MYCOPATHOGENS AND THEIR EXOTOXINS INFECTING THE GLASSY-WINGED SHARPSHOOTER: SURVEY, EVALUATION, AND STORAGE.

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ABSTRACT
A species of *Hirsutella,* the primary pathogen of glassy-winged sharpshooter (GWSS) in the southeastern US, has been the major focus of our research this past year. Due to the fastidious growth requirements of this fungus and the presence of numerous saprobic fungi associated with mycosed GWSS, a major effort has been made to design a series of gene-specific primers to be used to detect these diseases in field collected samples. Molecular-based diagnosis is being used to examine the hundreds of mycosed insects collected during the 2003 and 2004 regional surveys. A second effort has been directed at examining the seasonal incidence of this disease in an experimental crape myrtle plot. A number of parameters such as crape myrtle variety, host density, and mist irrigation (humidity) have been found to influence the onset of *Hirsutella* in GWSS populations. Current laboratory research is being directed at examining transmission of the lab culture to both GWSS and to alternate insect hosts. In addition, culture filtrates of all of the fungi collected from GWSS are being assessed for the presence of active metabolites.

INTRODUCTION
We are not aware of any studies that have examined the dynamics of pathogens associated with populations of GWSS in its native range. In general, the lack of pathogens (viral, bacterial, or protozoan) in leafhopper populations may be related to their piercing-sucking feeding behavior. In most cases, these pathogen groups are transmitted orally and would likely need to inhabit the xylem tissue to infect leafhoppers. Pathogens that are transmitted *per os* are typically affiliated with insects with chewing mouthparts. Thus, entomopathogenic fungi, which do not need to be ingested in order to infect insects, are considered to contain the primary pathogens of sucking insects. Indeed, the primary pathogens operating against insects such as whiteflies, scales, aphids, spittlebugs, plant hoppers, and leafhoppers are insect fungi (for listing see USDA-ARS Collection of Entomopathogenic Fungal Cultures at http://www.ppru.cornell.edu/mycology/catalogs/catalog). We commonly observe all mobile stages of GWSS exhibiting mycoses in north Florida and we are identifying them and assessing their impact. Since the last report we have concentrated on an assessment of the population dynamics of fungi and GWSS in the field. The incidence of *Hirsutella homalodisca* (*H. homalodisca*) in a GWSS population on crape myrtle was monitored in a preliminary manner in 2004 and then as part of a more exhaustive approach in 2005. Disease incidence was significantly lower in 2005. GWSS population levels were moderately lower at all sample dates available for comparison. One cultivar group was preferred to a significant level, but it is suspected that this was due to the effects of immigration. Most diseased cadavers appeared after the conclusion of the F_1 generation population peak, but at much lower incidence than in 2004. *Pseudogibellula formicarum* was found to colonize a high proportion of GWSS cadavers after mycosis by *H. homalodisca.*

OBJECTIVES
1. Identify and archive all the major pathogens affiliated with GWSS populations.
2. Estimate the distribution, frequency and seasonality of the major diseases of GWSS.
3. Screen the pathogens for exotoxins with potential toxicity to GWSS and other arthropods.
4. Confirm infectivity of the isolates and the exotoxins and determine which if any pathogens may serve as microbial controls of GWSS and other leafhopper vectors.

RESULTS
The presence of various opportunistic fungi on field-collected samples has limited our abilities to culture the more fastidious slow growing species of *Hirsutella*, *Sporothrix*, and *Pseudogibellula.* The aforementioned fungi were identified last year to be key entomopathogens isolated from GWSS populations. We have developed and optimized PCR primers within unique intron motifs of both the actin and tubulin genes that have been matched with primers from the open-reading frame. Control reactions have demonstrated that these primer combinations are able to specifically amplify the GWSS *Hirsutella* from DNA extracted from mummies. This technology is being used to screen the more than 250 DNA samples extracted from mycosed GWSS collected from throughout the southeastern U.S. This work has been summarized and submitted for publication.

A crape myrtle field plot was utilized to track a population of GWSS over the course of the 2005 summer season. The plot consisted of 4 replicates of 14 crape myrtle cultivars, with each cultivar represented by 4 adjacent trees in each replicate. Based on data collected in 2004, 4 cultivars were selected for intensive sampling and observation of both live and diseased GWSS. "Biloxi", "Osage", "Miami", and "Tonto" cultivars were selected, as they had demonstrated the highest incidence of mycosed GWSS in the previous season. Cultivar group position was completely randomized within the plot. The 4 replicates were divided into two treatments, misted and ambient. In the misted replicates, a 6' diameter emitter was staked above each tree in 10 of the 14 cultivars. This system was controlled by an automatic timer, which allowed the misters to run the first 15 min. of every hour, 24 hours a day, 7 days a week. This ensured that each misted tree remained under very high humidity conditions. The remaining two replicates were subjected only to the prevailing environmental conditions. Each replicate was sampled on a weekly basis. The individual trees were visually sampled for live GWSS by running a curved tool behind each branch and counting the insects as they displayed evasive behaviors. Sampling was performed between 08:00 and 12:00, a period of lower GWSS activity. Immediately following live sampling, each branch of the tree was visually inspected on all sides for the presence of mycosed insects. Those found were marked by tying a piece of surveyor's tape around the branch 10-15 cm below the cadaver. The tape was then marked with a number. This enabled development of detailed records on each individual mummy and monitoring of any change in its condition.

In addition, a 229 m grid with 51 locations was set up in the 60 ha area surrounding the field plot and 27 yellow sticky traps consisting of 7.5 x 15 cm mailing tubes on 1 m stakes were distributed to half the grid points at random. Trap placement was randomized each week among the 51 locations. The GWSS on the traps were counted weekly. Sticky trap counts indicated two peaks in GWSS numbers over the course of the 2005 study, the highest coming in week 7 and a much smaller peak at week 16. Visual GWSS counts in the misted portion of the first replicate closely mirror this trend with a delay of approximately 1-2 weeks. Counts in the dry portion of replicate 1, as well as both portions of replicate 2 show little homology to background numbers. Least squares means analysis of significant effects found in the repeated measures procedures revealed significant differences between both cultivar and humidity treatments were primarily due to the action of the crape myrtle cultivar "Biloxi" in the misted portion of the first replicate. This cultivar group held the highest numbers of leafhoppers of any group throughout the entire first population peak, often by a factor of 3. Most significant differences between treatment and cultivar were found within this peak. The exception to this phenomenon was that within the misted portion of the first replicate, leafhopper numbers on the cultivar "Tonto" were significantly lower when compared to "Osage" in weeks 16 through 18 and when compared to "Biloxi" in week 19. These differences, however, were not of great enough magnitude to affect between-treatment interactions. It is possible that the preference for "Biloxi" in the first peak was due to its placement in the plot, as it was located at the corner closest to a natural forest habitat and may be an immigration point for GWSS entering the plot. If this group of four trees is discounted there were no recognizable trends for preference of cultivar or irrigation treatment.

In 2005, the incidence of cadavers killed by *Hirsutella homalodisca* within the field plot did not closely follow fluctuations in GWSS populations. Mycosed cadavers appeared with greatest regularity beginning at the tail of the first population peak, until week 20. This time period was characterized by lower GWSS populations, but also by almost weekly shifts in host cultivar preference. Sticky trap data show much lower populations after week 11, but while plot populations were lower, the difference was not as pronounced. Mean cadaver numbers per tree were significantly higher in 2004 than 2005 ($t = 7.43$, $p < 0.0001$). The last sample time for the plot in 2004 occurred from 8/17/04 to 8/28/04. During this time, mean GWSS cadavers per tree was 4.44 ± 3.74 ($N = 64$). The same trees sampled on 8/24/05 had 0.31 ± 0.48 mean cadavers per tree. Total cadaver number for all trees at this time in 2004 was 284 compared to 20 for 2005. There is no obvious explanation for this, though host/pathogen interactions are often cyclical in insect systems. On all sample dates in 2004 where live GWSS counts were taken, leafhopper numbers were higher than in 2005, suggesting a higher total population in 2004. Whether a critical host density threshold was met in 2004 but not in 2005, is unknown, but may represent the most likely explanation for 2004 cadaver counts surpassing those from 2005 by more than an order of magnitude. Differences in cadaver numbers between misted and dry replicates were pronounced in 2004, but slight in 2005. This slight difference was probably an artifact owing to the much higher host numbers on the "Biloxi" cultivar in the first misted replicate. By revisiting the same GWSS cadavers every week and noting their condition, it was possible to ascertain that individuals initially displaying the *Hirsutella homalodisca* phenotype frequently developed *Pseudogibellula formicarum* morphologies later in the season. Currently, September 25, 2005, 46% of those cadavers found in the 2005 study present some degree of *P. formicarum* morphology, with the expected proportion to be higher once removed from the field and examined with the microscope. All of these cadavers originally sporulated as *H. homalodisca*, with *P. formicarum* probably acting as a secondary saprophyte of the cadaver. These findings run counter to a previous report identifying *P. formicarum* as a primary fungal pathogen of GWSS in the southeastern U.S. (Kanga et al. 2004).

CONCLUSIONS

We have identified and have in culture several isolates of a primary pathogen and potential GWSS biological control agent, *Hirsutella homalodisca*. Molecular methods have been established and are being used to diagnosis GWSS collected from sites throughout the southeastern US. This past two field season the dynamics of *H. homalodisca* has been examined in replicated crape myrtle plots. Mycosed GWSS developed throughout the mid-later part of the growing season in both years.

A large proportion of the mycosed GWSS infected with *H. homalodisca* later showed symptoms of *Pseudogibellula formicarum* suggesting that the later fungi may not be a primary pathogen of GWSS.

REFERENCES

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