Transmission of the Mycopathogen, Hirsutella spp., to Nymphs and Adults of the Glassy-Winged Sharpshooter, HomalodiscaVitripennis (=Coagulata), in the Greenhouse

Authors: Lietze, Verena-Ulrike, Mizell, Russell F., and Boucias, Drion G.

Source: Florida Entomologist, 94(1) : 106-108

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.094.0114

TRANSMISSION OF THE MYCOPATHOGEN, *HIRSUTELLA* SPP., TO NYMPHS AND ADULTS OF THE GLASSY-WINGED SHARPSHOOTER, *HOMALODISCA VITRIPENNIS (=COAGULATA)*, IN THE GREENHOUSE

VERENA-ULRIKE LIETZE[1], RUSSELL F. MIZELL III[2] AND DRION G. BOUCIAS[1]
[1]Entomology and Nematology Department, University of Florida, Gainesville, FL 32611

[2]North Florida Research and Education Center, University of Florida, Quincy, FL 32351

The mycopathogen *Hirsutella* spp. produces regular epizootics in Florida populations of the glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis*, a polyphagous, xylem-feeding cicadellid native to the southeastern U.S. and northeastern Mexico (Boucias et al. 2007). The GWSS transmits a lethal phytopathogenic bacterium, *Xylella fastidiosa*, and thereby threatens the production of economically important plants (Redak et al. 2004). Recent introduction of GWSS into southern California, French Polynesia, Tahiti and Hawaii has stimulated interest in identifying potential biocontrol agents against invasive populations (Boucias et al. 2007; Hunnicutt et al. 2008). During a field survey in Quincy, FL, a new species of *Hirsutella*, *H. homalodiscae* nom. prov., was detected in ~50% of mycosed sharpshooters (Boucias et al. 2007). Virtually nothing is known about the transmission of *Hirsutella* within GWSS populations. Our field observations have shown that *Hirsutella*-infected sharpshooters attach via rhizoids to the plant bark in the summer and often remain attached throughout the winter. Placed in a moist environment, overwintered cadavers produce conidiospores suggesting they serve as microhabitats protecting *Hirsutella* and facilitating transmission to the new generation of GWSS. This study examined potential transmission routes of *Hirsutella* under greenhouse conditions. Experimental treatments included topical spore application and choice-exposure to sporulating cadavers on host plants.

For insect rearing, field-collected, healthy adults without hyphal bodies (HBs) in their hemolymph were identified by non-destructive antennal bleeding (Breaux 2005) and maintained on caged, potted plants (soybean, *Glycine max*, cotton, *Gossypium hirsutum*, and cowpea, *Vigna unguiculata*) in a greenhouse (temperature 26-30°C, 14:10 h light:dark photoperiod). Leaves with egg masses were removed and hatching neonates were transferred to caged lemon basil (*Ocimum basilicum*). Soil was watered to saturation once daily. In each bioassay replicate, individual cotton or basil plants housing groups of 10-20 adults or nymphs, respectively, were covered with clear, gauze-covered acryl cylinders (15 cm diameter × 45 cm high). Mortality and infection were recorded daily by removing dead individuals from the soil surface and examining their hemolymph for HB propagation. Cadavers were maintained on water agar to record mycosis. After 3 weeks, each plant was examined for mycosed cadavers, and surviving GWSS were subjected to hemolymph examination. Statistical analyses were conducted using the Proc Genmod procedure and Ls means statement of the Statistical Analysis System (SAS) for Windows to compare mortality or infection responses by logistic regression (Neter et al. 1990; SAS 2004).

For topical application of *Hirsutella* spores, healthy adults were treated in three replicate assays by touching their ventral surface to either sporulating *in vitro* colonies of strain 3A (maintained at the UF Entomology/Insect Pathology lab, Gainesville, FL) (total n = 46 adults), to GWSS cadavers displaying spores of *Hirsutella* (n = 33), or to a nutrient agar plate (control, n = 35). Mortality in control, *in vitro*, and cadaver treatments was similar with 56 ± 11%, 74 ± 12%, and 60 ± 8%, respectively (χ^2= 2.48, P = 0.1155). Contact with agar, *in vitro* colonies or cadavers produced 0%, 13 ± 6% and 42 ± 5% infection, respectively, and only the latter treatment produced mycosis (28 ± 6%). Infection transmitted from cadavers was significantly higher than that transmitted from *in vitro* colonies (χ^2 = 8.02, P = 0.0046).

To examine whether co-existence of healthy and mycosed GWSS on a plant would result in disease transmission, either overwintered cadavers collected in Jan (stored at -80°C) or new cadavers collected in Jul/Aug were pinned to plants (10 per plant) and groups of healthy nymphs or adults were maintained on each plant for up to 3 weeks. Controls were conducted on plants without cadavers. The majority of pinned cadavers displayed sporulating *Hirsutella* mycelium within one week. New cadavers developed an unusually thick, white mycelium overgrowing the entire insect (Fig. 1A). Disease transmission was observed to varying degrees (Table 1). Dead exposed nymphs and adults (Fig. 1B and C), attached to the plant and displaying *Hirsutella*-induced mycosis, were seen as early as 7 and 12 d after exposure, respectively. Hemolymph-borne HBs were detected as early as 8 d after exposure. New cadavers were more efficient in disease transmission when compared with overwintered cadavers (Table 1). When nymphs were exposed to new cadavers, no survivors were found and all dead insects were overgrown with thick mycelium

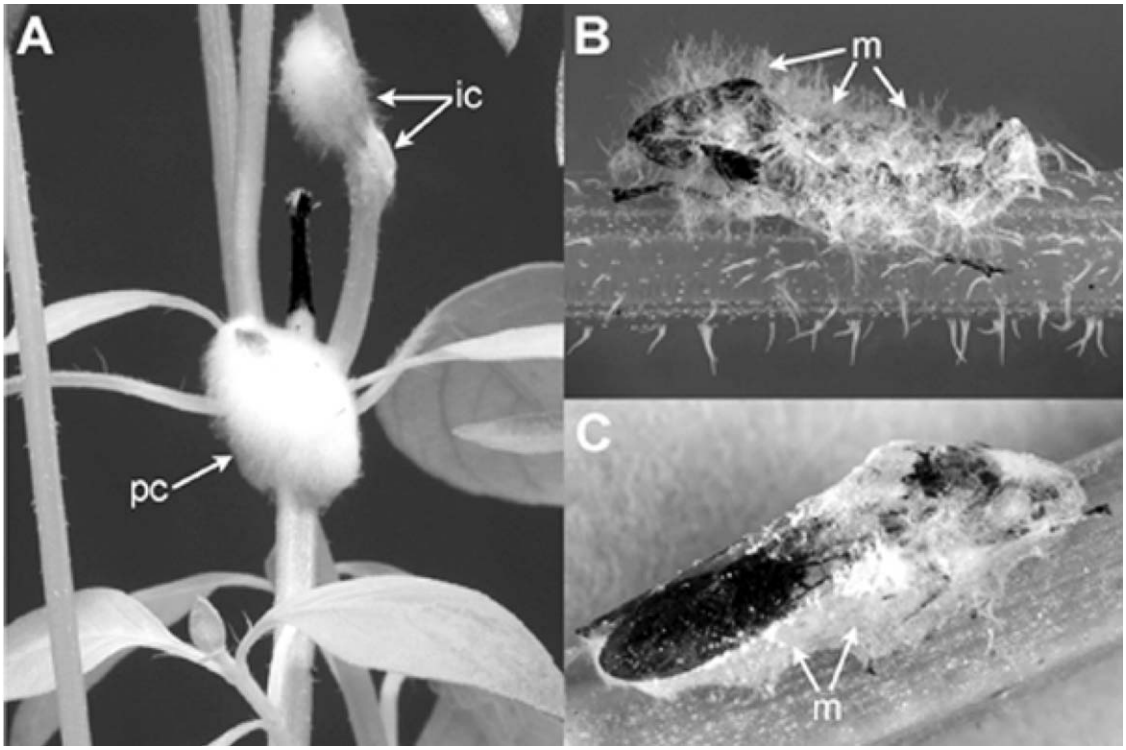

Fig. 1. Cadaver exposure experiments with *Homalodisca vitripennis*. (A) Part of a lemon basil plant spiked with new cadavers 3 weeks after introduction of nymphs. Note the white, thick mycelium overgrowing the pinned cadaver (pc) in the center and the introduced, mycosed nymphs (ic). (B and C) Mycosed nymph and adult, respectively, displaying thin *Hirsutella* mycelium (m) 3 weeks after release on a plant spiked with overwintered cadavers.

(Fig. 1A). Exposure of nymphs to overwintered cadavers induced 13 ± 8% mortality and 8 ± 5% infection producing a light and flat mycelium (Fig. 1B). Adult mortality after exposure to new and overwintered cadavers was 97 ± 2% and 76 ± 8%, respectively, and significantly higher than in the controls (47 ± 14%) (Table 1). Infection of adults induced by new cadavers (60 ± 9%) was significantly higher than that induced by overwintered cadavers (13 ± 9%).

Potentially, *Hirsutella* is one of the major mortality factors in endemic GWSS populations (Boucias et al. 2007). Our results clearly demonstrated that disease transmission from mycosed cadavers to healthy conspecifics efficiently occurs in undisturbed, small host populations. Identifying attractive cues that would cause foraging sharpshooters to contact infectious spores will be the next step for developing this pathogen as microbial control agent against *H. vitripennis*.

TABLE 1. MEAN (±SE) PERCENT MORTALITY AND INFECTION OF *HOMALODISCA VITRIPENNIS* 3 WEEKS AFTER INTRODUCTION TO CAGED PLANTS HARBORING *HIRSUTELLA*-MYCOSED CADAVERS.

Life stage exposed	Cadavers	Mortality[a,b]	Infection[a]	Induced mycosis[c]
Nymphs	None (control)	0 ± 0 a (139)	0 ± 0 a	No
	Overwintered	13 ± 8 b (68)	8 ± 5 b	Yes (2)
	New	100 ± 0 f (53)	100 ± 0 d	Yes (53)
Adults	None (control)	47 ± 14 c (103)	0 ± 0 a	No
	Overwintered	78 ± 6 d (61)	13 ± 5 b	Yes (1)
	New	97 ± 2 e (61)	60 ± 9 c	Yes (30)

[a]Numbers followed by different letters indicate significant differences (SAS proc genmod and lsmeans statement, $P < 0.05$).
[b]Numbers in parentheses indicate the total number of GWSS exposed in ≥4 replicate assays.
[c]Numbers in parentheses indicate the number of GWSS displaying mycosis within the 3-week observation period.

SUMMARY

For the first time, transmission of *Hirsutella* from mycosed, sporulating *H. vitripennis* to healthy conspecifics was demonstrated. In addition, methods were established to amplify infectious material *in vivo* for potential inoculative release.

REFERENCES CITED

BOUCIAS, D. G., SCHARF, F. W., BREAUX, S. E., PURCELL, D. H., AND MIZELL, R. E. 2007. Studies on the fungi associated with the glassy-winged sharpshooter *Homalodisca coagulata* with emphasis on a new species *Hirsutella homalodiscae* nom. prov. Biocontrol 52: 231-258.

BREAUX, S. E. 2005. Fungi associated with the glassy winged sharpshooter, *Homalodisca coagulata* in its native range. University of Florida (Master's thesis), Gainesville, 108 pp.

HUNNICUTT, L. E., MOZORUK, J., HUNTER, W. B., CROSSLIN, J. M., CAVE, R. D., AND POWELL, C. A. 2008. Prevalence and natural host range of *Homalodisca coagulata* virus-1 (HoCV-1). Arch. Virol. 153: 61-67.

NETER, J. WASSERMAN, W., AND KUTNER, M. H. 1990. Applied linear statistical models: regression, analysis of variance, and experimental designs. Richard Irwin Inc., Illinois.

REDAK, R. A., PURCELL, A. H., LOPES, J. R. S., BLUA, M. J., MIZELL, R. F., III, AND ANDERSEN, P. C. 2004. The biology of xylem fluid-feeding insect vectors of *Xytella fastidiosa* and their relation to disease epidemiology. Annu. Rev. Entomol. 49: 243-270.

SAS. 2004. User's guide, version 9.1. SAS Institute, Cary, North Carolina.