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CROP PROTECTION

Association of adjuvants and *Beauveria bassiana* fungus to control of Paraguay tea ampul

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ABSTRACT: *Aiming to increase the activity of the fungus Beauveria bassiana (Unioeste 44) against adults of Gyropsylla spegazziniana, the combination of the fungus with adjuvants Aureo® and Assist® was evaluated for application. The bioassays were carried out in direct and residual contact with insects on yerba mate seedlings. The effects on some biological parameters of the fungus were also evaluated in vitro. The combination of the fungus and the adjuvants in direct contact resulted in mortality rates of 65 and 75% (Aureo® and Assist®, respectively), with no difference between them; however, the mortality rate was greater than that obtained with the fungus alone (47 %) (P < 0.05). Mortality from residual contact was lower, but the association with adjuvant Assist® (48%) compared to Aureo® (16%) was still advantageous. Conidiogenesis in cadavers was not affected by adjuvants in the treatment by direct contact. However, in residual contact, conidiogenesis increased with the Assist® addition. Despite affecting the germination and growth of the fungus, the adjuvants were considered compatible. In addition to the efficiency of their combination, neither adjuvant caused phytotoxicity to yerba mate seedlings, representing advantages of its use in G. spegazziniana population management.*
Key words: *entomopathogenic fungi, yerba mate, Ilex paraguariensis, Gyropsylla spegazziniana, biological control.*

Associação de adjuvantes e fungo *Beauveria bassiana* visando ao controle da ampola da erva-mate

RESUMO: *Visando incrementar a atividade do fungo Beauveria bassiana Unioeste 44 contra adultos da ampola-da-erva-mate (Gyropsylla spegazziniana), avaliou-se a associação do fungo com adjuvantes Aureo® e Assist®, para calda de aplicação. Os bioensaios foram relizados em contato direto e residual sobre insetos. Foram também avaliados in vitro os efeitos sobre alguns parâmetros biológicos do fungo. A associação fungo + adjuvantes resultou em mortalidade de 65 e 75%, sem diferença entre eles, porém, significativamente maior que a obtida com o fungo isoladamente. A mortalidade no contato residual foi menor, mas também houve vantagem na associação com adjuvantes, principalmente Assist® (48%), em relação ao Áureo® (16%). A conidiogênese nos cadáveres não foi afetada pelos adjuvantes no tratamento de contato direto. Contudo, houve incremento na conidiogênese no tratamento de associação com Assist® no contato residual. Apesar de afetarem a germinação e o crescimento do fungo, os adjuvantes foram considerados compatíveis com o fungo. Além da eficiência da associação, ambos adjuvantes não causaram fitotoxidade para a erva-mate, representando vantagens do seu uso no manejo da ampola.*
Palavras-chave: *fungo entomopatogênicos, erva-mate, Ilex paraguariensis, Gyropsylla spegazziniana, controle biológico.*

INTRODUCTION

Gyropsylla spegazziniana Lizer & Trelles (Hemiptera: *Aphalaridae*), also named Paraguay tea ampul, is the most important nursery and planting pest of yerba mate (*Ilex paraguariensis* St.-Hil.) (*Aquifoliaceae*). Insect nymphs develop inside the galls in the buds of yerba mate plants, which are formed by the toxicogenic action of the saliva during the female feeding on the plant. As a consequence, the plant suffers leaf fall and grows new branches, affecting the productivity and quality of the final product. It should be noted that the galls are closed until the nymphs reach the fifth instar to finish development. Thus, the immature insects are protected, and control methods should target the adults (LEITE & ZANOL, 2001).

Beauveria bassiana (Bals.-Criv.) Vuill. (Hypocreales: *Cordycipitaceae*) Unioeste 44 strain was proven to be effective against *G. spegazziniana*

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nymphs (mortality over 80%) (ALVES et al., 2013; FORMENTINI et al., 2015). Adults were less susceptible when conidia were prepared with an aqueous suspension (LOEBLEIN, 2019). Conversely, adjuvants in suspension can increase the wettability and dispersal, distribution and mixing of conidia in water, and its adhesion and retention on pests, improving fungal activity in insect control (HOLLOWAY et al., 2000; ALVES et al., 2002; COWLES et al., 2020; ARNOSTI et al., 2019).

It is noteworthy that before adding any product, it is first necessary to know its effects on the biological parameters of the entomopathogenic fungus. Adjuvants such as CitrusWrap, Sylgard, Orocit, and Oroboost used in citrus cultivation are incompatible with the entomopathogenic fungus *Isaria fumosorosea* (Wize) (=*Paecilomyces fumosoroseus*) (Hypocreales: *Cordycipitaceae*), which can control *Diaphorina citri* Kuwayama (Hemiptera: *Liviidae*) (AVERY et al., 2013). Other products, such as Orchex, Sun Pure, Conoco Blend-1, Conoco Blend-2, and JMS adjuvants, improved the pathogenicity of *I. fumosorosea* on *D. citri* (KUMAR et al., 2017). In addition, both germination of *I. fumosorosea* conidia and their adhesion on *D. citri* adults increased with the addition of the adjuvants Silwet L77 0.025% and KBRAjd 0.075%, demonstrating the importance of these previous studies in biocontrol programs based on fungal pulverization (ARNOSTI et al., 2019).

Thus, this study evaluated and compared the combination of adjuvants with the fungus *B. bassiana* for Paraguay tea ampul control on yerba mate seedlings, as well as the *in vitro* effects of these products on the biological parameters of the fungus.

MATERIALS AND METHODS

Organisms and adjuvants

Infested branches with developed galls were manually collected in a commercial yerba mate plantation, Cascavel, PR (24°58'00''S; 54°23'27''W) without phytosanitary management. The galls were insert in a plastic bag. In the laboratory, the galls were opened, and those with fourth- and fifth-instar nymphs based on description from LEITE & ZANOL, (2001) were transferred to plastic boxes with a screened lid and a bottom filled with filter paper. The insects were kept in controlled conditions (26 ± 1 °C; 60±5 % relative humidity and a 12-h daylight cycle) to complete the development until adult emergence. In the bioassays, non-sexed adult insects were used at 24-36 h postemergence.

Yerba mate seedlings (approximately 20 cm high) were cultivated in 700 mL plastic pots with organic compost (earth, earthworm humus, charcoal, and ground pine bark) and kept under a 50% shading level, kept under 50% shade screen, and irrigated every two days.

The *B. bassiana* (*sensu lato*) strain Unioeste 44 was identified by analysis of the Bloc nuclear intergenic region sequence (REHNER et al., 2011) and the sequence was deposited at GenBank (OK004060). This strain was previously selected as virulent against *G. spegazziniana* (FORMENTINI et al., 2015). The fungus was grown on conidia production medium (KH_2PO_4 0.36 g, $NA_2HPO_47H_2O$ 1.05 g, $MgSO_47H_2O$ 0.60 g, KCl 1.0 g, glucose 10.0 g, $NaNO_3$ 1.58 g, yeast extract 5.0 g, agar 20.0 g, 1000 mL distilled water) in Petri dishes at 26 ± 1 °C; 12-h daylight cycle for 7–10 days. Conidia were scraped off with a spatula and kept in glass tubes in a desiccator with silica gel for seven days (humidity ~ 18%). The material was sieved (32 mesh), and the obtained conidia were stored at -20 °C (hermetic containers; 1.1×10^{11} conidia g^{-1}; minimum viability of 90%) until use.

The adjuvants Aureo® (https://www.agro.bayer.com.br/produtos/aureo) and Assist® (https://agriculture.basf.com/br/pt/protecao-de-cultivos-e-sementes/produtos/assist-ec.html) were tested. A distilled water + Tween 80 0.01% solution was used for comparison. The control only contained distilled water (Table 1).

Bioassays

In vivo evaluation

Treatments were performed with control (distilled water), both adjuvants alone at the recommended concentration (RC), 75% RC, 50% RC, 25% RC, Tween 80 (standard for comparison) was used at 0.01%, *B. bassiana* Unioeste 44 conidia suspension (1×10^9 conidia mL^{-1}) alone and the fungus in combination with each adjuvant.

For direct contact, 20 adults were confined in transparent plastic cups with 1 mm perforations at the bottom (5 cm high × 6 cm ∅). Each cup was sprayed with 200 µL from an airbrush sprayer connected to an air compressor (under a constant pressure of 0.5 kgf cm^{-2}) inserted into the opening of the container's lid. After spraying, the insects were transferred to cylindrical transparent polyvinyl chloride (PVC) cages (30 cm high × 12 cm ∅) with side openings (6 cm height × 5 cm length), and the upper part was covered with voile-type fabric containing a yerba mate seedling (LOEBLEIN et al., 2019). For each treatment and control, five replicates were prepared (20 insects per cup; n = 100).

Table 1 - Adjuvants used in the bioassay with the fungus *Beauveria bassiana* Unioeste 44 aiming to control of Paraguay tea ampul.

Product	Active ingredient	Chemical group	Concentration A.I. (g L^{-1})	Recommended concentration (RC) (%)
Assist®	Mineral oil	Aliphatic hydrocarbons	756	1.0
Aureo®	Vegetable oil	Soybean oil methyl ester	720	0.25
Tween 80	Polysorbate	Ethoxylated sorbitan esters	-	0.01

To assess the residual contact, the same procedures as for direct contact were adopted; however, seedling treatment occurred prior to insect release (1 ml of each treatment/seedling, a volume sufficient for a uniform distribution on the seedlings without runoff). After drying, the seedlings were transferred to PVC cages each with 20 adults.

In both bioassays, after the treatments, the plants were kept in a climate-controlled room (25 ± 2 °C, 70 ±10 % relative humidity and a 12-h daylight cycle). In both bioassays, mortality was assessed daily for 10 days after the treatments. Dead insects were immersed in an ethanol solution (70%) for five seconds and then in distilled water and kept for five days in a Petri dish plate with a cotton plug moistened with distilled water (humid chamber) to allow the development of the external mycelium and conidiogenesis. The confirmation of mortality by the fungus was achieved by observing the insects with a stereoscope (×40). A randomized experimental design. The bioassays were performed twice.

In vitro evaluation

The interaction of the fungus with the adjuvants was evaluated by conidial germination, vegetative growth, and conidial production (SILVA & NEVES, 2005; OLIVEIRA et al., 2015), as described below.

1) Viability: 300 µL of the conidial suspension (1.0×10^7 conidia mL^{-1}) was centrally placed using a micropipette on the surface of the PDA medium in a Petri dish. After drying, 250 µL of distilled water (control) and each adjuvant solution were applied with an airbrush sprayer connected to an air compressor (under a constant pressure of 0.5 kgf cm^{-2}). After an 18-hour incubation period (26 °C ± 1 °C, 12-h daylight cycle), germinated and non-germinated conidia were counted under a 400× optical microscope. Conidia were considered germinated if a germ tube larger than the conidial length was visible.

2) Vegetative growth: the fungus was inoculated at three points on the surface of the PDA medium in Petri dishes, and plates were incubated (26 °C ± 1 °C, 12-h daylight cycle). After 48 h, the plates were sprayed as described previously and incubated for seven days under the same conditions. Colonies were measured (two perpendicular measurements) to obtain the mean colony diameter and area.

3) Conidia production: After measurement, each colony was cut out of the culture medium with a spatula and suspended in a glass tube with distilled water + Tween 80 0.01%. The tubes were vortexed for 2-3 minutes to dislodge conidia from the colony. Thus, the concentration of conidia in the final suspension was determined using a Neubauer chamber, and the number of conidia $colony^{-1}$ was estimated.

Eventual phytotoxic effects of the adjuvants on the yerba-mate plants were analyzed, based on visual alteration on the colour or on the leaf morphology.

Statistical analysis

Bioassays were conducted in a completely randomized design. Normality was assessed in all treatments (Shapiro-Wilk test). Mortality data (total and confirmed) were analysed by one-way analysis of variance (ANOVA). Means were compared (Fisher LSD test; $P < 0.05$). To compare the mortality data (total and confirmed) obtained by direct and residual contact data, Student's t-test was used ($P < 0.05$). The biological parameter data were analysed for variance (ANOVA) and compared with each other (Tukey test; $P < 0.05$).

The effect of the adjuvants on the fungus was analyzed based on the toxicity calculation, according to the formula BI =, where BI = biological index; VG = percentage of vegetative growth in relation to the control; ESP = percentage of conidia production in the colonies in relation to the control; GER = germination percentage, with BI> 66

compatible; moderately toxic 42 <BI <66, and toxic BI <42 (0–41) (ROSSI-ZALAF et al., 2008).

The synergistic, additive, or antagonistic interaction between the fungus and the adjuvants was determined using a χ^2 test (KOPPENHÖFER & KAYA, 1997).

RESULTS

In direct contact, the combination of the adjuvants Aureo and Tween (standard) at RC concentration with fungus caused higher mortality than fungus and the adjuvant at RC alone ($P < 0.05$). In the combination of the adjuvants at the RC concentration, there was no significant difference between the products, with 65 and 74% mortality (for Assist® and Aureo®, respectively). Both products with fungus had a higher mortality than Tween 80 was used (47%). At the other concentrations, the Assist® + fungus was superior to the other treatments. (Table 2). In addition, the adjuvants alone, at most concentrations tested, also showed insecticidal activity.

In assessing the residual contact, higher activity was observed for Assist® RC, both alone (31% mortality) and in combination with the fungus (48%). With the other adjuvant, mortality was not expressive (ranging from 5 to 17%) ($P < 0.05$).

In addition, the low mortality obtained with Tween 80 (4 to 12%), both in direct and residual contact, emphasizes its safety for insects, thus justifying its choice as a standard for comparison (Table 2).

Comparing both types of contact (direct and residual), total mortality was significantly higher in direct contact ($P < 0.05$) using adjuvants at RC ($P < 0.05$). There was a significant effect of Tween 80 at all concentrations in direct contact (12% mortality). The exception was Assist®, which was more effective in residual contact in 50 RC (Table 2).

The interaction of adjuvants Assist® and Aureo® with the fungus was generally positive. In direct contact, an additive effect was reported with

Table 2 - Total mortality of *Gyropsylla spegazziniana* adults submitted to treatment with conidia of the fungus *Beauveria bassiana* Unioeste 44 (1×10^9 conidia mL^{-1}) alone or combined with adjuvants at different concentrations with direct and residual contact.

Treatment	Adjuvants						
	N	Assist®	Assist® + fungus	Aureo®	Aureo® + fungus	Tween 80	Tween 80 + fungus
Direct contact							
RC[2]	20	57 ± 7.24 Ab*	65 ± 7.07 Aab* (AD)	43 ± 10.72 Ac*	74 ± 6.58 Aa* (AD)	12 ± 1.58 Ad*	47 ± 6.32 Abc*
75RC	20	18 ± 5.98 Bbc	45 ± 12.74 Ba (A)	11 ± 3.76 BCc	24 ± 3.76 Bb (AD)	12 ± 1.58 Ac*	--[1]
50RC	20	5 ± 1.29 Cc	47 ± 8.80 Ba (AD)	16 ± 3.76 Bb	18 ± 6.32 Bb (AD)	12 ± 1.58 Abc*	--
25RC	20	23 ± 6.64 Bb	41 ± 5.16 Ba (A)	19 ± 6.58 Bbc	27 ± 7.79 Bb (A)	12 ± 1.58 Ac*	--
Control	20	4 ± 2.41 Ca	4 ± 2.41 Ca	4 ± 2.41 Ca	4 ± 2.41 Ca	4 ± 2.41 Aa	4 ± 2.41 Ba
Residual contact							
RC	20	31 ± 5.16 Ab	48 ± 4.83 Aa (AD)	6 ± 2.41 Ad	16 ± 4.74 Ac (S)	4 ± 1.29 Ad	8 ± 1.29 Ad
75RC	20	24 ± 5.55 Bb	49 ± 3.76 Aa (S)	8 ± 3.29 Ad	15 ± 4.56 Abc (S)	4 ± 1.29 Ad	--
50RC	20	22 ± 4.37 Bb*	37 ± 2.58 Ba (AD)	8 ± 2.58 Ac	17 ± 3.29 Ab (S)	4 ± 1.29 Ac	--
25RC	20	14 ± 3.16 Cb	36 ± 6.89 BaA (S)	5 ± 2.04 Ac	14 ± 2.41 ABb (S)	4 ± 1.29 Ac	--
Control	20	5 ± 2.04 Da	5 ± 2.04 Ca	5 ± 2.04 Aa	5 ± 2.04 Ba	5 ± 2.04 Aa	5 ± 2.04 Aa

[1]Means (± MSE) followed by the same capital letter in the column and lower case in the line do not differ statistically (Fisher LSD test - $P < 0.05$); N = number of insects in each repetition; *Significant difference for the same treatment, in the respective concentration, between contact strategies (Student's t test - $P < 0.05$); [1]-- = unassessed concentration. [2]Recommended concentration (RC); AD = additive; A = antagonistic; S = synergistic.

Assist® RC and 50 RC. Antagonism occurred with this adjuvant at 75 and 25 RC. In addition, Aureo® was only antagonistic at the lowest concentration. In residual contact, the positive interaction of the adjuvants was more evident. The interaction with the Assist® adjuvant was additive (RC and 50RC) or synergistic (75 and 25RC). Aureo® at all concentrations showed synergistic interactions with the fungus (D.F. = 1; P = 0.05) (Table 2).

Assessing the effect of the combination on confirmed mortality, in direct contact, significantly higher fungal outgrowth was observed on the cadavers from Assist® 50 and 25 RC treatments (29 and 26%, respectively). There was not a clear trend for the effect of Áureo® combined with the fungus. Only RC and 25 RC were different from the control. Comparing both adjuvants there was a significant reduction in confirmation with Áureo® 50 and 25 RC combined with fungi (Table 3). In addition, Tween 80 (standard for comparison) showed no difference in relation to the other adjuvants tested at the RC concentration.

However, in residual contact, Assist®-confirmed mortality ranged from 17 to 29%. It was significantly higher than observed with Áureo® in most of concentrations. There was not a clear effect of Assist® on the confirmed mortality. Conversely, there was no difference between Áureo® concentration on the confirmed mortality by the fungus, ranging from 6 to 11%. In contrast, no mortality was confirmed from the fungus in association with Tween 80 (Table 3).

Viability was affected by the adjuvants, especially Assist® (P < 0.05). By 18 hours, 96% of conidia had germinated in the control, whereas germination rates for Assist® and Aureo® were 90.8 and 94.7%, respectively. Vegetative growth was also affected by Assist®. Nevertheless, conidiogenesis was not affected (Table 4). In addition, both adjuvants showed a BI value well above the minimum for compatibility.

DISCUSSION

Both adjuvants are based on oil as an active ingredient and have surfactant action to improve surface coverage in the application of pesticides (BASF, 2020; BAYER, 2020). This oily nature may explain the high mortality in direct contact obtained with both adjuvants. Oily emulsions adhere to wings and other insect structures, leading to death. They also

Table 3 - Confirmed mortality (%) of *Gyropsylla spegazziniana* by the fungus *Beauveria bassiana* Unioeste 44 (1×10^9 conidia mL^{-1}) alone or combined with adjuvants at different concentrations with direct and residual contact.

Treatment	Adjuvants			
	N	Assist® + fungus	Áureo® + fungus	Tween 80 + fungus
Direct contact				
RC[2]	20	19 ± 6.25 Ba	21 ± 5.55 Aa*	24 ± 6.89 Aa*
75RC	20	13 ± 6.32 Ba	13 ± 5.62 ABa	--[1]
50RC	20	29 ± 6.89 Aa	6 ± 2.41 Bb	--
25RC	20	26 ± 6.89 ABa	17 ± 4.37 Ab	--
Control	20	0.0 ± 0.00 Ca	0.0 ± 0.00 Ba	0.0 ± 0.00 Ba
Residual contact				
RC	20	21 ± 8.51 ABa	6 ± 1.29 ABb	0.0 ± 0.00 Ab
75RC	20	29 ± 9.44 Aa	9 ± 3.76 Ab	--[1]
50RC	20	17 ± 4.37 Ba	11 ± 3.76 Aa	--
25RC	20	21 ± 8.99 ABa	7 ± 2.58 ABb	--
Control	20	0.0 ± 0.00 Ca	0.0 ± 0.00 Ba	0.0 ± 0.00 Aa

[1] Means (± SD) followed by the same uppercase letter in the column and lowercase letter in the line do not differ statistically from each other (Fisher LSD test - P < 0.05). *Significant difference for the same treatment, in the respective concentration, between contact strategies (Student's t-test - P < 0.05); [1]-- = unassessed concentration; [2]Recommended concentration (RC).

Table 4 - Effect of adjuvants on *in vitro* growth, sporulation, viability, and compatibility with *Beauveria bassiana* Unioeste 44 and its biological index.

Treatments	Viability (%)	vegetative growth (cm^2)	Conidiogenesis ($\times 10^6$)	BI (%)	Classification
Assist®	90.8 ± 1.75 c	1.8 ± 0.04 b	112.7 ± 6.96 a	89.6	Compatible
Aureo®	94.7 ± 1.38 b	1.9 ± 0.04 a	117.2 ± 17.01a	91.9	Compatible
Control	96.0 ± 1.03 a	1.9 ± 0.05 a	135.0 ± 7.28 a	--	--

Means (±MSE) followed by the same letter in the column do not differ significantly (Tukey's test - $P < 0.05$); BI = biological index; - = not evaluated.

form a thin layer on the surface of the insect body, which leads to the closure of spiracles, preventing gas exchange (TAVERNER, 2002; LEONG et al., 2012).

As demonstrated, both adjuvants were compatible with the fungus and increased insect total mortality when associated with the fungus at most concentrations tested. In addition to compatibility, the presence of adjuvants increases the amount of inoculum on the insects, especially in residual contact (AUSIQUE et al., 2017), as we observed here. They also allow better dispersion and solubilization of the active ingredient as well as increased deposition, dispersal, wetting, adhesion, and retention of conidia on the insect or plant structure, which consequently increases the activity of the pathogen (ARNOSTI et al., 2019).

In addition, BUTELER & STALLER (2011) proved that adjuvants reduce the resistance of the integument to the penetration of pathogens and contribute to water loss due to their lipidic nature. The effect of adjuvants on insect integument surfaces was previously observed by ARNOSTI et al. (2019). *D. citri* adults sprayed with the adjuvants KBRAdj® and Silwet L-77® showed spots on the cuticle that they were not observed in the treatment with Tween 80. A scanning electron microscope observation elucidated that the spots were lesions on the insect epicuticle. Also, ARNOSTI et al. (2019) reported a positive effect of the association of the fungi *B. bassiana* and *I. fumosorosea* with adjuvants (KBRAdj® and Silwet L-77®), which also have insecticidal action when applied alone, as we observed in this study.

As observed here, MOTA el al., (2017) previously observed that Tween 80 in water solution was the least effective adjuvant in combination with *B. bassiana* when aiming to control *D. citri*. This confirmed the observation that fungal formulations in emulsifiable adjuvante oils are more efficient than conidial suspension sprays in Tween 80 solution (ALVES et al., 2002).

The higher total mortality in treatments with adjuvants combined with the fungus, plus the synergistic effect shown by Assist® (75 and 25 RC - concentrations at residual contact) and Áureo® (all concentrations - residual contact), reinforces the benefits of their addition, even at a lower concentration. This demonstrated the importance of adjuvants for the biological control of sucking insects with fungal spraying (KOPPENHÖFER & KAYA, 1997; ARNOSTI et al., 2019).

The compatibility of adjuvant oils with entomopathogenic fungi requires both the absence of damage to the fungus and greater efficiency in controlling the insect (AVERY et al., 2013; KUMAR et al., 2017). SILVA et al. (2006) observed a great number of compatible adjuvants based on mineral or vegetal oil (among them Assist®) for *B. bassiana*. Microorganisms metabolize active ingredients, and molecules are released and can be used as secondary nutrients, promoting vegetative growth and conidiogenesis (MOINO Jr. & ALVES, 1998). It is noteworthy that reduction of viability was significant by both adjuvants (4 to 6% less viable conidia than control treatment). Germination is in the initial step in fungus infection. Reducing the germination is possible to affect the fungus activity (SILVA & NEVES, 2005). However, the reduction of viability observed here was not sufficient to affect the fungus activity. Also, BI obtained to Aureo® and Assit® (91,9 and 89,6, respectively) was at least twice the minimum established for compatibility based on BI calculation (BI = 42). The compatibility of Assist® with *B. bassiana* was also previously verified with germination above 98% (LUZ et al., 2004).

Laboratory tests have the advantage of exposing the pathogen to the maximum possible

activity of the evaluated products, which does not occur under field conditions. Thus, when a treatment is compatible *in vitro*, there is strong evidence of its selectivity in field conditions. Additionally, products considered compatible in the laboratory can be considered safe on the fungus (ALVES et al., 1998).

Thus, the combination of the fungus *B. bassiana* Unioeste 44 and the adjuvants Assist® or Aureo® has great potential for the control of *G. spegazziniana*. In addition, the association is favoured by the fact that no phytotoxic effects were caused for the yerba mate plants by the adjuvants. Further studies are needed to confirm the ampul control efficiency with the fungus pulverization in yerba mate nurseries and crops. In addition, it is necessary to evaluate the action of the formulation in protecting the conidia against adverse conditions in yerba mate crops.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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