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Effect of Formulated Entomopathogenic Fungi on Red Gum Lerp Psyllid *Glycaspis brimblecombei*

M.H.F.A. Dal Pogetto, C.F. Wilcken, R.S. Christovam, E.P. Prado and M.J. Gimenes
Department of Vegetal Production, UNESP-College of Agronomic Sciences, P.O. Box 237, 18610-307, Botucatu, Sao Paulo, Brazil

Corresponding Author: Mario Henrique Ferreira do Amaral Dal Pogetto, Department of Vegetal Production, UNESP-College of Agronomic Sciences, P.O. Box 237, 18610-307, Botucatu, Sao Paulo, Brazil

ABSTRACT

The objective of this study was to evaluate the potential use of formulated entomopathogenic fungi to be used as alternative method for the management of red gum lerp psyllid *Glycaspis brimblecombei*. The bioassay was carried out in laboratory using three species of fungi and six commercial brands: *Beauveria bassiana* (Boveril WP, Toyobo and Mycotrol O); *Metarhizium anisopliae* (Metarril WP and Toyobo); *Lecanicillium longisporum* (Vertirril WP). Five concentrations of each product were sprayed over the nymph kept on eucalyptus leaves. The results of mortality due to concentrations and time were used to calculate the LC_{50} and LT_{50}. All tested products were pathogenic to the red gum lerp psyllid and the products Metarril WP, Vertirril WP and Mycotrol O were more virulent. For these products the average death rate was above 90% in the highest concentration while LT_{50} varied between 1.4 and 2.3 days. The results indicate that the microbial control of red gum lerp psyllid with entomopathogenic fungi is viable and has potential to be used as an alternative control to this pest in eucalyptus forests.

Key words: Mycoinsecticides, *Beauveria bassiana*, *Metarhizium anisopliae*, *Lecanicillium longisporum*, eucalyptus

INTRODUCTION

The red gum lerp psylhid *Glycaspis brimblecombei* (Hemiptera: Psyllidae) is a native insect from the Australian continent which feeds on some species of eucalyptus (Dahlsten *et al*., 2003). In the last years the occurrence of this insect has been registered in various countries where it has been causing considerable damage to eucalyptus plantations (Brennan *et al*., 1999; Rosales *et al*., 2008; Valente and Hodkinson, 2008). Both nymphs and adults of psyllid feed on leaf juice. Under high infestation these feeding results in leaf fall, growth drop and death of the plant. Without the use of control measures this death rate can reach up to 15% in the first year of attack and 40% in the second year (Gill, 1998). The main characteristic of the presence of this insect is the formation of small white cones on the surface of the leaves which are formed by the excretions of the insect's nymphs, also protecting them until adulthood (Halbert *et al*., 2001).

Among the methods of control used on this pest, now a days stands out the biologic control though the breeding and liberation of the *Psyllaephagus bliteus* (Hymenoptera: Encyrtidae) parasitoid, whose presence in Brazil was detected right after the red gum lerp psyllid detection (Berti Filho *et al*., 2003). However, in spite of the efforts, as yet not an ideal level of population control of the plague was reached, probably due to the parasitoids inadaptability to some Brazilian regions. The research with chemical control related to red gum lerp psyllid are scarce and the available results are about the use of systemic insecticides, whose application has been considered effective only for short periods, attacking only a small population of the insect (Paine and Dreistadt, 2007). Besides, the demand for the restrict use of chemical insecticides on certified forests in growing and the use of some kinds of products is conditioned to the derogation period proposed by Forestry Stewardship Council (Forest Stewardship Council, 2008). Therefore, the search for alternative control methods, such as entompathogenic fungi, should be investigated because besides being less harmful to the environment, many bioinsecticides are produced on commercial scale (Burges, 1998; Khan *et al*., 2008). The use of entomopathogenic fungi to control some sucking pests has shown satisfactory results with significative reduction in the population of *Cacopsylla pyricola* (Puterka *et al*., 1994); *Diaphorina citri* (Padulla and Alves, 2009); *Agonoscena pistaciae* (Alizadeh *et al*., 2007). However, to obtain success in the development of microbial insecticides it is necessary for appropriate and careful selection of the species and isolated from the pathogens through the study of pathogenicity and virulence carried out in laboratories (Landa *et al*., 1994; Lacey, 1997; Soundarapandian and Chandra, 2007). This research has evaluated the pathogenicity and virulence of commercial formulations of mycoinsecticides based on *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium longisporum* on nymphs of red gum lerp psyllid.

MATERIAL AND METHODS

The present study was carried out at the Laboratory of Pest Forestry Biological Control, Department of Vegetal Production, College of Agronomic Sciences, Botucatu, Brazil during December 2008 to March 2009.

Mycoinsecticides preparation: The entomopathogenic fungi conidia used in this bioassay were from mycoinsecticides commercially produced (Table 1). Firstly was determined the conidia concentration of each bioinsecticide in Nuebauer chamber to obtain the stock solution. The viability of the conidia was determined by the spread of 0.1 mL of the stock solution of each product (1×10^8 conidia mL^{-1}) + 0.02% of Tween 20 on petri dishes (90 mm) containing potato dextrose agar and incubated in 26±1°C for 24 h. The viability was estimated by the counting of germinated conidia under an optic microscope.

Insects: The nymphs of red gum were obtained from laboratory rearing according to the methodology proposed by Wilcken *et al*. (2010). Forty couples of *G. brimblecombei* were liberated in rearing cages (80×40×44 cm) containing seedlings of *Eucalyptus camaldulensis* for oviposition

Table 1: Description of mycoinsecticides used against red gum lerp psyllid.

Manufacturer	Pathogen	Strain	Formulation[a]	Trade name
Itaforte Industrial de Bio Produtos	*B. bassiana*	PL 63	Technical concentrate	Boveril WP
Agro-Florestais Ltda, Brazil	*M. anisopliae*	ESALQ-1037	Technical concentrate	Metarril WP
	L. longisporum	1300	Technical concentrate	Vertirril WP
Laverlam International	*B. bassiana*	GHA	Oil dispersion	Mycotrol O
Corporation, USA	*B. bassiana*	IBCB 66	Technical concentrate	No trade name
Toyobo do Brasil Ltda., Brazil	*M. anisopliae*	IBCB 425	Technical concentrate	No trade name

[a]Formulations defined as Faria and Wraight (2007)

to happen. After fourteen days leaves with nymphs of psyllid were removed from the seedlings and carried out the removal of the shells produced by the insects and the excess of nymphs, so only ten nymphs remained between the third and fourth instar pear leaf on the adaxial part.

Bioassay: The virulence of the mycoinsecticides was evaluated by the calculation of LC_{50} and LT_{50} through spraying of five concentrations (1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8 conidia mL^{-1} +0.02% of Tween 20) from each one of the six products (Table 1) on the psyllid nymphs. In the control, treatment was sprayed an aliquot of 2.0 mL of distilled water + 0.02% of Tween 20. The sprayings were done in a sprayer tower with a continuous flux air exit on top with pressure of 15 lb pol^{-2}. For each spraying were carried out four replicates of three leaves with 10 psyllid nymphs (n = 120 nymphs per treatment). After spraying they were put to dry naturally on paper filter before being packed on petri dishes (90 mm). The plaques were prepared with a solution of hydroretentive gel (Hydroplan-EB, Empresa de Base and Distribuidora Ltd, Sao Paulo, Brazil) in a concentration of 5 g L^{-1} of water, providing a humid and gelatinous surface, enough for the leaves not to submerge and keep their turgidity. This technique was previously tested and did not show a deleterious effect on the red gum nymphs. Therefore the insects could be kept on the same leaf during the trial because the transferring of this insect's nymphs from one leaf to another may cause damage to the bucal system and kill them, besides being less time consuming. These plaques were incubated under 26°C and a 12:12 h photoperiod. The maintenance of the gelatinous state of the gel was kept by water reposition. The counting of dead psyllid was done daily, starting on the first day after spraying per seven days. The cadavers were transferred to petri dishes containing humid paper filter for 3-5 days for observation of the fungi development and confirmation of the insect death by the tested pathogens. The effect of mycoinsecticides was evaluated through nymphs' mortality in function of the concentration and of the time after the sprayings.

Statistical analysis: The data of mortality were submitted to Probit's analysis (Finney, 1971). To verify the data adjustments to Probit's model the qui-square test was used with 95% of probability. The difference of LC_{50} between the mycoinsecticides formulation and the difference of LT_{50} among the concentration in each formulation and among formulations was obtained through the overlapping of the confidence intervals (95% of probability). For LC_{50} the confidence interval was calculated using the log concentration (conidia mL^{-1}). The insect mortality was corrected by the Abbot's formula (Abbott, 1925).

RESULTS

Mycosis on *Glycaspis brimblecombei*: All tested pathogens were exteriorized over the psyllid nymphs' cadavers. The mycoses happened more rapidly in the higher concentrations of the pathogens (1-2 days). The product based on *L. longisporum* colonized the body of the insects still on the leaf, before transferring to the humid chamber. Before the extrusion of *B. bassiana* the body of insects were swollen and with pinkish color, no matter which product used.

***Glycaspis brimblecombei* mortality:** The percentage of dead insects was corrected according to the mortality obtained in the control treatment (average of 16.5%). There was a tendency of mortality growth with the increase of conidia concentration, except for the fungus *M. anisopliae* (Toyobo). The mortality varied from 54.1-100% in the higher concentrations, with prominence for the fungi *M. anisopliae* (Metarril WP) and *L. longisporum* (Vertirril WP). This variation was of

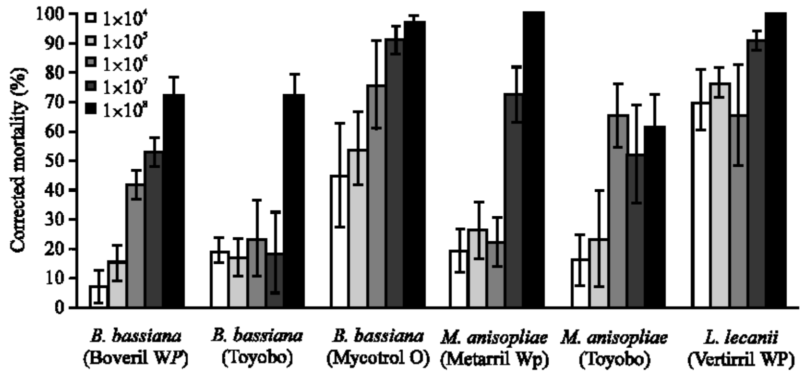

Fig. 1: Corrected mortality of *G. brimblecombei* seven days after spraying of fungi concentrations; error bar: ±SE

7.1-70.4% in the lower concentration for the fungi *B. bassiana* (Boveril WP) and *L. longisporum* (Vertirril WP) (Fig. 1). These results shown that all pathogens tested were pathogenic to the red gum lerp psyllid.

Virulence of the mycoinsecticides: Mycoinsecticides virulence determination was based on the analysis of LC_{50} on the seventh day after spraying while the LT_{50} considering the seven day period after spraying the psyllid nymphs. The difference of the LC_{50} and the LT_{50} among the mycoinsecticides was obtained through the comparison of the confidence intervals, with 95% of probability. All mycoinsecticides were able to kill at least 50% of psyllid nymphs in the interval of the concentration studied. The LC_{50} for *L. longisporum* (Vertirril WP) was not calculated because the mortality in all concentration was above 50%, indicating a high virulence of this pathogen against this insect. The mortality values obtained with the mycoinsecticides *B. bassiana* (Toyobo) and *M.anisopliae* (Metarril WP and Toyobo) did not fitted to the Probit model, because the values of the chi-square test for these products were significative. Due to the stimulus-response curve of these products did not follow a linear pattern, because the mortality of nymphs practically doubled among subsequent concentrations, not presenting gradual mortality according to the increase of the concentrations as observed with the mycoinsecticides Boveril WP and Mycotrol O (Fig. 1).

These results indicated that the isolate ones acted in a different way in the concentration intervals tested. Among the mycoinsecticides concentrations that fitted to the Probit model, Mycotrol O was the most virulent against the psyllid nymphs (LC_{50} = 0.1×10^5 conidia mL^{-1}) in comparison to Boveril WP (LC_{50} = 11.2×10^5), with LC_{50} approximately a hundred times lower than the other products (Table 2).

The nymphs treated with *B. bassiana* (Mycotrol O), *M. anisopliae* (Metarril WP) and *L. longisporum* (Vertirril WP) started to die two days after spraying due to the infection while in the remaining formulations the mortality started 3-4 days after the spraying. The concentrations that are not on Table 3 were not submitted to probit analysis for they presented a 50% lower nymph mortality. Based on the confidence intervals, the LT_{50} values reduced significantly with the increase of the concentrations of the products Boveril WP, Mycotrol O and Metarril WP (Table 3). The lowest LT_{50} value was obtained by the product Metarril WP, killing 50% of the insect's nymphs after 1.4 days (concentration of 1×10^8 conidia mL^{-1}). From the analysis of the over positioning of the confidence intervals this value did not differ significantly from the LT_{50} obtained by the product Vertirril WP (2.1 days) in the concentration of 1×10^7 conidia mL^{-1}.

Table 2: Estimative of LC_{50} (conidia mL^{-1}) of the mycoinsecticides for *G. Brimblecombei* nymphs

Pathogen (product)	LC_{50} (con. mL^{-1})	log.LC_{50}	log.IC (95%)	± log.SE	χ^2
B. bassiana (Boveril WP)	11.2×10[5]	6.05	(5.8; 6.3)	0.15	1.09
B. bassiana (Toyobo)	53.1×10[5]	7.73	(3.9; 8.1)	1.22	8.17*
B. bassiana (Mycotrol)	0.1×10[5]	3.99	(3.5; 4.3)	0.22	1.10
M. anisopliae (Metarril WP)	3.0×10[5]	5.48	(4.0; 7.0)	0.47	19.44*
M. anisopliae (Toyobo)	3.8×10[5]	5.58	(3.8; 7.3)	0.55	8.78*
L. longisporum (Vertirril WP) [a]	-	-	-	-	-

*χ^2 significant ($p<0.05$). [a]LC_{50} could not be calculated due to the death of more than 50% of the insects in each coucentration tested

Table 3: Estimative of LT_{50} (days) of the mycoinsecticides for *G. brimblecombei* nymphs

Pathogen (product)	Concentration (conidia mL^{-1})	LT_{50} (days)[a]	IC (95%)	±SE	χ^2
B. bassiana (Boveril WP)	1.0×10^7	6.0	(5.5; 6.4)	0.2	3.1
	1.0×10^8	3.9	(3.4; 4.3)	0.2	0.7
B. bassiana (Toyobo)	1.0×10^8	5.2	(4.5; 5.9)	0.4	1.2
B. bassiana (Mycotrol O)	1.0×10^5	5.9	(5.4; 6.5)	0.3	4.2
	1.0×10^6	3.8	(3.5; 4.3)	0.2	2.5
	1.0×10^7	3.1	(2.8; 3.4)	0.2	0.3
	1.0×10^8	2.3	(2.0; 2.7)	0.2	0.7
M. anisopliae (Metarril WP)	1.0×10^7	3.0	(2.5; 3.6)	0.3	0.6
	1.0×10^8	1.4	(1.0; 1.8)	0.2	0.1
M. anisopliae (Toyobo)	1.0×10^6	4.2	(3.8; 4.7)	0.2	5.6
	1.0×10^7	5.2	(4.7; 5.7)	0.3	2.7
	1.0×10^8	4.5	(3.9; 5.0)	0.3	1.8
L. longisporum (Vertirril WP)	1.0×10^4	4.0	(3.5; 4.5)	0.2	0.4
	1.0×10^5	3.9	(3.5; 4.3)	0.2	0.8
	1.0×10^6	4.5	(4.1; 4.9)	0.2	4.0
	1.0×10^7	2.1	(1.7; 2.5)	0.2	1.3
	1.0×10^8	2.4	(2.1; 2.7)	0.1	0.7

[a] LT_{50} calculated ouly for the concentrations which killed more than 50% of the insects

DISCUSSION

The results of this study show that all the mycoinsecticides were pathogenic to the *G. brimblecombei* and that the products Mycotrol O, Metarril WP and Vertirril WP were highly virulent against the nymphs of the insect in the concentration 1×10^7 and 1×10^8 conidia mL^{-1}. These concentrations, besides providing a high mortality of the insect, they're economically viable to field application. In others researches the mortality percentage of 42.1-46.5% in whiteflies nymphs obtained with *B. bassiana* at concentration of 1×10^6 conidia mL^{-1} was considered sufficient to control of pest (Al-Deghairi, 2008, 2009). These factors, in addition to supporting our results, are indicating that the microbial control of this psyllid is promising especially because these products are already formulated in commercial scale. This is interesting, because this insect is spreading rapidly and causing serious damage to eucalyptus forest worldwide (Brennan *et al.*, 1999; Wilcken *et al.*, 2003; Rosales *et al.*, 2008; Valente and Hodkinson, 2008). Literature is abundant in relation to the study of isolates selection from microorganisms for the determination of biopesticide activity but few authors have considered the use of formulated fungi (Hynes and Boyetchko, 2006). In comparison to the results of microbial control of other species of homopterous, the red gum lerp psyllid was highly susceptible to the pathogens. While our results of LC_{50} varied between 0.1×10^5 conidia mL^{-1}, the same isolated from the product Boveril WP used in this experiment needed from 2.3×10^7 conidia mL^{-1} to kill 50% of the population of citrus psyllid

Diaphorina citri (Padulla and Alves, 2009). The same study observed that the mortality of *D. citri* by fungi *M. anisopliae* and *L. longisporum* were inferior to 30%. Regarding the results of LT_{50} our results varied between 1.4 to 6 days while the use of *B. bassiana* to control the pear psyllid had a LT_{50} average of about 3 days (Puterka *et al.*, 1994). In other cases this values can be greater than seven days, being still considered viable to use for biological control (Sharififard *et al.*, 2011).

According to the results obtained by some researchers, the mycoinsecticides formulated in oil presents higher efficiency and are faster in insect control (Ibrahim *et al.*, 1999; Prior *et al.*, 1988; Wraight and Ramos, 2002). However, the products formulated in technical concentration and oil dispersion was similarly virulent against the *G. brimblecombei* nymphs. The lower performance of some mycoinsecticides against the psyllid nymphs may be justified by the lower susceptibility of this host to certain isolates, because there was a high mortality variation of insects to mycoinsectides formulated with the same species of fungus. In bioassays of selection this variation of pathogenicity has been observed quite often, being associated to factors such as virulence degree of the species or isolates from pathogenic and specificity and tolerance of the host (Vestergaard *et al.*, 1995; Milner, 1997; Ibrahim *et al.*, 1999; Chapple *et al.*, 2000; Wraight and Ramos, 2002).

In spite of the high variation of pathogenicity shown by the formulations on the red gum lerp psyllid, all the tested isolates were capable of development on the host. The growth of the fungi started initially between the femur articulations, tibia and antennas till totally covering the insect's body, similar to what happens in other insects (Wright and Chandler, 1991). Compared to chemical insecticides, the reproduction of the pathogen on the host is favorable for rendering the horizontal transmission of the illness (Scholte *et al.*, 2004; Quesada-Moraga *et al.*, 2008). In the case of the red gum lerp psyllid this transmission may be more efficient, because the insect establishes itself on the plant in an aggregated form (Ferreira-Filho *et al.*, 2008), with more than a hundred nymphs/leaf (Valente and Hodkinson, 2008). This can contribute to a better control of pest in field condition.

In conclusion, nymphs of *Glycaspis brimblecombei* are highly susceptible to entomopathogenic fungi *B. bassiana, M. anisopliae* and *L. longisporum* indicating that microbial control with formulated entomopathogenic fungi is promising as an alternative form to handle this pest in forests. Because the fungi is already formulated and applied the same way as other pesticides their use in the field becomes simple. Moreover, further studies regarding the compatibility of these microbial agents with the red gum lerp psyllid parasitoid *P. bliteus* are needed.

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