BIOLOGICAL CONTROL

Control of *Triatoma infestans* (Klug) (Reduviidae: Triatominae) with *Beauveria bassiana* (Bals.) Vuill.: Preliminary Assays on Formulation and Application in the Field

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Controle de *Triatoma infestans* Klug (Reduviidae: Triatominae) com *Beauveria bassiana* (Bals.) Vuill.: Testes Preliminares sobre a Formulação e a Aplicação em Campo

RESUMO - O fungo *Beauveria bassiana* (Bals.) Vuill., isolado CG 306, foi testado contra o *Triatoma infestans* (Klug) através de aplicação indireta por contato do inseto com papel filtro tratado com conídios. O patógeno foi aplicado sobre papel filtro através de filtragem a vácuo de uma suspensão de conídios. Foram feitas estimativas do tempo médio de sobrevivência entre 15 e 21 dias nas densidades de $3x10^6$ e 10^7 conídios/cm^2, respectivamente, sem diferenças significativas entre as concentrações. A concentração de $2,4x10^6$ conídios/cm^2 de CG 306 foi necessária para matar 50% de ninfas de terceiro ínstar de *T. infestans* 25 dias após inoculação e incubação a 25°C e 50% de umidade relativa. A CL_{50} foi estatisticamente maior ($2,0x10^7$ conídios/cm^2) quando os insetos foram expostos por uma hora aos conídios distribuídos sobre papel filtro. Um emulsificante à base de óleo mineral (2%) não afetou a germinação de conídios *in vitro*. Um número menor de conídios foi necessário para matar 50% dos insetos após exposição constante ao papel filtro tratado, em comparação com os conídios não formulados com o emulsificante. Quando a exposição foi limitada a 1 h, os conídios não formulados foram mais efetivos do que conídios formulados. Após aplicação de *B. bassiana* (10^7 conídios/cm^2) em pequenas casas experimentais de madeira, seguida de liberação de ninfas de terceiro ínstar de *T. infestans*, o nível de infecção de insetos recuperados 25 dias após aplicação com o fungo foi significativamente inferior as encontrado na casa-testemunha. A mortalidade atribuída a infecção fúngica de insetos recuperados e mantidos em laboratório foi de 38,1 a 93,8%. A mortalidade de insetos expostos ao papel filtro tratado e mantido em copos cobertos com gaze dentro das casas, foi de 35 a 78,8% 25 dias após aplicação. A persistência dos conídios foi superior a 98% durante os experimentos de campo. Não houve desenvolvimento de *B. bassiana* sobre cadáveres originados de infecção provocada em laboratório e posteriormente expostos às condições de campo no interior das casas.

PALAVRAS-CHAVE: Insecta, doença de Chagas, controle biológico, Hyphomycetes.

ABSTRACT - *Beauveria bassiana* (Bals.) Vuill., isolate CG 306, was assayed against *Triatoma infestans* (Klug) using indirect application by contact with a treated filter paper. The fungus was deposited on filter paper by vacuum-filtration of a conidial suspension. Estimates of 50% survival time of insects were between 15 and 21 days at $3x10^6$ and 10^7 conidia/cm^2 of treated support, respectively, without significant difference between concentrations. A concentration of $2.4x10^6$ conidia/cm^2 of CG 306 was necessary to kill 50% of *T. infestans* third instar nymphs 25 days after inoculation at 25°C and 50% relative humidity, when insects were continuously exposed to conidia. The LC_{50} was significantly higher ($2.0x10^7$ conidia/cm^2) when insects were exposed for 1 h to the treated filter paper, than with continuous exposure. A mineral oil based emulsifier (2%) had no effect on germination of conidia *in vitro*. Fewer formulated conidia were necessary to kill 50% of insects after constant exposure to the treated surface, compared with unformulated conidia. After a 1 h exposure, unformulated conidia were more effective than formulated. After spraying *B. bassiana* (10^7 conidia/cm^2) and releasing third instar nymphs of *T. infestans* in small wooden test houses, the rate of insect recovery after 25 days was significantly lower in the fungus treated houses compared to the control house. Mortality due to fungal infection of recovered insects transferred to the laboratory was between 38.1 and 93.8%, compared with no mortality in the control group. The rate of mortality of insects exposed to treated filter paper in gauze covered cups in the houses was between 35 and 78.8% after 25 days. Persistence of conidia on filter paper exposed inside the houses was > 98% during the field tests. There was no development of CG 306 on cadavers originating from laboratory infection and exposed to field conditions in the houses.

KEY WORDS: Insecta, Chagas disease, biological control, Hyphomycetes.

Beauveria bassiana (Bals.) Vuill. has been shown to be a promising candidate to control *Triatoma infestans* (Klug) (Reduviidae: Triatominae), a serious vector of Chagas disease in Southern Latin America (Luz *et al.* 1998a). Little data on the effectiveness of entomopathogenic fungi against triatomine vectors under field conditions are published. Climatic conditions are one constraint of fungal activity. Romaña (1992) tested a *B. bassiana* isolate on *T. infestans* under field conditions in Argentina and found that fungal activity was limited by high and low temperatures, which varied between 10 and 30°C. Luz (1994) reported on the limiting effect of humidity on the infection of *Rhodnius prolixus* Stål with *B. bassiana* and sporulation on cadavers in different typical domestic and peridomestic habitats in Colombia. Humidity in microhabitats of triatomine bugs, particularly in the domestic habitats, can be low and unfavorable for fungal development. The *B. bassiana* isolate CG 306 was shown to be virulent against *T. infestans* at a temperature range between 15 and 30°C and at the low humidity of 50% (Luz *et al.* 1998a). Formulation of fungal propagules may help to overcome unfavorable climatic conditions and may increase effectiveness of control. We report on the formulation and application of this *B.*

bassiana isolate in the laboratory, using a mineral-oil based emulsifier. Moreover, CG 306 was tested on *T. infestans* under field conditions, focussing on infectivity, sporulation on cadavers and persistence of conidia.

Material and Methods

Insect rearing. *T. infestans* were mass-reared in the laboratory. Insects were held at 25 ± 0.5°C and 75 ± 5% humidity with a photophase of aproximately 12 h. They were allowed to feed on chickens every two weeks. The insect colony originated from Paraná state, Brazil.

Fungal culture. *B. bassiana* (CG 306), from the fungal culture collection of Embrapa-National Research Center for Genetic Resources and Biotechnology, was originally isolated from *Thyanta perditor* (Fabr.), a hemipteran insect, in Brazil. To maintain its virulence, the fungus was passed on *T. infestans* and reisolated before each experiment. For laboratory tests, the fungus was subsequently cultured for 10-15 days on complete medium (0.001 g $FeSO_4$; 0.5 g KCl; 1.5 g KH_2PO_4; 0.5 g $MgSO_4.H_2O$, 6 g $NaNO_3$, 0.001 g $ZnSO_4$, 1.5 g hydrolysed caseine, 0.5 g yeast extract, 10 g glucose, 2 g peptone, 20 g agar and 1 000 ml distilled water) at 27 ± 0.5°C and a photophase of 12 h. For field application, the fungus was cultured on 100 g parboiled rice with 5% molasses and 60 ml distilled water in Roux bottles for 10 days under conditions mentioned above. Before field tests, conidia were harvested directly by scraping and sieving, dried over silica gel and stored at 4°C.

***In vitro* germination of formulated conidia.** The possible detrimental effect of a mineral-oil emulsifier (Assist, BASF) on the germination of *B. bassiana* was tested in a liquid complete medium. Increasing concentrations of the emulsifier (0; 0.006; 0.02; 0.06; 0.2; 0.6; 2 and 6%) were tested at 25°C. Germination was examined 6, 12, 24 , 36 and 48 h after inoculation. Conidia were considered to have germinated when the length of the germ tube exceeded the conidial diameter. Assays were repeated four times. In each replicate, 100 conidia were scored for germination.

Insect infection in the laboratory. Conidia were harvested and suspended in a) 10 ml of 0.1% Tween 80 solution and b) an aqueous solution of 2% mineral oil based emulsifier (Assist). Conidial suspensions were adjusted to provide 8 different conidial densities (10^4, $3x10^4$, 10^5, $3x10^5$, 10^6, $3x10^6$, 10^7, $3x10^7$ conidia/cm^2) on the treated surface after subsequent vacuum-filtration on filterpaper, Millipore 0.22 µm (47 mm diameter). For each bioassay, 20 unfed, newly emerged third instar nymphs were placed on the dried filter paper in gauze-covered transparent cups (47 mm diameter x 75 mm) and exposed to the fungus either constantly during the experiment or for just 1 h and then transferred to an untreated filter paper. Assays were repeated four times. Control insects were placed on filter paper treated with 0.1% Tween 80. Cups were closed with gauze and held in a climate chamber, set at 25 ± 1°C and 50 ± 5% humidity. Mortality was recorded daily.

Insect infection in the field. Conidia were suspended in 2 l of 0.1% Tween 80. The concentration was adjusted to 10^7 conidia/cm^2 of treated surface. The experimental plots were small, windowless houses (about 2x2x2 m) with an inner wall surface of about 15 m^2 built with wooden laths and covered by roofing tiles. Four experimental houses were treated with the fungal suspension and one control house only with 0.1% Tween 80 (2 l/house), using a manual backpack sprayer (Jacto PJ 16 l, 10 ml/sec). After drying of the suspension on the walls, 100 unfed recently molted third instar *T. infestans* nymphs were liberated in each house. Simultaneously, filter papers (7 cm diameter) were treated using the backpack sprayer as mentioned above, transfered to plastic cups and covered with gauze. Twenty third instar nymphs were exposed to each filter paper and the cups were placed in the five treated houses with four replicates each. The walls and floor of the inner

house were checked daily for dead triatomines during 25 days. Mortality of insects in the cups was also recorded daily. Temperature and humidity were monitored constantly in one house and in a meteorological shelter next to the houses, using two hygrothermographs. Houses were then dismantled and checked for dead and living insects. Living insects were captured and transferred to laboratory conditions at 25°C and 50% relative humidity. Triatomines inside the cups were also transferred to the laboratory. Mortality in laboratory was observed daily for 15 days.

Persistence of conidia in the field. Viability of conidia applied in the houses was tested every 3 days for 25 days after treatment, simultaneously with the insect infection. A strip of filter paper (20 x 3 cm) was fixed on the inner house wall and treated as mentioned above. For every test of viability, a piece of 2 cm (6 cm^2) was cut from the strip, vortexed for 5 min in 5 ml 0.1% Tween 80, and 0.2 ml were plated on complete medium to which dihydrostreptomycin (1 g in 1000 ml) had been added. Germinating conidia were examined about 10 h after inoculation, counting 100 conidia in 4 different Petri plates for every house.

Sporulation on cadavers. Fungus killed third instars, originating from laboratory infection with CG 306, were placed in Petri plates and exposed to field conditions in the houses. In each house, four dishes each with 25 cadavers were used. Appearance of external fungal growth on the cadavers and sporulation were recorded daily.

Data analysis. Angular transformed cumulative mortalities were analysed by ANOVA and means compared by cluster analysis (Scott and Knott 1974). The product-limit estimates of 50% survival time were calculated (Lee 1980) and analysed by ANOVA. Lethal concentrations (LC_{50} and LC_{90}) were calculated by probit-transformation and compared by Wald-test. (SAS Institute Inc. 1989).

Results

Germination in vitro. First formation of germ tubes was detected after 6 h of incubation of the conidia in liquid complete medium with no emulsifier (2.8% germination) and with the lowest concentration, 0.006% (0.5% germination). After 12 h, conidia had germinated in all emulsifier concentrations with lower germination rates at increasing concentrations of the emulsifier (Table 1). No difference in the rates of germination was observed after 24 h of incubation and germination was higher than 98% for all treatment levels.

Insect infection in the laboratory. Mortality of *T. infestans* third instars exposed to conidia treated surface increased with the dose and was higher after a constant exposure to the treated surface than for one h exposure (Fig. 1). However, at the highest concentra-

Table 1. Effect of an emulsifier on conidial germination of *B. bassiana* (CG 306) in liquid culture at 25°C.[1]

	Concentration of emulsifier (%)							
Exposure time (h)	0	0.006	0.02	0.06	0.2	0.6	2.0	6.0
12	70.8 (2.3) a	46.0 (2.5) bc	54.8 (2.5) ab	51.0 (2.5) abc	35.8 (2.4) bc	40.3 (2.5) bc	35.8 (2.4) bc	24.5 (2,2) c

[1]Four different counts of one hundred conidia per sample. Percentage of germination (± standard error), means followed by different letters are significantly different ($P<0.05$), SNK-test.

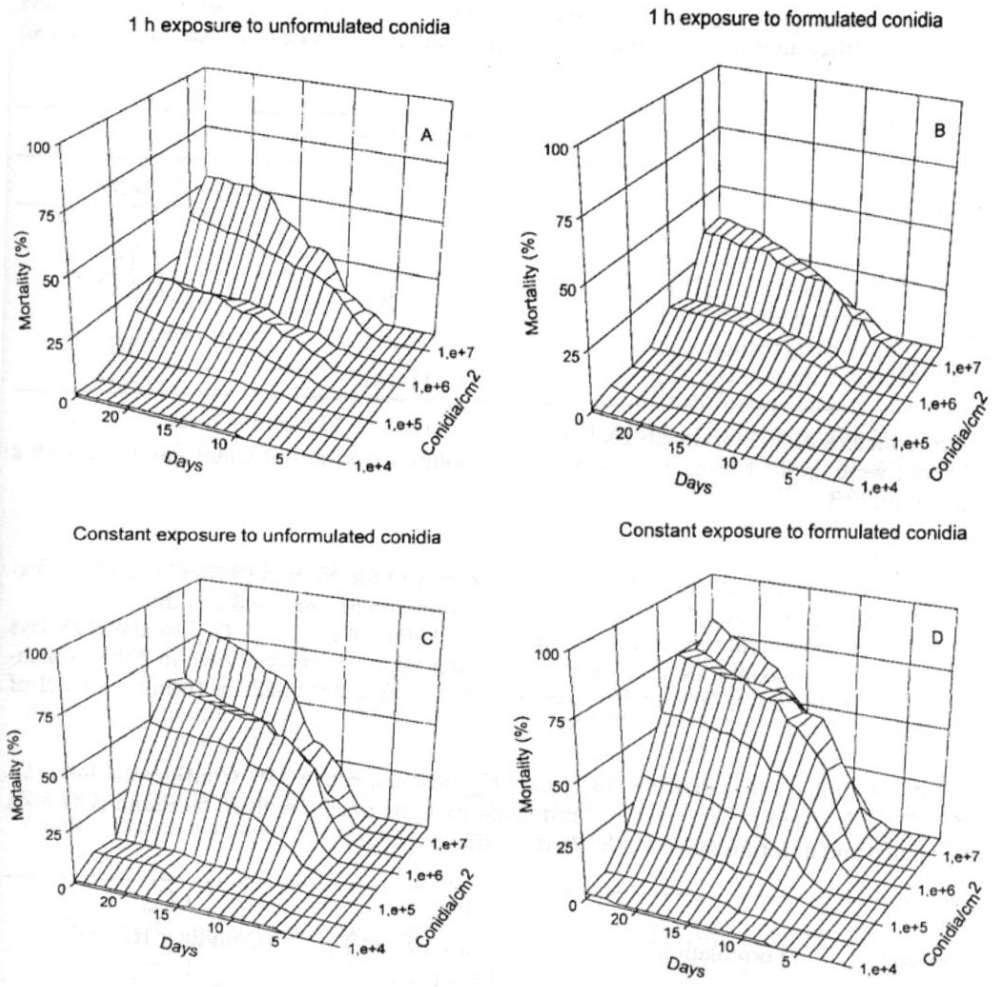

Figure 1. Cumulative mortality (%) of *T. infestans* third instar nymphs after one h exposure (A; B) or constant exposure (C; D) for 25 days to *B. bassiana* conidia on treated surface at 8 different doses (10^4 to $3x10^7$ conidia/cm^2) and subsequent transfer to untreated support. Conidia were applied unformulated (A; C) and formulated (B; D) with a mineral oil-based emulsifier (2%).

tion of conidia ($3x10^7$ conidia/cm^2), insect mortality started later and less insects were killed in the first days after treatment compared to lower concentration ($1x10^7$ conidia/cm^2). This was particularly noticeable after a constant exposure of insects to formulated conidia. At the lowest concentrations of conidia (10^4 up to 10^5 conidia/cm^2), mortality was < 20% after 30 days, independently of the period of exposure to the treated support. Estimates of 50% survival time of insects exposed constantly to doses between $3x10^6$ and $3x10^7$ conidia/cm^2 are shown in Table 2. ANOVA on estimates of survival time showed no significant effect of formulation ($F = 0.23$; $P = 0.6358$; $df = 1$) nor density of conidia (F

Table 2. Estimates of 50% survival time in days (95% C.I.) of *T. infestans* third instars after exposure to different doses of formulated and unformulated conidia of *B. bassiana* (CG 306) on filter paper. [1]

	Density of conidia/cm^2		
Formulation[2]	3x10^6	10^7	3x10^7
Water (Tween 80)	15 (12-25)	21 (16-30)	15 (15-17)
Oil-water (emulsion)	13 (11-16)	12 (11-18)	16 (15-18)

[1]Four replicates of 20 individuals each were tested.
[2]Conidia were applied using a 0.1% Tween 80 solution and an oil water emulsion with a mineral oil (2%).

= 1.51; P = 0.2481; df = 2) applied. Mortality after 40 days was not enough to calculate estimates of 50% survival time at a 1 h exposure, except at a maximal dose of 3x10^7 conidia/cm^2. At this dose, 50% of the insects survived for 20 days (95% C.I. = 17-40) exposure to unformulated conidia.

Values of LC_{50} and LC_{90} calculated 25 days after application are shown in Table 3. Estimates were obtained by fitting a model of

Table 3. Lethal concentrations (LC_{50} and LC_{90}) and respective 95% Confidential Intervals (95% CI) calculated for *T. infestans* third instar nymphs treated with *B. bassiana* (CG 306) formulated and unformulated conidia 25 days after exposure[1].

Time of exposure[2]	Formulation[3]	LC_{50} (Conidia x 10^7/cm^2) (95% C.I.)	LC_{90} (Conidia x 10^7/cm^2) (95%C.I.)
1 h	Water (Tween 80)	2.0 (1.0-4.4)	120.0 (43.0-410.0)
1 h	Oil-water (emulsion)	4.8 (2.2-12)	280.0 (94.0-1.1)
Constant	Water (Tween 80)	0.2 (0.1-0.4)	14.0 (6.1-39.0)
Constant	Oil-water (emulsion)	0.1 (0.07-0.2)	7.0 (3.2-18.0)

[1]Eight doses between 10^4 and 3x10^7 conidia/cm^2 were tested with 4 replicates of 20 individuals each.
[2]Insects were exposed to the treated filter paper constantly or for one h and then transferred to untreated filter paper.
[3]Conidia were applied unformulated or formulated using an oil water emulsion with a mineral oil (2%).

probit regression with a single slope and different intercepts for each treatment. The choice of this model was preceded by a likelyhood ratio-test in order to compare it with a reduced model which considers a single slope and different intercepts for the principal effects, being time of exposure and presence/absence of emulsifier. The reduced model was rejected ($\chi^2 = 15.63$, $P < 0.001$, df = 1), indicating a significant difference between the principal effects. The adjusted model indicates that a significant difference exists between 1h and constant exposure. The model also shows that the presence of the emulsifier caused a higher LC_{50} after 1 h exposure ($P = 0.098$) but a lower LC_{50} after constant exposure of insects to the treated support ($P = 0.069$).

Insect mortality in the field. The estimates of 50% survival time of the insects held in gauze-covered cups on fungus treated filter paper in the different houses varied between 14.5 and 34 days (Table 4). Survival time in the first, second and fourth houses differed significantly from that in the third house ($\chi^2 = 87.6$, $p < 0.001$), due to the reduced survival time in the third house. Mean mortality differences between treated and untreated insects in the experimental houses was 43.4% (SEM = ± 3.6%) after 25 days and 72.8% (± 3.3%) after 40 days.

During the assay some insects were observed moving on the inner walls, but no dead insects were found. After 25 days, recovery of living insects was 21% in the first and second houses, 12% in the third house, 16% in the fourth and 31% in the control house. A significant difference was observed between the recovery rates in the fungus treated houses and the control house ($\chi^2 = 8.30$, $P = 0.0040$). One and 4 cadavers were found in the first and fourth house respectively. *B. bassiana* developed on all cadavers after transfering to a humid chamber. Mortality among recovered living insects transferred to laboratory conditions of 25°C and 50% humidity, was 38.1% for insects from the first house, 47.6% for the second, 66.7% for the third and 93.8% for the fourth house after 15 days exposure. Most cadavers showed sporulation after incubation at saturated humidity. In the same period, only 9.7% of the insects found in the control house died and showed no fungal development on cadavers. A highly significant difference was observed between cumulative mortalities of insects originating from treated houses and the control house at day 40 ($\chi^2 = 49.59$, $p <$

Table 4. Cumulative mortality after 25 and 40 days and estimates of 50% survival time (days) of *T. infestans* third instar nymphs, exposed to *B. bassiana* (CG 306) treated filter paper and kept in gauze-covered cups inside the houses[1].

Houses	Mortality % (mean ± SE)		Estimates of 50% survival time[2] (95% C.I.)
	Day 25	Day 40	
1	37.5 (± 5.4)	67.5 (± 9.2)	34 (27-38)
2	35 (± 5.3)	68.8 (± 5.2)	31 (29-38)
3	78.8 (± 4.6)	100	14.5 (12-19)
4	42.5 (± 5.5)	75 (± 4.8)	28.5 (25-34)
Control	5 (± 2.4)	5 (± 2.4)	-[3]

[1]Four replicates of 20 individuals each were tested. Living insects were transferred to laboratory conditions (25°C and 50% RH) after day 25 and observed on day 40.
[2]Estimates of 50% survival time calculated over 40 days.
[3]Mortality within 40 days was not enough to calculate an estimate of 50% survival time.

0.0001).

Survival of conidia and sporulation on cadavers. Survival of conidia on treated filter paper tested every three days was maximal throughout 25 days of the test. Conidia in all four treated houses germinated with a rate of > 98% during this period. No sporulation of *B. bassiana* on the fungus-killed cadavers exposed inside the houses was observed during the 25-day period of the experiment.

Temperature and humidity during field tests. Minimum daily temperatures in the experimental house, where temperature was monitored, varied between 15 and 22°C with a mean of 17.7°C and were higher than those in the adjacent meteorological shelter which had temperatures between 11 and 20°C and a mean of 15.6°C. Maximum temperatures were also higher in the house than in the meteorological shelter and varied between 25 and 32°C with a mean of 26.7°C compared to 22 to 31°C in the shelter with a mean of 25.5°C. Maximum humidity was higher in the shelter varying from 85 to 99% compared to humidity between 67 and 98% in the experimental house. Periods of humidity > 97% never exceeded 8 h in the shelter and 4 h in the experimental house. Due to heavy rainfall in the first three days after treatment, humidity during these days was elevated, compared to the following days. Minimum humidities were found to be lower in the shelter from 28 up to 80% with a mean of 46.6%. In the houses, minimum humidities varied between 33 and 76% with a mean of 53%.

Discussion

High mortalities in *T. infestans* third instars treated with *B. bassiana* were observed in the laboratory assays. However, much lower mortality was observed when insects were exposed to treated filter paper even at high conidial densities ($3x10^7$ conidia/cm^2) than when insects were treated by submersion in the conidial suspension (10^7 conidia/ml), as shown by Luz *et al.* (1998a) under similar conditions of temperature and humidity. Doses of $2.4x10^6$ conidia/cm^2, after constant contact to the treated support, and $2.0x10^7$ conidia/cm^2, with 1-h exposure, were necessary to kill 50% of third instars compared to $8.9x10^5$ conidia/ml after submersion in the conidial suspension (Luz *et al.* 1998a). Application method and exposure time on the treated filter paper obviously influenced the amount of conidia contacted by tested insects.

In our tests, doses higher than 10^6 conidia/cm^2 did not enhance the fungal effectiveness. Interestingly, fungal effectiveness was retarded at the maximal doses tested. Luz *et al.* (1999) also reported a retarded emergence and conidial production of CG 306 and other *B. bassiana* isolates on cadavers of *T. infestans* insects treated with higher conidial doses, compared to lower doses. Compounds inducing self-inhibition of conidial germination at high inoculum dose have been demonstrated in different fungal species (Garraway and Evans 1984) and may be the reason for retarded mortality at the highest doses tested.

Triatomine vectors can survive long periods without feeding, as shown by Luz *et al.* (1998b) for *R. prolixus* under fluctuating temperatures and humidities. During the present assays, insects were not fed to avoid the impact of alimentation on insect susceptibility to fungal infection. The influence of nutritive status on *R. prolixus* susceptibility to infection with *B. bassiana*, particularly when fungus was inoculated 1-2 days before molting was observed (Luz 1998). However, insects starved for prolonged periods were as susceptible to *B. bassiana* as those recently molted.

The mineral oil-based emulsifier had no detrimental effect on conidial germination even at 6%. At 2% emulsifier, the recommended dose for field application in pest control, formulated conidia were more effective against *T. infestans* than unformulated conidia after constant exposure to the treated support. Survival of formulated conidia seems to be higher than unformulated conidia. However, unformulated conidia were more active after a 1 h exposure than formulated conidia.

In preliminary field tests, mortality of *T.*

infestans treated with unformulated conidia was reduced and retarded in comparison to laboratory tests. The low recovery of third instars liberated in the experimental houses was probably due to the small size of the insects, which were able to hide in the small cracks of the wooden house. Predators such as ants, spiders and lizards continuously observed during tests in the houses may have lowered the populations. Moreover, ants may be one reason for the low recovery of dead insects. The larger size of later instars may facilitate detection of living insects and dead specimens may be more difficult to be carried away by ants.

There is no information currently available about conidial doses of entomopathogenic fungi applied under field conditions to control triatomine bugs. The dose of 10^7 conidia/cm^2 used in the present study in the field was distinctly higher than doses normally used in the control of insect pests. Ponce *et al.* (1992) reported the most effective dose to be 10^{13} *B. bassiana* conidia/ha (10^5 conidia/cm^2) to control the banana pest *Cosmopolites sordidus* (Germ.). However, doses of $7.5x10^{10}$ conidia/plant were also used to control the sugar cane pest, *Antitrogus consanguineus* (Blackburn), with *Metarhizium anisopliae* (Metsch.) Sorok. (Allsopp *et al*. 1994).

During the field test, maximal humidity of 97% or higher in the fungus treated houses was never maintained longer than 8 h during one diurnal cycle. Under these conditions, the fungus was not able to emerge and sporulate on cadavers. This was also observed by Luz *et al.* (1994), who reported that a minimum period of at least 12 h at 97% RH in the insect microhabitat under fluctuating conditions was necessary to enable sporulation on cadavers and to allow rapid and total kill of *B. bassiana*-treated *R. prolixus*. Survival of conidia on filter paper inside the houses was high during assay, demonstrating that conidia can remain infective during extended periods after treatment. Romaña (1992) also showed 60% mortality of *T. infestans* kept at high humidity after exposure to Pampas grass from a house roof treated with *B. bassiana* 21 months before insect exposure.

Methods of fungal formulation and application may enhance survival of conidia and effectiveness against target pests under field conditions. Inglis *et al.* (1997) obtained low field efficacy of *B. bassiana* against grasshoppers using a 1.5% emulsifiable oil-emulsion and presumed unfavorable environmental conditions for the fungus. Other formulations, such as one using cotton seed oil used to formulate *Metarhizium flavoviride* Gams & Rozsypal conidia, have been shown to be effective at low humidity in locust control (Bateman 1997). However, ultra low volume application of oil-formulated conidia used against locusts does not seem adequate for use in houses. Spraying methods with improved penetration of conidial formulation into the treated zone seem to be more promising in triatomine insect control. Nocturnal triatomine insects are hidden in their domestic habitats and this makes it hard to spray the insects directly with a conidial suspension during treatment of houses. Triatomine insects must be contaminated with conidia while moving on treated surfaces.

Results found in laboratory and field conditions confirm that *B. bassiana* is a promising candidate to control the vector of Chagas disease, *T. infestans*. However, to enhance and stabilize infectivity, more investigations on formulation, application and evaluation under field conditions are necessary.

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