Pathogenicity of Hirsutella citriformis (Ascomycota: Cordycipitaceae) to Diaphorina citri (Hemiptera: Psyllidae) and Bactericera cockerelli (Hemiptera: Triozidae)

Authors: Casique-Valdes, R., Reyes-Martinez, A. Y., Sanchez-Peña, S. R., Bidochka, M. J., and Lopez-Arroyo, J. I.

Source: Florida Entomologist, 94(3) : 703-705

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.094.0341

PATHOGENICITY OF *HIRSUTELLA CITRIFORMIS* (ASCOMYCOTA: CORDYCIPITACEAE) TO *DIAPHORINA CITRI* (HEMIPTERA: PSYLLIDAE) AND *BACTERICERA COCKERELLI* (HEMIPTERA: TRIOZIDAE)

R. Casique-Valdes[1], A. Y. Reyes-Martinez[1], S. R. Sanchez-Peña[1], M. J. Bidochka[2] and J. I. Lopez-Arroyo[3]
[1]Departamento de Parasitología, Universidad Autónoma Agraria Antonio Narro (UAAAN), Saltillo, Coahuila, 25315, Mexico

[2]Department of Biological Sciences, Brock University, St. Catharines, ON, L2S 3A1, Canada

[3]INIFAP, Campo Experimental General Terán, N.L., 67413, México

Insect-pathogenic fungi are important regulators of populations of their hosts, and often cause epizootics. Fungi are essentially the only lethal entomopathogens capable of horizontal transmission in the insect order Hemiptera, whose sucking mouthparts prevent ingestion of microbial propagules of other entomopathogens that must infect the host orally (Ferron 1978).

Several psyllids (Hemiptera: Psylloidea) have emerged as vectors of the fastidious bacterium *Liberibacter*, which causes plant diseases of great concern. One such insect is the potato psyllid, *Bactericera* (= *Paratrioza*) *cockerelli* Sulzer, which vectors the *Liberibacter* causing "zebra chip" of potatoes; this insect is emerging as the main insect pest of solanaceous crops in the United States, Mexico, Guatemala, Honduras, New Zealand, and other countries (Munyaneza et al. 2007; Lacey et al. 2009). Also, the Asian citrus psyllid, *Diaphorina citri* Kuwayama, vectors the devastating *Liberibacter* causing the Huanglongbing (HLB) disease of citrus plants. This last insect is exotic to the American continent, where it has successfully invaded all major citrus-producing areas, from the USA to Argentina. At many locations in Mexico, conspicuous epizootics on *D. citri* have been attributed to the fungus *Hirsutella citriformis* Speare (Clavicipitales: Hypocreales). However, little research has been conducted there on this fungus besides reports on its distribution. Lacey et al. (2009) asserted that *Hirsutella* spp. should be tested on *B. cockerelli* due to the importance of the disease that this insect transmits.

We are interested in the infectivity of *Hirsutella* strains against both aforementioned insect species. In this work, fungal pathogens of the Asian citrus psyllid were collected in northeastern Mexico (states of Nuevo Leon, Tamaulipas and northern Veracruz) (Casique & Sánchez, 2010). In Sep-Oct 2009, numerous specimens of adult *D. citri* infected by *Hirsutella* cf. *citriformis* were collected, especially in southern Tamaulipas state (municipalities of Gómez Farías and Llera). For isolation of cultures, fructifications (synemmata) were washed 3 times with gentamicin at 0.025 mg/mL and placed in potato dextrose agar plus 0.5% yeast extract (PDAY). The slow-growing fungal cultures produced typical fructifications harboring conidia. Cultures will be deposited at the USDA-ARSEF Collection of Entomopathogenic Fungal Cultures, Ithaca, NY. Synnemata from insects and cultures in water were mounted on slides in lactoglycerol-cotton blue or water, and were observed under phase contrast with an Olympus CX41 microscope (Olympus, Mexico City).

For molecular identification, one *Hirsutella citriformis* strain inoculated on insects (HC817, see below) and additional strains (HC8D0, HC8D15 and HC8D16) were grown in liquid media (1% peptone, 1% dextrose, 0.5% yeast extract, and 0.3% malt extract) for 14 d (strain HC8D13 was not grown in liquid). The mycelia were removed by vacuum filtration onto filter paper and stored in 96% ethanol. The samples were subsequently crushed in liquid nitrogen using a mortar and pestle and DNA was extracted with the Qiagen DNeasy plant mini kit (Qiagen Inc., Mississauga, Ontario). For PCR reactions, we used forward primer F63 (5'-GCATATCAATAAGCGGAGGAAAAG) and reverse primer LR3 (5'-GTCCGTGTTTCAAGACGG) of the ribosomal large subunit D1-D2. PCR conditions and reactions were performed as described by Bidochka et al. (1994). PCR products were sequenced; sequences were multiple-aligned using MEGA 4.1 (Tamura et al. 2007) with sequences accessed in GenBank (www.ncbi.com).

To confirm the infectivity of *Hirsutella* strains to *D. citri* and *B. cockerelli*, live insects specimens were collected from orange and pepper plants in Cadereyta, state of Nuevo León, Mexico. Adults of both species were anesthetized with compressed carbon dioxide (Wenninger et al. 2009). Using a soft paintbrush with few bristles, anesthetized insects were introduced onto Petri dishes containing fungal cultures. Insects were placed over the synnemata in order to inoculate conidia onto the insect cuticle. In this way, *D. citri* was exposed to sporulating cultures of HC8D17 and HC8D13, each grown on agar (PDAY) in petri dishes, and on autoclaved wheat grains incubated in plastic bags. *B. cockerelli* was exposed to spores of both strains produced on PDAY. After 1minute, insects were transferred to fresh, tender leaves of host plants (citrus or pepper), and these leaves were

placed on disks of moistened yellow furniture sponge (3 cm thick) in 500-ml plastic containers. Control insects were handled similarly without exposure to fungus. Free water did not reach the leaves in the containers. Anesthetized, inoculated insects usually awoke within 2 min and started feeding within 24 h or less. Mortality was recorded every 24 h after inoculation.

Morphological traits (see Fig. 1) confirmed the identity of the fungus as *Hirsutella citriformis* Speare (Mains 1951; Meyer et al. 2007). Conidia measured 6.8-7.0 × 1.5-2 m (n = 10). Sequencing data and multiple-alignment analysis showed that strain HC8D17 (used in inoculations) as well as additional strains (HC8D0, HC8D15 and HC8D16, all identified initially as *H. citriformis* using microscopy) showed 97-100% identity to and maximum scores with GenBank sequence DQ075678 (partial sequence of the 28S ribosomal RNA gene), obtained from *Hirsutella citriformis* strain ARSEF 2346 (Humber et al. 2011). Strain ARSEF 2346 was found to infect the brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae) collected by M. Rombach in rice fields in Java (Indonesia) (Rombach et al. 1986; Humber et al. 2011).

Healthy *D. citri* and *B. cockerelli* adults inoculated with conidia of isolates HC8D13 and HC8D17 of *H. citriformis* died of mycoses by these fungi (Fig. 1). No control insects died of *Hirsutella* infections. *D. citri* and *B. cockerelli* inoculated with conidia from agar and wheat cultures were killed after 6 d, and synemmata emerged from the insect cadavers after 10 d. The macroscopic development of the fungus was very similar in both insect hosts, but on *Bactericera*, some synnemata (Fig. 1) appeared to be longer, thinner and more ramified than on *Diaphorina*. Few reports have verified Koch's postulates in a *H. citriformis*-insect host system. *H. citriformis* was reported as a pathogen of *D. citri* by Meyer et al. (2007) using field-collected infected insects and fungal cultures on rice as source of infective conidia. They reported that all insects died by 6-9 (-10) d post-inoculation. To our knowledge this is the first report of *H. citriformis* as a pathogen of *B. cockerelli*.

The potential of *H. citriformis* for the management of *B. cockerelli* is unknown. An approach towards its use could be inundative (= bioinsecticidal approach) by repeated application of *Hirsutella* spores. Another possibility is to induce epizootics by the introduction of the fungus into an agroecosystem, where subsequent reproduction on insects (as shown herein), and horizontal transmission of the fungus occur. *Hirsutella* has a longer life cycle

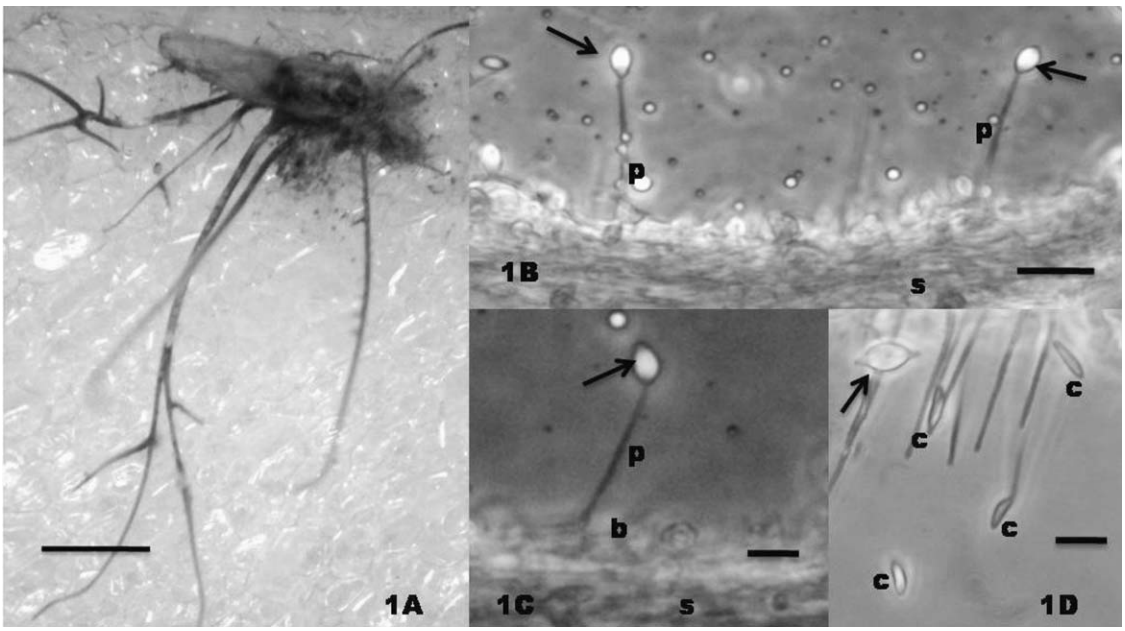

Fig. 1. Morphology of *Hirsutella citriformis*. A, ramified, sporulating fructifications (synnemata) of *Hirsutella citriformis* HC8D13 growing from infected *Bactericera cockerelli* 15 d after inoculation. Bar = 1.4 mm. B-D, phase contrast micrographs of the fungus grown on PDAY. B-C, surface of the synnema (s) of *H. citriformis*, with elongated conidiogenous cells (phialides); these consist of neck (p) and swollen base (b); conidium in mucilaginous ball (arrows). Bar B = 10 µ; Bar C = 5 µ. D, single conidia (c) without mucilaginous material; conidia shaped as orange segments. Bar = 7 µ.

(about 10 d) than other entomopathogenic fungi, such as *Beauveria* and *Isaria* (=*Paecilomyces*) (about 5 d). However, the production cycle of solanaceous crops (lasting close to 120 d) can be long enough for horizontal transmission and several replication cycles of *H. citriformis*, provided favorable conditions for sporulation and infection exist. In this case, *H. citriformis* could be a management tool for *B. cockerelli*.

Summary

This report confirms the identification of Mexican strains of *Hirsutella citriformis* isolated from Asian citrus psyllid, *Diaphorina citri*. Two *H. citriformis* strains were pathogenic to adults of *D. citri* and adults of the potato psyllid, *Bactericera cockerelli*.

Acknowledgments

The comments of Drion Boucias (University of Florida) and 2 anonymous reviewers improved this report. Supported by item 0202-0202-7104 from Dirección de Investigación, UAAAN, and CONACYT-INIFAP funds (FONSEC 108591).

References Cited

Bidochka, M. J., McDonald, M. A., St Leger, R. J., and Roberts, D. W. 1994. Differentiation of species and strains of entomopathogenic fungi by random amplification of polymorphic DNA (RAPD). Curr. Genet. 25: 107-113.

Casique-Valdés, R., and Sánchez-Peña, S. R. 2010. Entomopathogenic fungi attacking the Asian citrus psyllid, *Diaphorina citri*, in the Gulf citrus zone of Mexico, pp. 2-3 *In* Proc. 58th Ann. Mtg. Southwestern Branch, Entomol. Soc. Am., Cancun, Mexico. 60 pp.

Ferron, P. 1978. Biological control of insect pests by entomogenous fungi. Annu. Rev. Entomol. 23: 409-442.

Humber, R. A., Hansen, K., and Wheeler, M. 2011. Catalog of species. ARSEF, ARS Collection of Entomopathogenic Fungal Cultures. USDA-ARS, Ithaca, New York.

Lacey, L. A., de la Rosa, F., and Horton, D. R. 2009. Insecticidal activity of entomopathogenic fungi (Hypocreales) for potato psyllid, *Bactericera cockerelli* (Hemiptera: Triozidae): Development of bioassay techniques, effect of fungal species and stage of the psyllid. Biocontrol Sci. Tech. 19(9): 957-970

Mains, E. B. 1951. Entomogenous species of *Hirsutella*, *Tilachlidium* and *Synnematium*. Mycologia 43: 691-718.

Meyer, J. M., Hoy, M. A., and Boucias, D. G. 2007. Morphological and molecular characterization of a *Hirsutella* species infecting the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), in Florida. J. Invertebr. Pathol. 95(2): 101-109.

Munyaneza, J. E. 2010. Psyllids as vectors of emerging bacterial diseases of annual crops. Southwest. Entomol. 35(3): 471-477.

Rombach, M. C., Aguda, R. M., Shepard, B. M., and Roberts, D. W. 1986. Infection of rice brown planthopper, *Nilaparvata lagens* (Homoptera: Delphacidae), by field application of entomopathogenic Hyphomycetes (Deuteromycotina). Environ. Entomol. 15(5): 1070-1073.

Tamura, K., Dudley, J., Nei, M., and Kumar, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24: 1596-1599.

Wenninger, E. J., Stelinski, L. L., and Hall, D. G. 2009. Relationships between adult abdominal color and reproductive potential in *Diaphorina citri* (Hemiptera: Psyllidae). Ann. Entomol. Soc. Am. 102(3): 476-483.