MICROBIAL CONTROL OF THE GLASSY-WINGED SHARPSHOOTER WITH ENTOMOPATHOGENIC FUNGI

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ABSTRACT
Objectives of our study were to search for fungal pathogens of the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say) and evaluate their potential against the host. Searches within citrus orchards in Tulare and Riverside counties revealed no natural infections of entomopathogenic fungi in GWSS populations. Entomopathogenic fungi were also absent in cadavers of GWSS periodically collected from Riverside citrus orchards (courtesy CDFA) when incubated in the laboratory under ideal conditions for fungal emergence. However, about 140 isolates of *Beauveria bassiana* (Balsamo) Vuillemin and four isolates of *Metarhizium anisopliae* (Metschnikoff) Sorokin, both hyphomycetous fungi, were isolated from soil in GWSS habitats and other insect hosts. Some of these isolates along with a Weslaco isolate of *B. bassiana* from GWSS and a commercial *B. bassiana* isolate have been tested against GWSS. Preliminary results indicate that GWSS is susceptible to high concentrations of these fungi.

INTRODUCTION
The glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say), native to the southeastern United States, is a serious pest of the California grape industry because it vectors *Xylella fastidiosa* (Wells et al. 1987), a xylem-limited bacterium that causes Pierce's disease (PD). Although PD has been in California for a long time, the introduction and rapid spread of GWSS made the situation worse. In addition to grapes, GWSS has a wide host range and spreads various diseases in those hosts caused by *X. fastidiosa*. Vector control or avoidance has been a key tactic in controlling PD. Widely practiced chemical control with imidacloprid and application of kaolin particles have their limitations. While kaolin particles, although non-toxic, can leave unwanted deposits on the harvested grape bunches, chemical insecticides have undesirable effects including human health, impact on non-target organisms, and environmental concerns. Moreover, use of chemical insecticides in citrus disrupts the successful, long-term control afforded by IPM of many different citrus pests (Grafton-Cardwell and Kallsen 2001). Use of microbial agents, such as entomopathogenic fungi, can be a viable alternative that is compatible with IPM practices. Entomopathogenic fungi invade the host by penetrating through the integument and are appropriate candidates for GWSS that has piercing and sucking mouthparts.

Entomopathogenic fungi have been isolated from GWSS (Mizell and Boucias 2002, Jones - personal communication) and other cicadellids (Galaini-Wraight et al. 1991, Hywel-Jones et al. 1997, Magalhaes et al. 1991, Matsui et al. 1998, McGuire et al. 1987). The purpose of our study is to discover additional isolates of entomopathogenic fungi active against GWSS.

OBJECTIVES
1. Conduct surveys to find fungal infections in GWSS populations or insects closely related to GWSS and isolate soilborne entomopathogens from GWSS habitats.
2. Culture and isolate the fungi and evaluate their pathogenicity against GWSS.
3. Evaluate the host range of fungi that infect GWSS.
4. Conduct small-scale field tests to evaluate selected pathogens against GWSS on citrus in fall and winter.

RESULTS
Natural Infections in GWSS Populations
Citrus orchards in Tulare and Riverside counties were surveyed, in vain, for infected GWSS. GWSS cadavers from CDFA collections in the Riverside area were periodically obtained and incubated in the laboratory for fungal development. No entomopathogenic fungus has so far been found from these cadavers. However, cultures of *Beauveria bassiana* (Balsamo) Vuillemin from infected GWSS collected in Texas by Jones and *Hirsutella* spp collected in Florida by Mizell and Boucias were received in the past two months for testing against California GWSS.

Isolation of Fungal Pathogens

Soil samples were collected from an organic citrus orchard and a conventional pomegranate orchard in Tulare Co, CA and a citrus orchard at AgOps at UC Riverside. Fungal pathogens were isolated using larvae of the greater wax moth, *Galleria mellonella* L. and by soil plating on selective media. Waxworms were incubated in Petri plates with moist soil samples and fungal pathogens were isolated from cadavers. Alternatively, aliquots of soil suspensions were plated on media selective for *B. bassiana* and *Metarhizium anisopliae* (Metschnikoff) Sorokin. So far, 140 *B. bassiana* isolates and 4 *M. anisopliae* isolates have been isolated (Table 1). Additionally, *B. bassiana* was also isolated from the California harvester ant, *Pogonomyrmex californicus* Buckley, collected in Shafter, CA and the three-cornered alfalfa hopper, *Spissistilus festinus* (Say), collected in Parlier, California. Fungal isolates were cultured on selective and non-selective media to multiply the inoculum.

Table 1. Fungal pathogens isolated from citrus and pomegranate orchards and infected insects

Source	Method	*B. bassiana*	*M. anisopliae*
Organic citrus in Tulare Co	Waxworm bait	37	-
Pomegranate in Tulare Co	Waxworm bait	3	4
Riverside citrus	Waxworm bait	78	-
Riverside citrus	Selective media	22	-
California harvester ant	Selective medium	1	N/A
Three-cornered alfalfa hopper	Selective medium	1	N/A

Pathogenicity of Entomopathogenic Fungi to GWSS

Laboratory-reared or field-collected GWSS adults supplied by CDFA, Arvin were used for the bioassays. GWSS were either placed at -5^{o} C for 5 min or exposed to CO_2 for 15 sec to immobilize them and were inoculated by rolling them in a 10 µL drop of conidial suspension. Controls were treated with 0.01% of SilWet, an adjuvant used for preparing conidial suspensions. GWSS were individually incubated in a Petri plate with an excised citrus leaf and a moist filter paper. Petri plates were placed in a plastic box with moist paper towels and incubated at 27^{o} C and 16:8 L:D photophase. GWSS were observed daily for mortality. Dead GWSS were surface sterilized in 3% sodium hypochlorite solution followed by rinsing in deionized water and incubated in sealed Petri plates on water agar or moist filter paper at 27^{o} C in the dark.

Bioassay 1

The isolate of *B. bassiana* from *P. californicus* (PcBb1) was tested against laboratory-reared GWSS at four concentrations 10^1, 10^3,10^5, and 10^7 conidia/ml in comparison with controls. Each treatment and control had 10 adult GWSS. Infections were observed only at higher concentrations with 50% infection in GWSS treated with 10^7 conidia/ml and 10% in those treated with 10^5 conidia/ml.

Bioassay 2

Five *B. bassiana* isolates and a *M. anisopliae* isolate were tested against field-collected GWSS at four concentrations of 10^3, 10^5,10^7, and 10^9 (or 10^8 in case of *M. anisopliae*) conidia/ml along with untreated and SilWet (0.01%) treated controls. Isolates of *B. bassiana* included one from *P. californicus* (PcBb1), two from soil samples from citrus orchards in Tulare (GmBb25) and Riverside (GmBb41) counties, CA, one from *H. coagulata* in Weslaco, TX (TxBb) and a commercial isolate (designated GHA). The isolate of *M. anisopliae* (GmMa1) was from a soil sample from the pomegranate orchard in Tulare Co, CA. Each treatment and controls had 20 GWSS. Although all tested isolates were infective (Figures 1 and 2), all GWSS in this bioassay, including controls, suffered from a high mortality.

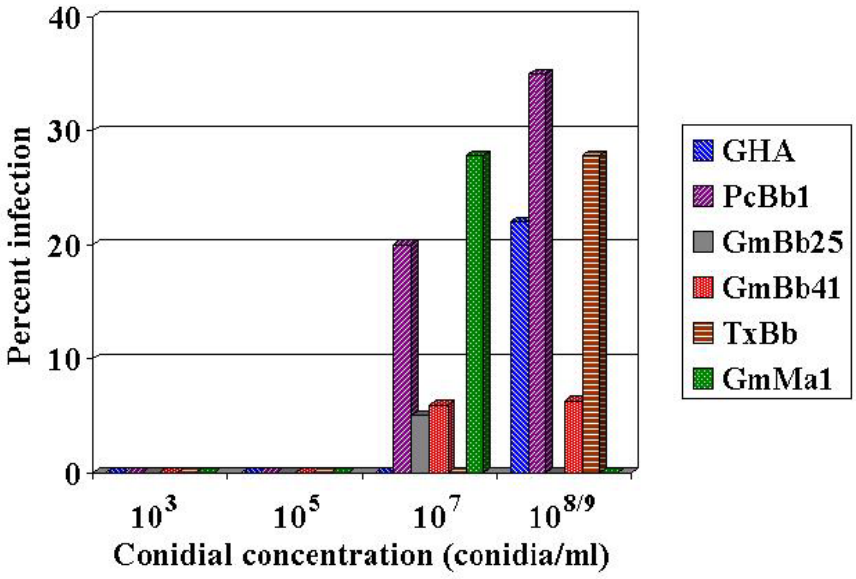

Figure 1. Pathogenicity of *B. bassiana* and *M. anisopliae* to GWSS

Figure 2. GWSS killed by *B. bassiana* and *M. anisopliae.*

Bioassay 3

This assay was conducted using only 10^9 conidia/mL concentration and 10 laboratory-reared GWSS per isolate. All the isolates from the previous bioassay were used in this assay except for PcBb1, which was replaced by the *B. bassiana* isolate from *S. festinus* (SfBb1). This assay had also suffered from very high mortality and all the insects died within 5 days after the treatment. Fungal infection was seen in only one GWSS cadaver treated with SfBb1.

CONCLUSIONS

The fact that GWSS is susceptible to entomopathogenic fungi such as *B. bassiana* is promising. Although infections occurred only at relatively high concentrations, there is enough variability in *B. bassiana* as a species to suggest other isolates may be more virulent. Efforts will continue to obtain isolates from collaborators and from likely GWSS host habitat in California for further laboratory evaluation and eventual field application.

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