First report of *Beauveria bassiana* in the *in vivo* control of *Eriosoma lanigerum* in Brazilian apple trees

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ABSTRACT

This study was conducted in a commercial apple orchard (*Malus domestica*) with high woolly apple aphid (*Eriosoma lanigerum*) incidence, which is highly harmful to apple culture and infects the trunk, branch, roots, shoot, and fruit. Although the fungus *Beauveria bassiana* is widely reported as a control agent, there is no evidence of its control action against this pest. This case study was conducted under natural infestation conditions to evaluate the effective action of *B. bassiana* in *E. lanigerum* nymph mortality and aimed to evaluate the control potential of *E. lanigerum* treated with different formulation doses based on *B. bassiana* strain ICBBb252. The experimental unit consisted of ten plants in four randomized blocks, where one colony per plant was previously marked in an orchard (2500 plants/ha). The treatments consisted of applying the formulate in 100, 200, and 300 mL/ha dosages at a 10^9 spore/mL concentration and one control group; 10, 20, 30, and 40 days after application, the treatments were evaluated by counting the number of colonies alive. The control of woolly apple aphids by *B. bassiana* strain ICBBb252 was more effective at 200 and 300 mL/ha dosages and has no phytotoxic effects on plants.

Keywords: *Malus domestica;* woolly apple aphid; entomopathogenic fungi; biological control.

INTRODUCTION

Brazil is the third-largest fruit producer globally and produces roughly 45 million tons per year, 65% of which are consumed domestically and 35% are exported (Embrapa, 2021). In 2021, Brazil reached an all-time record in fruit exports, totaling 1.24 billion tons (Mapa, 2022). Given the need for cold weather to complete its phenological cycle, apple (*Malus domestica*) production is concentrated in Rio Grande do Sul, Santa Catarina, São Paulo, and Minas Gerais States, reaching 32,468 ha of harvested areas in 2020 (IBGE, 2020). The crops contribute significantly as a valuable source of nutrition and income, albeit apple production is hampered by pest insects, causing economic losses like *Grapholita molesta* (Monteiro & Niederheitmann, 2022) and *Anastrepha fraterculus* (Monteiro *et al.*, 2021).

The woolly apple aphid *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae) is native to eastern North America and has been found in most apple-growing areas of the world (Zhou *et al.*, 2021). These insects indirectly damage apples as they inject toxins when they feed on the plant sap, inducing the formation of nodes or wrinkles; this can also affect the root system since they form galls and weaken the affected plant. In Brazil, these insects have been reported on apple trees in Espírito Santo and Santa Catarina States (Madalon *et al.*, 2020; Monteiro *et al.*, 2004). Different chemical defensives have been adopted to reduce the

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damage of this aphid in apple tree crops, although there has been a growing demand for organic products, contributing to the adoption of environmentally friendly pest control methods. Biological control is an alternative, where it uses actions to combat pests using other live agents (Stenberg *et al.*, 2021).

Biological control has been adopted in Brazil since 1921 as an alternative to the high toxicity of chemical pesticides (Parra, 2014). The fungus *Beauveria bassiana* has mechanisms of action to control insect pests; it can produce some enzymes (e.g., proteases, chitinases, and lipases) that lead to the degradation of the cuticle of the target insect, as well as using its appressors to penetrate the host cell. In this way, toxins such as beauvericin and bassianin can be secreted and act via cytotoxic and insecticidal activities (Wang *et al.*, 2021). Moreover, the genus *Beauveria* acts as a biocontrol agent and is commonly used against a plethora of pest insects, including silverleaf whiteflies (*Bemisia tabaci*) (Bhadani *et al.*, 2021), and banana root borers (*Cosmopolites sordidus)* (Fancelli *et al.*, 2013), among others. Despite the vast applicability and knowledge of this fungus, little is known about its action against *E. lanigerum* nymphs.

Given the above, this study aimed to confirm the efficiency and agronomic feasibility of different doses of a formulation based on the *B. bassiana* strain ICBBb252 in controlling nymphs of woolly apple aphids (*E. lanigerum*) in apple trees (*M. domestica*) in Rio Grande do Sul State, southern Brazil. To the best of our knowledge, this is the first study to evaluate the *in vivo* effects of the entomopathogenic fungus *B. bassiana* on controlling *E. lanigerum* nymphs in apple orchards. Our study focused not only on evaluating the control potential of *B. bassiana* but also the phytotoxicity of different doses of the formulation and guidelines concerning the application of the biological product according to the phenology of the plant and aphid life cycle.

MATERIAL AND METHODS

'Fuji' apple plants with a high natural *Eriosoma lanigerum* incidence from a commercial orchard implemented in 2007 in the municipality of Vacaria (Rio Grande do Sul, southern Brazil) were used for the experiment from May 16 to June 25, 2017. This period represents the end of the productive cycle of the apple tree and the beginning of the physiological dormancy period, in which the trees still have leaves.

The treatments consisted of applying a formulation based on the fungus *Beauveria bassiana* strain ICBBb252 and belonging to the fungus collection of ICB BIOAGRITEC Ltda., with a concentration of 1.0 x 10^9 spores/mL in three different dosages (100, 200, and 300 mL/ha), namely T1, T2, and T3, respectively. A characteristic colony of *E. lanigerum* was initially selected and demarcated for each of the ten plants in four random blocks, totaling 40 colonies per treatment. Each treatment had 40 sampling points, and only water was applied in the control group. The orchard had a 2500 plant/ha density and spacing of 4 m between rows and 1 m between plants.

The application was directed at the branches' segments containing the previously marked colonies by sprinkling with a CO_2 pressurized knapsack sprayer equipped with an adapted Spraying Systems bar, 1.5 m long with four TeeJet 110015 nozzles, spaced at 0.5 m. The working pressure was 40 Lbs/inch^2, and the volume of syrup applied was 800 L/ha.

Then, 10, 20, 30, and 40 days after application (DAA), the treatments were evaluated by counting the number of colonies that remained alive. The percentage of control (PC) generated by each treatment was evaluated based on the mortality values (V) presented by the control and the other treatments. The final value was calculated using the formula:

$$PC = [(VControl - VTreated) / VControl]\ x\ 100$$

The experimental design of randomized blocks was applied to the data and contemplated three treatments and the control in four repetitions. The incidence results were submitted to square root transformation of x + 0.5 for statistical analyses using the Scott-Knott test to separate the means with a 5% probability of error.

The phytotoxicity was evaluated using a visual scale in percent for the crop and ranged from 0 to 100%, in which 0% corresponds to the absence of phytotoxicity and 100% represents total plant injury (death).

RESULTS

In the evaluation performed before applying the treatments to the apple trees, the insect population presented a uniform distribution on the previously marked plants, with no statistical difference between the blocks (Table 1). In the first evaluation (10 DAA), the treatments presented means between 2.75 and 5.75 colonies of *E. lanigerum* per block evaluated, whereas the control presented the same initial value of aphid colonies. Treatments T2 and T3 obtained 72.5% PC of the pest and were statistically identical. For this same evaluation, T1 obtained PC of 42.5%, differing statistically from the control and the other treatments ($p \leq 0.05$; Table 1).

In the second evaluation (20 DAA), the treatments presented means between 1.5 and 4.75 colonies of *E. lanigerum* per block evaluated, while the control presented a mean of 10 colonies. Moreover, T1 obtained PC of 52.5% and differed statistically from the control and other treatments ($p \leq 0.05$). The T2 and T3 dosages obtained PC of 80 and 85%, respectively, being statistically similar to each other but differing from T1 and the control ($p \leq 0.05$; Table 1).

In the evaluation performed at 30 DAA, the treatments presented means between 0.5 and 3.75 colonies of *E. lanigerum* per block evaluated, whereas the control presented a mean of 10 aphid colonies (Table 1). Treatments T2 and T3 obtained 87.5 and 95% PC, respectively, and were statistically similar to each other, differing concerning the control and T1 ($p \leq 0.05$), which presented 62.5% PC (Table 1).

At 40 DAA, the treatments averaged between 0.5 and 3.5 *E. lanigerum* colonies per block, while the control averaged 10 colonies of the woolly apple aphid. The treatments T2 and T3 obtained PCs of 92.5 and 95%, respectively, and were statistically similar to each other, differing compared to the control and T1 (65%) ($p \leq 0.05$; Table 1).

The formulation did not show any phytotoxic effects on 'Fuji' apple plants at the tested doses.

DISCUSSION

Biological control using microorganisms against pest insects is an ecologically suitable strategy. *Beauveria bassiana* is an important entomopathogen and has shown positive results regarding biological control of Coleoptera, Lepidoptera, and Hemiptera, demonstrating effective control in insects of agricultural importance in recent years (Figure 1; Table 2). Although the order Hemiptera is one of the most used in research, there are no known studies of *E. lanigerum* and its susceptibility to *B. bassiana* (Figure 1; Table 2).

The susceptibility of *E. lanigerum* to *B. bassiana* strain ICB06Bb was evaluated under in vivo conditions, and was observed a control rate above 40% in the first 10 DAA. The integument in the Insecta class is composed of chitin, protein, water, a complex mixture of lipids, metal ions, and calcium carbonate. *Beauveria bassiana* initiates the expression of its pathogenicity by conidia that attach to the epicuticle of the host insect. The metabolism of this fungal possesses molecular and biochemical mechanisms of virulence, including hydrolases, proteases, lipases, and phosphatases and the production of numerous toxic metabolites such as beauvericin, oosporein, and oxalic acid. These molecules degrade and allow the fungi to penetrate into the insect cuticle, followed to germination and formation of the appressorium (Shin *et al.*, 2020; Pedrini *et al.*, 2013; Vincent & Wegst, 2004). Upon reaching the hemolymph, *B. bassiana* can kill the host, again causing the mycelial growth of the fungus and the cycle restart (Shin *et al.*, 2020). This flexibility of *B. bassiana* against targets means it to be considered a potent entomopathogen that it can be used to a wide range of organisms (Figure 1; Table 2).

At 40 DAA, the dosages applied in T2 and T3 showed the highest suppression rates of the pest insect, with rates exceeding 90% mortality, differing statistically only from T1, which showed the lowest effective action against the pest (65% of control) (Table 1). These findings are consistent with previous research, in which *B. bassiana* was effective in controlling *Macrosiphum rosae* (rose aphids) when applied at a concentration of 4.6 x 10^6 conidia/mL, guaranteeing 95% of insect control after 45 days of applica-

Table 1: Mean number of colonies and mean control of *E. lanigerum* nymphs on apple trees by *B. bassiana* ICB06Bb

Treatment	Control (no. of colonies)	Mean colonies (mean insect control %)			
		10 DAA	20 DAA	30 DAA	40 DAA
T1 (100 mL/ha)	10	5.75^b (42.5)	4.75^b (52.5)	3.75^b (62.5)	3.5^b (65)
T2 (200 mL/ha)	10	2.75^c (72.5)	2.0^c (80)	1.25^c (87.5)	0.75^c (92.5)
T3 (300 mL/ha)	10	2.75^c (72.5)	1.5^c (85)	0.5^c (95)	0.5^c (95)
Control	10	10^a	10^a	10^a	10^a

DAA: Days after application; * Means followed by the same letter do not differ by the Scott-Knott 5% test. The data were transformed by the square root of x + 0.5.

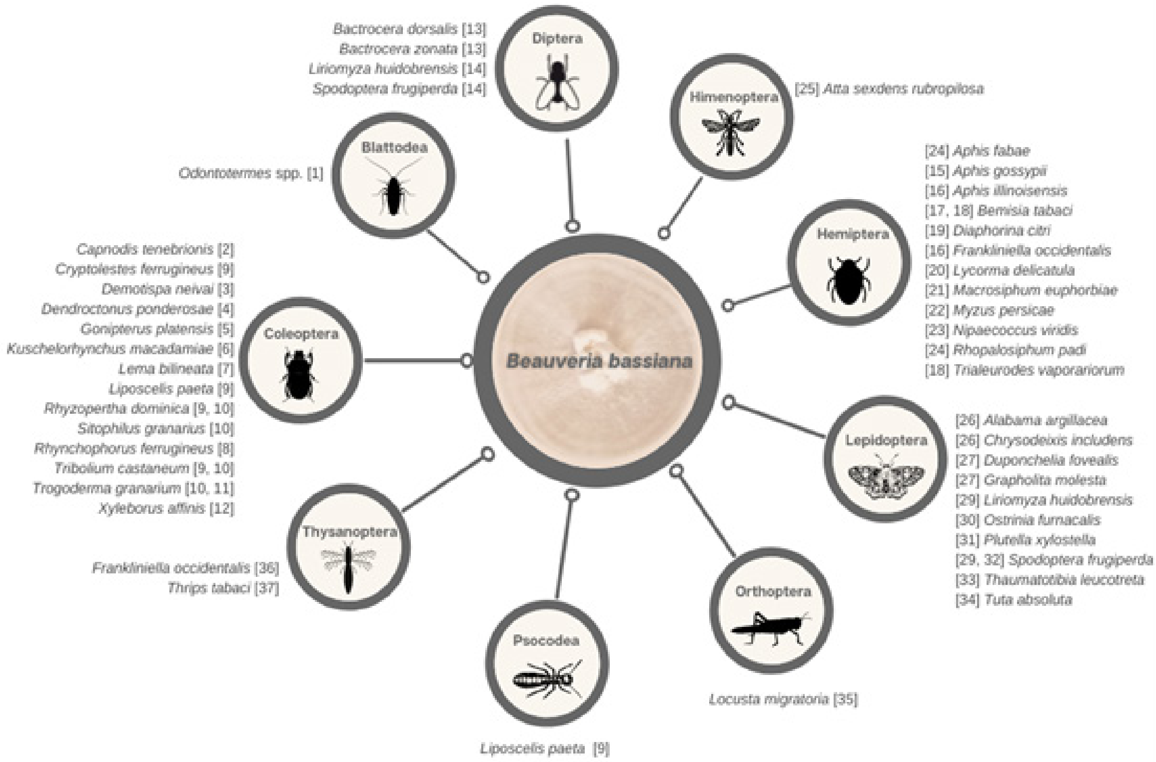

Figure 1: Research published worldwide evaluating the action of *B. bassiana* against insects in 2019-2022, classified by taxonomic order.

tion of the formulation (Sayed *et al.*, 2019). In cases where 100% effectiveness of pest suppression is not achieved, monitoring of aphids in the adult stage is highly indicated as the remaining nymphs complete their life cycle at higher temperatures because they go through their four developmental stages. This situation makes a new application of the formulation necessary to avoid the pest's prevalence, development, and proliferation.

The integument of insects is considered a highly efficient physicochemical barrier against external factors. Nevertheless, it becomes very vulnerable to pathogen penetration during the changes of stages due to the incomplete sclerotization of the insect (Alves & Pereira, 1998). *Beauveria bassiana* has metabolic arsenals that favor penetration into the insect, such as chitinases and lipases. Combined with the fragility of the insect cuticle in the nymph stage, the fungus may present greater virulence and thus lead to higher nymph control rates. In fact, Xavier & Avila (2006) revealed a higher nymph mortality than adult insects when entomopathogenic fungi were inoculated with *Scaptocoris carvalhoi* in a greenhouse. The adult insect has higher aldehyde concentrations in its tegument than the nymph stage since aldehyde increases according to the insect's age; this factor is important since this compound is associated with inhibiting germination of the fungus conidia. In *E. lanigerum*-infested orchards, a dose-dependent application of biological control is recommended in the early summer.

The *E. lanigerum* has been described in some studies, and its life stages may have adaptive characteristics for each region that inhabits (Orpet *et al.*, 2019; Zhou *et al.*, 2015; Timm *et al.*, 2005; Ruiz-Montoya *et al.*, 2015; Madalon *et al.*, 2020). In North America, its primary host is *Ulmus americana* L., followed by the apple tree (*Malus domestica* Borkh). It is on these hosts that sexual reproduction of the aphid occurs (Sandanayaka & Bus, 2005). As the elm is not commonly cultivated in Brazil, *E. lanigerum* uses apples as its main host, and it is believed that the insect reproduces only parthenogenetically (Kovaleski, 2004). Due to the need for the direct contact of the fungal conidia with the insect cuticle to start the infection process, the biological characteristics of the pest insect are important factors to be studied and considered (Samuels *et al.*, 2016). Based on these characteristics, it is possible to design a more effective method of application and accurately determine

Table 2: Research published worldwide on the action of *B. bassiana* on insects

Order	Species	Reference	Figure 2
Blattodea	*Odontotermes* spp.	Ambele *et al.* (2020)	1
Coleoptera	*Capnodis tenebrionis*	Ment *et al.* (2020)	2
	Demotispa neivai	Martínez *et al.* (2022)	3
	Dendroctonus ponderosae	Rosana *et al.* (2021)	4
	Gonipterus platensis	Jordan *et al.* (2021)	5
	Kuschelorhynchus macadamiae	Khun *et al.* (2021)	6
	Lema bilineata	Furuie *et al.* (2022)	7
	Rhynchophorus ferrugineus	Sutanto *et al.* (2021)	8
	Tribolium castaneum, Rhyzopertha dominica, Cryptolestes ferrugineus, Liposcelis paeta	Wakil *et al.* (2021)	9
	Tribolium castaneum Rhyzopertha dominica Sitophilus granarius and *Trogoderma granarium*	Wakil *et al.* (2022)	10
	Trogoderma granarium	Iqbal *et al.* (2021)	11
	Xyleborus affinis	Castrejón-Antonio *et al.* (2020)	12
Diptera	*Bactrocera zonata, Bactrocera dorsalis*	Wakil *et al.* (2022)	13
	Liriomyza huidobrensis	Chebet *et al.* (2021)	14
Hemiptera	*Aphis gossypii*	Mseddi *et al.* (2022)	15
	Aphis illinoisensis, Bemisia tabaci, Frankliniella occidentalis	Sayed *et al.* (2021)	16
	Bemisia tabaci	Wari *et al.* (2020)	17
	Bemisia tabaci, Trialeurodes vaporariorum	Gebremariam *et al.* (2022)	18
	Diaphorina citri	Awan *et al.* (2021)	19
	Lycorma delicatula	Clifton *et al.* (2020)	20
	Macrosiphum euphorbiae	Sinno *et al.* (2021)	21
	Myzus persicae	Mantzoukas *et al.* (2022)	22
	Nipaecoccus viridis	Olabiyi *et al.* (2022)	23
	Rhopalosiphum padi, Aphis fabae	Rasool *et al.* (2020)	24
Hymenoptera	*Atta sexdens rubropilosa*	Stefanelli *et al.* (2021)	25
Lepidoptera	*Alabama argillacea, Chrysodeixis includens*	Galdino *et al.* (2021)	26
	Duponchelia fovealis	Stuart *et al.* (2020)	27
	Grapholita molesta	Sarker *et al.* (2020)	28
	Spodoptera frugiperda	Chebet *et al.* (2021)	29
	Ostrinia furnacalis	Batool *et al.* (2020)	30
	Plutella xylostella	Soth *et al.* (2022)	31
	Spodoptera frugiperda	Rajula *et al.* (2021)	32
	Thaumatotibia leucotreta	Mondaca *et al.* (2020)	33
	Tuta absoluta	Aynalem *et al.* (2021)	34
Orthoptera	*Locusta migratoria*	Tan *et al.* (2021)	35
Psocodea	*Liposcelis paeta*	Wakil *et al.* (2021)	9
Thysanoptera	*Frankliniella occidentalis*	Sayed *et al.* (2021)	36
	Thrips tabaci	Gulzar *et al.* (2021)	37

the target and ideal concentration of the biological control to be used.

The attack of *E. lanigerum* on plant roots is characterized by gall formation and root system reduction (Fachinello *et al.*, 2008). In addition, it can serve as a constant source of canopy reinfestation. Thus, control measures must simultaneously target the roots and canopy of orchards (Lordan *et al.*, 2015); the root area was not assessed during the present experiment, although this could be a shelter for the aphids. Thus, a new application of *B. bassiana* is indicated for cases of root infestation since it would make pest control more efficient.

Given the pioneering nature of this study, it was pivotal to evaluate the phytotoxicity of different concentrations of *B. bassiana* on apple trees. The biological formulation did not show phytotoxicity adverse effects on the plants evaluated in any of the dosages applied. Probably this result was obtained because *B. bassiana* has metabolic mechanisms favorable to becoming endophytic and beneficial to plants, such as increased root development of *Vitis vinifera* and promoted growth of *Phaseolus vulgaris* L. (Mantzoukas *et al.*, 2021; Afandhi *et al.*, 2019).

From a financial perspective, agriculture needs biopesticides that can regenerate their mode of action to re-infect target pests for more than one generation (Glare *et al.*, 2012) and viable cost benefit. The persistence of fungi in host tissues coupled with plant growth promotion, protection against pest insects, and induction of systemic resistance make biological control more popular in agriculture (Bamisile *et al.*, 2021). And economically, biological control has been proving to be effective and cheaper than other treatments (Monteiro *et al.*, 2006). Therefore, further studies are needed to test gradual doses of *B. bassiana* and its virulence against *E. lanigerum* at the adult stage, since these pests have a different integument composition from the nymph stage and may require additional effort applications of the active control agent at different apple tree development stages.

CONCLUSIONS

This is the first report demonstrating that the biological formulation based on *B. bassiana* ICBBb252 is effective for controlling *E. lanigerum* colonies in apple trees without causing any phytotoxic effect on the plants. A treatment containing 300 mL/ha of the formulation is recommended since this dosage showed 95% efficiency at 40 days after application. New applications are necessary to eradicate the remaining insects from the root system to the stem of the infected plants. For the application of biological control agents, it is necessary to consider characteristics such as temperature, humidity, quality of the strain, the formulation used, and associating the application to the phenological stage of the plant and the cycle of the pest.

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Authors declare there is no conflict of interests in carrying the research and publishing this manuscript.

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