd Over 60% of CLB were attracted to extracts of bark, phloem and leaf in this study. Field trapping with the above-mentioned potential candidate volatiles as lures were carried out in Fujian province of China in a forest stand with 15-18 year old *Casuarina equisetifolia* trees where CLB was causing significant tree mortality. Unfortunately, no CLB was caught in traps baited with any lures in field during July to August 2011.

Figure 1: *Assemble and placement traps at a site in Fujian province.*

Trap and Lure for Walnut Twig Beetle

Project Lead: David Lance

Contributors: Damon Crook and Vic Mastro (Otis Laboratory)

Collaborator: Steve Seybold (USDA Forest Service, Davis, CA)

Walnut twig beetle (WTB), *Pityophthorus juglandis*, vectors the fungus Geosmithia morbida, resulting in a condition known as thousand cankers disease (TCD) (**Fig. 1**). TCD is responsible for the mortality of black walnut trees in the western U.S. and threatens walnut plantations in California. The beetle and the disease have recently been spread by human activities and now threaten walnuts in central and eastern areas of the U.S. Efficient methods for detecting and monitoring populations of WTB would greatly facilitate efforts to contain or manage this pest.

The CPHST Otis Laboratory initiated a Farm Bill project in 2010 to accelerate development of survey methods for WTB by providing support to scientists from the USDA Forest Service and University of California at Davis, CA. This group had already developed evidence of beetle-produced compounds that appeared to have potential as a lure. In 2010, Farm Bill funding was provided both through an interagency agreement between CPHST and USFS and a cooperative agreement with the Plant Pathology department at UC-Davis. The farm bill project, and support to the CA researchers, was renewed in 2011 through a single interagency agreement with USFS, "Development and implementation of a pheromone-baited trap for the walnut twig beetle, *Pityophthorus juglandis*." The Forest Service subsequently used a portion of these funds to support the work at UCD through a cooperative agreement.

Major accomplishments of the project through September 2011 include:

- A single male-produced compound was found to dramatically increase numbers of beetles of both sex caught in traps.
- Some female-produced compounds have been shown to be antennally active, but these did not enhance capture, and some appeared inhibitory or repellent.
- A number of different types of traps were shown to be effective for WTB, including Lindgren funnels and sticky (panel) traps.
- Initial testing was completed to optimize trap effectiveness using color, trap position, and loading rate of the attractant. Damon Crook of the Otis laboratory is working with the California team on issues related to trap color and color vision in the WTB.

Work to date has demonstrated that traps baited with pheromone-based lures have great potential as tools for detecting and monitoring populations of WTB.

Figure 1: *Walnut twig beetle (left), thousand cankers disease (center) and a walnut tree that has succumbed to attack (right) Photo credit: Steve Seybold.*

Providing Invasive Insect Specimens for Training Programs and Outreach

Written by: Hannah Nadel

Rearing of insects from infested host plant material, and maintenance of insect colonies, creates the opportunity to amass specimens of insects and damaged host material for use in training programs, outreach, and taxonomy.

In 2011, Otis Laboratory scientists and technicians maintained colonies of several exotic *Lymantria* moth species and subspecies (European gypsy moth, *L. dispar dispar*; Asian gypsy moth, *L. dispar asiatica; L. dispar japonica*; rosy moth, *L. mathura*; casuarina moth, *L. xylina*), European grapevine moth (*Lobesia botrana*), light brown apple moth (*Epiphyas postvittana*), Emerald ash borer (*Agrilius planipennis*) and its parasitoids *Spathius agrili* and *Spathius* sp., and Asian longhorned beetle (*Anoplophora glabripennis*). The invasive wood wasp *Sirex noctilio* was reared out of logs collected in New York State. All insects were held in the Otis Laboratory Containment Facility except for a large colony of European gypsy moth (*Lymantria dispar dispar*), which required no containment in Massachusetts.

Figure 1: *(a) Preparation of gypsy moth specimens, and (b) Riker display of Asian longhorned beetle.*

Specimens were sent upon request in the form of pinned and unpinned adults, pupae, larvae preserved in alcohol, irradiated (killed) eggs, Riker displays, and autoclaved branches or slices of wood bearing insect galleries (**Fig. 1**). This year, in addition to the usual types of specimens, samples of frass from Asian longhorned beetle were collected for training dogs to detect infestations in trees.

A summary of specimens prepared and distributed by Otis Laboratory personnel in 2011 is presented in **Table 1**. The majority were of European gypsy moth and Asian longhorned beetle that were destined for federal and state identification, detection, and interception training programs. These programs include, among others, courses for Customs and Border Protection (CBP) Officers, PPQ identifiers, technicians, and interns, Cooperative Agricultural Pest Survey (CAPS) personnel, and Coast Guard Officers (**Fig. 2**). Outreach programs acquired specimens of European gypsy moth, Asian longhorned beetle, and emerald ash borer along with samples of the beetles' galleries in wood. The Entomology Department at Cornell University requested live larvae of European gypsy moth for use in laboratory courses. A local nature center used live gypsy moth larvae to teach children about insect growth and behavior. Specimens were also sent to PPQ's counterparts in Australia and Republic of Korea to expand their reference collections. Lastly, specimens of *Sirex noctilio* were sent to the University of Minnesota Insect Collection to refresh its color-faded collection of *Sirex*.

Acknowledgments. Thanks are due to the many people who contributed to insect rearing and specimen preparation at the Otis Laboratory in the 2011 fiscal year (**Table 2**).

Figure 2: *Customs and Border Protection training exam. Photo supplied by William Tang.*

Table 1: *Preserved specimens of invasive insects sent for training and outreach purposes in 2011.*

Purpose[1]	Agency and Location[1]	Species[2]	Specimens
CBP teaching tools, reference collection, school outreach	US Department of Homeland Security, Customs and Border Protection, Blaine, WA	EGM ALB	Adults
Teaching tools for CBP, CG, and APHIS identifiers, reference collection	APHIS PPQ Plant Inspection Station, Miami, FL	EGM	All life stages
Teaching tools	Gypsy Moth Trapping Unit, Minnesota Dept. of Agriculture, Plant Protection Division, St. Paul, MN	EGM	Adults, larvae
Showcasing of agency's products and programs	USDA ARS PSI Systematic Entomology Laboratory, Beltsville, MD	EGM	Eggs, larvae, pupae
Outreach and training, reference collection	Forest Pest Program, Emergency and Domestic Programs, USDA, Riverdale, MD	EGM	All life stages
Teaching tools, reference collection	USDA APHIS PPQ Urbandale, IA	EGM	All life stages
Training	AHIS PPQ Pest Survey – ND/MT, Bismarck, ND	EGM	All life stages
Training, US military	APHIS PPQ QPAS, International Service, Heidelberg, Germany	EGM, EAB	All moth life stages; EAB adults, larvae
Taxonomic collection	University of Minnesota Insect Collection, St. Paul, MN	*Sirex noctilio*	Adults
CBP training, publicity/outreach video	APHIS PPQ Riverdale, MD	ALB, EAB	Adults, larvae, wood damage
Reference collection	Australian Quarantine and Inspection Service, Melbourne, VIC, Australia	AGM, rosy moth, casuarina moth	Adults
Reference collection	Korean Quarantine and Inspection Agency, Plant Quarantine Technology Center, Yeongtong gu Suwon, Republic of Korea	EGM, AGM, rosy moth, EGVM, LBAM, EAB, ALB	Adults
Teaching tools – university level	Department of Entomology, Cornell University, Ithaca, NY	EGM	Larvae
Outreach - children	Greenbriar Nature Center, Sandwich, MA	EGM	Larvae
Teaching tool	USDA APHIS PPQ, Boston, MA	ALB	Wood damage
Outreach, teaching	Massachusetts Wood Producer's Association, Phillipston, MA	ALB	All life stages
Teaching tool	USDA APHIS PPQ, Ripley, WV	ALB	All life stages
Teaching tool	USDA APHIS LPA, Riverdale, MD	ALB	Wood damage
Dog detector training	National Detector Dog Training Center, Newnan, GA 30265	ALB	Frass
Teaching	USDA APHIS PPQ, Aurora, CO	ALB	Wood damage

[1]CBP: Customs and Border Protection; CG:Coast Guard.
[2]AGM: Asian gypsy moth; ALB: Asian longhorned beetle; EAB: emerald ash borer; EGM: European gypsy moth; EGVM:

Table 2: *Many people contributed to insect rearing and specimen preparation at Otis Lab in 2011.*

Employees	Role	Species[1]
Hannah Landers, Sue Lane, Christine Lokerson, Alyssa Pierce	Rearing	EGM
Alyssa Pierce	Specimen preparation	EGM, other *Lymantria* spp.
Kevin Anderson	Rearing and specimen preparation	AGM, other *Lymantria* spp.
Tracy Ayer	Rearing	EAB
Everett Booth, Barbara Holske, Natalie Leva	Rearing	ALB
Audra Baker	Insect and wood specimen preparation	ALB
Tanya Dockray, Sue Lane	Rearing	EGVM
Tanya Dockray	Specimen preparation	EGVM, EAB
Ashley Hartness, Daniel Lafoon, Miriam Cooperband	Specimen preparation	*Sirex noctilio*

[2]AGM: Asian gypsy moth; ALB: Asian longhorned beetle; EAB: emerald ash borer; EGM: European gypsy moth; EGVM: European grapevine moth; LBAM: light brown apple moth.

FY 2011 Publications

1. Bauer, L., J. Gould, J. Duan. 2010. Can biological control of emerald ash borer save our ash? *Newsl. Mich. Entomol. Soc.* 55: 26-27.
2. Domingue, M. J., G. Csóka, M. Tóth, G. Vétek, B. Pénzes, V. Mastro and T. C. Baker. 2011. Field observations of visual attraction of three European oak buprestid beetles toward conspecific and heterospecific models. *Entomol. Exp. Appl.* 140: 112-121.
3. Duan, J. J., L. S. Bauer, M. D. Ulyshen, J. Gould, R. Van.Driesche. 2010. Development of methods for the field evaluation of *Oobius agrili* (Hymenoptera: Encyrtidae) in North America, a newly introduced egg parasitoid of the emerald ash borer (Coleoptera: Buprestidae). *Biol. Control* 56(2): 170 – 174.
4. Duan, J.J., M. D. Ulyshen, L. S. Bauer, J. R. Gould, and R. Van Driesche. 2010. Measuring the impact of biotic factors on populations of immature emerald ash borers (Coleoptera: Buprestidae). *Environ. Entomol*. 39: 1513-1522.
5. Elkinton, J. S., D. Lance, G. Boettner, A. Khrimian, and N. Leva. 2011. Evaluation of pheromone-baited traps for winter moth and Bruce spanworm (Lepidoptera: Geometridae). *J. Econ. Entomol*. 104(2): 494-500.
6. Francese, J. A., I. Fraser, D. R. Lance, and V. C. Mastro. 2011. Efficacy of multifunnel traps for capturing emerald ash borer (Coleoptera: Buprestidae): effect of color, glue, and other trap coatings. *J. Econ. Entomol*. 104(3): 1235-1241.
7. Francese, J. A., I. Fraser, M. L. Rietz, D. J. Crook, D. R. Lance and V. C. Mastro. 2010. Relation of color, size, and canopy placement of prism traps in determining capture of emerald ash borer (Coleoptera: Buprestidae). *Can. Entomol*. 142(6): 596-600.
8. Gould, J. R., T. Ayer, and I. Fraser. 2011. Effects of rearing conditions on reproduction of *Spathius agrili* (Hymenoptera: Braconidae), a parasitoid of the emerald ash borer (Coleoptera: Buprestidae). *J. Econ. Entomol*. 104(2): 379-387.
9. Hallman, G. J., S. W. Myers, A. J. Jessup, and A. Islam. 2011. Comparison of in vitro heat and cold tolerances of the new invasive species, *Bactrocera invadens* (Diptera: Tephritidae), with three known Tephritids. *J. Econ. Entomol.* 104(1): 21-25.
10. Khrimian, A., A. Cossé, and D. J. Crook. 2011. Absolute configuration of 7-epi-sesquithujene. *J. Nat. Prod*. 74, 1414–1420.
11. Lelito, J. P., M. J. Domingue, I. Fraser, V. C. Mastro, and J. H. Tumlinson. 2011. Field investigation of mating behaviour of *Agrilus cyanescens* and *Agrilus subcinctus*. *Can. Entomol*. 142: 370-379.
12. Marshall, J. M., A. J. Storer, I. Fraser, and V. C. Mastro. 2011. A predictive model for detection of *Agrilus planipennis* (Col., Buprestidae) larvae in girdled ash (*Fraxinus* spp.). *J. Appl. Entomol*. 135:91-97.
13. Marshall, J. M., A. J. Storer, I. Fraser, and V. C. Mastro. 2011. Addendum to a predictive model for detection of *Agrilus planipennis* (Col., Buprestidae) larvae in girdled ash (*Fraxinus* spp.). *J. Appl. Entomol*. doi: 10.1111/j.1439- 0418.2010.01582.x
14. McCullough, D. G., T. M. Poland, A. C. Anulewicz, P. Lewis, and D. Cappaert. 2011. Evaluation of *Agrilus planipennis* (Coleoptera: Buprestidae) control provided by emamectin benzoate and two neonicotinoid insecticides, one and two seasons after treatment. *J. Econ. Entomol*. 104(5): 1599-1612.
15. Myers, S. W. and S. M. Bailey. 2011. Evaluation of the ISPM-15 heat treatment schedule for Asian longhorned beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae). *Forest Prod. J*. 61(1): 46-49.
16. Silk, P.J., K. Ryall, P. Mayo, M. A. Lemay, G. Grant, D. J. Crook, A. Cossé, I. Fraser, J. D. Sweeney, D. B. Lyons, D. Pitt, T. Scarr, and D. Magee. 2011. Evidence for a volatile pheromone in *Agrilus planipennis*

Fairmaire (Coleoptera: Buprestidae) that increases attraction to a host foliar volatile. *Environ. Entomol*. 40(4): 904-916.

17. Taverner, P. D., C. Sutton, N. M. Cunningham, C. Dyson, N. Lucas, and S. W. Myers. 2011. Efficacy of several insecticides alone and with horticultural mineral oils on light brown apple moth (Lepidoptera: Tortricidae) eggs. *J. Econ. Entomol*. 104(1): 220-224.
18. Ulyshen, M. D., J. J. Duan, L. S. Bauer, J. Gould, P. Taylor, D. Bean, C. Holko, and R. Van Driesche. 2011. Field-cage methodology for evaluating climatic suitability for introduced wood-borer parasitoids: Preliminary results from the emerald ash borer system. *J. Insect Sci*. 11:141 available online: insectscience.org/11.141.

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5. Miriam Cooperband
6. Carrie Crook
7. Damon Crook
8. Tanya Dockray
9. Peggy Elder
10. Joe Francese
11. Jason Hansen
12. Daniel Laffoon
13. Dave Lance
14. Hannah Landers
15. Sue Lane
16. Phil Lewis
17. Chris Lokerson
18. Ron Mack
19. Julia MacKay
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21. Vic Mastro
22. Dave Mills
23. John Molongoski
24. Scott Myers
25. Hannah Nadel
26. Alyssa Pierce
27. Baode Wang
28. Dave Williams
29. Deborah Winograd

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30. Charyl Calfina
31. Dave Cowan
32. Mike Garrity
33. Juli Gould
34. Helen Hull-Sanders
35. Natalie Leva
36. Bill Panagakos
37. Carl Wilcox

Brighton Lab (not pictured)

38. Bethany Boegler
39. Mike Rietz
40. Benjamin Sorensen

Syracuse Lab (not pictured)

41. Michael Crawford
42. Kelley Zylstra

2011 Former Otis Lab Staff

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44. Sian Bailey (pictured)
45. Jeff Cunningham
46. Ashley Hartness
47. Barbara Holske
48. Dyanna Louyakis (pictured)
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