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MICROBIOLOGY

Cordyceps cateniannulata and *Cordyceps javanica*: first report of pathogenicity to *Glycaspis brimblecombei* (Hemiptera: Aphalaridae)

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Abstract: Cultivation of species of the genus *Eucalyptus* is important for the Brazilian economy, with 6.97 million hectares planted. *Glycaspis brimblecombei* Moore (Hemiptera: Aphalaridae), detected in Brazil in 2003, has dispersed and now damages *Eucalyptus* crops in all regions of this country. The location and identification of entomopathogenic fungi isolates may increase the options for integrated pest management. The objective of this research was to evaluate the pathogenicity of *Cordyceps cateniannulata* and *Cordyceps javanica* isolates to *G. brimblecombei*. Ten nymphs of *G. brimblecombei*, with or without lerps, were placed per *Eucalyptus* leaf cut with one of its edges on hydroretentive gel inside Petri dishes. The fungi isolates were suspended in a solution of Tween 80 (0.1%) at the concentration of 1.0×10^8 conidia mL^{-1} and sprayed on the *G. brimblecombei* nymphs. The mortality of this insect was evaluated daily for seven days, and the dead individuals were transferred to humid chambers. The conidia viability of the isolates was greater than 93%. The mortality of *G. brimblecombei* nymphs, seven days after the application of the fungi, was 100%. This is the first report of the pathogenicity of *C. cateniannulata* and *C. javanica* isolates, occurring naturally in the field, to *G. brimblecombei*.

Key words: biological control, entomopathogenic fungi, *Eucalyptus,* pest management.

INTRODUCTION

The area of forest cultivation in Brazil reached nine million hectares in 2019, with an increase of 2.4% in relation to 2018, 77.4% of which planted with species of the genus *Eucalyptus* (IBÁ 2020). Native and exotic pests can reduce the productivity of *Eucalyptus* crops (Paine et al. 2011). The red gum lerp psyllid, *Glycaspis brimblecombei* Moore (Hemiptera: Aphalaridae), was reported in Brazil in 2003 in Mogi-Guaçu, São Paulo, in *Eucalyptus camaldulensis* and *Eucalyptus tereticornis* plantations, and has dispersed throughout the country, reducing *Eucalyptus* productivity. Biological control using the parasitoid *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae) (Berti-Filho et al. 2003) and entomopathogenic fungi, especially *Beauveria bassiana* and *Metarhizium anisopliae* (Dal-Pogetto et al. 2011), and the use of chemical products acetamiprid, acetamiprid + bifenthrin and etophenproxy (AGROFIT 2021), are the main strategies for managing this pest.

The search for and identification of entomopathogenic fungi isolates can complement the integrated management of insect pests in agricultural and forest crops. The objective of this research was to evaluate

the pathogenicity of *Cordyceps cateniannulata* (LCBPF 17) and *Cordyceps javanica* (LCBPF 11) isolates, occurring naturally in the field, to *G. brimblecombei*.

MATERIALS AND METHODS

Site of study

The present research was developed in Botucatu, São Paulo, Brazil, in BOD-type incubators at a temperature of 25 ± 1 ºC, RH of 70 ± 10% and a photophase of 12 h.

Rearing *Glycaspis brimblecombei* (Hemiptera: Aphalaridae)

Glycaspis brimblecombei was reared in a laboratory at a temperature of 25 ± 2 ºC, 60 ± 10% RH and a photophase of 13 h on *Eucalyptus camaldulensis* and on saplings of the hybrid clone 3025 (*Eucalyptus grandis* x *E. camaldulensis*), both highly susceptible to this pest. Two *Eucalyptus* saplings planted per 1 L pot were placed in a standard cage (40 cm x 45 cm x 80 cm). A total of 80 to 100 *G. brimblecombei* adults were released per cage. These *Eucalyptus* saplings were irrigated daily, using a 500 ml laboratory wash bottle with water, and were changed at each insect life cycle, around 25 days (Wilcken et al. 2010).

Source of the fungi isolates

Isolates of the tested fungi *Cordyceps javanica* and *Cordyceps cateniannulata* were collected from soil in soybean crops (SO) and in native forest (NA), respectively, in the municipality of Botucatu, São Paulo state, Brazil. These fungi species were catalogued as LCBPF 11 (*C. javanica*) and LCBPF 17 (*C. cateniannulata*), and they were preserved in a freezer (-18 °C) in a Castellani medium.

Viability of the fungi isolates

The number of viable conidia was determined 14 days after the fungi cultivation at 25 °C in a Potato-Dextrose-Agar (PDA) medium. After this period, the conidia were suspended in 0.05% Tween 80 at a dilution of 1.0 x 10^6 conidia mL^{-1}. The percentage of conidia viability, per isolate, was calculated by counting them after 18 hours in a Neubauer chamber (Wraight et al. 2007).

Pathogenicity of the fungi isolates to *Glycaspis brimblecombei* (Hemiptera: Aphalaridae)

The conidia were obtained by superficial scraping of the fungus colonies in the PDA culture medium 14 days after their onset. The material was suspended in Tween 80 (0.1%) and adjusted to the concentration of 1.0 x 10^8 conidia mL^{-1} in a hemocytometer with an optical microscope. A total of 125 µL of the conidia suspension was sprayed with a DB134K airbrush (Fenghua Bida Machinery Manufacture Co., China) mounted on top of acrylic cylinder tubes 25 cm apart and with a working pressure of 68.95 kilopascal on *G. brimblecombei* nymphs with or without *G. brimblecombei* lerps. The control was a spray of only water + Tween 80 (0.1%). The equipment was washed in 70% alcohol and autoclaved with distilled water after each application.

Ten *G. brimblecombei* nymphs per replication in a Petri dish (90 x 15mm) were sprayed with the treatments and control and kept in BOD-type incubators (Eletrolab, model EL202/4), at the temperature of 25.0 ± 1.0 °C, relative humidity of 83.0 ± 2.0% and 12 h photophase with a total of 10 replications. A piece of approximately 5 cm^2 was removed from each leaf of the clone 433 (*E. urophylla* var. *platyphylla*) and placed on hydroretentive gel for each replication, reducing the loss of turgor and preventing *G. brimblecombei* from escaping. Each replication (a Petri dish) was evaluated daily for seven days, and the dead insects were

counted and transferred to moist chambers to stimulate the fungi development and to evaluate the insect mortality. The mortality values were corrected using the Schneider-Orelli formula. The equation (r^2) was estimated by adjusted polynomial trendline.

The datasets generated during and/or analyzed during the current study are available in the UNESP repository [https://repositorio.unesp.br/handle/11449/204462].

RESULTS

The *C. cateniannulata* and *C. javanica* isolates were identified by BLAST search using GenBank, with 100% homology. The viability of the conidia of the isolates, used in the pathogenicity bioassay for *G. brimblecombei*, was higher than 93% (Table I).

The virulence, conidia production and infection rate of *G. brimblecombei* nymphs were similar between the *C. cateniannulata* and *C. javanica* isolates, causing mortality of this insect mainly from the third day after their application (Figures 1 and 2). Mortality in the control was low, starting on the third day after application, with an average of two nymphs killed per replication, without evidence of fungal infection. The mortality of *G. brimblecombei* nymphs, seven days after the fungi application, was 100% (Figure 2).

DISCUSSION

The high viability of the conidia of the fungus isolates is similar to that observed for those of *B. bassiana* and *C. javanica*, above 92%, for *Duponchelia fovealis* Zeller (Lepidoptera: Crambidae) (Baja et al. 2020). The infection and sporulation of *C. cateniannulata* and *C. javanica* on *G. brimblecombei* nymphs agrees with that reported for *C. javanica* on *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) (Scorsetti et al. 2008) nymphs and adults, and on *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) (Ou et al. 2019), *C. cateniannulata* on *Tetranychus urticae* Koch (Acari: Tetranychidae) (Zhang et al. 2016) and *Beauveria bassiana* on *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Aphalaridae) (Alizadeh et al. 2007), indicating that these fungi can be used as mycoinsecticides.

The high mortality of *G. brimblecombei* nymphs, seven days after the application of the *C. cateniannulata* and *C. javanica* isolates, confirms their pathogenicity to this pest, with better results than those of *B. bassiana* and *Metarhizium anisopliae,* causing mortality above 90% of the *G. brimblecombei* nymphs (Dal-Pogetto et al. 2011), of *B. tabaci* by *B. bassiana*, *Cordyceps* sp. and *M. anisopliae* (Sani et al. 2020), *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) by *C. cateniannulata* (Zhou et al. 2020) and *D. fovealis* larvae by *Cordyceps javanica* (Baja et al. 2020). The pathogenicity of these fungi is due to the production of mycotoxins that affect the host immune system, such as the bassianolide by *C. fumosorosea*, with mortality of *D. citri* nymphs and adults of 70% and 80%, respectively (Qasim et al. 2020).

This is the first report of pathogenicity of isolates of the fungi *C. cateniannulata* and *C. javanica*, of natural occurrence, to *G. brimblecombei*. This research is the initial step towards new formulations and products for the management of this forest pest, indicating the potential for using isolates of entomopathogenic fungi of the genus *Cordyceps* as a new tactic for the integrated management of *G. brimblecombei*.

Table I. Molecular identification code (Code), species, host, culture (Cul.), coverage (Cov.), identity (Ident.), genBank access code (AC) and percentage viability (Viab.) (Mean$^{\pm SE}$) of the entomopathogenic fungi *Cordyceps javanica* isolates collected in soybean (SO) and *Cordyceps cateniannulata* in native forest (NA) in Botucatu, São Paulo, Brazil.

Code	Species	Host	Cul.	Cov.	Ident.	AC	Viab.(%)
LCBPF 11	*C. javanica*	*B. tabaci*	SO	100%	100%	MW138089	$97.63^{\pm 0.65}$
LCBPF 17	*C. cateniannulata*	*T. molitor*	NA	100%	100%	MW131688	$95.61^{\pm 3.06}$

Figure 1. Nymphs of *Glycaspis brimblecombei* (Hemiptera: Aphalaridae) infected by *Cordyceps cateniannulata* (a) and *Cordyceps javanica* (b); Healthy nymph (c).

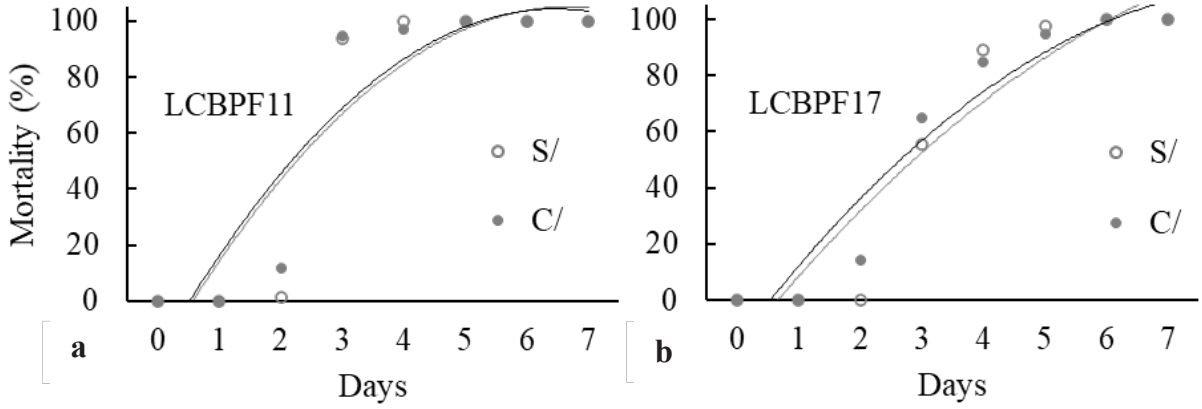

Figure 2. Accumulated corrected mortality of *Glycaspis brimblecombei* (Hemiptera: Aphalaridae) nymphs with (C/) or without (S/) lerp, over the days after application of the LCBPF 11 *Cordyceps javanica* (a) and LCBPF 17 *Cordyceps cateniannulata* (b) isolates with trendline.

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