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Scientific Communication

Occurrence of *Purpureocillium lilacinum* in citrus black fly nymphs

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Abstract - Black fly is a pest of Asian origin that causes direct and indirect damages to citrus, damaging the development and production of plants. For the development of efficient management strategies of the pest, the integration of control methods is necessary, and biological control is the most appropriate. Among the agents that can be used, entomopathogenic fungi are considered one of the most important and wide-ranging use. This work investigated the occurrence of *Purpureocillium lilacinum* (Thom.) Luangsa-ard et al. (= *Paecilomyces lilacinus*), attacking nymphs of citrus black fly, *Aleurocanthus woglumi* Ashby (Hemiptera: Aleyrodidae). The fungus was isolated from infected Black fly nymphs, present on *Citrus* spp leaves in the municipality of Morros, Maranhão. After isolation, purification and morphological and molecular characterization, pathogenicity test was performed with *A. woglumi* nymphs. Morphological and molecular correspondence was verified between inoculum and the reisolated, proving the pathogenicity of *P. lilacinum*.
Index terms: biological control, *Aleurocanthus woglumi*, entomopathogenic, fungi.

Ocorrência de *Purpureocillium lilacinum* em ninfas de mosca-negra-dos-citros

Resumo - A mosca-negra é uma praga de origem asiática que causa danos diretos e indiretos aos citros, prejudicando o desenvolvimento e a produção das plantas. Para o desenvolvimento de estratégias de manejo eficientes da praga, é necessária a integração de métodos de controle, sendo o controle biológico o mais indicado. Entre os agentes passíveis de utilização, os fungos entomopatogênicos são considerados um dos mais importantes e com largo espectro de utilização. Neste trabalho, é relatada a ocorrência de *Purpureocillium lilacinum* (Thom.) Luangsa-ard et al. (=*Paecilomyces lilacinus*), atacando ninfas da mosca-negra-dos-citros, *Aleurocanthus woglumi* Ashby (Hemiptera: Aleyrodidae). O fungo foi isolado de ninfas da mosca negra infectadas, presentes em folhas de *Citrus* spp. no município de Morros, Maranhão. Após isolamento, purificação e caracterização morfológica e molecular, realizou-se um teste de patogenicidade com ninfas de *A. woglumi*. Constatou-se correspondência morfológica e molecular entre o inóculo e o reisolado, comprovando-se a patogenicidade de *P. lilacinum*.
Termos para indexação: controle biológico, *Aleurocanthus woglumi*, entomopatógeno, fungos.

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Citrus black fly *Aleurocanthus woglumi* Ashby (Hemiptera: Aleyrodidae) is a threat to the fruit growing sector, as it is a polyphagous pest with great potential for economic damage. This insect, besides citrus, attacks the main Brazilian fruit species, such as cashew (*Anacardium occidentale* L.), avocado (*Persea americana* Mill.) and mango (*Mangifera indica* L.). However, in high population density, adults disperse to other host plants such as jambeiro-vermelho [*Syzygium malaccense* (L.) Merr. & L. M. Perry] and grumixameira (*Eugenia brasiliensis* Lam.). In general, the species has about 300 host plants, causing direct (sap sucking) and indirect damages (development of sooty mold), thus reducing plant respiration and photosynthesis, compromising production and fruit quality (MELLO; MAIA, 2008).

Currently, citrus black fly control is carried out with sprays of insecticides, being effective in the control of nymphs and adults. However, the use of biological control is a valid alternative to restore functional biodiversity in agricultural ecosystems, since every insect population in nature is attacked in some way by one or more natural enemies. Thus, entomopathogens act as natural control agents that, when properly managed, can determine the regulation of herbivore populations in a particular agroecosystem (NICHOLLS, 2004).

In this paper, the occurrence of fungus *Purpureocillium lilacinum* (Thom.) Luangsa-ard, Houbraken, Doorn, Hong, Borman, Hywel-Jones and Samson (Hypocreales: Ophiocordycipitaceae) was reported to cause epizootics in *A. woglumi* nymphs.

The fungus was isolated from infected, dead, mottled, mummified-looking *A. woglumi* nymphs present on *Citrus* spp. leaves collected on April 2014 in the municipality of Morros, Maranhão (S 02 ° 51 '52 ", W 44 ° 02' 22"). In the laboratory, fungi that colonized citrus black fly cadavers were transferred to plates containing potato-dextrose-agar medium (BDA) by platinum needle replate. After growth, microcultures of the fungal isolates were carried out to observe the morphological structures.

To confirm the entomopathogenic potential of the fungus, 150 µL of a suspension of 1 x 10^7 conidia per mL of the isolate was sprayed into 40 2nd and 3rd instar nymphs (four replicates, each replicate represented by ten nymphs) in *Citrus latifolia* leaves collected directly from the field.

Tests were performed on Petri dishes (15 x 90 mm) containing one layer of circular filter paper sterilized and moistened with distilled water, where one citrus leaf per plate was placed. The suspension was sprayed with airbrush under pressure of 69 kPa with distance of 5 to 10 cm from the nozzle to the plant. Plates were then covered with plastic film, punctured and incubated in BOD (25° ± 1°C, 86 ± 2% and 12 hour photophase) for seven days.

Mortality was evaluated daily for seven days and recorded on the basis of insects that showed external mycelial growth or sporulation, because it was not possible to quantify dead and non-sporulated nymphs due to the lack of mobility and because they did not present color differentiation when dead, and only confirmed mortality was evaluated.

Cadavers were transferred to Petri dishes containing wet cotton (humid chamber) and placed in a BOD germination chamber (25 ± 1°C and 12 hour photophase) for 10 days to confirm the mortality caused by the pathogen through mycelial growth and fungus colonization.

Identification was made based on the morphological characteristics of conidia, phialides and hyphae (BARNETT; HUNTER, 1998; LUZ, 2012) and the molecular analysis with sequencing carried out at the Laboratory of Molecular Biology of the Biological Institute, São Paulo, Brazil.

The genomic DNA of the fungus was extracted from pure culture, and the ITS (internal transcribed spacer) region, located between the 18S and 28S ribosomal genes was amplified by PCR. The amplified products were sequenced and compared to sequences of authentic specimens deposited in the GenBank - NCBI.

After bioassay, it was confirmed that it was the same species, previously morphologically and molecularly identified, confirming the ability of *P. lilacinum* to infect *A. woglumi* nymphs. Nymphs died up to the seventh day after inoculation, reaching 100% mortality.

The isolate was included in the collection of fungi belonging to the "Prof. Gilson Soares da Silva" mycological collections from the State University of Maranhão, São Luís, MA, under number MGSS 136.

P. lilacinum is a fungus that presents cosmopolitan distribution, commonly isolated in most agricultural soils, decaying vegetation, insects, nematodes, in the air and even in some vertebrates, including man (MEDRANO-LÓPES et al., 2015). It is a parasite of eggs and cysts of *Meloidogyne incognita*, and other species of nematodes, including *Radopholus similis*, *Heteredora* spp., *Globodera* spp., *Rotylenchulus reniformes* (KANNAN; VEERAVEL, 2012; CARRION; DESGARENNES, 2012; ALZATE et al., 2012; CASTILLO et al., 2013). In addition, there are also reports of its pathogenicity on some insect species such as *Aphis gossypii, Trialeurodes voporariorum, Thrips palmi, Tribolium confusum, Triatoma infestans*, and *Tetranychus urticae* and *Rhipicephalus microplus* mites (MARTI et al., 2006; FIEDLER; SOSNOWSKA, 2007; WAKIL et al., 2012; ANGELO et al., 2012; HOTAKA et al., 2015; BARRA et al., 2015).

Fiedler and Sosnowska (2007), showed the efficiency of *P. lilacinum* on 3rd and 4th instar nymphs of *Trialeurodes vaporariorum* after 7 days of application of the agent at concentration of 1 x 10^6 spores / mL, causing 84% of nymphal mortality, agreeing with Wakil et al. (2012), who reported the effectiveness of the fungus on *Aphis gossypii*.

This communication reports for the first time the occurrence of fungus parasitizing *A. woglumi* nymphs. The use of entomopathogenic fungi is another promising strategy for the integrated management of citrus pests. Information on the behavior of *P. lilacinum* as a parasite

of different species is necessary, so its use in agriculture is recommended.

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