# Biocontrol of Citrus Blackfly, Aleurocanthus woglumi Ashby (Homoptera: Aleyrodidae), by Spraying Aschersonia sp. Conidia Collected from Infected Nymphs in Quintana Roo, Mexico

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# Scientific Notes

# Biocontrol of citrus blackfly, *Aleurocanthus woglumi* Ashby (Homoptera: Aleyrodidae), by spraying *Aschersonia* sp. conidia collected from infected nymphs in Quintana Roo, Mexico

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The citrus blackfly, *Aleurocanthus woglumi* Ashby (Homoptera: Aleyrodidae), has been present in the Americas since 1913 after being reported in Jamaica (Ashby 1915). Citrus blackfly is a polyphagic pest that has more than 300 hosts, particularly citrus fruits, whose development is favored by temperatures ranging from 28 to 32 °C, and relative humidity between 70% and 80%. This pest does not survive at temperatures higher than 40 °C and feeds on plant sap, causing plant weakening and withering. In addition, excretion of sugary substances favors fungi development (mostly sooty mold) on leaves and fruits, damaging surfaces, which decreases their quality, resulting in higher economic losses (Nguyen & Hamon 1993).

Mexico grows 589,758 ha of citrus fruits, distributed in 24 states, with an estimated production of 8.2 million tons (SIAP-SADER 2017). It is the fifth largest citrus fruit producer in the world, representing a value of around USD $1,167 billion annually (SENASICA 2020). Citrus blackfly originally was found in the state of Sinaloa, Mexico, in 1935 (Myartseva 2005), where it is considered a secondary pest because it is found in population levels that do not represent economic distress due to introduced biological control agents, including the citrus blackfly parasitoid *Encarsia opulenta* (Silvestri) (Hymenoptera: Aphelinidae). This parasitoid originally was released in Mexico in 1971, in addition to the tiny parasitic wasp *Amitus hesperidium* (Silvestri) (Hymenoptera: Platygastridae), released earlier in Mexico (around the 1960s) (Smith et al. 1964; SENASICA 1999). However, populations of this insect pest increase or decrease due to climatic factors (Velázquez 1984) as well as indiscriminate application of chemical insecticides (Smith et al. 1964) that also affect parasitoid populations.

*Amitus hesperidium* was reported in 13 Mexican states (SENASICA 2020) in the mid-1920s. A report by SENASICA (2019) provided similar results for citrus blackfly control in Oaxaca, Chiapas, and Morelos states. After applying *Moelleriella libera* (formally known as *Aschersonia* cf. *aleyrodis* Webber) (teleomorph: *Hypocrella libera*) (Clavicipitaceae: Hypocreales) (Liu et al. 2006) in combination with the parasitoid wasps *Encarsia perplexa* Huang & Polaszek (Hymenoptera: Aphelinidae) and *A. hesperidium*, the citrus blackfly population was significantly reduced. The aim of the present study was to apply a simple technique to increase *Aschersonia* sp. distribution in orchards by collecting and spraying its conidia on citrus that are heavily infested with citrus blackfly nymphs to assess its potential for control in 1 Quintana Roo location.

In Aug 2018, after chemical application to control the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), the blackfly population increased among citrus orchards in high humidity areas of the Jose Maria Morelos locality in Quintana Roo, Mexico (19.7500°N, 88.7000°W) (Fig. 1a). Furthermore, in some orchards the entomopathogenic fungus *Aschersonia* sp. was detected along with this insect infecting citrus blackfly nymphs. Fungus conidia were collected from *Aschersonia* sp. infected nymphs (Fig. 1b) in a Petri dish, detaching the conidia from the cuticle with a previously sterilized needle (Fig. 1c) and transferring them to a 0.1% Tween 80 solution (Fig. 1d). To homogenize the conidia suspension, it was manually and vigorously shaken for 5 to 10 min. This conidia suspension was sprayed onto some shoots infested with uninfected citrus blackfly. For this, collected conidia were suspended in a 0.1% Tween solution at a concentration of about $1.0 \times 10^8$ conidia per mL and sprayed on citrus blackfly nymphs uninfected by *Aschersonia* sp. (Fig. 1e). This suspension was applied manually by spraying with a portable backpack sprayer (Lola 15 L, Torke S.A. de C.V., Mexico City, Mexico) on 30 leaves infested with citrus blackfly nymphs until moist when relative humidity was about 95%. Tween (1%) solution was used as a control.

Between Oct and Nov 2018, and after detecting citrus blackfly population infesting other citrus orchards from the same region, we used the same technique to treat nymphs, collecting conidia from infected nymphs and spraying them with a motorized sprayer (Honda Wjr4025, Gx35, 1.0 kw, 4.0 mpa, ARISA Maquinaria S.A. de C.V., Mexico City, Mexico), where a single application of about $1.0 \times 10^8$ conidia per mL was applied in 30 ha of a Jose Maria Morelos location in Quintana Roo until moist. During the application period, the average temperature was 23 °C with ≤ 95% relative humidity. In 2019, fungus conidia were not applied because we did not find a citrus blackfly pest population.

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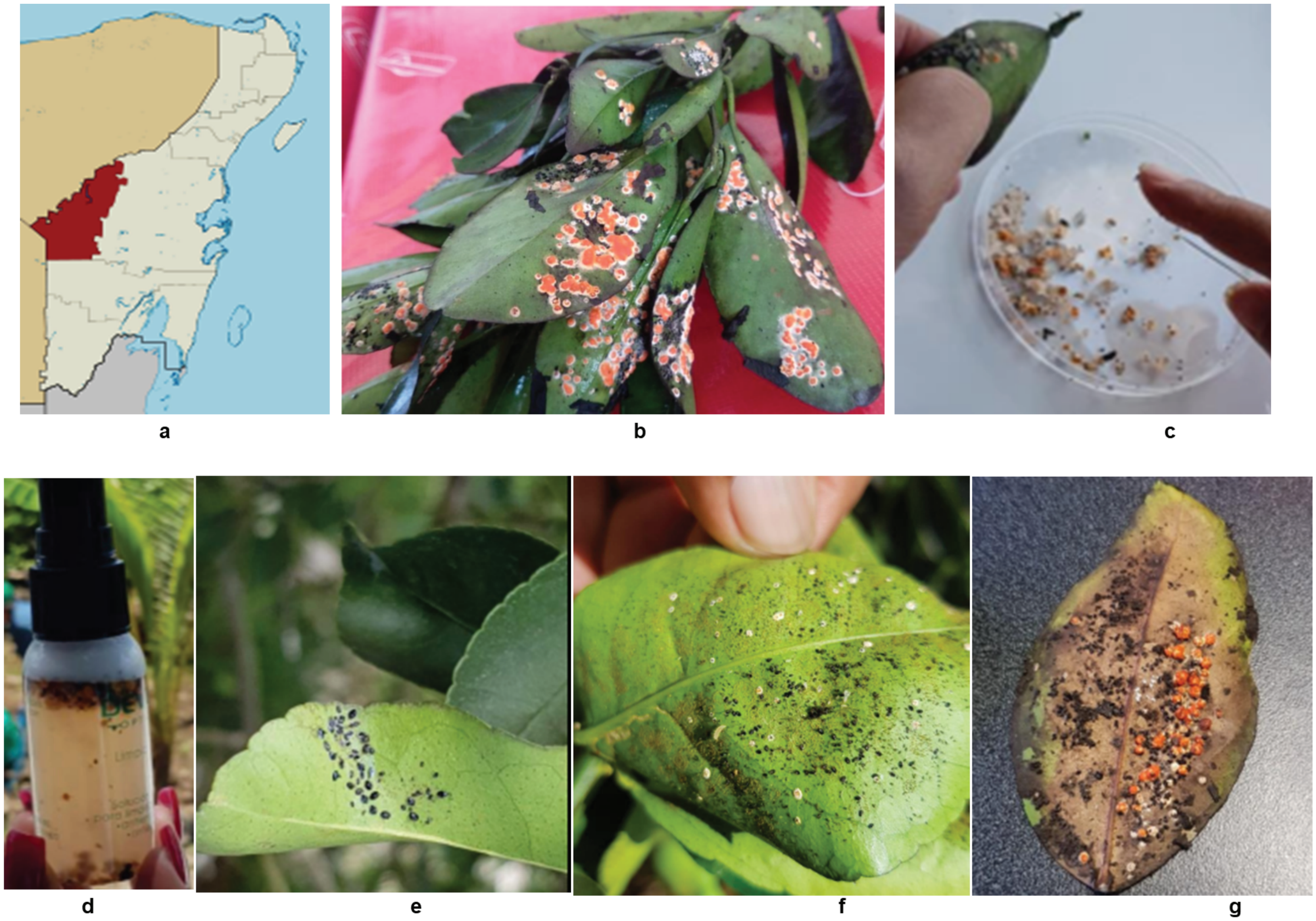


**Fig. 1.** (a) *Aschersonia* sp. conidia application site, Jose Maria Morelos, Quintana Roo, Mexico; (b) selection of citrus blackfly nymphs infected with *Aschersonia* sp. from citrus leaves; (c) conidia extraction with a needle from infected nymphs; (d) conidia suspension; (e) selected citrus leaves with citrus blackfly nymphs uninfected with *Aschersonia* sp. for conidia application; (f) citrus blackfly nymph infection by *Aschersonia* sp. and mycelia development after fungus application in 30 leaves infested with citrus blackfly nymphs in Jose Maria Morelos locality at 7 d after application; (g) at about 55 d after application. Photographs provided by Cipriano Villarreal-Rizo.

Following application of fungal conidia in Aug 2018, citrus blackfly control was achieved by late Sep 2018, about 55 d after application, where fungi were disseminated to other individuals of this species present along untreated shoots (Fig. 1f & 1g). After the application of fungi in 30 ha in Jose Maria Morelos, Quintana Roo, an epizootic of citrus blackfly nymphs infected with *Aschersonia* sp. was detected, and this pest control method was assessed (Fig. 2a). The following year (2019), rain was scarce and the average relative humidity in this location was lower than 60%. We did not detect the pest nor the fungi. In Sep 2020, temperatures averaged 30 °C and relative humidity was about 80%, which increased the citrus blackfly population. We did not apply conidia despite detection of *Aschersonia* sp. Citrus blackfly biocontrol actually was achieved as in 2018, resulting in an epizootic, without further control application (Fig. 2b). Citrus blackfly adults and nymphs are phloem feeders, which cause direct damage to plants. In addition, indirect damage is caused by the presence of sooty mold on leaves, branches, and fruits. Damage affecting plants and fruits causes great economic losses.

Integrated pest management is a sustainable ecosystem-based approach for pest control that combines different management strategies to minimize chemical pesticide application and grow eco-friendlier crops (FAO 2020). The use of parasitoid wasps is a common procedure for citrus blackfly biological control (Hart et al. 1978; Nguyen et al. 1983; Huang & Polaszek 1998; Meagher & French 2004; White et al. 2005; SENASICA 2019). The entomopathogenic fungi *Aschersonia* cf. *aleyrodis* and *Aegerita webberi* Fawcett have been associated with citrus blackfly (Vieira Lima et al. 2017) but their application for biological control in the field is rare.

*Aschersonia* sp. conidia collection from infected nymphs, to be sprayed in citrus orchards to achieve citrus blackfly infection and control, represents a simple and inexpensive technique to reduce this pest population. By 2013, the citrus blackfly population significantly decreased in the Mexican states of Morelos (92% reduction), Oaxaca (77% reduction), and Chiapas (45% reduction) by using the parasitoids *E. perplexa* and *A. hesperidium* in combination with the fungus *Aschersonia aleyrodis* from orchards where they were present (SENASICA 2019).

In our study, we did not release any parasitoids but the pest population was significantly reduced after conidia application in field, where *Aschersonia* sp. led to an epizootic on citrus blackfly in 2018. In 2019,

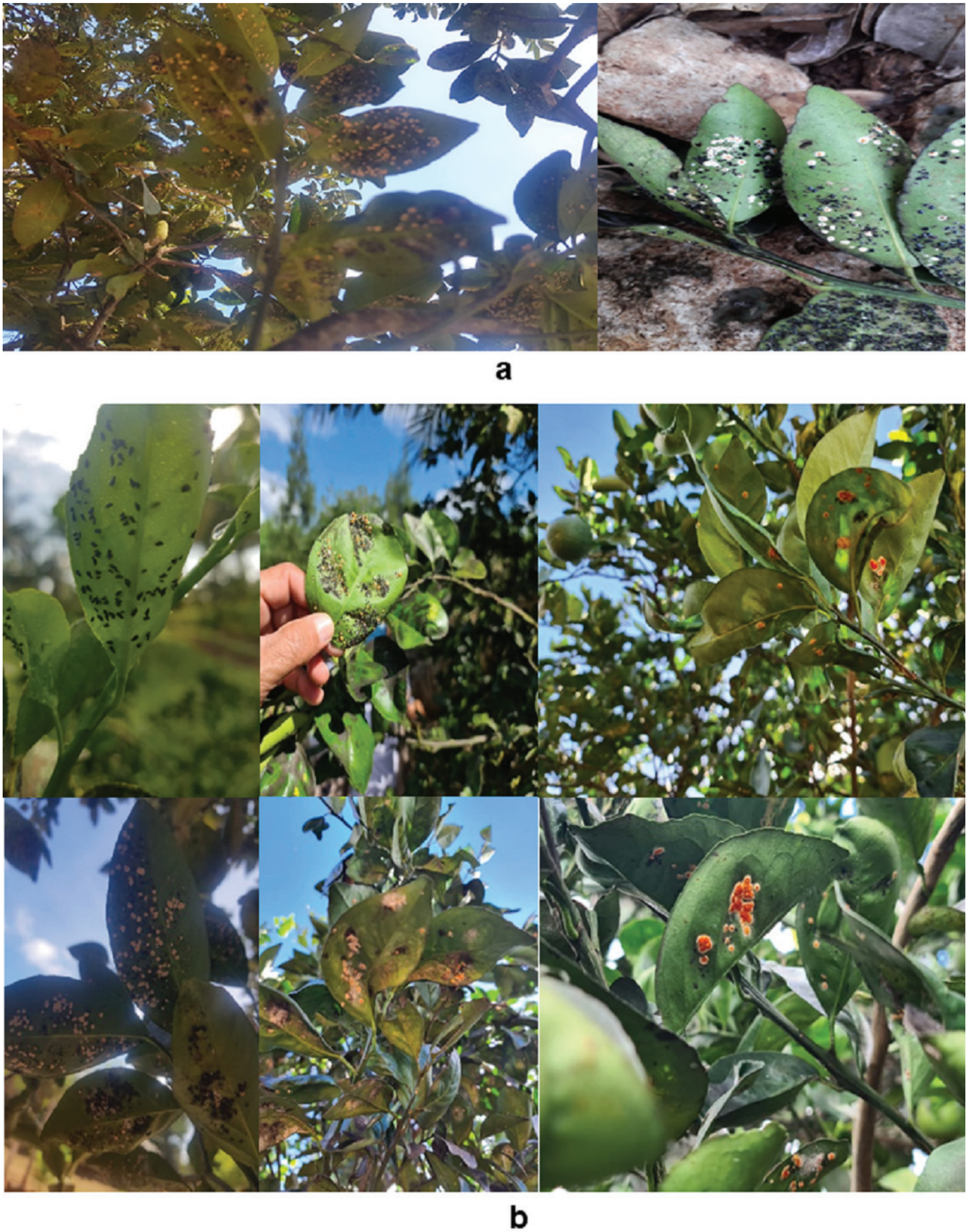

**Fig. 2.** (a) Epizootic caused by *Aschersonia* sp. on citrus blackfly nymphs after conidia application on 30 ha in Jose Maria Morelos, Quintana Roo, Mexico (late Nov 2018); (b) presence of *Aschersonia* sp. on citrus blackfly nymphs (Sep to Nov 2020) without conidia application in Jose Maria Morelos, Quintana Roo, Mexico. Photographs provided by Cipriano Villarreal-Rizo.

*Aschersonia* sp. conidia were not applied because the citrus blackfly population was rather low, probably due to environmental factors where the recorded temperature increased (higher than 35 °C) and the relative humidity decreased (lower than 60%). In 2020, after the citrus blackfly population recurred at higher numbers, when environmental conditions temperatures averaged 30 °C and relative humidity was higher than 80%, a second *Aschersonia* sp. epizootic infection of citrus blackfly nymphs was recorded at the same location of previously applied conidia. Based on the results reported for the region in 2020, fungus was still present in the sprayed area and became active again after citrus blackfly numbers increased. In summary, field results showed that it is possible to control citrus blackfly nymphs using a simple technique found to naturally control pests that may be used by citrus producers and biological control agents.

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## Summary

Among the citrus production industry, Mexico represents the fifth largest producer worldwide as of 1 Mar 2022 data (Producción de cítricos en México, Biblioteca de Publicaciones Oficiales del Gobierno de la República, Gobierno, www.gob.mx [last accessed 3 Jun 2022]). Among insect pests affecting citrus orchards, the citrus blackfly *Aleurocanthus woglumi* Ashby (Hemiptera: Aleyrodidae) causes great economic losses to this industry. Biological control with parasitoids is used principally to manage this pest. In this study, we evaluated a simple and inexpensive technique for citrus blackfly biocontrol in citrus orchards. Conidia from the entomopathogenic fungus *Aschersonia* sp. were collected from citrus leaves with *Aschersonia* sp. infecting citrus blackfly nymphs in 1 locality in Quintana Roo State, Mexico. Conidia were suspended with vigorous shaking in a 0.1% Tween solution to achieve a concentration of about $1.0 \times 10^8$ conidia per mL and sprayed on citrus blackfly uninfected nymphs in 1 selected (high citrus blackfly population) Quintana Roo locality when relative humidity was about 95%. After about 55 d, fungus had killed the treated nymphs, and had infected and killed other citrus blackflies causing an epizootic in the application area. This technique was scaled up by citrus producers to 30 ha with similar results. During 2019, relative humidity was lower than 60%, and citrus blackfly-uninfected or *Aschersonia* sp.-infected nymphs were not detected. During 2020, the relative humidity was higher than 80% and we did not detect *Aschersonia* sp. Infected- or uninfected-citrus blackfly nymphs in the previously treated area. In conclusion, collection and application of *Aschersonia* sp. conidia on uninfected citrus blackfly nymphs may result in an epizootic, if relative humidity is 80% or higher, and *Aschersonia* sp. remains and disseminates along with this insect pest.

Key Words: entomopathogenic fungus; conidia field collection; conidia field application; citrus orchard; natural dissemination; epizootic

## Sumario

Entre la industria de producción de cítricos, México representa el quinto productor a nivel mundial según datos del 01 de marzo del 2022 (Producción de cítricos en México, Biblioteca de Publicaciones Oficiales del Gobierno de la República, Gobierno, www.gob.mx [último accedido 3 Jun 2022]). Entre las plagas de insectos, la mosca prieta de los cítricos, *Aleurocanthus woglumi* Ashby (Hemiptera: Aleyrodidae) puede provocar grandes pérdidas económicas a esta industria. El control biológico con parasitoides es el método más utilizado para controlar esta plaga. En este estudio, evaluamos una técnica sencilla y económica en huertos de cítricos para el control biológico de la mosca prieta de los cítricos. Se recolectaron conidios del hongo entomopatógeno *Aschersonia* sp. de hojas de cítricos con *Aschersonia* sp. infectando ninfas de mosca prieta de los cítricos, en una localidad del estado de Quintana Roo. Los conidios se suspendieron con agitación vigorosa en una solución de Tween al 0.1% para lograr sobre $1.0 \times 10^8$ conidios por mL y se aplicaron mediante aspersión sobre ninfas no infectadas con mosca prieta de los cítricos, en una localidad seleccionada de Quintana Roo (alta población de mosca prieta de los cítricos), cuando la humedad relativa era sobre el 95%. Después de 55 días, el hongo ya habían matado a las ninfas tratadas e infectado y matado a otras mosca prieta de los cítricos, causando una epizootia en el área de aplicación. Se escaló esta técnica por los productores de cítricos en 30 hectáreas, lo que resultó en un biocontrol similar. Durante 2019, la humedad relativa fue inferior al 60% y la mosca prieta de los cítricos y no se detectaron ninfas sin infectar o infectadas por *Aschersonia* sp. Para 2020, la humedad relativa fue superior al 80% y se detectaron tanto la mosca prieta de los cítricos como ninfas infectadas por *Aschersonia* sp. en la zona previamente tratada. En conclusión, la recolección y aplicación de conidios de *Aschersonia* sp. sobre ninfas de mosca prieta de los cítricos no infectadas, podrían provocar una epizootia si la humedad relativa es del 80% o mayor y *Aschersonia* sp. podría permanecer y diseminarse entre esta plaga de insectos.

Palabras Clave: hongo entomopatógeno; colección de conidios en campo; aplicación de conidios en campo; huerto de cítricos; diseminación natural; epizootia

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