Improving the Monitoring, Trapping and Management Tactics of Ambrosia Beetles in the Nursery Agroecosystem

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PROJECT OBJECTIVES:

1. Identify attractive volatiles to be used as lures for improved detection and monitoring of ambrosia beetles.
2. Optimize trap design and develop a technique for inducing attacks by ambrosia beetles on specific trees.
3. Screen the efficacy of conventional insecticides and botanical formulations for controlling ambrosia beetles.
4. Characterize the relationship between seasonal activity and degree-days of *Xylosandrus germanus* and *Xylosandrus crassiusculus* to develop the most accurate system for timing insecticide treatments.
5. Improve application technology of insecticidal trunk sprays, and assess the efficacy of chemigation tactics for controlling ambrosia beetles and fungal pathogens.
6. Determine the effects of stress factors on nursery stock attractiveness and susceptibility to ambrosia beetles.
7. Identify symbiotic fungi associated with *Xylosandrus* species and determine their genetic diversity and pathogenicity among populations from eastern United States.
8. Determine the effectiveness of available microbial control agents against ambrosia beetles and their symbiotic fungi.

ACCOMPLISHMENTS RELATED TO OBJECTIVES:

1. Identify attractive volatiles to improve detecting and monitoring ambrosia beetles.

- Ethanol is currently the most effective attractant for monitoring ambrosia beetles. Trapping studies conducted in Ohio and Virginia demonstrated a positive correlation between ethanol emission rate and trap counts of *X. germanus* and *X. crassiusculus*. Thus, the higher the amount of ethanol being released from the lure, the more effective the trap will be for monitoring ambrosia beetles.
- Efforts are focused on developing an optimal blend of attractants superior to ethanol alone that can be used for monitoring ambrosia beetles. Extensive field trapping studies conducted in Ohio have demonstrated that a combination of ethanol and alpha-pinene typically catches significantly more *X. germanus* than ethanol alone. A variety of stress-related volatiles other than ethanol have also been tested for their attractiveness to *X. germanus*, but none are more attractive than ethanol or demonstrated a synergistic relationship.

- To assist in the identification of attractants, a volatile collection procedure was developed in Ohio that allows for volatiles to be collected from the trunk and foliage of individual trees. Volatile components from hosts are currently being identified using analytical chemistry techniques, and will subsequently be incorporated into a lure to develop a blend of attractants superior to ethanol.
- An electrophysiological instrument has been assembled at the ARS Horticultural Insects Research Laboratory in Wooster, Ohio, that allows for measuring ambrosia beetle antennal responses to plant and insect-derived volatile compounds. The instrument will aid in the identification of attractants. The first known electroantennogram recordings of *X. germanus* were conducted in 2009 in Ohio.
- Role of team members: Dr. Ranger (Ohio) has been handling the collection and identification of ambrosia beetle volatile attractants, along with electroantennogram and behavioral experiments. Drs. Reding (Ohio), Oliver (Tennessee), and Schultz (Virginia) have been contributing to field trapping experiments involving attractants.

2. Optimize trap design and develop a technique for selectively inducing attacks by ambrosia beetles on specific trees.

- Trapping experiments conducted in Ohio, Tennessee, and Virginia determined that traps low to the ground capture the largest number of *X. germanus* and *X. crassiusculus* compared to elevated traps. In particular, ethanol-baited traps should be deployed at heights of 1.7 meters or less for optimal collection of either *X. crassiusculus* or *X. germanus*.
- A novel strategy optimized by the USDA-ARS in Wooster, Ohio, rapidly triggers attacks by ambrosia beetles on specific trees. The procedure utilizes an Arbor Jet® tree injection system to forcibly inject ethanol into specific trees, thereby leading to ethanol emission. Injection of ethanol induces the largest number of attacks by *Xylosandrus germanus* compared to other stress-related volatiles. No attacks have occurred on water-injected or non-injected control trees throughout the course of numerous experiments conducted in Ohio. Complementary studies conducted in Tennessee and Virginia confirmed the ethanol injection technique is also effective at inducing attacks by *X. crassiusculus.* Ethanol-injection triggers dramatically more attacks than simply irrigating with ethanol solutions or hanging ethanol lures from trees. Using the ethanol-injection technique to reliably induce ambrosia beetle attacks on specific trees will allow for conventional and reduced risk insecticides to be screened, and for the development of improved application technology methods.
- Trapping studies conducted in Ohio, Tennessee, and Virginia demonstrated that bolts prepared from ethanol-injected trees hung in traps caught dramatically more ambrosia beetles than ethanol-dispensing lures. Volatiles emitted from the bolts will be collected and identified to ultimately develop an improved lure.
- Role of team members: Drs. Reding (Ohio), Schultz (Virginia), Oliver (Tennessee), and Ranger (Ohio) are collaborating to optimize trapping design and conditions. Dr. Ranger is collaborating with Drs. Reding, Schultz, and Oliver to maximize the ethanol-injection strategy for detecting and mass trapping ambrosia beetles.

3. Characterize the relationship between seasonal activity, plant phenology, and degree-days of *Xylosandrus germanus* and *Xylosandrus crassiusculus* to develop the most accurate system for timing insecticide treatments.

- Extensive field-based trapping studies conducted in Ohio, Tennessee, and Virginia are providing key details on ambrosia beetle seasonal activity. Peaks typically occur in late spring with the exact period varying from year to year. *X. germanus* is the predominant species caught in Ohio, while *X. crassisculus* is the predominant species in Tennessee and Virginia. Seasonal activity is being correlated with degree-day information to develop a predictive model for timing insecticide applications. It is anticipated that soil depth temperatures and/or plant phenological observations will be a consistent predictor of ambrosia beetle emergence and flight periods. We anticipate that one of the soil depth temperatures may simulate the temperature conditions in ambrosia beetle overwintering harborages better than air temperatures, and therefore, could lead to better predictions of ambrosia beetle flight activity. This study will require several years of field-trapping experiments to identify a consistent predictor of ambrosia beetle flight activity.
- Ethanol-injected trees positioned at cooperating nurseries in Ohio demonstrated the timing of initial attacks by *X. germanus* coincides with captures in ethanol-baited traps. In contrast, studies conducted in Tennessee indicated that ethanol-injected trees were a better indicator of ambrosia beetle initial activity than ethanol-baited traps.
- Role of team members: Drs. Reding (Ohio), Schultz (Virginia), Oliver (Tennessee), and Ranger (Ohio) are working cooperatively to characterize ambrosia beetle seasonal activity.

4. Screen the efficacy of conventional insecticides and botanical formulations/repellents for controlling ambrosia beetles.

- Efficacy trials of conventional insecticides have been hampered by a lack of consistent ambrosia beetle attacks on untreated control trees. Since the ethanol-injection technique ensures experimental trees will be attacked, insecticide efficacy trials were successfully conducted in Ohio, Tennessee, and Virginia with a variety of contact and systemic products. Permethrin (i.e. Perm Up) provided the best control, but no product provided 100 percent protection from ambrosia beetle colonization. Neonicotinoid drenches (i.e., dinotefuran [Safari 20SG] or chlorantraniliprole [Acelepryn]) applied to the trunk were completely ineffective at preventing ambrosia beetle attacks.
- The ethanol-injection technique also allowed for multi-state efficacy trials to be conducted with botanical formulations. A few botanical formulations provided a very promising barrier against ambrosia beetle colonization. Electrophysiological and laboratory-based behavioral experiments also provided insight into the efficacy of botanical formulations.
- Role of team members: Dr. Ranger has been conducting electrophysiological and behavioral experiments to identify botanically-based ambrosia beetle repellents. Drs. Reding (Ohio), Schultz (Virginia), Oliver (Tennessee), and Ranger (Ohio) are working cooperatively to evaluate the efficacy of conventional insecticides and botanical formulations.

5. Improve application technology of insecticidal trunk sprays, and assess the efficacy of chemigation tactics for controlling ambrosia beetles and fungal pathogens.

- Results from multi-state insecticide efficacy trials targeting ambrosia beetles have demonstrated that systemic neonicotinoid products do not provide adequate protection

against ambrosia beetles. Thus, chemigation is not considered a promising insecticide delivery method for ambrosia beetles. Instead it is presently believed that thorough coverage of the trunk is critical for protecting vulnerable nursery stock. Research currently being conducted with a sensor-based spray system that uses intelligent technologies to continuously match system operating parameters to crop structures. As such, the sprayer maximizes target coverage and minimizes non-target application by timing insecticide application based on presence or absence of the target (i.e. trunk).

- Role of team members: Dr. Zhu (Ohio) has been developing the sensor-based spray system. Drs. Ranger and Reding are collaborating with Dr. Zhu to evaluate and optimize the technology.

6. Determine the effects of stress factors on woody ornamental attractiveness and susceptibility to ambrosia beetles.

- Experiments were conducted to evaluate the impact of flood-stress and prolonged dormancy on the attractiveness of dogwoods to ambrosia beetles. In 2008, specimens of *X. germanus* and other ambrosia beetles were observed on the flood and dormancy-stressed trees, but no attacks occurred. In 2009, significantly more *X. germanus* were attracted to flood-stressed dogwoods compared to non-stressed trees, and a few of the flood-stressed dogwoods were colonized multiple times. None of the unstressed trees were attacked in the 2008 or 2009 trials. Volatiles were collected from the flood-stressed and unstressed trees and are being analyzed for compounds emitted only by the stressed trees to identify ambrosia beetle attractants.
- Extensive evaluations of stress factors on tree attractiveness to ambrosia beetles were also conducted in Tennessee. In 2008, stress treatments of Chinese chestnut trees included root drowning, 50 percent crown defoliation, 100 percent crown defoliation, water deficiency, no fertilization, and prolonged dormancy. Despite imposing the stress factors, none of the treatments triggered ambrosia beetle attacks. In 2009, redbud, tulip poplar, and Okame cherry were stressed with 100 percent crown defoliation, root drowning, planting too deep, water deficiency, and prolonged dormancy. Significant differences in amount of attacks were not detected between any of the stress treatments, but there was a trend for more ambrosia beetle attacks on the flood-stressed and non-treated control trees compared to the delayed dormancy and drought stress treatments for both tulip poplar and redbud trees. *X. crassiusculus* was among the ambrosia beetles attacking the trees.
- Role of team members: Drs. Oliver, Ranger, Schultz, and Reding are collaborating to evaluate the influence of stress factors on the attractiveness and susceptibility of nursery stock to ambrosia beetles.

7. Identify symbiotic fungi associated with *Xylosandrus* species and determine their genetic diversity and pathogenicity among populations from eastern US.

- Isolated ambrosia fungi from field-collected *X. germanus* adult females from New York, Ohio and Virginia. Seventy-seven new fungal isolates were obtained from dissections of mycangia from 90 beetles. Isolates have been successfully grown on four different media and preserved for long-term storage. All samples are maintained at the USDA-ARS in Ithaca, New York.

- In order to identify and characterize fungal strains, colony morphology and cycloheximide tolerance were determined. Extracted DNA from each isolate in preparation for amplification and sequencing of internal transcribed spacer (ITS) region and β-tubulin genes. These sequences will be compared with those known from fungi from other ambrosia beetles to verify species identification.
- Fungal symbionts associated with *X. germanus* were tested for their pathogenicity to *Magnolia virginiana*. The strains tested to date did not produce terminal die-back at low inoculation levels, but necrosis and poor healing were observed around the site of inoculation. Subsequent pathogenicity studies will be conducted using strains collected by Drs. Castrillo and Vandenberg.
- Light microscopy, and scanning and transmission electron microscopy are also being used to characterize the morphology of the mycangium and fungal symbionts of *X. germanus.* On-going studies are also attempting to document the temporal variability in fungal symbiont incidence.
- Role of team members: Drs. Castrillo and Vandenberg are isolating and identifying symbiotic fungi and determining their genetic diversity. Dr. Ranger is collaborating with Drs. Castrillo and Vandenberg to assess fungal pathogenicity to nursery stock.

8. Determine the effectiveness of available microbial control agents against ambrosia beetles and their symbiotic fungi.

- A rearing protocol was developed for *X. germanus* on a sawdust-based artificial diet. The ambrosia fungus readily grew within brood chambers on the diet within rearing tubes, resulting in overlapping batches of developing brood. More than 60 adult progeny (from single females) were obtained from some tubes. The adult progeny obtained were healthy and remained viable for at least 2 weeks. This rearing system should allow the production of sufficient numbers of beetles for laboratory bioassays of microbial control agents.
- An in-vitro competition assay was used to test three strains of *Ambrosiella* spp. (isolated from *X. germanus* and *X. crassiusculus*) against the commercially-available fungal biocontrol agents *Beauveria bassiana* and *Metarhizium anisopliae*. These assays allow evaluation of fungal competition in three ways. In preliminary tests, each of the biocontrol fungi limited the growth of ambrosia fungi. Further studies will quantify effects among an expanded set of fungal strains and will evaluate competition within wood or sawdust-diet galleries.
- Mortality of field-collected adult female *X. germanus* was 100 percent within 8 days of exposure to a single dosage of commercial strains of either *B. bassiana* or *M. anisopliae*. Additional multi-dose bioassays are currently underway using laboratory-reared beetles. Future studies will include the effect of low dosages on brood survival and examination of interactions between biocontrol fungi, ambrosia fungi, and beetles.
- Role of team members: Drs. Castrillo and Vandenberg are assessing microbial control agents against ambrosia beetles and their symbiotic fungi.

TECHNOLOGY TRANSFER/IMPACT:

- Growers are implementing the use of ethanol-baited traps for monitoring ambrosia beetle seasonal activity to accurately pinpoint insecticide applications. The benefits of elevated ethanol release rates are being realized, along with combining ethanol and alpha-pinene for increasing trap counts of *X. germanus.*

- The trap height studies will allow more effective monitoring and trapping of *Xylosandrus* and other ambrosia beetle species by deploying the traps at optimal heights (<1.7m). Improved trapping will lead to better monitoring and timing of insecticide sprays.
- The ethanol-injection technique optimized by the USDA-ARS in Wooster has been adopted by several labs for rapidly inducing ambrosia beetle attacks on specific trees, and now allows for conventional and botanical insecticides to be evaluated. The following manuscript describing the technique has been accepted for publication:
    - Ranger, C. M., M. E. Reding, A. B. Persad, and D. A. Herms. Ability of Stress-Related Volatiles to Attract and Induce Attacks by *Xylosandrus germanus* and other Ambrosia Beetles (Coleoptera: Curculionidae, Scolytinae). Agricultural and Forest Entomology.
- Insecticide efficacy experiments have assisted growers in choosing the most effective product, namely, permethrin. The promise of botanically-based repellents for preventing ambrosia beetle colonization is also being conveyed to growers.
- 78 new isolates of *Ambrosiella* sp. from ambrosia beetle *X. germanus*, representative of beetle populations in New York, Ohio, and Virginia, are now in storage at the USDA-ARS in Ithaca, New York. A protocol has also been developed for rearing *X. germanus* on artificial diet, along with an assay for testing microbial control agents against ambrosia beetles and symbiotic fungi.
- Numerous presentations, abstracts, and proceedings on our FNRI-funded ambrosia beetle research have been presented at international and national meetings scientific meetings, along with grower-based extension meetings.

ADDITIONAL FUNDING/EXTERNAL SUPPORT:

- Schultz, P. B. Virginia Nursery and Landscape Association. 2007- 2009. $17,500.
- Oliver, J.B., Ochieng, S., and C. M. Ranger. Tree Injection and other Methods to Improve Trapping of Buprestid, Cerambycid, and Scolytid Borers. USDA-APHIS, Request for Technology Development Proposals to Improve Detection and Survey for Exotic Wood Borers. 2009. $100,000.
- Zhu, H., and C. M. Ranger. Effects of Atmospheric Humidity on Evaporation Rates of Pheromone. USDA Forest Service. 2009. $50,000.
- Vandenberg, J. D., Castrillo, L. A., Ranger, C. M., and M. E. Reding. Testing and development of fungal biocontrol agents against ambrosia beetles and their symbiotic fungi. Submitted to the Horticultural Research Institute in 2009.