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Entomopathogenic fungi associated with citrus blackfly (*Aleurocanthus woglumi* Ashby) in Southern Bahia

*Fungos entomopatogênicos associados à mosca-negra-dos-citros (**Aleurocanthus woglumi** Ashby) na região Sul da Bahia*

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ABSTRACT: The Brazilian citrus industry is the world leading producer of concentrated orange juice. The presence of citrus blackfly *Aleurocanthus woglumi* Ashby (Hemiptera: Aleyrodidae) in Brazilian citrus orchards has led to agribusiness problems due to damages and inadequate control measures. Entomopathogenic fungi act as natural control in high precipitation regions, being important components of integrated management. The objective of this work was to identify entomopathogenic fungi associated with citrus blackfly in the southern region of the state of Bahia. Orange (*Citrus sinensis* L. Osbeck) and Tahiti lime (*Citrus latifolia* Tann.) leaves infested with citrus blackfly eggs, nymphs and pupae were collected in orchards in the municipalities of Ilhéus and Laje, Bahia, Brazil. Fragments of fungi colonies were disinfected with 70% alcohol and 2% sodium hypochlorite, and then washed with distilled water. After drying, the material was placed in Petri dishes with PDA and chloramphenicol, which were placed in bio-oxygen demand (BOD) incubators (27 ± 2 ºC, 12-hour photophase) until spore production. After isolation, the fungi were replaced in PDA Petri dishes and kept under continuous fluorescent light for 30 days to stimulate growth and spore production. The fungi isolates were preserved in test tubes containing PDA and mineral oil, and then stored at 18 ºC in a refrigerated incubator. Entomopathogenic fungi *Aschersonia* cf. *aleyrodis* Webber and *Aegerita webberi* Fawcett were associated with citrus blackfly in Southern Bahia.

KEYWORDS: Aleyrodidae; citriculture; microbial control; conservation biological control.

RESUMO: A citricultura brasileira lidera mundialmente a produção de suco concentrado de laranja. A presença da mosca-negra-dos-citros *Aleurocanthus woglumi* Ashby (Hemiptera: Aleyrodidae) em pomares citrícolas brasileiros tem gerado problemas ao agronegócio, devido aos danos e medidas inadequadas de controle. O controle natural por ação de fungos entomopatogênicos é comum em regiões com alta precipitação, sendo esses agentes importantes componentes do manejo integrado. O objetivo deste trabalho foi identificar fungos entomopatogênicos associados à mosca-negra-dos-citros na região Sul do estado da Bahia. Foram coletadas folhas de laranjeira (*Citrus sinensis* L. Osbeck) e de lima-Tahiti (*Citrus latifolia* Tann.) infestadas com ovos, ninfas e pupários da mosca-negra-dos-citros em pomares nos municípios de Ilhéus e Laje, Bahia. Fragmentos de colônias dos fungos foram desinfetados com álcool 70% e hipoclorito de sódio a 2% e, posteriormente, com água destilada. Após secagem, o material foi colocado em placas de Petri com BDA e cloranfenicol, em seguida alocadas em câmaras climatizadas do tipo BOD (27 ± 2 ºC, 12 horas de fotofase) até a produção de esporos. Após o isolamento, os fungos foram recolocados em placas de Petri com BDA, as quais ficaram sob luz fluorescente contínua durante 30 dias para estimular o crescimento e a produção de esporos. Isolados dos fungos obtidos foram preservados em tubos de ensaio contendo BDA e óleo mineral, e armazenados a 18 ºC em câmara climatizada. Os fungos entomopatogênicos associados à mosca-negra-dos-citros na região Sul da Bahia foram *Aschersonia* cf. *aleyrodis* Webber e *Aegerita webberi* Fawcett.

PALAVRAS-CHAVE: Aleyrodidae; citricultura; controle microbiano; controle biológico conservativo.

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Brazil is the world's biggest citrus producer and stands out as the greatest orange producer (ZULIAN et al., 2013). The state of Bahia ranks second in Brazil in orange production, and citrus blackflies were detected in 2010 by technicians of the Agricultural Defense Agency of the state of Bahia (ADAB) in far south regions, in the municipalities of Caravelas and Teixeira de Freitas. The first events in nurseries, orchards and urban areas were probably associated with the intense traffic of propagating material entering the State via roads (ADAB, 2010). The introduction of citrus blackfly *Aleurocanthus woglumi* Ashby (Hemiptera: Aleyrodidae) in Brazil in 2001 raised concern about future impacts on citriculture. The Brazilian law regulated interstate traffic, demanding permits and phytosanitary certification to market host fruits. Currently, the infestation has been detected in almost all Brazilian states and is considered a polyphagous exotic disease with preference for citrus, not classified as A-2 quarantine disease. The incidence of infestations brings difficulties to the sector due to damage and inadequate control measures (PENA et al., 2009; RAGA et al., 2013a; ALVIM et al., 2016). Although this insect has slow-action dispersion, new records have been found in places far from those previously infested, probably due to transport and commercialization of fruit tree seedlings or citrus fruits containing a peduncle with leaves from infested regions (RAGA et al., 2013a). Citrus blackflies can also have negative impacts on economics due to foreign trade barriers (MEDEIROS et al., 2009).

In tropical areas, natural epizootics are responsible for the natural control of a variety of plague-insect species. Species genus *Aschersonia* Montagne, *Cordyceps* Fries, *Hirsutella* Patouillard, *Isaria* Persoon, *Lecanicillium* W. Gams & Zare, *Paecilomyces* Bainier, *Synglіocladium* Petch, *Nomuraearileyi* (Farl.) Samson, *Metarhizium anisopliae* (Metsch.) Sorok and *Beauveria bassiana* (Bals.) Vuill are mentioned in the literature as important entomopathogenic fungi (ALVES; LOPES, 2008; MYCOBANK, 2015).

This paper aimed at identifying entomopathogenic fungi associated with citrus blackfly in Southern Bahia.

Ten leaves of sweet orange [*Citrus sinensis* (L.) Osbeck] and ten leaves of Tahiti lime (*Citrus latifolia* Tann.), pertaining to the family Rutaceae and containing blackfly eggs, nymphs and pupae associated with fungi, were collected from orchards in the municipalities of Laje [(13º09'49,3"S; 39º20'8,6"W; 204 m) and (13º12'8,7"S; 39º20'4,3"W; 236 m)], and Ilhéus (14º47'51,1"S;39º10'21"W; 225 m), Southern Bahia, in October 2014. The leaves were placed in Petri dishes lined with filter paper moistened in distilled water, sealed with plastic film, and sent for identification.

Fragments of fungi colonies collected from the leaves went through superficial disinfection with 70% alcohol and 2% hypochlorite solutions, washed in distilled water, and then were placed on paper towels to dry. After drying, they were put in Petri dishes containing PDA culture medium (potato, 200 g; dextrose, 20 g; agar, 20 g; distilled water, 1 L; chloramphenicol, 250 mg/liter of PDA) and placed in a biooxygen demand (BOD) incubator at 27 ± 2 ºC, until spore production. The fungi were replicated in PDA Petri dishes, and subsequently placed on shelves under controlled environmental conditions and continuous fluorescent light for 30 days to stimulate growth and spore production. Fungus isolates were preserved in 10 mm × 100 mm test tubes containing PDA culture medium and mineral oil, and stored in BOD at 18 °C until identification (PENA et al., 2009). Fungi were identified with a stereoscopic microscope at the Instituto Biológico (IB) by Dr. José Eduardo Marcondes de Almeida, and sent to the Entomopathogenic Fungi Collection of the IB, Campinas.

Two species of entomopathogenic fungi from sample citrus leaves collected in Southern Bahia were associated with citrus blackfly, *Aschersonia* cf. *aleyrodis* and *Aegerita webberi* (Figs. 1 and 2).

The entomopathogenic fungus *A. aleyrodis* is present in many Brazilian states. It infects citrus blackfly nymphs and has orange coloring. This fungus has been reported every month of the year and is a relevant natural enemy widely found in citrus orchards in the state of São Paulo, Brazil. Another fungus found in citrus blackfly nymphs in the inner and lower part of citrus tree crowns is the reddish-brown fungus *Aegerita webberi*, which seems to be more sensitive to fungicides (RAGA et al., 2013b). *A.* cf. *aleyrodis* fungi were found on citrus blackfly nymphs in the states of Pará and São Paulo (Artur Nogueira), Brazil, under favorable epizootic conditions (BATISTA et al., 2002; RAGA; COSTA, 2008). A massive growth of *A.* cf. *aleyrodis* associated with citrus blackflies was found in orange and tangerine leaves in the state of Amazonas, Brazil, mainly during rainy seasons and in higher humidity conditions (PENA et al., 2009; GONÇALVES, 2013). In the state of Rio de Janeiro, *Aegerita* sp. colonizes young forms of citrus blackflies. The formation of more concentrated colonies only on nymphs, not spreading over the leaf surface, is a characteristic of this fungus, as it seems not to cover the stomata on the abaxial leaf surface (ALVIM, 2014).

Nymphs infected with *A. aleyrodis* in orchards were found and accounted for in order to evaluate population fluctuation and spatial dispersion of citrus blackfly. There were 10,435 infected nymphs on 2,910 leaves, with at least one nymph infected by fungus. The presence of nymphs infected with *A. aleyrodis* was observed in 10.69% of leaves. *A. webberi* was also observed in citrus blackfly nymphs on Tahiti lime leaves, totaling 14,533 nymphs infected among 1,709 leaves, which represents 6.28% of all leaves collected (SALDANHA, 2016). *A. aleyrodis* is tolerant to low relative humidity (about 50%) and has long-term persistence on leaves (MEEKES et al., 2002). A reduction in citrus blackfly population was observed in late winter and early spring due to the incidence of entomopathogenic fungi *A. aleyrodis* and *A. webberi* (RAGA et al., 2016).

A. aleyrodis fungus grown in laboratory had 65, 84, 42 and 25% mortality rates in 1st, 2nd, 3rd and 4th instar citrus blackfly nymphs, respectively, showing slow growth in culture medium and efficacy for citrus blackfly control in initial phases (eggs, first and second instar nymphs), with mortality beginning on the fourth day after fungus inoculation.

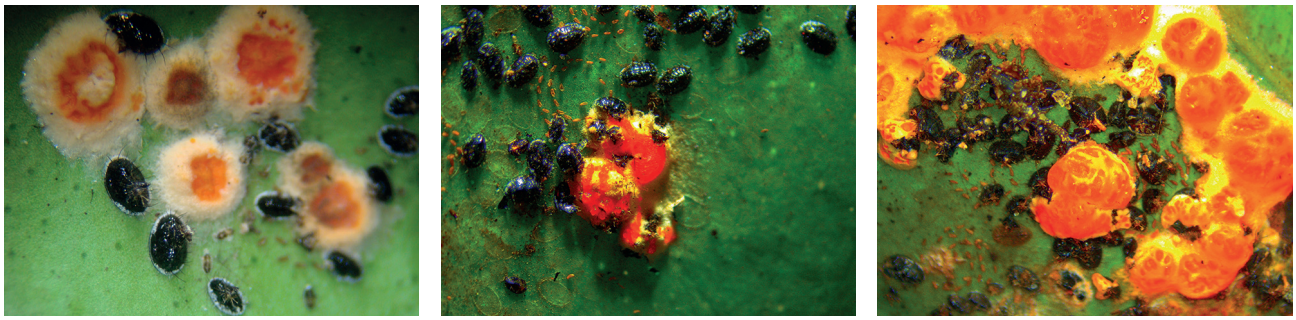

Figure 1. *Aschersonia* cf. *aleyrodis* colonies associated with citrus blackfly nymphs and pupae in sweet orange leaves collected in Laje, Southern Bahia.

Figure 2. *Aegerita webberi* colonies associated with citrus blackfly nymphs and pupae in leaves collected in Ilhéus, Southern Bahia.

Better control efficiency starting from 2.3×10^7 conidia/mL has contributed to disease reduction in the states of Pará and São Paulo, considering that the highest occurrence of this fungus in São Paulo took place in the lower third of Tahiti lime trees (BATISTA et al., 2002; PENA et al., 2009; RAGA et al., 2013a).

A. aleyrodis fungus was shown to have high pathogenicity on citrus blackflies at the highest concentrations (1×10^7 and 1×10^8), with mortality rate of 100% in the second evaluation, carried out six days after inoculation. The conidia suspension had mortality rate of 93.84 and 99.10% at the highest concentrations in the first evaluation, carried out three days after inoculation. Consequently, high contamination rates of this fungal disease can be related to the nymphs being immobile and very close together, which characterizes uniform fungus growth on the insect in a cottony aspect, with the nymphs totally covered by fungi and showing mummified appearance and/or signs of light-orange histolysis (LEMOS, 2008).

The present work described the occurrence of entomopathogenic fungi *A.* cf. *aleyrodis* and *A. webberi* for the first time associated with citrus blackflies in Southern Bahia, being a viable alternative for natural biological disease control, mainly due to the region's climate conditions (temperature 28.5 ± 5 ºC and RH 75 ± 5%). Entomopathogenic fungi *Aschersonia* cf. *aleyrodis* and *Aegerita webberi* are associated with citrus blackflies in Southern Bahia.

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