S-1058 MEETING MINUTES
MARCH 2, 2014, Greenville, SC

ACCOMPLISHMENTS:

ARKANSAS

Objective 1. Characterize and evaluate the effect of established natural enemies.
Arkansas efforts under this objective involved development of techniques to evaluate the impact of established, introduced natural enemies of spotted knapweed. We also evaluated the presence/impact of established natural enemies of Japanese beetle in Arkansas.

One biological control agent (*Urophora quadrifasciata*) spread naturally from releases in the northern US, but previous studies indicated it had limited impact on the weed. We verified that *U. quadrifasciata* was the only knapweed biological control agent naturally established in Arkansas, and our studies described 3 generations each season, although only 2 generations occur elsewhere. In Arkansas, the fly reduces the total number of seeds produced by spotted knapweed, but only late in the season when knapweed is environmentally stressed. Therefore, we determined that the redistribution of additional knapweed natural enemies was warranted.

Japanese beetle larval densities are significantly lower than densities noted in northern states, although the pathogens and parasitoids currently in Arkansas offer little control. Surveys of natural enemies of Japanese beetles in Arkansas indicate that few natural enemies are present, suggesting that classical biological control has potential.

Soils from apiaries in Arkansas were tested against larvae and adults of small hive beetle, *Aethina tumida*, to determine the presence of naturally occurring entomopathogenic nematodes or fungi. The small hive beetle is an important pest of bees in apiaries, and the larvae of the beetle are highly resistant to the nematodes and fungi naturally present. A new obligate pathogen of the small hive beetle was discovered Arkansas. This pathogen is a spore-forming protozoan infecting the Malpighian tubules of the small hive beetle. The effect of this pathogen on the host and its biological control potential are still unknown, although it may provide some level of biological control of the small hive beetle in apiaries in the southern region.

Objective 2. Exploration, importation, and assessment of natural enemies for invasive pests.

Based on our determination that additional species were necessary, a program was developed to redistribute two additional biological control agents, the flower head weevil *Larinus minutus* and the knapweed root weevil *Cyphocleonus achates* to Arkansas. *Larinus* adults were released each year from 2008-2012 and field nurseries were established at 37 locations in northwestern Arkansas. Establishment of *Larinus* in Arkansas was first documented in 2010, with recoveries at all release locations. *Cyphocleonus achates*, was introduced for the first time in Arkansas (north central Arkansas) from collections in Colorado in 2010-2012. Recoveries at all release sites indicate establishment in Arkansas is likely. These are the first establishments of either knapweed weevil in the southern US. Together, these two new species are expected to help reduce the spread of this invasive species and suppress established knapweed populations, thus reducing herbicide use in both public and agricultural lands. Plots evaluating the long-term impact of these beneficial agents on local and regional knapweed populations were established using traditional plant stand counts, photographic evidence and remote sensing tools at the landscape level. Reflectance signatures of knapweed were described to allow tracking knapweed

spread (and decline) through satellite imagery. These remote sensing tools will allow further development of long-term evaluation study methodologies. These new methods can be used not only in monitoring the success of the spotted knapweed program in the southwest, but they can be adopted for use on other weeds in other locations.

Over the last decade, decapitating phorid flies in the genus *Pseudacteon* have been released across fire ant infested areas of the south (including Arkansas) for biological control of *Solenopsis invicta, S.richteri* and their hybrids. Recent surveys indicate *Pseudacteon curvatus* is established across most fire ant infested areas of Arkansas. *Ps.tricuspis* is only established in a 2 county region of SW Arkansas, despite multiple releases.

A redistribution effort for Japanese beetle natural enemies was initiated due to our determination that additional natural enemies are needed because the pathogens and parasitoids currently in Arkansas offer little control. Releases in Arkansas of the microsporidian, *Ovavesicula popilliae*, were made using material from Michigan. Recovery surveys and studies to determine the effectiveness of the pathogen against Japanese beetle continue.

Objective 3. Implementation, evaluation, and enhancement (e.g., conservation) of biological control.
The presence of adults of both knapweed biological control agents *Larinus minutus* and *Cyphocleonus achates* at virtually all of the release locations signifies a high quality of the collected and released material. This longevity and retention of adults at release sites over multiple years was a characteristic of successful establishments of these species in the Canada, the Pacific Northwest and Colorado, thus fueling our optimism about the establishment of species and the ultimate reduction of spotted knapweed throughout northwestern Arkansas. Long-term evaluation plots are established at locations across northern Arkansas, and previously-reported remote sensing technologies may be deployed to provide a thorough analysis of agent impact.

During 2013, the fire ant parasitoids *Ps. obtusus* and *Ps. cultellatus* were released in two locations in central Arkansas. Although a few specimens have been recovered, establishment of these species is unknown at this time. Also during 2013, different trapping and phorid fly recovery methods were evaluated at sites (in SW Arkansas) where both *Pseudacteon* species were present. This evaluation (still in progress) is aimed at identifying the optimal method to monitor multiple *Pseudacteo*n species.

UTILITY OF FINDINGS:

Three new natural enemies attacking the invasive spotted knapweed are now known from the southern US. One (*Urophora quadrifasciata*) spread naturally from releases in the northern US, but studies indicate it has limited impact on the weed. Two other beneficial insects (the flower head weevil, *Larinus minutus* and the knapweed root weevil, *Cyphocleonus achates*) were intentionally established by this project in Arkansas via their redistribution from Colorado. Together, these species will help reduce the spread of this invasive species and suppress knapweed populations already established, thus reducing herbicide use in both public and agricultural lands. Reductions in knapweed populations will allow native species to reclaim the habitats invaded by this pest. Long-term evaluation study methods were developed as were remote sensing tools. Successful development and implementation of remote sensing tools to track the reduction of knapweed populations at the local and regional levels will allow their application for terrestrial weed biological control programs in Arkansas. These new methods

will be essential in the cost-effective, long-term monitoring of the success of the spotted knapweed program in the southwest. Remote sensing tools that are developed will also be useful in other terrestrial weed biological control programs in Arkansas.

Survey results indicate that future *Pseudacteon curvatus* releases are not necessary at this time and that additional release efforts should concentrate on other *Pseudacteon* species. The scarcity and limited expansion of *Ps. tricuspis* indicates that either biotic or abiotic factors or trapping bias may be at play and require further investigation.

A new obligate pathogen of the small hive beetles, *Aethina tumida*, was discovered Arkansas. This new pathogen may provide some level of biological control of the small hive beetle, an important pest of honey bees.
The results of these research efforts were disseminated a broad array of outlets. The scientific community was informed of these findings through the numerous previously-reported publications appearing in refereed journals and through delivery of study details at scientific meetings through presentations delivered by principle investigators and graduate students. Producers (particularly in the fruit industry) were notified of project results via grower-organized production meetings as well as those organized through the Cooperative Extension service. Results were also provided to directly impacted private landowners through personal visitation by project personnel on numerous occasions.

BRAZIL

The invasive scale insect *Maconellicoccus hirsutus* (pink hibiscus mealybug) was found for the first time in Espírito Santo (Southeastern Brazil) in 2012 and in 2013 we conducted research to determine what if any natural enemies of the pest are present in this area.

UTILITY OF FINDINGS

Our research results will be useful for preparing for establishment of and for management of invasive pests such as the mealybug *M. hirsutus* and *Q. erythrinae* in Espírito Santo and other parts of Brazil and South America.

FLORIDA

ACCOMPLISHMENTS

OBJECTIVE 1. Characterize and evaluate the effect of established natural enemies

Air potato: Studies are underway to describe the overwintering biology of two biotypes (Nepalese and Chinese) of *Lilioceris cheni*, a leaf feeding beetle released in Florida in 2012 for biological control of air potato.

OBJECTIVE 2. Exploration, importation, and assessment of natural enemies for invasive pests

Cogongrass: Foreign exploration for natural enemies of cogongrass was conducted in Tanzania,

the Philippines and Japan. In all three countries, potentially useful insect herbivores were found, including an *Acrapex* sp. in Tanzania, an *Atherigona* sp. in the Philippines and a *Contarinia* sp. in Japan. *Acrapex* sp. from Tanzania was introduced into a quarantine laboratory in Florida but a colony could not be established.

Brazilian peppertree: Host range testing on two natural enemies of Brazilian peppertree, the thrips *Pseudophilothrips ichini*, and a psyllid, *Calophya latiforceps*, are nearly completed. Petitions for field release of both agents in Florida are being prepared. Established a laboratory colony of a third natural enemy, the psyllid *Calophya terebinthifolli*, and maintaining an established colony of the stem boring weevil *Apocnemidophorus pipitzi* for biological studies and supplemental host range tests.

OBJECTIVE 3. Implementation, evaluation, and enhancement (e.g., conservation) of biological control.

Hydrilla: The colony of the hydrilla miner *Cricotopus lebetis* produced approximately 2000 eggs per day and 14,000 per week following a recent introduction of new field material. A laboratory experiment was set up to assess compatibility of *C. lebetis* with predator (*Gambusia*) and the adventive moth *Parapoynx diminutalis*. Preliminary findings indicate that *Gambusia* fish may negatively impact emergence of the tip miner *C. lebetis* and the defoliator *P. diminutalis* However, it appears that in the absence of *Gambusia* fish, the midge *C. lebetis* and the adventive moth *P. diminutalis* are compatible with each other, causing greater damage to hydrilla (reduced biomass) than either herbivore alone. A laboratory test tube experiment was designed to assess *C. lebetis* larval competition for the host plant hydrilla. Results showed that the average adult emergence were highest when only a single larva (1) was placed with one tip. These finding suggests that larvae do compete for the tip resource and have greater developmental success with limited competition for a host plant tip. Another experiment was designed to define the cold tolerance of *C. lebetis* eggs in terms of duration rather than temperature for establishing release protocols, i.e., storage time or shelf life. There was a significant effect of cold storage on egg hatch (after 7 days) and adult emergence (after 1 day). Experiments were conducted in 55-L aquaria located in a controlled-environment growth chamber at the U.S. Army Engineer Research and Development Center (ERDC) to determine the compatibility of the hydrilla tip miner *C. lebetis* with the hydrilla fungus *Mycoleptodiscus terrestris* (Mt) and the ALS inhibiting herbicide imazamox. Results showed that the tip miner *C. lebetis* is compatible with the Mt fungus, and a synergistic effect was seen within 28 days; hydrilla biomass was reduced by almost 80% when compared with the untreated control. Results also showed that the tip miner *C. lebetis* is compatible with the herbicide imazamox; in fact, adult emergence was higher in some of the tanks treated with imazamox compared to the controls without imazamox.

Whiteflies: Whiteflies are major insect pests of vegetables and horticultural crops. There is increasing interest in use of non-chemical methods to control whiteflies especially in organic and sustainable farming. Several cooperators (J. Pinero, Lincoln University of Missouri; A. Simmons, USDA-ARS, Charleston, DSC; J. Sivinski, USDA-ARS, Gainesville, FL) were involved in various research studies. We tested various plants and commercial oils that are easily available to growers. We documented that giant red mustard plants, mustard oil and squash trap crops are most promising deterrents of whitefly populations in vegetables. These tactics may be

used as the "push" component in a "push-pull" management strategy. For the "pull" component, hover fly generalist predators were collected from several perennial and annual weedy plants and the most abundant hover flies species were recorded in the blue mistflower, *D. carota* and *M. punctata*. These native perennial plants may be used to attract beneficial insects in a farmscaping approach to control whiteflies. We conducted olfactometer bioassays to determine mechanism of repellency. We documented that giant red mustard is a promising companion plant to repel whiteflies. Other mustard varieties such as caliente and nemat will be tested next. Cucumber plants served as the control. We also conducted assays to determine plant attractiveness to predators (e.g. plant volatiles, food sources, plant morphology). We documented the most abundant hover fly predator species in perennial plants such as the blue mistflower, *D. carota* and *M. punctata*. A common ladybeetle whitefly predator showed highest survival when fed whitefly prey and honeydew from the whitefly and honey solution in laboratory conditions.

Filth flies: Research was conducted on chemical cues and the influence of colony generation on host location by *Spalangia cameroni* (Hymenoptera: Pteromalidae). Laboratory assays were developed to assess host searching. Olfactometer bioassays were conducted with a 2012 colony of *Muscidifurax raptor*. Olfactometer bioassays of *Spalangia cameroni* were completed and volatile chemicals were extracted from substrates that elicited a positive response by the wasps.

Asian citrus psyllid: Quality control criteria are being developed for mass rearing *Tamarixia radiata* to maximize parasitism of Asian citrus psyllid, *Diaphorina citri*. Research was initiated on key QC procedures for the *T. radiata* rearing system, including production, process and product quality control parameters and protocols. A *T. radiata* mass-rearing SOP was drafted. Preliminary data was obtained on the effects of size and longevity on levels of parasitism.

Crop pests: A project was designed to establish and evaluate selected cover crops on small farms to increase the impact of beneficial arthropods on crop pests.

Mosquitoes: Research was conducted on the potential for the essential amino acid methionine as a mosquito larvicide. Laboratory assays were developed to assess the effect of methionine on mortality and pupation. Assays were conducted with three pestiferous mosquito species in three different genera: *Culex tarsalis, Anopheles quadrimaculatus* and *Aedes albopictus*. The results demonstrated that methionine was an effective larvicide for all three species and was particularly toxic to *An. quadrimaculatus*.

Training:

- High school students: One conducted study on the effect of cold temperature on adult emergence of the hydrilla tip miner *Cricotopus lebetis*. Two high school students were mentored for science fair projects on invasive plants.

- Undergraduate students: Four students conducted studies on invasive plants during the reporting period; one on the effect of Brazilian peppertree leaf extracts on growth of three species of mangrove; the second on overwintering survival of Brazilian peppertree in different latitudinal zones in Florida, the third tested artificial lighting conditions for rearing the psyllid *C. latiforceps*; and the fourth on improving mass rearing of the

hydrilla miner *C. lebetis*.

- Graduate students: One UF student conducted research on the biology of the leaflet-galling Brazilian peppertree psyllid of *C. terebinthifolii*. An MSc student from the Federal University of Blumenau in Brazil conducted research on the biology of *C. latiforceps,* a candidate biological control agent of Brazilian peppertree.

- Teacher training: Twenty-four secondary school teachers in Florida participated in a program on biological control of invasive plants.

UTILITY OF FINDINGS

Cogongrass: Identification of natural enemies is the first step towards classical biological control of cogongrass. The discovery of potentially useful insect natural enemies in Tanzania, the Philippines and Japan provides a foundation for future efforts to examine the biologies of selected species.

Brazilian peppertree: Significant progress has been made on defining the host ranges of the stem boring weevil *A.pipitzi*, the thrips *P. ichini* and the psyllid *C. latiforceps*; the effect of the latter two agents on the growth of Brazilian peppertree also is being assessed. Based on our findings to date, these Brazilian peppertree insects appear to be specialists, and may prove to be sufficiently host specific for eventual field release.

Air potato: Studies on the overwintering biology of two biotypes of *L. cheni* may prove useful in developing release strategies at different latitudes in Florida.

Hydrilla: The recent discovery of fluridone and endothall resistant hydrilla is forcing aquatic plant managers to consider alternative control methods. Integrating the midge *C. lebetis* with the fungus Mt and the herbicide imazamox, or IPM, may provide a viable alternative to traditional herbicide tools.

Whiteflies: These research areas relate to the use of "push-pull" strategies to control whiteflies and other insect pests in vegetables in an integrated pest management program.

Filth flies: The primary purpose of the filth fly parasitoid project is to better understand the chemical cues and effects of colony generation associated with host location in an effort to improve release strategies for *S. cameroni*, a common pupal parasitoid released in livestock facilities.

Asian citrus psyllid: *Tamarixia radiata* is being mass reared and released in unsprayed citrus groves in Florida for biological control of Asian citrus psyllid, *D. citri*. This project will provide improvements in rearing and effectiveness of this parasitoid.

Crop pests: The purpose of this research is to determine the effectiveness of annual cover crops and associated natural enemies in reducing insecticide use on small farms in the southern U.S.

Mosquitoes: The amino acid methionine has potential for use in mosquito IPM. As an essential amino acid, methionine is natural and likely to be non-toxic to vertebrates.

GEORGIA

Paratelenomus saccharalis (Hymenoptera: Platygastridae) was found parasitizing eggs of *Megacopta cribraria* (Hemiptera: Plataspidae) in several locations in the southeastern U.S. in 2013. Parasitism was first discovered in eggs deposited on kudzu foliage on the UGA Griffin Campus (Spalding Co., GA) in late May. Parasitism was subsequently confirmed in 18 additional counties in Georgia, 9 counties in Alabama, and 1 location in Mississippi in June and July. Elijah J. Talamas of the Hymenopteran Unit of the Systematic Entomology Laboratory (USDA-ARS, Smithsonian Institute, Washington, DC) confirmed the identity of the parasitoid. The sudden appearance of this parasitoid over a wide geographic area will likely not be explained. However, genetic comparison of specimens collected from the wild over this geographic range shows that all are genetically related and are not of the same clade as those held in quarantine (USDA-ARS, National Biological Control Laboratory, Stoneville, MS).

Pesticides commonly used in commercial greenhouse management were evaluated for compatibility with two biological control agents: a leafminer parasitoid (*Diglyphus isaea* [Walker]) and a predatory mite (*Neoseiulus californicus* [McGregor]). These natural enemies were exposed to miticides, fungicides, and insecticides targeting leafminers, thrips, and whiteßies, according to label directions in laboratory vial assays, after which mortality at 12, 24, and 48 h was recorded. Greater mortality of predatory mites than leafminer parasitoids was observed overall, illustrating that fewer pesticides were compatible with predatory mites compared with the parasitoid. However, some commonly used pesticides
were found to cause high mortality to both the leafminer parasitoid and predatory mites.Two spotted spider mite (Tetranychus urticae Koch) infestations often disrupt leafminer (*Liriomyza trifolii* [Burgess]) biocontrol programs. Therefore, potentially compatible miticides (bifenazate, hexythiazox, spiromesifen,acequinocyl, etoxazole,andclofentezine) identified in laboratory trialswerealso evaluated in agreenhouse study and found to be compatible with leafminer biocontrol.

UTILITY OF FINDINGS

The discovery significant and widespread parasitism of *M. cribraria* eggs suggests immediate expanded management options for this invasive species.
Augmentative biocontrol in greenhouse production will be facilitated by expanded knowledge of compatibility with insecticides and miticides commonly used in cutflower production.

KENTUCKY

OBJECTIVE 1: Characterize and evaluate the effect of established natural enemies

Understanding the foraging behavior of predators is a key component of integrating these species into biological control programs. Sit-and-wait foraging is a distinct strategy that involves significant investments prior to prey encounters. However, the complexity of decisions involved

in selecting, maintaining and abandoning ambush sites provides considerable opportunity for learning and flexibility, particularly for trap-building predators, such as web-building spiders. In one study, we sought to determine whether flexible trap-construction is a means of real-time decision making during foraging, and test a new hypothesis that site selection is not a single decision, but a cumulative series of several, distinct decisions based on evaluation of relevant site characteristics. In a laboratory assay, web-building spiders were allowed to choose between microhabitats with and without prey, and site utilization and web construction behaviors were evaluated over a foraging period, to determine how prey cues impact the evaluation and selection of foraging sites by predators. Spiders responded to prey availability across successive phases of site searching and web construction, demonstrating that multiple, distinct decision-making steps are involved in foraging site selection. These results indicate that site maintenance behaviours, such as multiple phases of trap construction, are an integral part of the decision-making framework of foraging predators.

Furthermore, we examined the foraging behavior of a web-building spider in response to pest (aphids) and non pest prey (springtails) aiming to assess the potential role of predator foraging behavior in biological control. Spiders preferred microhabitats with aphid prey over paired microhabitats with no prey but showed no preference for springtail prey. Spiders rarely constructed webs in response to aphids but frequently utilized web-construction tactics in the presence of springtails. These findings demonstrate that predators can regulate their foraging activity to maximize the consumption of specific prey items. In the many cases where pest and non pest prey differ in ecology, prey-specific foraging tactics such as these may limit the usefulness of natural enemies in pest suppression: high-quality, non pest prey may cause shifts in predator behavior that reduce consumption of pest prey. Therefore, non pest prey can have behavior mediated, indirect effects on biological control.

In another study, we examined diet flexibility and prey preference of a common group of generalist predators in winter wheat. Determining diet breadth of a predator is crucial to understanding its potential role in biological control. We developed a molecular framework to study the feeding habits of an epigeal spider community in a winter wheat agroecosystem, and correlated these results with prey availability. We found that our assemblage of spiders appear to prefer Collembola over other suitable-sized prey that are also frequently encountered which were under-utilized in relation to their abundance. Aphid predation was surprisingly high and was not correlated with web size or prey availability. Considering the scarcity of aphids in this system, non-web foraging was likely responsible for the levels of aphid predation recorded. We concluded that there was sufficient abundance of suitable prey (Collembola) to sustain epigeal spider populations in winter wheat such that they were available to prey on pests during immigration into the crop. Our results show that these spiders are not truly polyphagous, but appear to exhibit specialized feeding habits, feeding on jumping (not flying) prey such as Collembola or slowly-crawling prey such as aphids.

The phenomenon of early season predation was examined in a soybean crop, using molecular tools to delineate interactions between predators and prey. The objectives of this study were to assess the incidence of predation by *Pterostichus melanarius*, a common carabid species in North America soybeans, using molecular gut-content analysis, and to quantify its impact on aphid populations through laboratory and field cage experiments. Throughout the growing season

between 16.8% (during low aphid density) and 33.7% (at times of high aphid density) of *P. melanarius* tested positive for aphid DNA. Furthermore, although laboratory feeding trials confirmed that *P. melanarius* preyed on aphids, short-term field cage experiments failed to demonstrate a significant reduction of pest populations by carabid beetles. These results suggest a relatively weak interaction between *P. melanarius* and aphids when pest densities are high, but the high predation rate when aphid densities are particularly low suggests these natural enemies may function as important early-season predators.

Soybeans were also sampled by sweep net during vegetative and reproductive plant growth stages for pests and natural enemies (predators). The most abundant pest species were bean leaf beetles, with lesser numbers of green cloverworms and stink bugs. Green stink bugs accounted for the majority (74%) of stink bug pests, with brown stink bugs making up most of the rest. The invasive brown marmorated stink bug was not collected. The most abundant predators included damsel bugs, minute pirate bugs, big-eyed bugs, lady beetles, and spiders. Lady beetles were more abundant than expected, given the low numbers of aphids, and consisted primarily of *Harmonia axyridis* and *Coleomegilla maculata*, with the former accounting for more than 80% of the lady beetles. All collected *Harmonia* were adults and the only lady beetle species collected in the larval stage was *Coleomegilla maculata*. Predominant spiders included orb weavers, crab spiders, jumping spiders, and lynx spiders. The striped lynx spider accounted for 24.6% of all spiders collected. When spiders in Kentucky soybeans were intensively sampled three decades ago over a three-year period, striped lynx spiders accounted for less than one per cent of the total foliage inhabiting spiders. This species traditionally has been abundant in crops in the southernmost USA but it may be increasing in abundance at more northerly locations.

OBJECTIVE 3: Implementation, evaluation, and enhancement (e.g., conservation) of biological control

Biological control can be enhanced through the supplementation with alternative food resources, driven through conservation management in/around field crops. Uncertainties exist about the value of non-prey food for predators that are commonly food-limited. Prior studies show that large quantities of pollen grains are intercepted in the webs of web-building spiders. Research examined the nutritional benefits of pollen as a non-prey food for a common ground-dwelling, sheet web-building spider, *Mermessus fradeorum*. Supplementing diets with pollen increased both juvenile and adult survival, and the greatest survivorship and offspring production was observed when spiders were provided diets of Collembola supplemented with pollen. Our results show that Collembola are high-quality prey for spiders and pollen has positive effects on nutritional status and survival of a carnivorous species. Foraging on plant material potentially promotes population growth at early and late developmental stages by supplementing diets of poor-quality prey, and preventing starvation when prey are scarce.

UTILITY OF FINDINGS

Research utilizing molecular tools to study foraging behavior of generalist predators has enhanced our understanding of the mechanisms of foraging and the role of alternative prey biodiversity in biological control. This research also provided evidence for the occurrence of high levels of early season predation upon pests, supporting similar findings from previous

research in wheat agroecosystems. Ultimately our ability to discern the strength of all trophic linkages in agricultural food webs, particularly those with alternative prey and intraguild predators that potentially disrupt levels of biological control, will contribute to identifying the role of indigenous natural enemies in management of pest species. New research will examine how field borders influence biological control in cucurbit and soybean crops.

LOUISIANA

Objective 1. Characterize and evaluate the effect of established introduced natural enemies.

The musk thistle head weevil, *Rhinocyllus conicus* Froelich, was introduced into Northwest Louisiana in 1975 for control of musk thistle, *Carduus nutans* L. Initially released near Hanna, LA (Red River Parish) only one subsequent survey was conducted in 1976 by the researchers who made that initial release. Beginning in 1994, periodic surveys (2001, 2004, 2008, and 2010) of musk thistle in Northwest Louisiana were conducted to determine the range expansion of the musk thistle weevil. Beginning in 2004 the surveys were expanded into southwestern Arkansas. Since our initial survey in 1994, *R. conicus* has continued to expand its range north and weevil numbers continue to increase in areas of expansion.

Infestations in Southwest Arkansas (primarily Hempstead, Lafayette and Miller counties) increased from 7.6 weevils per flowerhead in 2010 to 11.0 weevils per flowerhead in 2013. Percentage infested flowerheads increased from 43% to 96% during the same period.

Objective 3. *Implementation, evaluation, and enhancement of biological control.*

The salvinia weevil, *Cyrtobagous salviniae* Calder and Sands, was imported into the US for control of the invasive aquatic fern, giant salvinia (*Salvinia molesta* Mitchell). This weevil was released into Cross Lake in March of 2013 to investigate its potential as a biocontrol agent in Northwest Louisiana. Salvinia weevils were released at two sites on the west end of Cross Lake. The first release site was approximately 26,000 m^2 and 8300 weevils were released at 12 locations within the area. The second release site was about 52,000 m^2 and 13,600 weevils were released at 13 locations within that area. In subsequent samples taken from the release locations, weevil numbers increased from 0.15 weevils/kg of giant salvinia in early May to 10.8 weevils/kg in late September. During the same period the percentage of sites where weevils were recovered increased from 12.5% to 85.7%. Mass rearing of weevils for basic biological studies and for field releases is underway at the Red River Research Station.

UTILITY OF FINDIGS

The musk thistle head weevil continues to survive and spread across Northwest Louisiana and into southwestern Arkansas since its release in 1975. Monitoring biological control agents after their initial release is important to our understanding of these agents and their ability to establish and spread within their targets host range.

The salvinia weevil has been a successful biological control for giant salvinia worldwide. In northern Louisiana, efforts to establish the weevil have been less successful because of winter temperatures that can kill off the weevils but not the giant salvinia. It is hoped that with multiple release sites on Cross Lake, niches might be discovered within the lake that are more conducive to winter survival. Additionally, sampling release sites after periods of below freezing temperatures might provide us cold tolerant individuals that can be used to establish a cold tolerant colony.

OKLAHOMA

Objective 1. Characterize and evaluate the effect of established natural enemies.

Royer:
We are characterizing generalist predator assemblages in riparian zones heavily encroached upon by *Tamarix spp*. in western Oklahoma. Our primary objective is to describe the impact of native predators on *Diorhabda carinulata* populations as they move into Oklahoma. *Diorhabda carinulata* (Coleoptera: Chrysomelidae) was released in 2006 for biological control of *Tamarix spp*. in TX and has since established and is spreading northward into Oklahoma.

Giles:
Completed studies evaluating suitability of canola aphids for survival and development of *Hippodamia convergens* and *Chrysoperla carnea*. Aphids from winter canola are suitable for the survival of *H. convergens* and *C. carnea*, but significantly affect both the duration of larval and pupal development as well as adult weight. Both lady beetle and lacewing development took up to 11 days longer when provided turnip aphids relative to the control when daily prey was most limited, and adult weight was reduced by as much as 36% when fed turnip aphids at the 8 mg daily prey level relative to the pea aphid control.

No preferences for any aphid species were detected, but both predators often consumed more green peach aphids. The lack of clear preferences among winter canola aphids is an indication both predator species should readily feed on all three aphid species, and prey preferences should not negatively influence biological control of aphid pests in winter canola. While increased levels of daily turnip aphid and cabbage aphid prey resulted in faster development times, adult live weights of predators were only marginally improved at the highest prey levels, indicating a qualitative difference in these specialist aphids relative to green peach aphid and pea aphid prey. Despite demonstrated reduced suitability of *Brassica*-specialist aphids from winter canola for *H. convergens* and *C. carnea,* the results from this study are further indication that the addition of a high-energy biofuels crop (winter canola) to traditional winter wheat landscapes may benefit aphidophagous predators primarily because of consistent and abundant alternative prey resources. The sheer number of aphids that occur in late spring in winter canola can provide nearly unlimited resources for developing predators, and green peach aphids may be regarded as a high-quality prey species. Furthermore, floral resources of winter canola habitats are expected to benefit natural enemy populations by providing additional, non-aphid foods to species commonly utilizing pollen and nectar. In addition, delays in development rates may be reduced if

predators are consuming multiple aphid species, rather than developing on monospecific diets of *Brassica*-specialist aphids.

In our experiments, turnip aphid diets resulted in the slowest development times among both lady beetles and lacewings. Some green lacewings took more than 50 days to develop from a newly hatched larva to an adult, raising important questions about whether such delays to predator development could negatively impact biological control. Such a delay would be likely during fall and early spring months, as aphid resources are typically small, clustered populations of less than 50 individuals during this time. As aphid populations increase and predators begin pupation and adult emergence, they are likely to be sprayed with a broad-spectrum insecticide. While this may substantially reduce aphid populations, it is also likely to cause significant mortality to predators nearing adult stages. The overall contribution that natural enemies are expected to make to aphid control in winter canola may therefore be limited by their ability to develop rapidly enough to escape frequent broad-spectrum insecticide applications. A delay in insecticide applications made in early spring may have the potential to facilitate a numerical response of predators as they complete development and lay additional eggs in winter canola. This method of conserving natural enemies known to occur in high numbers in winter canola may further be enhanced with the incorporation of natural enemy presence into sampling protocols and economic thresholds for each aphid species.

Large aphid populations in winter canola have caused severe economic damage and regulation of these aphid populations by natural enemies has not yet been observed. These annual infestations of aphids in winter canola have resulted in regular use of synthetic pyrethroids, and mortality associated with spring applications of insecticides may be the greatest threat to predators within the Southern Great Plains. Detailed studies on natural enemy life histories and their relationship to pest management strategies within winter canola are needed to determine whether this crop may serve as an ecological source or sink habitat. Indeed, any delay made to insecticidal applications in early spring has the potential to facilitate predator development and enhance biological control of aphid pests.

Completed 3rd year of studies evaluating natural enemy abundance and movement within winter canola landscapes. Studies are designed to evaluate the impact of this expanding biofuel crop on insect predators, and pollinators. Data indicate that canola and winter wheat are significant sources of natural enemies.

Objective 3. Implementation, evaluation, and enhancement (e.g., conservation) of biological control.

Royer and Giles
Converted binomial sequential sampling plans for Russian Wheat Aphid to "Glance „n Go" format for compatibility with greenbug sampling. Unlike the greenbug sampling scheme, RWA sampling does not include simultaneous thresholds for parasitoids (frequency of sample units with a parasitized aphid).

Giles
Laboratory and greenhouse studies completed examining intraguild dynamics among aphid

parasitoids and Coccinellidae predators (native versus exotic) in winter wheat systems.

- *Coccinella septempunctata* is a dominant intraguild predator exhibiting greater tendency toward cannibalism and predation on *Hippodamia convergens*.
- Greater degree of cannibalism and IGP likely to occur in field settings with parasitism
- *C. sept.* (exotic) - Competitive edge, but only where alternative prey is available
- *H.con.* (native) – Likely to suffer in field settings where parasitism levels are high and C7 is present, but otherwise less likely to be negatively affected by parasitism

UTILITY OF FINDINGS:

Diorhabda carinulata populations are moving into Oklahoma at a rapid pace. We are continuing to evaluate whether native natural enemies pose a significant threat to *D. carinulata* and ultimately if suppression of *Tamarix* in Oklahoma is being disrupted. The RWA „Glance n‟ Go‟ sampling and management plan is currently not able to incorporate reliable suppression by natural enemies, primarily because natural enemies are not able to maintain RWA populations below EIL‟s. Studies on Intraguild dynamics among aphid parasitoids and Coccinellidae predators (native versus exotic) in winter wheat agroecosystems continue to reveal the competitive advantage displayed by *Lysiphlebus testaceipes* in the Southern Plains, but also why the native *Hippodamia convergens* is the most abundant coccinellid (fewer nutritional requirements when parasitism is high). Summarization of insect trap count data from canola and wheat indicate that these crops are likely sources of natural enemies in agricultural landscapes of the US Southern plains.

SOUTH CAROLINA

Objective 1. Characterize and evaluate the effect of established introduced natural enemies.

A multi-state collaborative project to characterize the phenology and the natural enemy associates of the native oak lecanium scale in GA (Kris Braman, UGA), SC (JH Chong, Clemson), NC (Steve Frank, NCSU) and VA (Peter Schultz, VT) was completed in 2013. The natural enemy associates of the oak lecanium scales were sampled with tissue collection and sticky cards from 2010 to 2013. A total of 16 parasitoid species (Aphelinidae, Encyrtidae, Eulophidae and Pteromalidae), 8 coccinellid species, *Chrysoperla rufilabris* (Neuroptera) and *Tricorynus confusus* were found to be associated with oak lecanium scale. *Coccophagus lycimnia*, *Cocophagus* sp. 2, *Eunotus* sp., *Blasthorix* sp., and *Encyrtus* sp. are the major parasitoid species that were responsible for 78% parasitism in nymphs and 66% parasitism in adults. *Pachyneuron* sp. was the major hyperparasitoid species, which *Chilocorus stigma* and *C. rufilabris* were the major predator species. Parasitoids were most active in late April and May, while the predators were present year round. The impact of predators was more difficult to assessed. This project demonstrated the diversity and importance of natural enemies in maintaining the population of soft scale insects below damaging level.

Objective 3. Implementation, evaluation, and enhancement of biological control.

The influence of foliar sprays of insecticides and selective application methods of neonicotinoids on the activity and impacts of natural enemies were investigated in selected urban landscapes in SC. The results demonstrated that foliar applications of organophaphates, pyrethroids and neonicotinoids reduce the populations of both the oak lecanium scale and its associated parasitoids, while horticultural oil and insect growth regulators had negligible impacts on the parasitoids and predators. Applications of imidacloprid and dinotefuran through indirect methods (soil drench, trunk spray and granule) were found to have minimal impacts on the activities and abundance of natural enemies than foliar applications of the same active ingredients. This project demonstrated that biological and chemical control could be integrated successfully through careful planning and selection of insecticide formulation and application method.

UTILITY OF FINDINGS

The results of this project were disseminated mainly through extension workshops, training and presentation, as well as the publication of extension articles in trade journals and newsletters. This project showed that the parasitoids and predators of oak lecanium scale are active during the time of scale insect crawler emergence, we will have to revise the typically recommendation of spraying insecticides during the time of crawler emergence for scale insect control in order to minimize the negative impacts of the insecticide applications on the natural enemy populations. Fortunately, some of the most effective insecticides in reducing scale insect crawlers, such as horticultural oil, insect growth regulators and neonicotinoid insecticides were shown to have minimal impacts on the natural enemies of the oak lecanium scale. Therefore, ornamental plant producers and landscape care professionals are advised to apply the aforementioned insecticides, through direct or indirect application methods, during the time of crawler emergence to reduce the density of scale insect crawlers on infested trees. Doing so will help reduce damage to the trees, reduce management costs and result in minimal impact on existing natural enemies.

TENNESSEE

[Most of the efforts reported in this Annual Report focus on biological control efforts against hemlock woolly adelgid. Activities also are underway against emerald ash borer and walnut twig beetle (vector of *Geosmithia morbida* which causes thousand cankers disease on black walnut).]

During 2013, research was concentrated against three invasive species (emerald ash borer [EAB], hemlock woolly adelgid [HWA], and walnut twig beetle [vector of thousand cankers disease - TCD]) affecting tree health in forests, nurseries, and urban areas. Two of these invasive threats (EAB and TCD) were first documented in the southern U.S. (Tennessee) in 2010, so research efforts have regional and national implications. Research continues to focus on four primary areas of management of these invasive species: 1) assessment and enhancement of natural enemies to improve tree health, 2) enhancement of chemical insecticides to suppress pest populations and to improve their use with biological control agents, 3) evaluation of a new, immediate threat (TCD and its associates [a fungus and the walnut twig beetle]), and 4) assessment of introduced parasitoids as a management tactic against EAB. Research investigated population establishment of two species of introduced predatory beetles (*Laricobius nigrinus* and *Sasajiscymnus tsugae*) following the use of large whole-tree canopy cages to enhance their survival, establishment, and reproduction for management of HWA on eastern hemlock.

Research demonstrated that this novel technology could enhance establishment of *L. nigrinus* and *S. tsugae* and that these two species, along with a native species (*L. rubidus*), could disperse and coexist on the same tree in the same area. This novel technology also was used to assess natural enemies of EAB. Research on a new natural enemy (*L. osakensis*) of HWA from Japan was initiated. Management studies in the Great Smoky Mountains National Park have documented persistence and efficacy of imidacloprid and its metabolites against HWA. Research has confirmed presence of TCD (and its vector [walnut twig beetle] and fungal associate [*Geosmithia morbidus*]) in numerous counties and, for the first time, documented its occurrence in forested settings. Future research will focus on enhancing our understanding and management of TCD and EAB, as well as the use of whole-tree canopy enclosures to assess impact of species complexes of biological control organisms against HWA and EAB.

Objective 1. Characterize and evaluate the effect of established introduced natural enemies. A Biological Control Demonstration Site was established near Cosby, TN, in the Great Smoky Mountains National Park (GRSM). Multiple species of natural enemies of hemlock woolly adelgid were released into four large, tree cages (one species/cage). The four predator species were: *Laricobius nigrinus* (already established at the site), *L. osakensis* – northern Japan strain, *L. osakensis* – southern Japan strain (both strains of *L. osakensis* were released as larvae), *Sasajiscymnus tsugae*, and *Scymnus coniferarum* (both *S. tsugae* and *S. coniferarum* were released as adults). Trees within these cages and within this area were sampled every week (beginning late September) for individuals of each species. The newly released *L. osakensis* was recovered in beat sheet sampling and from emergence traps. Recovery from emergence traps indicates that this introduced species was able to feed as larvae, undergo larval development on the plant, drop and pupate successfully in the soil, and emerge as adults.

An evaluation of the contributions of several biotic and abiotic factors to establishment of *S. tsugae* at recovery sites in GRSM was completed. Stepwise logistic regression analysis indicated a significant inverse relationship between year of release and recovery of *S. tsugae*. Regression analysis indicated average maximum temperature seven days following release was significant with *S. tsugae* establishment. A significant correlation was found between presence of *S. tsugae* and the average minimum and maximum temperatures seven days following release and the minimum and maximum temperature one day following release. Results indicate that the temperature immediately following releases is important to short-term establishment of predatory beetles; the longer the time since release, the greater likelihood of recovering beetles.

Efforts continued to assess seasonality and impact of established natural enemies following large whole-tree canopy releases at two locations (Blackberry Farm and Elkmont, GRSM). Weekly sampling continued during 2013. These data are being converted into graphs and tables, combined with data from other years, and interpreted to determine seasonality of biological control agents of hemlock woolly adelgid.

A study to investigate the use of spatial analyses as a tool to assess hemlock health and mortality in biologically-treated areas, chemically-treated areas, and control (i.e., not treated) areas was completed. Results, which are being compiled and analyzed, suggest that hemlock trees in areas where introduced biological control areas have been recovered are healthier than trees in areas where these same agents have not been recovered.

Objective 2. *Exploration, characterization and assessment of natural enemies for invasive species.*

No international activities were conducted during this reporting period; a trip was planned to collect additional predatory beetles of hemlock woolly adelgid from hemlock in the Nagano region of Japan. However, this trip was cancelled to enable efforts to focus on additional rearing and evaluation of previously collected organisms.

The newly renovated Beneficial Insects Containment Laboratory at the University of Tennessee is „open for business." However, federal government sequesters negatively impacted funding for research in this facility in 2013. Address is: East Tennessee Res. and Education Ctr., Plant Sci. Unit, 3215 Alcoa Highway, Knoxville, TN 37920.

Objective 3. *Implementation, evaluation, and enhancement of biological control.*

Biological Control Efforts against Hemlock Woolly Adelgid, *Adelges tsugae, Sasajiscymnus tsugae*

Production: Although fewer oviposition jars were used than ever before (50), 314,678 eggs were produced from 12/12/12 to 06/05/13 yielding 118,825 adults (38% survival) from 1/14 to 7/11. More than 22,000 eggs also were released.

Releases: Releases were made from 2/21 to 6/11, and from 10/29 to 11/15. 16,382 beetles were released in GRSM in 4 releases; other releases were in South Cherokee National Forest (SCNF) (22,275 beetles in 5 releases); Catoosa Wildlife Mgmt. Area (8,706, 2 releases); North Cumberland WMA (9,027, 2 releases); Bays Mtn. Park, Kingsport (4,000, 1 release); Lone Mtn. St. Forest (3,615, 1 release); Piney Falls St. Natural Area (5,000, 1 release); Cumberland Mtn. St. Park (2,204, 1 release); Cumberland Trail on Piney River (5,026, 1 release), at Double Bridges (5,968, 2 releases), and on N. Chickamauga Creek (6,247, 2 releases); and in the Log Haven Community near Knoxville as a demonstration (4,236, 2 releases). Occasionally an overabundance of eggs was produced so several egg releases were made including in the SCNF (4,100 eggs), Bays Mtn. Park (2,670 eggs), Cumberland Trail on Piney River (6,300 eggs), Piney Falls St. Natural Area (4,700 eggs) and Catoosa WMA (4,600 eggs). All egg releases were made in combination with an adult release. Potential release sites were evaluated.

Notes: In August and December, adults were supplied to predator beetle rearing labs at University of North Georgia-Dahlonega (1,810 beetles) and Young-Harris College (2,259 beetles), respectively, to augment their rearing colonies. About 20,000 beetles were held for summer storage and fall releases. Stored beetles were maintained on foliage with N1 HWAs which was replaced monthly, and were provided Wheast. Survival of beetles stored for >150 days was 66% (8 boxes) with an average storage time of 170 days and an average starting density of 1721 beetles per storage box.

Laricobius nigrinus

Production: Oviposition jars were maintained from 1/24 to 6/12 using adults collected from Banner Elk, NC and Seattle, along with lab-reared adults. 38,471 mature larvae were harvested from 2/11 to 7/16. Adults began emerging on 7/17 and as of 1/4/14 13,135 have emerged (34% survival).

Releases: 1,554 adults were released in GRSM in 3 releases; other releases were in South CNF (2,264 beetles, 5 releases); North CNF (471, 1 release); North Cumberland WMA (987, 2 releases); Catoosa WMA (1222, 3 releases); Piney Falls SNA (498, 1 release);

Frozen Head St. Park (457, 1 release); Cumberland Mtn. St. Park (956, 2 releases); and on/near Cumberland Trail on Piney River (519, 1 release), at Double Bridges (498, 1 release), on N. Chickamauga Creek (478, 1 release), and on Little Possum Creek (517, 1 release). Potential release sites were evaluated.

Field-collected and lab-reared progeny production: As reported last year, progeny production (mature larvae produced) for lab-reared and wild-caught (Banner Elk) adults was compared during the 2012 rearing season and was found to be essentially the same. Resulting adult emergence (not reported last year) differed, as survival to adult was 42% and 34% for colony and wild-caught progeny, respectively.

Establishment documented at Frozen Head State Park: Beat sampling at two release sites at Frozen Head State Park (about 35 miles NW of Knoxville) in early November yielded 4 *Laricobius* adults, assumed to be *L. nigrinus* or the hybrid, which were presented to the park superintendent and rereleased. Releases were made at these sites in 2008 (575 beetles released in November) and 2009 (291 released in December). The sites are only 400 yards apart, so establishment at one could result in *L. nigrinus* being at both sites now. Only 8 or 9 branches were beaten to collect the beetles indicating a well-established population.

***Laricobius osakensis* (Northern)**

Production: 247 adults with origins from northern Japan were obtained from the Virginia Tech lab in mid-December 2012 for rearing at LYBIL. Oviposition jars were maintained from 12/18 to 6/5 resulting in 8,095 mature larvae harvested from 3/9 to 7/20. Adults began emerging on 8/14 and currently 1,325 (16%) have emerged, a disappointing number considering the care (e.g., abundant food) the larvae were provided.

Releases: On 4/18 about 150 immatures (eggs and larvae) were released in a tree cage in the GRSM. Three adults were collected this fall from the release tree. 620 adults were released at one site in GRSM near Gatlinburg in three separate releases from 10/17 to 11/19. 290 adults were released near the Cumberland Trail in north Hamilton Co. in mid-December. Remaining adults will be released to supplement the release in Hamilton Co.

Notes: LYBIL will not rear the northern strain in 2014 but will focus on the Kansai strain.

***Laricobius osakensis* (Kansai)**

Production: Seventy-six adults with origins from the Kansai region of southern Japan were obtained from the Virginia Tech lab in mid-December 2012 for rearing at LYBIL. Oviposition jars were maintained from 12/18 to 6/5, and 1,593 mature larvae were produced from 3/9 to 6/12. Adult emergence began on 9/8 and 430 (27%) have emerged so far. As with the northern strain, adult emergence is less than expected considering the quality and quantity of food provided the larvae.

Releases: On 4/18 ca. 150 immatures (eggs and larvae) were released in a tree cage in the GRSM near the tree where northern strain immatures were released. No adults have been collected from this release tree yet. Due to limited number of adults produced, no other releases were made and all remaining adults are being held in the lab for rearing in 2014.

Scymnus coniferarum

Production: Using beetles collected from Tacoma/Seattle in early November 2012, about 530 adults were produced in the lab from 3/21 to late July. Although beetles were provided HWA beginning 11/27/12, oviposition (based on adult progeny emergence) did

not begin until about the 3rd week of January. Initially, gallon, plastic oviposition jars containing 20 beetles each were held at 12° C and 12:12 L:D. Foliage was removed and replaced weekly. Exposed foliage was held at 20° C and 13:11 L:D for immature development. In mid-January, oviposition temperature was increased to 20° C.
Releases: 160 lab-reared adults were released on 4/26 in a tree cage in GRSM where *L. osakensis* immatures were released. A mix of wild-caught and lab-reared beetles were released in sleeve cages (6 beetles/cage) at Catoosa WMA in Morgan Co. 96 beetles were released in 16 cages on 6/4; cages were removed on 6/20 and 16 more cages (with new beetles) were set up on the same trees (different branches), and removed 20 d later.
Notes: Remaining adults are in storage for rearing in 2014.

Biological Control Efforts against Emerald Ash Borer, *Agrilus planipennis*
The egg parasitoid *Oobius agrili* was released at three of the release sites (Cowan Park, Haven Hill and Miser Station) where *Spathius agrili* and *Tetrastichus planipennisi* were released in 2012; released *O. agrili* at three additional sites (Frank Lorino Park, Ramsey House, and Rutledge). More than 2,300 eggs of emerald ash borer were supplied from the parasitoid rearing laboratory in Brighton, MI, for studies designed to monitor *O. agrili*; thus, these eggs allowed us to assess recovery of 2013 releases of *O. agrili* using sentinel eggs at release sites where *S. agrili* and *T. planipennisi* had been released in 2012.

Releases of the two larval parastioids *S. agrili* and *T. planipennisi* continued at three release sites, Cowan Park, Haven Hill, and Miser Station, initiated during 2012. Additionally, three new sites, Frank Lorino Park, Ramsey House, and Rutledge, were assessed, and releases of *S. agrili* and *T. planipennisi* were conducted during 2013. No new parasitoid releases were made at one 2012 site (Rowe Road, Claiborne Co.), as many study trees at this site were dead. To monitor parasitoid establishment at this site, 10 pan traps were installed during June 2013 and monitored weekly. One *S. agrili* was recovered from a pan trap at the Rowe Road site on 12 August. Additionally, two native *Spathius* spp. (one *S. floridanus* and an unknown *Spathius* species) were recovered from pan traps.

Three trees that were caged at Cactus Cove with parasitoids in October 2012 were felled; ½ of each tree trunk was peeled and examined for parasitism; the remaining ½ of the trunk was placed in barrels and held to assess emerging parasitoids. Branches and limbs from these three trees were held in cages in the field; they were removed from these cages and placed in barrels and held to assess emerging parasitoids. During 2013, 24 trees (Cowan Park = 3, Haven Hill = 3, Cactus Cove = 3, Lorino Park = 2, Miser Station = 3, Ramsey House = 3, Rowe Road = 7) were felled and held in barrels and monitored for parasitoid emergence. One *S. agrili* was recovered on 22 April from a barrel containing tree limbs from Cactus Cove. Additionally, two native *Spathius* spp. (*S. floridanus* and an unknown *Spathius* spp. on 29 July) were recovered from wood from Cactus Cove.

Adults (n=2) of the native parasitoid *Atanycolus hicoriae* were recovered from wood collected from a caged tree described in #15 above; trees were caged in October 2012 but emerald ash borer larvae had been exposed to natural parasitism before cages were placed on the trees. Of parasitoids emerging from caged wood or found in pan traps, selected individuals were sent to Jonathan Lelito and/or Julie Gould for species identification.

A study initiated during Fall 2012 to assess overwintering and generations per year of parasitoids on small emerald ash borer-infested ash bolts in cups was discontinued in Spring 2013, due to an excess of moisture and increased temperatures in the cups. On 11 September, a second study was initiated, and 30 *S. agrili* females or 30 *T. planipennisi* females were placed into each of four cups (2 cups/species) containing a small emerald ash borer-infested bolt. As with the 2012 study, the tops of these cups were covered with netting and placed outdoors underneath a covered shelter. Cups were monitored daily for parasitoid emergence. No adults emerged during this reporting period.

Efforts continued to investigate the potential to rear parasitoids in screened cages in the field to enhance release and establishment. Additional „release bolts" containing parasitized emerald ash borer larvae were obtained from the Brighton laboratory; these larvae had been exposed to *S. agrili* and *T. planipennisi*. Also, oobinators (emerald ash borer eggs parasitized by *O. agrili*) were obtained (all specimens originated from the Brighton facility). These parasitized specimens were held in the outdoor rearing facility at the University of Tennessee to assess overwintering capabilities. On 5 June, three green ash trees on Cactus Cove Road (Blount Co.) were selected for use in a large cage study. Each tree was topped at 6 m from ground level by staff from the Tennessee Division of Forestry with the aid of a Genie Z 45/22 articulating boom truck. Once topped, the upper trunk and limbs were placed in plastic 125-liter drums, so that they could be supplied with water throughout the year. All drums containing the upper sections of the tree were attached to the standing trunk of the tree from which they were cut. A large cage (ca. 7 m tall, 5 m diameter at base) was placed over each of the three trees. On 12 and 13 June 2013, ca. 50 female and 45 male emerald ash borer adults were added to the cages, so that they could mate and lay eggs and larvae could begin development within the trees. On 20 June, 50 *O. agrili* were released in the cages. Approximately 160 emerald ash borer eggs on coffee filters were placed on the trunks of each tree at the time of release of *O. agrili* to supplement eggs laid on the bark of the cut ash trees within cages. On 2 August, 83 female *S. agrili* and 100 *T. planipennisi* (and a mixed number of males of each species) were released in the cages to parasitize the maturing emerald ash borer larvae. Additional *S. agrili* were added to cages on 6 August, so that two cages contained 99 and one cage 100 female *S. agrili*. All tree material will be left in the field throughout the winter to allow parasitoids to pupate/ complete their lifecycle.

Another large cage study was conducted at the Rutledge site (ca. 4.8 km south of Rutledge [Grainger Co.]). On 13 June, six clusters of green ash stump sprouts were selected; each cluster of trees was trimmed to a height of ca. 6 m from ground level. Between 13 and 19 June, large cages (ca. 7 m tall, 5 m diam. at base) were placed over each of the six tree clusters and labeled as #1-6. Cages 1-3 were designated for an egg parasitoid study; cages 4-6 were designated for a larval parasitoid study. Temperature/relative humidity logging devices (HOBOs) were placed in each cage and on an external tree on 15 July; HOBOs were set to record temperature and humidity every hour. These large cages were used to assess parasitism of egg parasitoids; on 26 June, 50 *O. agrili* adults were released into each cage and ca. 150 emerald ash borer eggs on coffee filters were placed on trunks of stump sprouts in each of the three cages. Shelter for the filters was provided using a paper plate fixed above to protect from rain. Each filter was photographed upon placement and then each Monday, Wednesday, and Friday until they were removed. It was observed that many eggs were missing after only two days. The decision was

made to substitute plastic plates and cut plastic boxes for subsequent releases to further protect the eggs. Subsequent egg placement and retrievals (each ca. 150 egg/cage) were performed. Photographs of each filter were saved and will be used to document each egg‟s parasitism over time, as parasitism alters the color of emerald ash borer eggs. Retrieved eggs were examined under the scope and assessed for parasitism. No eggs were parasitized, and the majority of eggs were either removed or eaten by ants or washed away by the elements. Later discussions with other collaborators yielded that emerald ash borer eggs may be used as sentinel eggs if laid on the bark surface of a small limb and covered loosely with a ribbon.

Large cages also were used to assess parasitism of emerald ash borer larvae; on 26 June, 40 female and 33 male emerald ash borer adults were released into each cage to insure infestation of the ash stump sprouts with larvae. Larvae were allowed to develop for 6 to 8 weeks into 3rd or 4th instars; 100 female *S. agrili* and 100 female *T. planipennisi* (and a mixed number of males per cage) adults were then released into the cage during the week of 12 August. These parasitoids were able to oviposit on emerald ash borer larvae present in the caged trees. All tree material will be left in the field throughout the winter to allow parasitoids to pupate/complete their lifecycle. Twelve cages (1 m tall by 65 cm^2) were designed and constructed of anti-virus mesh netting placed over a PVC frame. Cages were equipped with a wire grid 63.5 cm from the base to keep inserted wood pieces in a more vertical position. The netting was closed on the bottom of the cage by heaping gravel about the base. On 25 July, seven trees from a proposed open parasitoid release site located on the grounds of Historic Ramsey House, Knox Co, were cut and sectioned to obtain 108 branch sections (ca. 76 cm long and between 6.2 and 12.7 cm diam). Each wood section was sealed on each end with paraffin wax to reduce moisture loss within the wood. On 1 August, 12 cages were placed in a shade house with a tarp roof at ETREC. Nine wood pieces were placed into each cage. Cages were randomly selected for one of four different treatments (three cages/treatment). Treatment 1: one parasitoid release (20 *S. agrili* and 20 *T. planipennisi*) at time 0. Treatment 2: one parasitoid release at time 0 and one release at 0 + 2 weeks. Treatment 3: one parasitoid release at time 0, one release at 0 + 2 weeks, and one release at 0 + 4 weeks. Treatment 4: Control, no releases. Time 0 release occurred on 1 August 1, and further releases are on schedule.

Objective 4. *Evaluate the benefits and risks of introduced and indigenous natural enemies.* Although *L. nigrinus* (native to Pacific Northwest) has been approved and released against hemlock woolly adelgid, this species has been documented to hybridize with *L. rubidus*, a species native to the eastern U.S. Research continues to address hybridization of these species in our research areas, but results for 2013 are not yet available.

UTILITY OF FINDINGS:

Biological control is crucial to controlling hemlock woolly adelgid as it is the only viable management tool for hemlocks over vast areas, and also has the potential to permanently reduce population densities of hemlock woolly adelgid. Therefore, improving and expanding mass production of biological control agents, predaceous beetles, is of utmost importance. Discovering and utilizing new release areas, especially those on the western edge of expansion of hemlock woolly adelgid in Tennessee, will enable personnel to better plan predator releases to improve

the chances of reducing populations of this invasive pest in these newly infested areas, which should ultimately reduce the damage that this pest may cause to hemlock forests.

Hemlock woolly adelgid has killed hundreds of thousands of trees throughout the eastern U.S. The loss of this long-lived, foundation species in our forests has numerous ecological, environmental, social, and economical ramifications, as well as safety issues when dealing with so many dead trees in highly traveled and visited areas, such as the GRSM. Thus, the management of this invasive insect species introduced from Japan is critical to forest health, aesthetics, safety, and the environmental sustainability of forest systems. We evaluated the establishment, spread and coexistence of two introduced predators of hemlock woolly adelgid, and the factors responsible for their success. The results of our research provided new knowledge to enhance and revitalize biological control and management efforts against hemlock woolly adelgid. This new knowledge will improve establishment of released biological control agents, resulting in more successful release sites and improved tree health and survival.

The recovery of *S. tsugae* in new areas in GRSM and Blackberry Farm is promising and indicates this species is established at multiple locations. Understanding the role of biotic and abiotic factors in establishment and recovery of *S. tsugae* will greatly enhance their use in biological control efforts against hemlock woolly adelgid. Temperature and elevation are among the most important factors which influence establishment of *S. tsugae*. The documented inverse relationship of year of release to establishment and recovery indicates that *S. tsugae* requires more time to reach detectable levels after establishment. Releases of *S. tsugae* at freezing or below freezing temperatures may hinder their establishment. Releases of these introduced predators at the right time of year and at appropriate elevations will enhance biological control efforts against hemlock woolly adelgid, especially in GRSM.

Emergence traps have proven to be a useful and versatile tool to detect *L. nigrinus* in sites where it has been released and has established. This tool may be used to enhance recovery efforts and support the use of these sites as field insectaries, where beetles can be collected and relocated to new areas.

Documenting that hybridization occurs in areas where both the native and introduced *Laricobius* species occur is important to establish a comparative baseline for future studies. It is unclear if the level of hybridization (ca. 8.9% of specimens examined from Elkmont and Blackberry Farm) is yet biologically significant, and the rate of hybridization is currently unknown. Specimens of *Laricobius* continue to be collected and will be analyzed to gain a broader understanding of the prevalence and extent of hybridization between these predatory species in natural areas.

A complex of natural enemies has been released against the hemlock woolly adelgid in the southeastern United States. These species complement each other, as they feed on various life stages and at varying times of the year. Two predator species are established at numerous locations in the southeastern U.S.; research at the University of Tennessee has demonstrated that both species can survive together on the same infested hemlock tree. Predator compatibility will elicit a more efficient and successful program for control of the hemlock woolly adelgid. Research on recovery and establishment of introduced predators in GRSM continues to provide valuable information about *S. tsugae* and *L. nigrinus* establishment in the Park, as well as

expectations for recovery.

Emerald Ash Borer: A new invasive insect threat (emerald ash borer) has been documented in 21 counties in Tennessee, representing the southernmost distribution of this insect pest in the U.S. This insect threatens to greatly reduce, if not cause the extinction of, populations of ash in the U.S., leading to tremendous economic and ecological losses. Entomologists at the University of Tennessee are at the forefront of research focusing on emerald ash borer in the southern U.S., with efforts directed at implementation of biological control to protect ash trees in forests, nurseries, and urban areas. Results of this research have demonstrated that these introduced natural enemies can overwinter and survive in southern climates. The ultimate goal is to provide sufficient mortality of emerald ash borer to protect trees and minimize adverse environmental impacts. Studies on emerald ash borer and its parasitoids in the south are at early stages. Biological control is the most promising broad-scale management option, but, as with many biological control programs, these introduced parasitoids may take years to establish and exert population reduction. Thus, development and evaluation of management efforts may take time before they can lessen this pest‟s impact in the south. Additional parasitoid releases and evaluation of native parasitoids will continue in 2014.