

INFECTION BY *ENTOMOPHTHORA* SENSU STRICTO
(ENTOMOPHTHOROMYCOTA: ENTOMOPHTHORALES) IN *DIAPHORINA*
CITRI (HEMIPTERA: LIVIIDAE) IN VERACRUZ, MEXICO

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The Asian citrus psyllid, *Diaphorina citri* Kuwayama is one of the most important pests of citrus. It vectors the pathogenic bacterium causing the disease Huanglongbing (HLB) or Greening of citrus trees (Halbert & Manjunath 2004). Entomopathogenic fungi are important biological control agents of insects and often cause epizootics that reduce host populations dramatically (McCoy et al. 1988). Among the entomopathogenic fungi infecting *D. citri* in the field are *Beauveria bassiana* (Bals.) Vuill. (Rivero-Aragon & Grillo-Ravelo 2000; Yang et al. 2006), *Hirsutella citriformis* Speare, (Rivero-Aragon & Grillo-Ravelo 2000; Subandiyah et al. 2000; Meyer et al. 2007; Casique-Valdes & Sanchez-Peña 2010; Casique-Valdes et al. 2011), *Isaria fumosorosea* Wize (Samson 1974; Subandiyah et al. 2000; Casique-Valdes & Sanchez-Peña 2010), *Isaria javanica* (Friederichs & Bally) Samson & Hywel-Jones (= *Paecilomyces javanicus* (Friederichs & Bally) Brown & Smith) (Yang et al. 2006), and *Lecanicillium lecanii* (Zimm.) Zare & W. Gams (= *Acrostalagmus aphidum* Oudem., *Verticillium lecanii* (Zimm.) Viegas) (Rivero-Aragon & Grillo-Ravelo 2000; Yang et al. 2006; Casique-Valdes & Sanchez-Peña 2010). Aubert (1987) also lists *Cladosporium* nr. *oxysporum* Berk. & M.A. Curtis and the sooty mold, *Capnodium citri* Berk. and Desm. attacking *D. citri*, but the pathogenicities of these 2 usually saprophytic fungi are not clear.

In a survey of entomopathogenic fungi of *D. citri*, about 600 plants of *Citrus* spp. (Persian lime, sweet orange and tangerine) and orange jessamine (*Murraya paniculata* (L.) Jack) were examined for dead infested insects on foliage on 9-13 Oct 2012, across 90 km in Veracruz state, Mexico. The area (described below) includes the main production zone of Persian lime in Mexico (Sánchez-Torres et al. 2011). We also collected live *D. citri* adults in our search for fungal infections, On 12 October 2012, live adults were collected on *M. paniculata*, at the localities of Ixtacuaco, La Guadalupe, San Andres, San Pedro, Totomoxtle and Venustiano Carranza, in the municipalities of Papantla, San Rafael, and Tlapacoyan in north-central Veracruz state (highway distance: approx. 90 km). At Ixtacuaco, insects were collected from sweet orange (*Citrus × sinensis* (L.) Osbeck) also. Psyllids ($n = 210$) were placed in brown paper bags (38.5 × 16 cm) by shaking the

insects off infested branches into the bags. The opening of each bag was folded and sealed with masking tape. The puffed bags containing *D. citri* adults were carefully placed in large plastic bags, moistened slightly and held at 25-30 °C during transport to the laboratory in Saltillo. In this way live healthy insects can be maintained in bags for a few days. We expected to find mainly infections by *H. citriformis*, a slow-growing fungus that is commonly found attacking *D. citri* adults in the Gulf Coast region of Mexico (Casique-Valdes et al. 2011).

After 3 days of keeping insects in paper bags, dead ($n = 203$) and live ($n = 7$) insects were placed in petri dishes at room temperature, under diffuse fluorescent light (10:14 h L:D) with a piece of wet cotton to maintain high humidity. Insects were observed daily to detect fungal development. One dead *D. citri* showing the typical signs of infection by an *Entomophthora* species (Entomophthorales: Entomophthoraceae) was observed in a paper bag on day 3 after collection; on day 6, there were 2 additional similar dead insects (all from Venustiano Carranza, Papantla) in petri dishes (total 1.4% infection) (Fig. 1A-C). These insects had swollen abdomens, that angled away from the substrate; wings spread latero-dorsally and raised above the abdomen, and conspicuous masses of glutinous hyphae and conidiophores on the abdomen and thorax, similar to the well-known fungus *Entomophthora muscae* (Cohn) Fres., infecting flies (Diptera: Muscidae) (Mullens 1990; Krasnoff et al. 1995). All infected insects were collected at Venustiano Carranza, Papantla: N 20° 27' 18" W 97° 17' 06". No similarly killed insects were detected in the field survey. Fungi in the Entomophthorales forcibly eject their primary conidia (PC) (Keller 2007). Thus, slides were placed in the petri dish under infected insects in an attempt to collect discharged conidia.

Two of these insects became rapidly overgrown by saprophytic fungi (mainly *Cladosporium* sp.; Capnodiales: Davidiellaceae), which completely obscured the original pathogen. In petri dishes, some dead adults in typical *Entomophthora*-infected posture were also overgrown by fungal saprophytes. This rapid secondary fungal growth is frequently observed on other insects killed by Entomophthorales (Sanchez-Peña 1993). Thus the percent infection by *Entomophthora* is possibly underestimated.

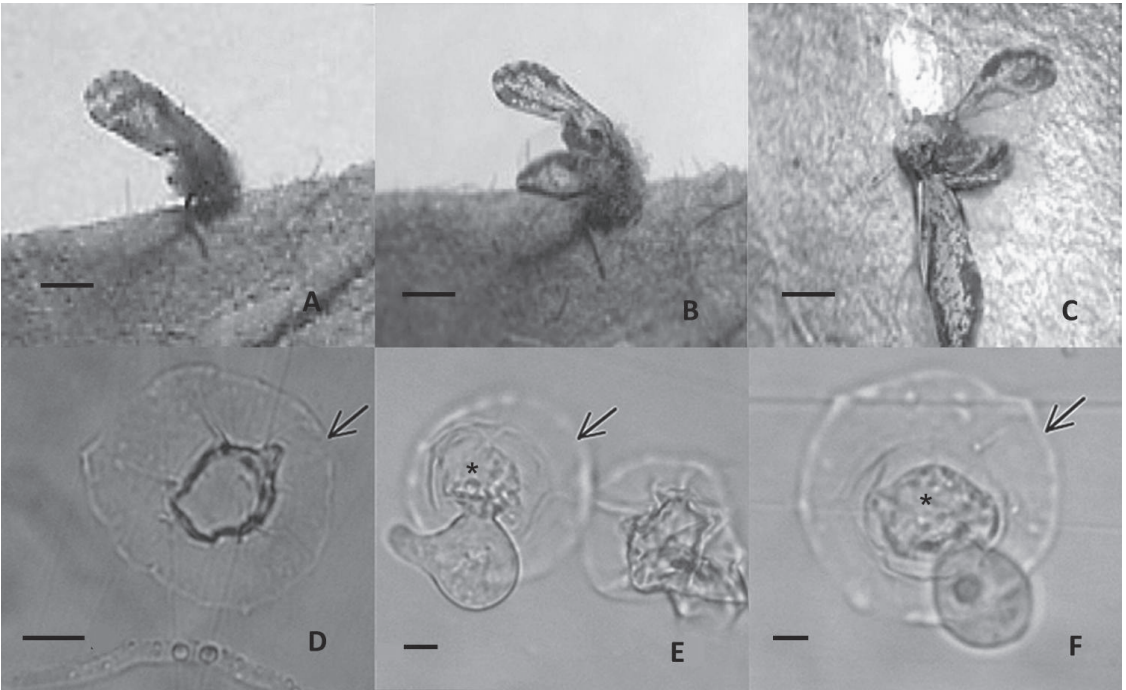


Fig. 1. *Entomophthora* sp. on *Diaphorina citri*. Figs. 1A-1C. Dead *D. citri* adults with typical position of *Entomophthora*-infected insects (see text); the same insect is in A and B. Figs. 1D-1F. Conidia of *Entomophthora* sp. Fig. 1D. Empty or dehydrated primary conidium ($22.7 \times 16.6 \mu\text{m}$) with typical shape of *Entomophthora* species. Fig. 1E. Empty primary conidium (*) and production of secondary conidium ($14.1 \times 11.6 \mu\text{m}$) with small germ tube. Fig. 1F. Empty campanulate primary conidium (*) and production of secondary conidium. The halos in D-E (arrows) formed upon conidial attachment to hard surfaces. Bar = 1mm (Figs. 1A-1C); Bar = $11 \mu\text{m}$ (Fig. 1D), Bar = $7 \mu\text{m}$ (Figs. 1E-1F).

Unfortunately no PC landed on the slides placed under infected insects for microscopical examination; they landed on the bottom of the petri dish. Pictures of unstained conidia shown were taken from the dish bottom under the compound microscope. Most PC observed had germinated, producing long germ tubes and/or secondary conidia (SC) (Humber 1981; Keller et al. 2007). The pictures herein are either of PC emptied of cytoplasmic contents, or perhaps slightly dehydrated, and measured with the software Axioma Vision 4.5 ($n = 4$). Average measurements are provided. Both PC and SC observed are typical of the genus *Entomophthora* Cohn in the strict sense (Humber 1981). PC are campanulate, with a flat base, surrounded with a halo of mucilage upon discharge (Fig. 1 D-F), with prominent apical point and broad basal papilla, $16.6 \times 22.7 \mu\text{m}$; SC were globose, with broad basal papilla, $14.1 \times 11.6 \mu\text{m}$, borne apically on a short conidiophore arising from PC (Fig. 2). This is similar to the spore size (PC: $11.5\text{-}20.5 \times 9\text{-}18.5 \mu\text{m}$; SC: $11.5\text{-}16 \times 9\text{-}11.5 \mu\text{m}$) of *Entomophthora philippinensis* Villacarlos & Wilding infecting the leucaena psyllid, *Heteropsylla cubana* Crawford, in the Philippines (Villacarlos & Wilding 1994).

We also observed the mucous cytoplasm halo around the discharged PC, typical of *Entomophthora* (Fig. 1D-F) (Eilenberg et al. 1986). Most PC were empty (had germinated) when detected, and no nuclei were observed; they also lysed a few hours later. Thus it is not possible to assign this fungus to a species with certainty since number and size of conidial nuclei are essential criteria for the identification of *Entomophthora* species (Humber 1981; Keller 2007). Entomophthorales have been occasionally reported to attack psyllids in temperate regions of Europe (Prischepa et al. 2011; Jankevica 2004) and the tropics of South Asia (see review in Villacarlos & Wilding 1994). In the New World tropics, Alves et al. (2009) reported *Zoophthora radicans* on psyllids (*Gyropsylla spegazziniana*) in Brazil; however, the globose spores and coarse hyphae shown do not correspond to the capillary conidia characteristic of *Zoophthora* and perhaps describe a mixed infection with the fungus, *Batkoa*, or other fungus. Felix-Alvarez et al. (2003) mentioned infections by Entomophthorales on *D. citri* in Matanzas, Cuba, but provide no further description of the fungi involved.

Most *Entomophthora* species are restricted to a narrow range of closely related host species

(Geden et al. 1993). It is possible that in the New World this fungus host-jumped to the exotic *D. citri* from another (psyllid?) host. This was possibly the case for *E. philippinensis* on *H. cubana*, which is exotic in the Philippines (Villacarlos & Wilding 1994). There are no records of Entomophthorales infections on *D. citri* from Mexico, possibly because *Entomophthora* has recently host-switched to this invasive host and/or because the fungus has not become widespread or abundant. Even though we did not observe epizootics of this fungus in this region of Veracruz, epizootics by a similar fungus have apparently occurred in the Ixtacuaco area in previous seasons (C. Hernandez-Torres, INIFAP, pers. comm.). This fungal species is of interest as a new potential biological control agent of *D. citri*. *Entomophthora* species can cause high mortality levels among hosts (Geden et al. 1993). Considering the inherently high infective potential of the Entomophthorales, this fungus could be considered (after host range studies) for introduction into other citrus-growing areas to enhance the natural enemy complex of *D. citri*.

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SUMMARY

In a survey of natural enemies of the Asian citrus psyllid, *Diaphorina citri*, live adults of this insect were collected at and near the municipalities of Papantla and Tlapacoyan, in central Veracruz state, Mexico, and held in the laboratory looking for individuals infected with entomopathogenic fungi. A species of the fungus *Entomophthora* Cohn *sensu stricto* (Entomophthoromycota: Entomophthorales) was observed infecting 1.4% adult insects (n = 210) collected from orange Jessamine, *Murraya paniculata*, at the town of Venustiano Carranza, municipality of Papantla. *Diaphorina citri* is a new host record for this entomopathogenic fungal genus.

Key Words: *Entomophthora*, fungus, new host

RESUMEN

En la búsqueda de enemigos naturales del psílido asiático de los cítricos, *Diaphorina citri*, se colectaron adultos vivos de este insecto dentro y cerca de los municipios de Papantla y Tlapacoyan, en el estado de Veracruz, México, y se mantuvieron en el laboratorio para detectar individuos infectados con hongos entomopatógenos. Se observó una especie del hongo *Entomophthora* Cohn *sensu stricto* (Entomophthoromycota: Entomophthorales) infectando 1.4% de insectos adultos (n = 210)

colectados de limonaria, *Murraya paniculata*, en el pueblo de Venustiano Carranza, municipio de Papantla. *Diaphorina citri* es un nuevo registro de hospedero para este género de hongo entomopatógeno.

Palabras Clave: *Entomophthora*, hongo, nuevo hospedero

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