EVALUATION OF SOME FUNGAL PATHOGENS FOR THE CONTROL OF THE GLASSY-WINGED SHARPSHOOTER

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REPORTING PERIOD: The results reported here are from work conducted October 2004 to September 2005.

ABSTRACT
Several isolates of the hyphomycetous fungi, *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin, were recovered from the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say), habitats and other insect hosts in southern California. Some of these isolates were evaluated against GWSS along with other fungal pathogens isolated from GWSS in Texas, Mississippi and Florida. Growth of the selected isolates was also evaluated at 15, 23, 28 and 32^{o}C. Two California isolates and a Texas isolate of *B. bassiana* were significantly more virulent to GWSS than other isolates. Although no natural fungal infections have been found in GWSS populations in California to date, we continue to search for them by periodical sampling in Kern, Riverside and Ventura counties.

INTRODUCTION
A collaborative project between UC Davis and USDA-ARS is aimed at identifying suitable entomopathogenic fungi for the control of the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say), a pest that threatens the grape industry in California as a vector of the Pierce's disease causing bacterium, *Xylella fastidiosa*. Entomopathogenic fungi, which enter the host through the cuticle, are ideal candidates for insects like GWSS with piercing and sucking mouthparts. Entomopathogenic fungi were isolated from GWSS in the southeastern United States (Mizell and Boucias 2002, Kanga et al. 2004), but no fungal pathogen has so far been reported in California GWSS populations. However, we recovered several isolates of two generalist fungi, *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin, from GWSS habitats in California and tested them against GWSS (Kaya et al. 2004). We also isolated *B. bassiana* from California harvester ant, *Pogonomyrmex californicus* (Buckley), three-cornered alfalfa hopper, *Spissistilus festinus* (Say) and a darkling beetle from Kern, Fresno and Riverside counties, respectively. Bioassay protocols were improved and experiments were conducted to compare efficacy of several fungi against GWSS.

OBJECTIVES
1. Conduct surveys to find fungal infections in GWSS populations or insects closely related to GWSS.
2. Culture and isolate the fungi and evaluate their pathogenicity against GWSS.
3. Assess environmental effects like temperature and sunlight on conidial survival and germination, fungal growth, and infectivity.
4. Evaluate the host range of fungi that infect GWSS.
5. Conduct small-scale field tests to evaluate selected pathogens against GWSS on citrus in fall and winter.

RESULTS
Natural infections in GWSS populations
We continue to search for natural infections in GWSS populations in southern California. GWSS adults were periodically collected from Kern, Riverside and Ventura counties on citrus, oleander and some weed hosts like mare's tail, mule-fat and Spanish tobacco. Insects were monitored in the laboratory for at least two weeks in attempts to recover infected individuals, but no entomopathogenic fungi have been found in these insects.
We tested several previously received isolates of *B. bassiana* recovered in Texas (Walker Jones, USDA-ARS now in Montpellier, France) and Mississippi (Russell Mizell and Drion Boucias, University of Florida) and a new species of *Hirsutella* recovered in Florida and Mississippi (Mizell and Boucias). We recently received GWSS infected with *Pseudogibellula formicarum* (Mains) Samson & Evans collected in Poplarville, MS by John Goolsby (USDA-ARS, Weslaco, TX) and have conducted preliminary experiments with the isolate.

Virulence of entomopathogenic fungi to GWSS

Beauveria bassiana

Laboratory-reared GWSS adults supplied by CDFA, Riverside were used for the bioassays. Seven isolates of *B. bassiana* - two from California GWSS habitats, one each from the California harvester ant, three-cornered alfalfa hopper, GWSS from Weslaco, TX, GWSS from Jackson, MS and a commercial isolate GHA (Emerald BioAgriculture) - were evaluated against GWSS. GWSS were anesthetized by exposing them to CO_2 for 20 sec and then inoculated by rolling them in a 10 µl drop of conidial suspension at 1 X 10^9 conidia/ml concentration. Controls were treated with 0.01% of SilWet, an adjuvant used for preparing conidial suspensions. GWSS were incubated on potted cowpea plants covered with cylindrical cages and their mortality was recorded daily for two weeks. Cadavers were surface sterilized in 3% sodium hypochlorite solution and incubated on water agar for fungal emergence. These assays were repeated four times. Three of the isolates – Texas isolate from GWSS (TxBb) and California isolates from three-cornered alfalfa hopper and soil (Bb 41) – caused significantly higher ($P < 0.01$) infections than others (Figure 1).

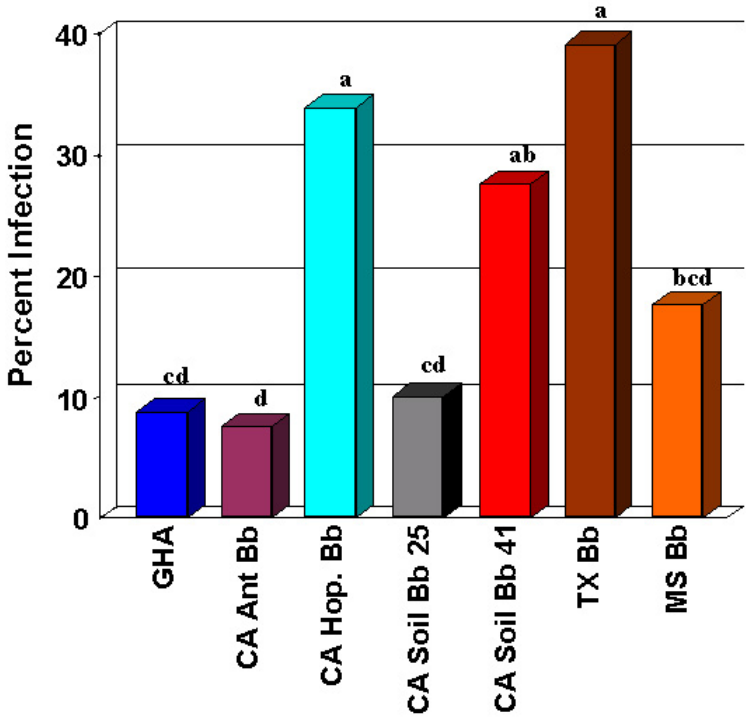

Figure 1. Virulence of *B. bassiana* to GWSS

***Hirsutella* spp.**

An assay was conducted to compare different isolates of *Hirsutella* spp., an isolate of *B. bassiana* and an unknown fungus, all recovered from natural infections in GWSS in Mississippi and Florida (provided by Mizell and Boucias). Due to poor conidial production of some of these isolates on standard culture media, hyphal bodies for all isolates were cultured on liquid glucose medium enriched with yeast extract. Treatments were administered either by injecting about 1 µl of the suspension at 1 X 10^9 hyphal bodies/ml through intersegmental membrane in the abdomen using a capillary tube or by rolling the insects in a 10 µl drop of the suspension (Figure 2). The rest of the procedure was similar to the one explained above. In general, higher mortality and infection resulted from injection than topical application (Figure 3).

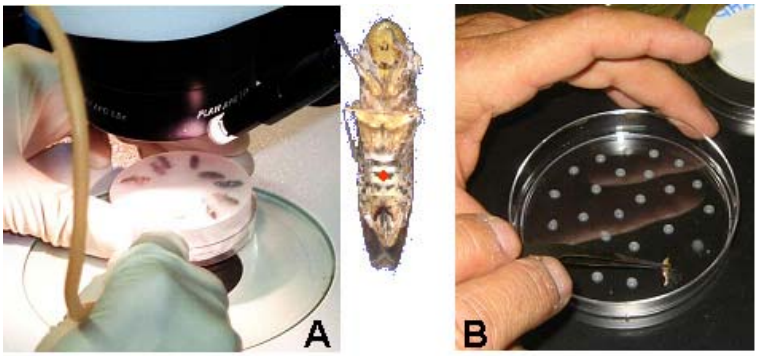

Figure 2. Injection (A) and topical application (B) of hyphal bodies.

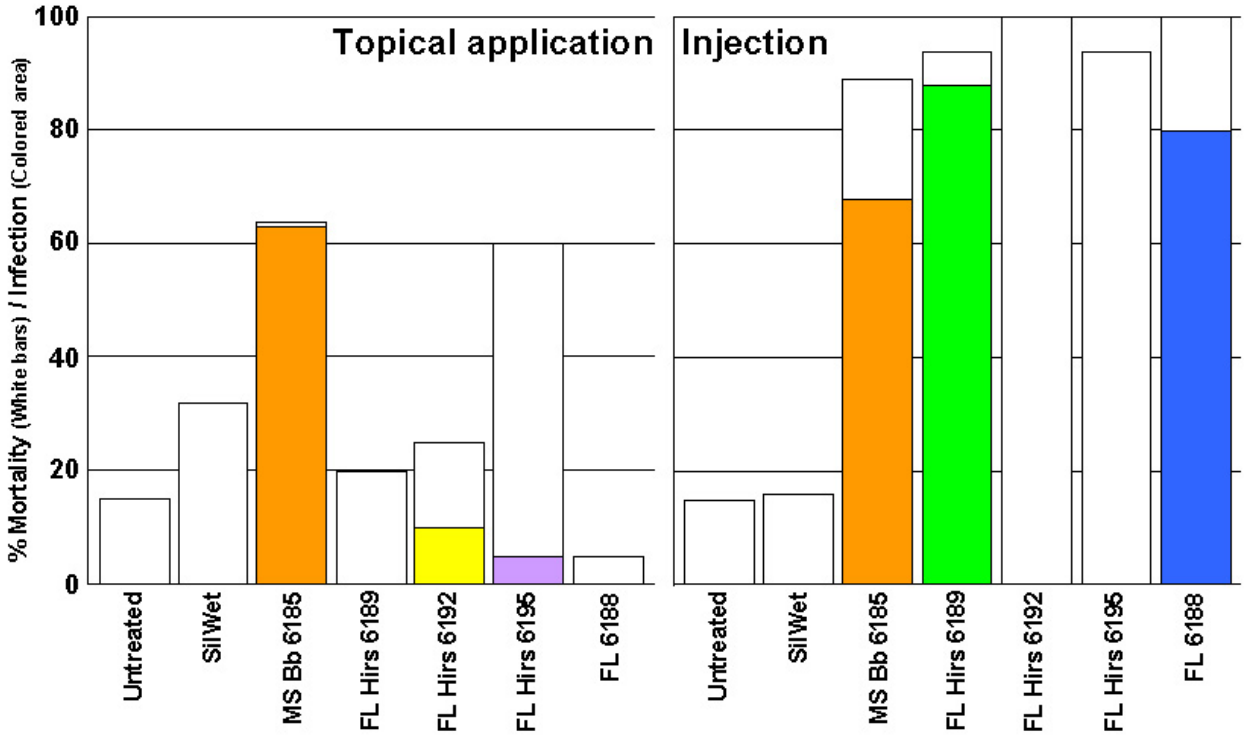

Figure 3. Pathogenicity of *B. bassiana* and *Hirsutella* spp to GWSS. White bars indicate percent mortality and colored area indicates percent infected among dead.

Pseudogibellula formicarum

Cadavers of GWSS with this fungus are frequently seen in the southeastern United States (Kanga et al. 2004, Mizell and Boucias, personal communication; Figure 4). Two small-scale assays were conducted where GWSS cadavers with sporulating *P. formicarum* were rubbed against healthy insects and incubated individually in clip cages attached to potted euonymus plants. So far no infection has been found in the treated insects.

Figure 4. GWSS infected with *P. formicarum*

Radial growth of some fungal isolates

An assay was conducted to determine the effect of temperature on the growth of some of the selected fungal isolates at 15, 23, 28 and 32 oC. A 9 mm disc was cut out from 3-5 d old fungal culture and incubated on Sabouraud dextrose agar medium enriched with yeast extract. Fungal growth was monitored for four weeks and average daily growth was determined. This assay was repeated thrice. Significant differences were found among the isolates ($P < 0.001$; Figure 5). Florida isolate of *Hirsutella* sp. (6192) outgrew *B. bassiana* isolates at higher temperatures. Among the three isolates that showed higher virulence against GWSS, growth rate of Bb 41 was lower than the other two isolates at all temperatures except 32oC while TxBb had the slowest growth rate at this temperature.

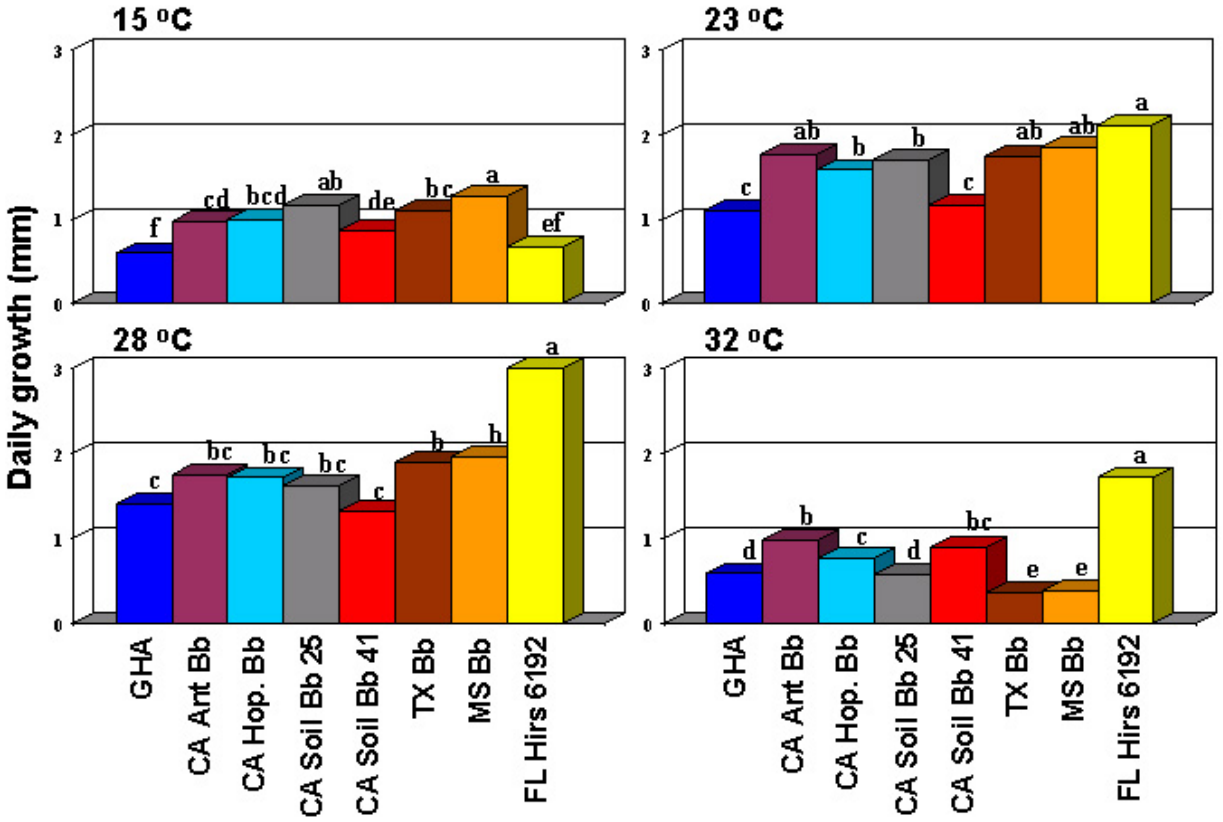

Figure 5. Radial growth of *B. bassiana* and *Hirsutella* sp. at different temperatures. Bars with the same letter are not significantly different (P <0.001).

CONCLUSIONS

The *B. bassiana* isolates from GWSS from Texas (TxBb) and three-cornered alfalfa hopper and soil (Bb 41) from California were significantly more virulent than other isolates against GWSS. However, their growth varied at different temperatures. These isolates will be thoroughly evaluated for their potential for GWSS control through various laboratory and field experiments. Search for natural infections in California populations of GWSS will continue.

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FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.