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FUNGAL PATHOGENS OF THE GLASSY-WINGED SHARPSHOOTER *HOMALODISCA COAGULATA* (HOMOPTERA: CICADELLIDAE)

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The glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Homoptera: Cicadellidae), is native to the southeastern United States, but was reported in California in 1990 (Sorensen & Gill 1996). This homopteran pest is now widely established in southern California (Blua et al. 1999), and it is also present in northern and central California (Phillips 1998). The sharpshooter feeds and reproduces on an extremely wide variety of plants (over 130 plant species) (Phillips 1998).

The major problem associated with the sharpshooter is its ability to vector and transmit several strains of the pathogenic bacterium, *Xylella fastidiosa* (Well & Raju), that cause diseases in many plants. One of the most important of these infections is Pierce's disease of grapevines. Bacterial pathogens clog the xylem, and xanthum gum production exacerbates water stress in susceptible hosts. Infected plants display scorch-like symptoms before dying of dehydration. This vector is a serious new threat to vineyards because it moves faster and further into vineyards than other known vectors (Phillips 1998).

Chemical control with pyrethroids and neonicotinoids looks promising against immature and adults, but it is associated with residue contamination and interferes with biological control strategies (Varela et al. 2001). The most commonly used compounds for protecting *Xylella*-susceptible plants is imidacloprid, which is registered for home and landscape, and for use on non-food crops. Insecticidal soaps and oils are the least toxic and disruptive chemical compounds to biological control, but these chemicals are only effective against soft-bodied nymphs of the sharpshooter (Varela et al. 2001).

Biological control methods are being investigated to reduce populations of the sharpshooter. Currently, the control strategies focus on several natives and imported egg parasitoids from South America. The most important parasite released against the sharpshooter is *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae). Although the rate of egg parasitism by *G. ashmeadi* can reach 80% in Florida and Louisiana, and up to 100% in California (Phillips 1998; Triapitsyn et al. 1998), large numbers of the sharpshooter remain throughout the season (Varela et al. 2001).

Very little is known about predators, parasitoids, and pathogens that attack the sharpshooter nymphs and adults. We examined specimens of several populations of the glassy-winged sharpshooter where an epizootic of fungal infections were widespread in the fall of 2002 in Poplarville, Mississippi. We collected samples of about 30 glassy-wing sharpshooter cadavers that had died on crape myrtle (*Lagerstroemia indica* L.) and holly (*Ilex myrtifolia* Walter) for identification of the pathogens. The fungus found on some cadavers of sharpshooters bearing conidiophores was identified to be *Trichothecium roseum* (Pers.) Link. Several other specimens were covered with *Beauveria bassiana* (Bals.) Vuillemin.

To confirm the identification of the primary pathogen, another set of twelve specimens of glassy-winged sharpshooters collected was surface-sterilized by dipping them successively in 65-70% ethanol (10-15 min), 2% sodium hypochlorite solution (2-3 min), and sterile water (20-40 seconds). Smears or whole specimens were then plated on Petri plates (10.0 × 1.5 cm) containing either oatmeal agar or Sabouraud maltose agar (Difco, Detroit, MI) supplemented with 1% yeast extract (SMAY), and incubated at 27 ± 1°C, 85% RH for 7-14 days. After incubation, both fungi, *T. roseum* and *B. bassiana* were not found on the Petri plates, suggesting these fungi probably invaded the specimens after they were killed by another fungal pathogen. Madelin (1996) reported that *T. roseum* is not a virulent pathogen of many insects while *Trichothecium acridiorum* (Trabut) Madelin appears to be the main entomogenous species of this genus (Madelin 1996). Both of these species grow and sporulate relatively easily on a wide range of media and should probably be best regarded as nonfastidious saprobes. Similarly, the fungus, *B. bassiana* occurs worldwide but its main natural hosts are usually Lepidoptera, Coleoptera, Hemiptera, Diptera, and Hymenoptera rather than Homoptera, although

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B. bassiana can affect a wide range of homopteran hosts (Li 1988).

Further, after incubation of the inoculum from the white ball of conidia from the cadavers of the glassy-winged sharpshooter, we found that the primary fungal pathogen is a species in the genus *Pseudogibellula* (Samson & Evans 1973). The morphology of the conidiophores is similar to those of *Pseudogibellula formicarum* (Mains) Samson & Evans (1973); there is a roughened stalk tapering towards the top end and forming a slightly bulbous swelling at the apex that then bears nearly globose cells in one or two layers. These cells bear a single, spherical outer layer of conidiogenous cells.

The overall characteristics of this species from Mississippi indicate that the fungus is *Pseudogibellula formicarum* (Mains) Samson & Evans (1973). In addition, we have isolated recently *P. formicarum* from dead glassy-winged sharpshooters collected from Weslaco, TX and tested the fungus for pathogenicity. Further, virulence and pathogenicity of *P. formicarum* to homopterans in the Ricaniidae and Cercopidae, mymicine and ponerine ants, spiders, and lagriid beetles has been reported (Samson & Evans 1973).

To determine the pathogenicity of *P. formicarum* (ARSEF, Ithaca, NY) to the sharpshooter, we cultured the fungus on SMAY, and incubated the Petri plates at 27 ± 1°C, 85% RH, and 13:11 (L:D) h photoperiod. We collected 14-day-old cultures and used them in each experiment. We also tested 14-day-old cultures of *Metarhizium anisopliae* (Metschinkoff) 5630 (EcoScience, New Brunswick, NJ) against the sharpshooter. Conidia concentration was determined with a hemocytometer. Both fungi were serially diluted in a solution of 0.02% Silwet L-77® (Loveland Industries Inc., Greeley, CO) to provide the concentrations needed for the bioassays.

Pseudogibellula formicarum was tested at concentrations of 2×10^8 and 2×10^9 conidia ml^{-1}, and *M. anisopliae* was tested at a single concentration of 2×10^8 conidia ml^{-1}. For each concentration, 5-7 sharpshooters were transferred to a glass Petri plate containing an ice-cold (4°C) and wet Whatman filter paper (90 mm diameter). The sharpshooters were sprayed with 1 ml of the conidial suspension in a Potter Precision Spray Tower (Burkhard Manufacturing, Rickmansworth, England) with 0.7 kg cm^{-2} pressure and a 0.25 mm orifice diameter nozzle. There were two replicates per fungal concentration and sharpshooters treated with deionized water containing 0.02% Silwet L-77® served as controls. After being sprayed, the sharpshooters were transferred to young seedlings of Cowpea (*Vigna unguiculata* L.) as food source. The seedlings were placed in Plexiglass cages (46 × 46 × 46 cm) (BioQuip Products, Gardena). The cages were maintained at 27 ± 2°C, 85 ± 2% RH in the greenhouse and in the Percival Scientific incubators (Percival Manufacturing Company, Boone, IA).

To determine conidia viability at the time of each experimental run, each concentration of fungal suspension was sprayed onto three Petri dishes containing SMAY. The conidia were incubated for 20 h at 27 ± 1°C, 85% RH. After incubation, three droplets of lactophenol cotton blue stain (0.5% cotton blue) were added to each Petri dish to fix and stain the conidia, preventing any further germination from occurring in the sample. The droplets were covered with a glass slide and evaluated with 400× phase-contrast magnification. The number of conidia that germinated in the first 100 conidia observed under the microscope was determined for each of the three droplets on each slide.

Dead sharpshooters were collected daily from the fungal treatments and the controls, and tested in the following way to determine if mortality was due to infection. The sharpshooters were surface-sterilized as described above. They were then transferred with a camel-hair brush to Petri dishes containing SMAY and incubated at 27 ± 1°C, 85% RH for 7-14 days. The Petri dishes were sealed with parafilm prior to incubation and the dead sharpshooters were observed daily for the presence of external fungal hyphae. The number of dead sharpshooters with external hyphae was counted, and to reduce the possibility of cross contamination, these sharpshooters were removed from the Petri dishes. Only sharpshooters that showed fungal growth were considered to have died of infection and used to compute the pathogenicity of the fungal pathogens.

Mean conidial germination was 97.6 ± 0.5% for *P. formicarum* and 98.6 ± 0.5% for *M. anisopliae.* Dead sharpshooters collected daily from the control samples showed no infection by *P. formicarum* after 21 days experimental period. In contrast, sharpshooters treated with 2×10^9 spores ml^{-1} and held in the incubators showed infection by the fungus ranging from 66% at day 7 to 93% 21 days posttreatments (Table 1). In greenhouse tests fungal infection ranged from 25% at day 7 to 81% at day 21 after the treatments were initiated (Table 1). A total of 76 dead sharpshooters collected from the treatment groups were investigated for fungal infection, and 70% of them showed mycosis at the end of 21 days of the experiments. Unlike the controls, the percentage of dead sharpshooters infected with *M. anisopliae* range from 20% at day 7, 50% at day 14, and 75% at 21 days after the fungal treatments (Table 1).

Overall, the glassy-wing sharpshooter was found to be suitable host for both *P. formicarum* (Figs. 1 and 2), and *M. anisopliae* (Fig. 3). However, a comprehensive study of virulence of the fungi has yet to be investigated. It appears that both fungal pathogens have the potential to control the glassy-wing sharpshooter.

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TABLE 1. RECOVERY OF *PSEUDOGIBELLULA FORMICARUM* AND *METARHIZIUM ANISOPLIAE* FROM THE GLASSY-WING SHARPSHOOTER (GWSS), *HOMALODISCA COAGULATA* AFTER TREATMENTS.

Site	Treatments	N[a]	Infection of GWSS by *P. formicarum* after[b]		
			7 days	14 days	21 days
In Incubators	Control	15	0%	0%	0%
	2×10^8	15	17%	60%	75%
	2×10^9	20	66%	91%	93%
In Greenhouse	Control	12	0%	0%	0%
	2×10^8	13	10%	33%	48%
	2×10^9	15	25%	52%	81%
			Infection of GWSS by *M. anisopliae* after[b]		
			7 days	14 days	21 days
In Incubators	Control	10	0%	0%	0%
	2×10^8	13	20%	50%	75%

[a]Number of glassy-wing sharpshooters tested.
[b]Dead sharpshooters collected daily from treated and control samples were surface-sterilized, plated onto Petri dishes containing SMAY, and incubated at 27 ± 1°C, 85% RH to investigate the recovery of the fungi.

SUMMARY

An epizootic of fungal diseases on glassy-winged sharpshooters was examined in the fall of 2002 in Mississippi. *Trichothecium roseum* and *Beauveria bassiana* were identified from the cadavers of the sharpshooter bearing conidiophores, but these fungi appeared to be either secondary pathogens or saprobes rather than primary pathogens of the sharpshooter. *Pseudogibellula formicarum* was determined to be the cause of the epizootics. However, additional studies are need to provide a better understanding of host-pathogen interactions, and identify the factors that enhance or limit disease increase in sharpshooter populations under natural conditions. In addition, the sharpshooter was found to be also a suitable host for *Metarhizium anisopliae.* Overall, *P. formicarum* and *M. anisopliae* could provide new avenues for the biological control of the glassy-winged sharpshooter and complement current control strategies.



Fig. 1. Mycelia of *Pseudogibellula formicarum* emerge from dead glassy-wing sharpshooters collected from the treated samples after 4 days incubation at 27 ± 1°C, 85% RH. The sharpshooters were surface-sterilized and plated on SMAY to investigate the recovery of the fungus.

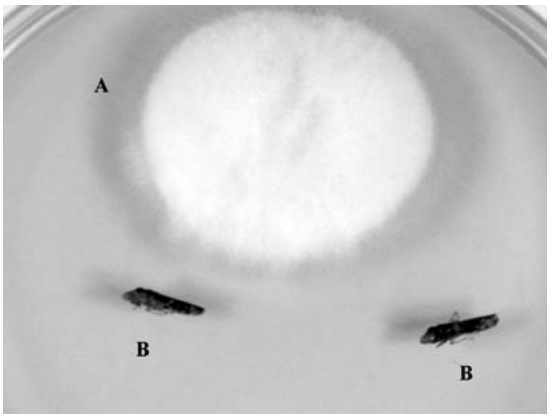


Fig. 2. A dead glassy-wing sharpshooter collected from the treated samples is covered with mycelia and conidia of *Pseudogibellula formicarum* (A), while control sharpshooters showed no fungal infection (B) after 7 days incubation at 27 ± 1°C, 85% RH. The sharpshooters were surface-sterilized and plated on SMAY to investigate the recovery of the fungus.

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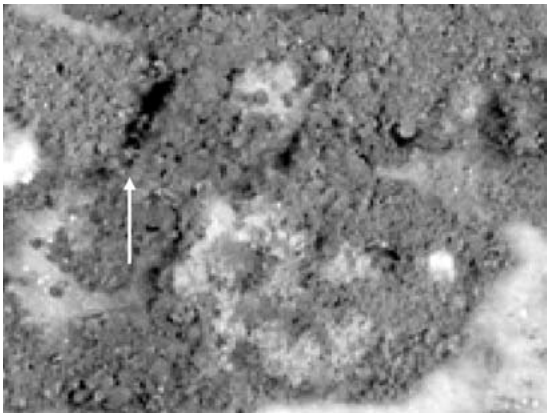


Fig. 3. Dry conidia of *Metarhizium anisopliae* emerge from dead glassy-wing sharpshooters collected from the treated samples after 14 days of incubation at 27 ± 1°C, 85% RH. The sharpshooters were surface-sterilized and plated on SMAY to investigate the recovery of the fungus.

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