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Selection and characterization of *Beauveria* spp. isolates to control the broad mite *Polyphagotarsonemus latus* (Banks, 1904) (Acari: Tarsonemidae)

C. C. Martins[a], L. F. A. Alves[a], A. P. Mamprim[a] and L. P. A. Souza[a]*

[a]Programa de Pós-graduação em Conservação e Manejo de Recursos Naturais, Laboratório de Biotecnologia Agrícola, Universidade Estadual do Oeste do Paraná – Unioeste, Rua Universitária, 2069, Jardim Universitário, CEP 85819-110, Cascavel, PR, Brazil

*e-mail: luis.alves@unioeste.br

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(With 1 figure)

Abstract

This study was performed under laboratory conditions to identify isolates of the fungus *Beauveria* spp. that can control *Polyphagotarsonemus latus* in the greenhouse and field. Thirty *Beauveria* spp. isolates were tested by spraying 1 mL conidia (1×10^8 conidia/mL) on pepper leaf discs containing 15 mites. Evaluations were performed on the 3rd and 6th day post application by counting the number of dead mites. Vegetative growth and conidial production were measured from the selected isolates, and bioassays were conducted in the greenhouse on bean seedlings in plastic pots. The isolate Unioeste 53 was selected, and a conidial suspension (1×10^8 conidia/mL) was applied with a backpack sprayer. The evaluation consisted of pre- and post-treatment counts of the number of live mites on ten leaflets in both the plots treated with the fungus and control plots, and the same procedure was followed for the field experiment. In the laboratory, the Unioeste 53 isolate resulted in total and confirmed mortality rates of 70% and 57.7%, respectively. In the greenhouse, the population decreased by 76.71% by the 16th day after application. In the field, the population decreased by 66% by the 12th day after application, demonstrating the potential of this fungus for mite management.

Keywords: microbial control, entomopathogenic fungi, broad mite.

Seleção e caracterização de isolados do fungo *Beauveria* spp. visando ao controle do ácaro branco *Polyphagotarsonemus latus* (Banks, 1904) (Acari: Tarsonemidae)

Resumo

Este trabalho teve por objetivo selecionar isolados do fungo *Beauveria bassiana* em condições de laboratório com potencial de uso em casa de vegetação e campo no controle do ácaro branco. Foi realizada uma seleção com 30 isolados de *Beauveria* spp. através de pulverização direta de conídios (1×10^8 conídios/mL) sobre discos foliares de pimenta contendo 15 ácaros. As avaliações foram realizadas no terceiro e sexto dia contando-se o número de mortos, e confirmação do patógeno em câmara úmida. Parâmetros de crescimento vegetativo e produção de conídios foram avaliados. No bioensaio em casa de vegetação foram preparados vasos com plantas de feijão e fez-se a aplicação do Unioeste 53 (1×10^8 conídios/mL), utilizando pulverizador costal, a avaliação constou da contagem prévia e posterior à aplicação do número de ácaros vivos em 10 folíolos, tanto nas parcelas destinadas ao tratamento com fungo, quanto na testemunha. A fase de campo seguiu os mesmos padrões, porém, com área experimental total de 225 m^2, com oito parcelas de 10,24 m^2, sendo 4 testemunhas e 4 onde foi aplicado o isolado Unioeste 53 seguindo metodologia de aplicação e avaliação já descritas para casa de vegetação. Em laboratório o isolado Unioeste 53 causou mortalidade total de 70% e 57,7% de mortalidade confirmada. Em casa de vegetação, apresentou redução da população de 76,71% 16 dias após aplicação, já em campo, a redução da população foi de 66% após 12 dias da aplicação, demonstrando o potencial do ácaro pelo fungo.

Palavras-chave: controle microbiano, fungos entomopatogênicos, ácaro branco.

1. Introduction

The broad mite *Polyphagotarsonemus latus* (Banks, 1904) (Acari: Tarsonemidae) is one of the most important species of phytophagous mites, and it is a cosmopolitan and polyphagous pest associated with various crops (Moraes and Flechtmann, 2008; Sarmento et al., 2011).

This pest damages crops by perforating the plant cell wall, which results in the leakage of intracellular contents, upon which the pest feeds. Breakage of the cell wall can lead to uneven leaf growth, decreased photosynthetic capacity and early leaf drop, which affects plant development and yield and provides a gateway for opportunistic pathogenic microorganisms (Moraes and Flechtmann, 2008; Alves et al., 2010).

Synthetic chemical acaricides are commonly used to control mite populations; however, improper chemical use has negative effects, including intoxication at the time of application and the presence of residues in food. In addition, these products can result in mite populations resistant to the active ingredients and can eliminate non-target organisms, thus affecting natural enemies and even vertebrates depending on the concentration and exposure time (Gallo et al., 2002; Alves et al., 2008).

The control of this mite becomes more difficult when *P. latus* attacks crops for which there are no registered chemical acaricides, such as yerba mate. This mite has been cited as a significant pest in yerba mate nurseries and can severely damage seedlings, which then become unviable for planting (Alves et al., 2010; BRASIL, 2012). The use of chemical acaricides is restricted to organic and agro-ecological farming, especially for crops that co-occur with *P. latus* and are fairly common on small farms, thus representing a large share of the income of small producers (Echer et al., 2002).

Within the context of natural resource conservation and management, studies have been conducted to reduce the impact of phytophagous mites on the environment and human health and develop alternatives for controlling such populations. Entomopathogenic fungi have produced positive results in the control of *P. latus*, and studies in the laboratory and greenhouses have demonstrated the potential of entomopathogenic fungi, particularly *Beauveria bassiana*, against this mite species (Peña et al., 1996; Nugroho and Ibrahim, 2007; Alves et al., 2010). However, field studies on using entomopathogenic fungi to control mites are scarce and non-existent for *P. latus*.

Because of the potential of *B. bassiana* for mite control, the objective of this study was to select, characterize and evaluate potential isolates of the fungus *Beauveria* spp. from Brazil for use in the control of the broad mite *P. latus* in the greenhouse and field.

2. Material and Methods

This study was conducted in three phases. The first phase was conducted in the laboratory, the second phase was conducted in a greenhouse, and the third phase was conducted in the field in the municipality of Marechal Cândido Rondon, PR, São Cristóvão (24°39'19.96 "S, 54°01'08.66"W, 302 m altitude) from January to March 2014.

2.1. Fungal isolates

Thirty isolates were obtained from the entomopathogenic fungi collection of the Unioeste Agricultural Biotechnology Laboratory - Cascavel *Campus*, PR (UNIOESTE, 2014), where these fungi are stored as pure conidia derived from monosporic cultures and maintained at –80 °C (Table 1).

The isolates were inoculated over seven days on Petri dishes containing a sporulation culture medium (SM) to produce conidia (20 g agar, 5 g yeast extract, 4.6 g salt mixture, 10 g glucose and 1000 mL distilled water) and incubated at 26 ± 1 °C with 14 hours of photophase. The conidia were then scraped from the surface of the culture medium and stored in sterile glass tubes that were closed and stored for a period not exceeding 15 days at –10 °C for later use in bioassays (Alves et al., 1998).

2.2. Source of mites

Mites of the species *P. latus* were obtained from mulberry trees (*Morus alba* L.) (Malvales: Moraceae). Mite identification was performed according to and Moraes and Flechtmann (2008) by fixing specimens and observing them under a microscope.

The mites were reared on Balloon pepper plants (*Capsicum baccatum* L.) (Solanales: Solanaceae) in pots and maintained in a greenhouse. When the leaves became wrinkled, new pepper seedlings were introduced into the stock for infestation (Echer et al., 2002).

2.3. Selection of isolates

The selection of the isolates was based on a study by Oliveira et al. (2004). *Beauveria* spp. strains were grown on artificial media as described above to produce the inoculum. The conidia were scraped and transferred into glass tubes to which distilled water + 0.01% Tween 80 was added. The suspensions were counted in a Neubauer chamber and then standardized to 1×10^8 conidia/mL. The isolates had a minimum viability of 90%, which was verified on artificial media as described by Alves et al. (1998).

In the preparation of bioassays, 226 mm^2 leaf discs were cut using a stainless steel punch. The discs were surrounded with moistened cotton and kept in Petri dishes that were lined with moistened foam with the abaxial side facing up, and they received ten female *P. latus* (Moraes and Flechtmann, 2008). The experimental design was completely randomized. For each isolate, three Petri dishes with three discs containing ten mite females were prepared for a total of 90 mites/treatment. Each plate was considered a replicate.

A suspension with 1 mL volume of the 1×10^8 conidia/mL was sprayed on each leaf disc using a Potter spray tower (Burkard Agronomics, Uxbridge, Middlesex, United Kingdom) (0.703 kgf/cm^2). Only sterile distilled water + 0.01% Tween 80 was sprayed on the controls. After spraying, the plates were incubated (26 ± 1 °C at 70 ± 10% RH for a 14-hour photoperiod). The evaluation was performed on

Table 1. Isolates of *Beauveria* spp. and respective hosts and places of origin.

ISOLATE	SPECIES	HOST	LOCATION
Unioeste 1	*Beauveria bassiana*	*Astylus variegatus* (Coleoptera, Chrysomelidae)	Cascavel, PR
Unioeste 2	*Beauveria sp.*	*Alphitobius diaperinus* (Coleoptera, Tenebrionidae)	Cascavel, PR
Unioeste 4	*Beauveria sp.*	*Alphitobius diaperinus* (Coleoptera, Tenebrionidae)	Cascavel, PR
Unioeste 5	*Beauveria sp.*	*Alphitobius diaperinus* (Coleoptera, Tenebrionidae)	Cascavel, PR
Unioeste 25	*Beauveria bassiana*	Soil, Yerba mate plantation	Cascavel, PR
Unioeste 26	*Beauveria bassiana*	Soil, Yerba mate plantation	Cascavel, PR
Unioeste 36	*Beauveria bassiana*	Chrysomelidae sp.	Cascavel, PR
Unioeste 37	*Beauveria bassiana*	*Bombyx mori* (Lepidoptera: Bombycidae)	Arapongas, PR
Unioeste 38	*Beauveria bassiana*	*Bombyx mori* (Lepidoptera: Bombycidae)	Ibaiti, PR
Unioeste 39	*Beauveria bassiana*	*Cosmopolites sordidus* (Coleoptera, Curculionidae)	São Miguel do Iguaçu, PR
Unioeste 40	*Beauveria bassiana*	Curculionidae sp.	Cascavel, PR
Unioeste 41	*Beauveria bassiana*	*Astylus variegatus* (Coleoptera, Chrysomelidae)	Cascavel, PR
Unioeste 42	*Beauveria bassiana*	Erotylidae sp.	Cascavel, PR
Unioeste 44	*Beauveria bassiana*	Pentatomidae sp.	Toledo, PR
Unioeste 46	*Beauveria bassiana*	*Euschistus heros*	Cascavel, PR
Unioeste 47	*Beauveria bassiana*	Pentatomidae sp.	Primavera do Leste, MT
Unioeste 48	*Beauveria sp.*	*Alphitobius diaperinus* (Coleoptera, Tenebrionidae)	Cascavel, PR
Unioeste 49	Beauveria bassiana	*Alphitobius diaperinus* (Coleoptera, Tenebrionidae)	Cascavel, PR
Unioeste 53	*Beauveria bassiana*	*Alphitobius diaperinus* (Coleoptera, Tenebrionidae)	Cascavel, PR
Unioeste 54	*Beauveria bassiana*	*Alphitobius diaperinus* (Coleoptera, Tenebrionidae)	Cascavel, PR
Unioeste 56	*Beauveria bassiana*	*Anthonomus grandis* (Coleoptera, Curculionidae)	Cascavel, PR
Unioeste 65	*Beauveria bassiana*	*Anthonomus grandis* (Coleoptera, Curculionidae)	Cascavel, PR
Unioeste 66	*Beauveria bassiana*	*Anthonomus grandis* (Coleoptera, Curculionidae)	Cascavel, PR
Unioeste 68	*Beauveria sp.*	*Alphitobius diaperinus* (Coleoptera, Tenebrionidae)	Pinhalzinho, SP
Unioeste 69	*Beauveria sp.*	*Hedypathes betulinus* (Coleoptera, Cerambycidae)	Ivai, PR
Unioeste 70	*Beauveria bassiana*	*Vatiga manihotae* (Hemiptera, Tingidae)	Marechal C. Rondon, PR
Unioeste 71	*Beauveria bassiana*	*Bemisia tuberculata* (Hemiptera, Aleyrodidae)	Marechal C. Rondon, PR
Unioeste 75	*Beauveria bassiana*	*Vatiga manihotae* (Hemiptera, Tingidae)	Marechal C. Rondon, PR
Unioeste 76	*Beauveria bassiana*	*Diabrotica speciosa* (Coleoptera, Chrysomelidae)	Marechal C. Rondon, PR
Unioeste 77	*Beauveria bassiana*	Aphid collected on brassicas	Marechal C. Rondon, PR

the 3rd and 6th day after fungus application, and dead mites were transferred to a humid chamber for conidiogenesis. The cadavers were examined using a stereoscopic microscope (250 × magnification) for signs of the fungus according to Humber (1997).

The data for the total and confirmed percent mortality at six days after application (DAA) was analyzed using descriptive statistics and plotted using Microsoft Excel. Isolates were selected based on their total and confirmed percentage of mortality values (minimum 50%), and the confirmed mortality on the 3rd day was also determined for these isolates.

The data were analyzed for normality with the Shapiro-Wilk test, and data homogeneity was assessed using the Levene test with the program Statistics 7.0. When the results were homogeneous and normally distributed, an analysis of variance (ANOVA) and Tukey test ($p < 0.05$) were used. At the end of the experiment, the isolate that presented the highest total and confirmed mortality was selected for field trials. This experiment was repeated twice, and the same tendency was observed in the results.

2.4. Vegetative growth and conidial production

The selected isolates were characterized by evaluating their vegetative growth and conidial production in a culture medium and on commercial whole white rice.

Vegetative growth was evaluated by growing the isolates on a PDA (potato, dextrose, agar) medium in Petri dishes. A platinum needle was used to inoculate thee equidistant points on the surface of the culture medium. Next, plates were incubated for seven days at 26 ± 1 °C and a 14-hour photoperiod. Subsequently, the colonies were measured (two perpendicular measurements) to obtain the mean colony diameter and area (Alves et al., 1998). For each isolate, four plates were prepared (each plate was considered a replicate). This experiment was repeated twice, and the same tendency was observed in the results.

After the vegetative growth evaluation, two colonies from each plate that exhibited uniform and homogeneous growth were selected to estimate the number of conidia per colony and per cm^2. The colonies were cut from the medium along the borders and individually transferred to sterile glass tubes to which 10 mL distilled water containing 0.01% Tween 80 was added and stirred until the conidia were released. Serial dilutions were performed, and conidia were counted in a Neubauer chamber. Four plates with two colonies were used for each isolate. Each plate was considered a replicate. The average production of conidia was calculated in each plate according to the arithmetic mean of the conidial counts of both colonies. To estimate the conidial production per cm^2, the total production of the colony was divided by its area.

Conidial production in rice was performed according to Alves and Faria (2010) with a completely randomized experimental design in which polypropylene bags were filled with 100 g commercial whole white rice and 40 mL distilled water, the mixture was homogenized manually, and the bags were then autoclaved for 30 min at 121 °C. After cooling, 10 mL conidial suspension (1×10^8 conidia/mL) was added to the mixture in the bags. The bags were then incubated for ten days (26 ± 1 °C over a 14-hour photoperiod) and stirred daily to prevent the formation of clumps. Five bags were prepared for each isolate (each bag was considered a replicate). After ten days, the cultures were evaluated by removing 1 g of the rice + conidia mixture from each bag. Each sample was suspended in 10 mL distilled water + 0.01% Tween 80. Serial dilutions and conidial counts were performed as previously descried to estimate the number of conidia per g rice.

The normality of the data was verified using the Shapiro-Wilk test, and the homogeneity was verified by the Levene test. The data were analyzed using the Kruskal-Wallis test and the Student-Newman-Keuls test for post hoc comparison of means using the program BioEstat 5.3.

2.5. Greenhouse experiment

The greenhouse experiment conducted on bean seedlings (*Phaseolus vulgaris* L., Tiziu variety) occurred in a plastic container with potting soil. When the seedlings reached 20 cm in height and had at least six leaves, the seedlings were subjected to mass broad mite rearing.

The fungus was multiplied once more on rice (Alves and Faria, 2010), and an emulsifiable oil formulation (provided by Novozymes BioAg S.A.) was prepared according to Oliveira (2009). The formulation was suspended in water (1×10^8 conidia/mL) and applied using a backpack sprayer at a volume equivalent to 800 L/ha on the adaxial and abaxial surfaces of the leaves in consideration of the habits of the mite and vegetation cover. In the control, distilled water + Tween 80 0.01% was applied.

The experimental design included completely randomized blocks. The data were analyzed by an ANOVA in a split-split plot in time design, with fungal presence/absence as the principal factor and time of evaluation as the secondary factor (before and 1, 2, 3 and 4 DAA). For each treatment, 50 pots with two plants were prepared. The pots were randomly distributed in five plots for each treatment (10 pots per plot) of distilled water (control) and Unioeste 53 isolate conidial suspension. The experiment was conducted in a randomly arranged split-plot in time design.

Prior to application, the mite population on the plants was assessed by randomly collecting ten leaves from each plot and counting the number of mites. This procedure was repeated at four, eight, 12 and 16 days after the fungal application.

The data were analyzed for normality with the Shapiro-Wilk test, and data homogeneity was assessed using the Levene test. When the results were homogeneous and normally distributed, an ANOVA and Tukey test ($p > 0.05$) were performed using the program Statistics 7.0.

2.6. Field experiment

The experimental area (225 m^2) was divided into eight plots of 10.24 m^2 and randomly distributed with bean plants over eight rows × eight columns as described by Emater (2000). Only the plants in the inner seven rows and seven columns were considered in the experiment. The plots were isolated using plastic sheeting to prevent drift during the fungal spraying. The experimental design was the same as for the greenhouse experiment. The *B. bassiana* Unioeste 53 isolate was applied to four plots (treatment), and water was applied to the other four plots (controls).

The plot infestation, production, fungal application and pre- and post-application evaluations were performed as described for the greenhouse experiment. This experiment was also conducted in a randomly arranged split-plot in time design.

The data were analyzed for normality with the Shapiro-Wilk test, and data homogeneity was assessed using the Levene test. The data were analyzed with the Wilcoxon test, and the mean number of mites on the treated and non-treated plants was compared. The data from different evaluation intervals were analyzed using the Friedman test ($p < 0.05$) (Statistics 7.0).

The efficiency of the treatment for the field experiment was calculated using Henderson & Tilton's formula (Bakr, 2002; Equation 1):

$$100 \times \{1- [(Cb \times Ta) \div (Ca \times Tb)]\} \quad (1)$$

where Tb and Ta represent the mean mite lifespan in the treatment group before and after treatment and Cb and Ca represent the mean mite lifespan in the control group before and after treatment.

3. Results and Discussion

3.1. Selection of isolates

The total mite mortality ranged from 0 to 82%, and a large variation was observed among the isolates. The confirmed mortality percentages were generally as low as 40% and varied among the isolates. Variation between the total and confirmed mortality values for the same isolate is quite common and may occur for many reasons (Figure 1). One of these reasons is that the fungus often causes death, but this cannot always be confirmed because the host may die from mechanical damage, such as by perforation of the integument or vital organs, or indirectly because of fungal growth leading to the production of toxins and the depletion of nutrients. Additionally, the fungus does not always encounter suitable conditions to complete its life cycle (Hajek and St. Leger, 1994; Alves, 1998).

Thus, a minimum confirmation of 50% at 6 DAA was used to select the Unioeste 26, Unioeste 53 and Unioeste 39 isolates (Figure 1). In addition, a comparison of the mortality values at 3 DAA showed that the greatest efficiency was exhibited by isolated Unioeste 53 at 33% mortality. Thus, Unioeste 53 was selected for the greenhouse and field experiments (Table 2).

The selection of isolates is the first step in using entomopathogenic fungi for pest control because of the close pathogen × host relationship and because genetic variability is among the main factors affecting fungus pathogenesis on the host (Chouvenc et al., 2009; Xiao et al., 2012). Thus, effective isolate selection in the laboratory improves the chances of success in the field.

Limited studies have used *B. bassiana* isolates to control *P. latus*, and these studies have primarily compared the effectiveness among different fungal species and not among isolates within the same species.

Penã et al. (1996) compared the mortality of *P. latus* using isolates of *B. bassiana, Hirsutella thompsonii* and *Isaria fumosorosea* and observed mortality of approximately 20% on the 2nd day and 70% by the 6th day.

The duration of *P. latus* female immature phases (from egg to adult) is approximately four days, and females begin oviposition at five days old, laying 5.6 eggs/day over ten days (Vieira and Chiavegato, 1998a, b). Thus, the mortality results for Unioeste 53 at six DAA (74%) correspond to just over half of the female lifespan and demonstrate that the isolate can greatly impact the development of the next mite generation by preventing large numbers of nymphs from reaching adulthood and preventing or reducing oviposition.

Table 2. Percent of confirmed mortality of *Polyphagotarsonemus latus* adult females sprayed with 1 × 10^8 conidia/mL suspension of different *Beauveria* spp. isolates at 3 DAA.

Treatment	Confirmed mortality
Unioeste 53	33.5 ± 2.17 a
Unioeste 39	26.6 ± 1.46 b
Unioeste 26	19.8 ± 0.95 c
CV = 9.16%	

DAA = days after application; CV = coefficient of variance; SEM = standard error of the mean; mean values (± SEM) followed by the same letter in the column do not differ in terms of Tukey test ($p > 0.05$).

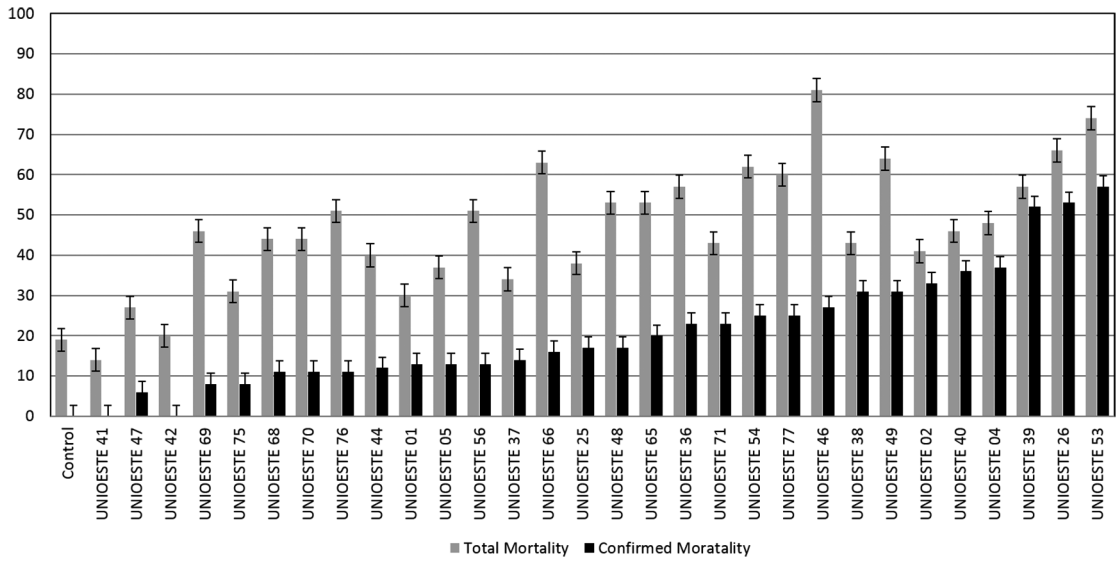

Figure 1. Percent total and confirmed mortality of *Polyphagotarsonemus latus* adult females at 6 DAA using *Beauveria* spp. conidial suspension at a 1 × 10^8 conidia/mL concentration.

Despite differences in the percentage values, Penã et al. (1996) observed results similar to those of this study, in which confirmed mortality values of 33% and 57.7% were found for Unioeste 53 isolates at 3 and 6 DAA, respectively.

Nugroho and Ibrahim (2004) also tested *B. bassiana*, *Metarhizium anisopliae* and *I. fumosorosea* against *P. latus*; these researchers found that *B. bassiana* caused the highest mortality rate (80.9% at a concentration of 1×10^8 conidia/mL) and observed large variation in the mortality caused among its isolates, which is consistent with the results presented here.

Differences in mortality observed in this study could be linked to genetic variation, which results in differences in the ability to produce enzymes, such as proteases and chitinases; cell growth mediators; cuticle-degrading fatty acids; certain hydrophobins that are extremely important for adhesion, infection and pathogenicity to the host; and compounds used as immunosuppressors and toxins, such as beauvericin, which contributes to suppressing the host immune system as well as inhibiting the growth of opportunistic microorganisms (Von Döhren, 2004; Valencia et al., 2011; Xiao et al., 2012).

Additionally, Chouvenc et al. (2009) demonstrated that the host defense system in other arthropods is effective in suppressing fungal infection and can affect fungal action. Moreover, the authors also observed a higher efficiency of the isolates in terms of reduced time to complete the infection cycle (adhesion to the host and colonization and death of the host), which explains variations between isolates.

These results indicate the importance of selecting isolates that have a high ability to kill the host in the shortest time possible and highlight the importance of performing isolate screening for virulence.

3.2. Vegetative growth and conidial production

A comparison of the parameters for growth and conidial production showed that the Unioeste 26 and 39 isolates had average diameters of 1.8 and 2.6 cm, respectively. The Unioeste 53 isolate had an intermediate diameter of up to an average of 2 cm (Table 3). These values are lower than but close to those found in other studies in which colony diameters ranged from 2.9 to 4.5 cm (Potrich et al., 2006; Rohde et al., 2006).

These differences were primarily caused by the distinct isolates investigated in these studies, and because this fungus has a broad spectrum of hosts and large genetic plasticity, physiological variations can be expected among isolates because of their different nutritional requirements. Methodological differences may also have affected the outcomes because the reported incubation times varied between seven and ten days.

Different levels of vegetative growth were reported by Potrich et al. (2006) and Rohde et al. (2006) at 4.8×10^8 and 33.5×10^8 conidia/colony production, respectively. A conidia/colony production of 26.2×10^8 was observed for the Unioeste 53 isolate (Table 3) in the present study, which was in between the values reported in the aforementioned studies.

The Unioeste 53 isolate also exhibited a very high production of up to 7.9×10^8 conidia/cm^2. In the previously cited studies, the highest observed values were approximately 3.0×10^8, showing that colony size is not correlated with conidial production.

Conidial production on rice was higher with the Unioeste 26 isolate (3.1×10^9 conidia/g rice), whereas the Unioeste 53 and 39 isolates were similar (1.6×10^9 and 1.7×10^9 conidia/g rice, respectively) (Table 3).

It is difficult to compare the production rates on rice observed here to those reported for other studies because a culture medium cannot be standardized because of differences in the origin of the rice, group or even harvest, and these effects can manifest in the nutritional quality of the substrate. Nevertheless, the production rates of the Unioeste 53 isolate were similar to those reported in other studies and were even higher in some cases (Londono et al., 1992; Vilas Boas et al., 1996; Potrich et al., 2006; Rohde et al., 2006).

Although the Unioeste 53 isolate was not the most productive on rice, it was chosen for the subsequent tests in the greenhouse and field because of the results for the overall mortality parameters that were evaluated as well as the characterization of the selected isolates, which are important factors for mass production (Petlamul and Prasertsan, 2012).

3.3. Greenhouse experiment

Prior to the fungal application, the mite population on the control plants was 11 mites/leaf, and this value remained statistically unchanged until the 3rd evaluation (12 DAA), when the mite population reached 17.3 mites/leaf and remained constant until the final evaluation. The population grew by 64% on the control plants from pre-application to the last evaluation at 16 DAA (Table 4).

Table 3. Vegetative growth (colony diameter) and conidial production for different *B. bassiana* isolates in sporulation medium (SM) and rice.

Isolate	Mean colony diameter (cm)	Conidial production /colony	[1] Conidial production/cm^2	[2] Production in rice
Unioeste 39	2.6 ± 0.07 a	2.33 ± 0.35 b	0.4 ± 0.06 b	1.7 ± 3.38 b
Unioeste 53	2.0 ± 0.02 b	26.2 ± 4.44 a	7.9 ± 1.43 a	1.6 ± 2.95 b
Unioeste 26	1.8 ± 0.09 b	0.2 ± 0.01 c	0.1 ± 0.00 c	3.1 ± 2.15 a

[1] mean number of conidia per cm^2 × 10^8; [2] mean number of conidia per g rice × 10^9; mean values (± SEM) followed by the same letter in each column do not differ in terms of the Kruskal-Wallis test and the Student-Newman-Keuls comparison ($p < 0.05$).

Table 4. Mean number of live *Polyphagotarsonemus latus* mites on untreated bean leaves and bean leaves that are treated with a conidial suspension of the Unioeste 53 isolate (1×10^8 conidia/mL) in the greenhouse (Cascavel, PR, January to March 2014).

Treatments	Pre-application	Evaluation 1	Evaluation 2	Evaluation 3	Evaluation 4
Control	11.0 ± 0.54 Bb	12.9 ± 0.31 Ab (+17)	11.4 ± 1.01 Ab (+4)	17.3 ± 0.58 Aa (+57)	18.1 ± 0.49 Aa (+64)
Unioeste 53	13.2 ± 0.59 Aa	9.2 ± 0.30 Bb (–31)	4.9 ± 0.31 Bc (–62)	4.3 ± 0.40 Bc (–67)	4.2 ± 0.37 Bc (–68)
CV 1 = 11.28%					
CV 2 = 11.11%					

CV = coefficient of variation; SEM = standard error of the mean; + = percent population increase, – = percent population decrease compared with the pre-application population; mean values (± SEM) followed by the same upper-case letter in a column and the same lower-case letter in a row are not different in terms of Tukey test ($p < 0.05$).

In contrast, the initial population in the treated plants of 13.2 mites/leaf was significantly lower than that for the control plants at the 1st evaluation (4 DAA) at 9.2 mites/leaf. In the 2nd evaluation (8 DAA), the population was reduced even further to less than five mites/leaf and remained at this level until the last evaluation (16 DAA). The population decreased by 68.12% between the pre-application and the final evaluation (16 DAA). The mite population in the treated plants with the fungus was statistically lower than that found in the control plants for all evaluations that were performed after the fungal application (Table 4).

At 8 DAA, the mite population was reduced by approximately 62% and by 68.12% at 16 DAA, which was consistent with the results reported by Penã et al. (1996) in which the *P. latus* population was reduced by 88% at 12 DAA in a greenhouse experiment using *B. bassiana* isolates.

Ashraf et al. (2011) observed a reduction of 87.8% in the broad mite population at 5 DAA using *B. bassiana*, demonstrating more rapid action by the isolate than in the present study, where the reduction was 31% at 4 DAA. However, in subsequent evaluations (7, 10 and 17 DAA), the same authors obtained mean population reductions of 59.89, 42.30 and 0.00%, respectively. In this study, however, the population reduction at 8, 12 and 16 DAA was 62, 67 and 68%, respectively, indicating that the action of the isolate Unioeste 53 was more persistent although slower than that of other *B. bassiana* isolates.

Despite the variation observed between the results of the present study and relevant studies available in the literature caused by differences in the experimental conditions (ambient conditions, such as daily temperature conditions of 22 ± 3 °C and relative humidity of 84 ± 10%; host plant species; and isolate and conidial concentrations, among others), the fungus applied in this study maintained a low mite population throughout the experiment, which demonstrates the potential of the Unioeste 53 isolate to control this pest.

3.4. Field experiment

The initial mite population at pre-application was homogeneous, and significant differences were not observed in the initial number of mites between plants.

The control mite population remained constant throughout the experiment and did not exhibit significant changes compared with the mite population in the plants that were treated with the fungus. The action of the fungus on the mite population resulted in significant variations of the treated populations, with the mite population gradually decreasing up to 12 DAA and increasing significantly at 16 DAA. However, these populations remained below the population size at pre-application, and mycosed mites were not observed on treated plants.

Significant differences in size were not observed between mite population in the untreated plants and those treated with fungus at each evaluation; however, the efficiency of the fungal treatment, which was obtained using Henderson-Tilton's formula, gradually increased over time from 28% after 4 DAA to a maximum value of 69% at 12 DAA and then decreasing to 49% at 16 DAA (Table 5).

Compared with the pre-application values, the fungal application resulted in a population reduction of approximately 26% at 4 DAA, whereas the population in the control remained virtually unchanged. In the 2nd evaluation at 8 DAA, the fungal application resulted in a population reduction of 47% compared with a reduction of only 6% in the control. In the 3rd evaluation at 12 DAA, the population decreased by 67% for the treated plants and increased by 4% in the control. However, in the final evaluation at 16 DAA, the population increased from that at 12 DAA, and the final population in the treated plants was 42% lower than the pre-application population in the treated plants; however, the final population exceeded the initial population by 10% in the control (Table 5).

In this pioneering study to assess the control of *P. latus* using pathogenic fungi under field conditions, pathogen efficiency was moderate compared with that of chemical acaricides, and the use of abamectin, multimethyl alkenol and dicofol in cotton reduced the mite population by up to 94% at 13 DAA or 90% at 14 days after Pyridathioben® and Cascade application (Silva et al., 1989).

Chemicals are immediately effective on hosts because the majority of chemicals act directly on the nervous system and cause motor activity to cease almost immediately, which affects the physiological functions of the hosts and causes death (Coutinho et al., 2005). In contrast, fungi require a longer time to develop and kill the host. However, according to the concept of augmentative biological control, successive applications of a fungus contribute to an

Table 5. Mean number per treatment of live *Polyphagotarsonemus latus* mites on untreated bean leaves and bean leaves that are treated with a suspension of conidia from the Unioeste 53 isolate (1×10^8 conidia/mL) in the field (Marechal Cândido Rondon, PR, January to March 2014).

	Pre-application	Evaluation 1 (4 DAA)	Evaluation 2 (8 DAA)	Evaluation 3 (12 DAA)	Evaluation 4 (16 DAA)
Control	16.1 ± 2.54 Aa	16.3 ± 1.85 Aa (+1)	15.5 ± 1.08 Aa (–7)	16.8 ± 0.64 Aa (+4)	17.7 ± 1.05 Aa (+10)
Unioeste 53	29.8 ± 5.80 Aa	22.0 ± 3.08 Ab (-26)	15.8 ± 2.47 Ab (–47)	9.9 ± 0.92 Ac (–67)	17.0 ± 0.81 Ab (–42)
Efficiency (%)	-	28	45	69	49

+ = percent population increase, – = percent population decrease compared with the pre-application population; means (± SEM) followed by the same upper-case letter in each column are not different in terms of the Wilcoxon test ($p < 0.05$); mean values (± SEM) followed by the same lower-case letter in each row are not different in terms of the Friedman test ($p < 0.05$). Efficiency % = percent efficiency over time (per evaluation) calculated using Henderson-Tilton's formula.

increased inoculum potential in the environment, thereby increasing the effectiveness of fungi over time (Alves et al., 2008). Nevertheless, sequential applications of chemicals promote selective resistance among populations, which increases the difficulty of pest control.

Furthermore, chemical acaricides are not allowed for all of the crops in which mites occur (BRASIL, 2012). *P. latus* is a polyphagous species; thus, entomopathogenic fungi are important for the control of this pest because there are no restrictions on the use of these fungi in crops for which chemical use is not allowed, including organic crops (Gallo et al., 2002), although fungal biocontrol products must be registered for such crops.

The 41.4% efficiency achieved in the 3rd evaluation shows the potential of the fungus to control the mite, and this efficiency could be increased by reapplication according to the incremental biological control concept developed by Alves (1998) and Alves et al. (2008). Compared with the effects of chemical acaricides, the effectiveness of the biological control of mites is increased by predatory mites, which are not affected by the fungus and thus are preserved in the agro-ecosystem (Cavalcanti et al., 2008).

In addition, under greenhouse and field conditions, the *P. latus* population size after application always remained below the original size before the fungal treatment, whereas in the control group, the population increased over time. These results demonstrate the isolate's potential for control, especially under greenhouse conditions, where *P. latus* is a major cause of damage to many crops.

Because of the absence of studies testing entomopathogenic fungi control of *P. latus* under field conditions, the results of the present study can be used to advance research on mite control and promote pest control alternatives to mitigate the negative impacts to crop production or natural resources and the health of farmers and consumers. However, additional studies are necessary to evaluate the effectiveness of the fungus in reapplications, which may increase fungal populations in the environment as an augmentative pest control technique.

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