Activity of Oil-formulated *Beauveria bassiana* against *Triatoma sordida* in Peridomestic Areas in Central Brazil

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Field tests were carried out during the rainy season of 2001/2002 in São Luís de Montes Belos, Goiás, Brazil, to evaluate the potential of the entomopathogenic fungus, Beauveria bassiana*, against peridomestic* Triatoma sordida*. An oil-water formulation of the isolate CG 14 (Embrapa) was applied in triatomine infested hen houses of four farms at a final concentration of 10^6 conidia/cm^2. Numbers of* T. sordida *decreased over the next 25 days, after application of the fungus, and* B. bassiana *developed on dead insects in one hen house. A high number of* B. bassiana *colonies was detected in substrates collected in treated hen houses 24 h after application of CG 14. In the following three months the presence of* B. bassiana *declined to values found before treatment.*

Key words: field test - *Triatoma sordida* - *Beauveria bassiana* - peridomestic - Central Brazil

Entomopathogenic fungi, such as *Beauveria bassiana* and *Metarhizium anisopliae* have been reported to be highly active under laboratory conditions against *Triatoma infestans* and other triatomine species (Romaña & Fargues 1987, Luz et al. 1998 a, b, Luz & Fargues 1999, Fargues & Luz 2000, Lecuona et al. 2001) and to reproduce on their cadavers (Luz & Fargues 1998, Fargues & Luz 1998). However, there is little information about activity of fungi against Triatominae under field conditions. Preliminary simulated field tests with a *B. bassiana* isolate in Brazil indicated that activity against *T. infestans* may be reduced compared to laboratory conditions (Luz et al. 1999). The recent development of effective oil-formulations of entomopathogenic fungi is opening new possibilities for environmentally safe control strategies (Lomer et al. 2001). We report here on field and semi-field tests with oil-formulated *B. bassiana* in Central Brazil to control peridomestic *T. sordida*.

MATERIALS AND METHODS

Tests were carried out in four different farms in the rural proximity of São Luis de Montes Belos, Goiás, in Central Brazil during the rainy season of 2001/2002. Farms were selected because of their peridomestic infestation with *T. sordida* and a comparable structure of the hen houses, which were generally built with loose bricks and roofing tiles and covered by wooden planks and fragments of black plastic bags. All hen houses covered an area of about 5 m^2. In previous studies, *M. anisopliae* and *B. bassiana* were detected two months before application of *B. bassiana* in substrates of all tested farms with the exception of the first farm where this fungus was not found (C Luz, LFN Rocha, and GV Nery unpublished observations). In each farm, one hen house was fungus treated and another hen house, 5 to 10 m distant, was used as a control without applying *B. bassiana*. Temperature and humidity were monitored constantly in one of the farms in a shelter close to the hen house, using a mechanical hygrothermograph.

Detection of fungi in hen houses - Prior to and during the trial, six samples of substrate that consisted of soil, organic material such as animal faeces, and plant litter were taken from each hen house at randomly selected locations inside, where triatomines probably circulate during the night. About 25 g substrate were scraped to a depth to 2-3 cm, transferred to plastic bags and stored in a styrofoam cooler at about 20°C until processing in the laboratory. Two different techniques to detect entomopathogenic fungi were used.

In vivo detection - Newly emerged and unfed third instar nymphs (N3) of *T. infestans*, were used to detect entomopathogenic Hyphomycetes in substrates and to test survival of conidia applied to filter papers and exposed to field conditions. Nymphs of *T. infestans* were used because they are easier to mass-rear than nymphs of *T. sordida*. The *T. infestans* colony was originally from the state of Paraná, Brazil, and has been maintained in the laboratory since 1981. Insects were fed on chickens every two weeks, and maintained at 25 ± 0.5°C, 75 ± 5% relative humidity (RH) and 12 h photophase (Silva 1985).

The substrates of each sample were homogenized and approximately 3 g were transferred to Petri dishes (90 x 15 mm). Ten N3 were set on the substrate and exposed during 15 days at 25°C and relative humidity close to saturation (RH > 98%). Mortality was monitored daily. Dead insects were dipped in 93% alcohol, their surface disinfected in 2.5% sodium hypochlorite for 3 min and then washed three times for 1 min in sterile water. Cadavers were then incubated during 10 days at 25°C and RH >

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98%. Fungal development on the cadavers was evaluated daily and sporulating fungi inoculated onto complete medium (CM) amended with chloramphenicol (1 g/1000 ml): 0.001 g $FeSO_4$, 0.5 g KCl, 1.5 g KH_2PO_4, 0.5 g $MgSO_4$.7 H_2O, 6.0 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 1000 ml distilled water.

In vitro detection - Hyphomycetes were isolated from the substrates using modified Chase medium (MCM): oatmeal infusion (2%), 20 g agar, 0.3 g dodine (N-dodecylguanidine monoacetate, Cyprex 65 WP), 5 mg chlortetracycline, 0.4 g penicillin, 1 g streptomycin, 10 mg crystal violet, and 1000 ml distilled water (Chase et al. 1986). Samples of 1 g substrate were suspended in 10 ml of sterile 0.1% Tween 80 and vortexed for 3 min. Each suspension was then diluted 1/100 in distilled sterile water, spread onto MCM and incubated for 20 days at 25°C. Developing CFU (colony forming unit) were examined daily and inoculated separately on CM after formation of conidia. Cultures of *B. bassiana* and *M. anisopliae* were identified by microscopic examination.

Field tests - The monosporal *B. bassiana* strain CG 14 (Embrapa Genetic Resources and Biotechnology, Brasília, Brazil) was used in the tests. This strain was obtained in 1988 in Londrina, Paraná, Brazil from a hemipteran *Podisus* sp. (Pentatomidae) and reported to be virulent against *T. infestans* under laboratory conditions (Luz et al. 1998a). The fungus was cultivated in culture bags with 500 g parboiled rice and 300 ml distilled water in each bag for 10 days at 25°C and 12 h photophase. Conidia were harvested directly by sieving and aspiration. They were then dried over silica gel during two weeks and stored at 4°C. Just before application conidia were resuspended in 10% aqueous emulsifier added with vegetable oil (Veget'Oil, Oxiquimica Agrociência Ltda). The final concentration was adjusted to give 10^6 conidia/cm^2 of treated surface. Viability of formulated conidia was tested inoculating 100 µl of the suspension on CM and evaluating quantitative germination within 24 h after inoculation at 25°C. Before application of conidia the approximate number of *T. sordida* and their instars were checked during 10 min in the hen houses. For this, hen houses were carefully dismantled and rearranged without removing the triatomines or altering the structure of the hen house. Four hen houses were then treated with 5 l of the formulation each and four control hen houses only with 5 l of the emulsion using a manual high pressure 5 l sprayer (Brudden Equipment Ltd). Covering materials were removed before application and the formulation sprayed on the bricks and the roofing tiles. Hen houses were then covered again. All hen houses were checked during 25 days every five days during 10 min for live and dead triatomines as mentioned before. Dead insects were transferred to Petri dishes and kept in the same location. Fungal development on cadavers was evaluated at five-day intervals during at least 10 days. Living insects detected 25 days after spraying were captured and transferred to laboratory at 25°C and RH > 98%.

Tests on survival of conidia in the field - A strip of filter paper (20 x 3 cm) and a 9 cm diameter circular filter paper were treated with CG 14 using the manual sprayer as mentioned above and fixed on the inner wall of a shelter close to the hen house in each farm. Viability of conidia was tested 24 h after application and their activity against *T. infestans* during three months. A piece of 2 cm was cut from the strip, vortexed for 5 min in 5 ml 0.1% Tween 80, and 200 µl plated on CM amended with chloramphenicol or MCM and incubated at 25°C. Germinating conidia were examined at 12 and 24 h after inoculation, counting 100 conidia for every strip. Formation of CFU on MCM was evaluated during 20 days. Activity of CG 14 was tested monthly by exposing 10 *T. infestans* N3 on the circular filter paper under laboratory conditions. Insects were incubated at 25°C, 75% RH and RH > 98%, respectively. Relative humidities of 75% and > 98% inside the test chambers were regulated with saturated solutions of NaCl and K_2SO_4, respectively (Winston & Bates 1960). Mortality and fungal development on cadavers were evaluated as mentioned for the laboratory tests with *T. sordida*.

Semi-field tests - Bricks (19 x 19 x 9 cm) with eight openings were treated at a dosage of 10^6 conidia/cm^2 surface by submersion into a formulation of conidia prepared as mentioned above. After drying, bricks were transferred to cloth bags (33 x 40 x 22 cm) with 10 field collected *T. sordida* of randomly selected nymphal instars (N2 - N5) and adults on the bricks. The insects had been captured the day before treatment in other farms of the same region. No first instar nymphs were used due to their vulnerability. The number of N2 and N3 in the bags varied between zero and one specimen and each bag contained one N4, five N5 and three adults. Bags were carefully closed and fixed at about 2 m height in a shelter close to the hen houses. In each farm four bricks treated with the fungal formulation and two bricks with the formulation without conidia which served as controls were tested separately. Mortality was evaluated during 25 days every five days. Dead insects were transferred to Petri dishes and exposed in the bags. Fungal development was evaluated as mentioned before.

Laboratory tests - Field-collected nymphs of different instars and adults of *T. sordida* were brought to laboratory. Ten randomly selected nymphs and adults were set on filter paper (9 cm diameter) and treated topically at a final dose of 10^6 conidia/cm^2 surface using a Potter sprayer (Burckard, Hertfordshire, UK). After drying, insects were transferred to untreated filter paper in a Petri dish and incubated during 15 days at 25°C, 75% RH and RH > 98%, respectively. Mortality was examined daily and fungal development on cadavers evaluated in a humid chamber as mentioned above. Four replicates were assayed for both humidities.

Characterization of B. bassiana isolates - A total of 17 *B. bassiana* isolates detected before and after application of the isolate CG 14 were analyzed by RAPD and compared with CG 14 and an outgroup isolate, CG 478, from *Anthonomus grandis* (Coleoptera: Curculionidae), Campinas, São Paulo, Brazil in 1983. One isolate from the second and third farm and two isolates from the fourth farm originated from substrates which had been collected before application of CG 14. Another 13 isolates detected 24 h, 25 days or one month after application of CG 14 in substrates, or on *T. sordida* were analyzed. Mycelium used for RAPD analysis was obtained from a submersed cul-

ture of conidia in CM without agar shaking at 150 rpm at 25°C for three days. Mycelium was harvested by filtration through filter paper (Whatman No. 1), lyophylized and stored at –80°C. Genomic DNA was extracted using a simple method described by Al-Samarrai and Schmid (2000). The polymerase chain reaction (PCR) reactions were performed in 30 µl volume, with 15 ng of each template, using the PTC-100 programmable thermal controller (MJ Research), and a temperature profile described by Tigano et al. (1995). The amplifications were done using the following reaction mix: 1 unit of Taq polymerase (Life Technologies), 1 x Taq polymerase reaction buffer, 4 mM of $MgCl_2$, 200 µM of each deoxynucleotides triphosphate (Pharmacia Biotec), and 1 µM of 10-mer primer (Operon Technologies). Twenty primers were used: OPA-09, OPA-13, OPA-14, OPA-16, OPB-05, OPB-06, OPB-10, OPB-17, OPE-01, OPE-02, OPE-03, OPE-04, OPE-07, OPE-14, OPE-15, OPE-16, OPE-19, OPE-20, OPR-08, and OPR-14. Amplified products were electrophored in 2% agarose gel dissolved in 0.5 x Tris-borato-EDTA (TBE) buffer. After electrophoresis, gels were stained with ethidium bromide (Sambrook et al. 1989) and photographed under UV light. DNA fingerprints were scored directly from the photographs. The presence or absence of each fragment was considered as an independent character.

Data analysis - Insect mortality and number of CFU were analyzed by ANOVA (F) or Kruskal-Wallis analysis (H) and the multiple Student-Newman-Keuls range test of comparison of means. Means were considered significantly different at $P < 0.05$. Values of recovery of live *T. sordida* in the hen houses were analyzed with the T-test (t) or Mann-Whitney-Rank-Sum test (T) at $\alpha \leq 5\%$ (SAS Institute Inc. 2000). RAPD markers were analyzed using NTSYS-pc V2.1 (Exeter Software, Setauket, NY). A similarity matrix was calculated using Jaccard similarity coefficients. Clustering was done by UPGMA.

RESULTS

All hen houses were found infested with *T. sordida* before application of CG 14. No other triatomine species was identified among captured specimens. Highest rates (up to 97 specimens) were observed in the hen houses of the first farm. The number of captured insects in the other farms was between 15 and 29 specimens. The number of different instars of *T. sordida* found in all farms is presented in Table I. Temperature and relative humidity monitored in the shelter during 25 days after treatment varied between 17°C and 31°C and 37% and > 98%, respectively. Highest temperatures with lowest humidities were registered between 12 and 4 p. m. and lowest temperatures with highest humidities during the night between 10 p. m. and 6 a. m. Periods of constant relative humidity > 98% in this shelter did not exceed 10 h.

Field tests - Germination of formulated conidia tested on CM either directly without application in the field or after application on filter paper and exposure under field conditions during one day was > 98%, 24 h after inoculation. After suspending one month old filter paper in 0.1% Tween and inoculation of 100 ml of the suspension on CM a high number of bacteria and fungi, but no *B. bassiana* was found. However, inoculating the same suspension on MCM, *B. bassiana* colonies developed. No *B. bassiana* was observed when testing two or three months old filter papers independently of the medium utilized.

Mean recovery rates of live insects decreased in most fungus-treated and control hen houses compared to the number of individuals found directly before application of *B. bassiana*. Higher rates of live *T. sordida* were found in the control hen houses, especially of the first farm (Fig. 1) compared to the rates found in the fungus-treated hen houses. In the other farms with lower initial insect infestation, percentages of live individuals in the treated hen

TABLE I

Composition of live *Triatoma sordida* instars found in the hen houses before and after application of *Beauveria bassiana*, up to 25 days after treatment

Time after application (d)	Hen houses	N1	N2	N3	N4	N5	Ad	Total
		Total number of nymphal instars and adults in the hen houses before application						
0	*a*	9	8	23	17	26	40	123
	b	0	0	6	13	12	4	35
		Relative number of instars found after fungal application (%)						
5	*a*	0	25	4.4	52.9	53.9	20	27.6
	b	0	0	83.3	7.7	50	0	34.3
10	*a*	0	37.5	21.7	17.7	15.4	15	17.1
	b	0	0	100	23.1	50	75	51.4
15	*a*	0	0	13	17.7	11.5	12.5	11.4
	b	0	0	16.7	61.5	50	50	48.6
20	*a*	0	0	0	5.9	15.4	12.5	8.1
	b	0	0	16.7	30.8	33.3	100	34.3
25	*a*	0	0	4.4	11.8	11.5	7.5	7.3
	b	0	0	33.3	38.5	25	125	42.9

a: fungus treated; *b*: control areas where no *B. bassiana* was applied in four different farms, located in the rural proximity of São Luis de Montes Belos, Goiás, Brazil, were checked during 10 min for live individuals. Rates of recovery were related to the number of live *T. sordida* detected immediately before fungus application. Nymphal instars: N1 - N5; adults: Ad

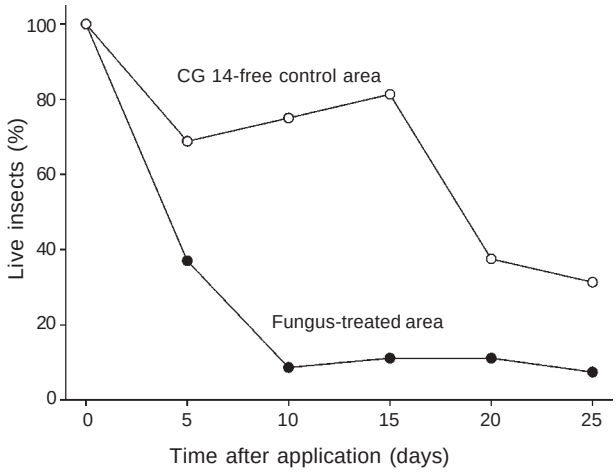

Fig. 1: recovery of live *Triatoma sordida* after application of *Beauveria bassiana*, CG 14, in a triatomine-infested farm compared to the number of insects found before treatment.

houses varied from 0% up to 130% related to the number of insects counted before treatment. A highly significant difference between values of recovery of live insects in the fungus-treated and control hen house during 25 days of observation was found for the hen houses of the first farm with the highest initial number of insects ($t = 3.7$, $P = 0.006$). The composition of *T. sordida* instars which were detected live in all hen houses after application of the fungus, related to the total number found before the treatment is shown in Table I. Twenty five days after application, nine *T. sordida* nymphs and adults were captured in the fungus-treated hen houses and 15 in the control areas and transferred to the laboratory. Mortality was 33.3% for the insects originating from fungus-treated areas and 6.7% from the control areas, 15 days after incubation at RH > 98%. On all cadavers, including one dead adult from a control hen house, development of *B. bassiana* was observed, 10 days after exposure in a humid chamber.

Dead insects were found only in the fungus-treated hen houses during the 10 first days after treatment, with highest rates in the hen house of the first farm. The recovery of live insects, as compared with the initial population before fungal application was as follows. At five days after application, 18.8% adults and 22.2% N5 were recovered; at 10 days after application, 21.9% adults, 38.9% N5, and 18.2% N4 were recovered. Most of the cadavers in the first farm were detected close to a colony of ants at the bottom of the hen house and ants were observed carrying entire dead individuals or fragments away into their colony. No cadavers were found between 15 and 25 days after treatment in this hen house. Distinct development of *B. bassiana* five days after transfer to Petri dishes and exposure in the fungus-treated hen house of the first farm was observed on one dead N4, three N5 and four adults. However, *B. bassiana* was not more visible on those cadavers, 25 days after treatment and could not be isolated with MCM. Other saprophytic fungi were observed on the cadavers. In the second and fourth farm, in the fungus-treated hen houses, one dead adult was detected five and ten days after treatment, respectively. No fungi had developed on these cadavers after exposure in Petri dishes in the hen houses up to 25 days after treatment.

Detection of B. bassiana and M. anisopliae in substrates, after application of CG 14 - Mortality of *T. infestans* N3 after exposure to substrates collected 24 h after fungal application in the four hen houses varied between 8% in the third hen house and 94% in the second hen house, 15 days after incubation under laboratory conditions. Mean values of mortality and fungi detected on cadavers are presented in Table II. *B. bassiana* was detected on 60.6% of the insects which had died after exposure to substrates originating from all hen houses. The highest number (80%) of mummified cadavers with only *B. bassiana* were found testing substrates from the hen house of the first farm. For the hen house of the third farm only one dead individual was found with *B. bassiana*. In addition, *M. anisopliae* was found on cadavers in the first (20%), third (50%) and fourth (4.8%) farms. One dead nymph showed a mixed development of both, *B. bassiana* and *M. anisopliae* on the integument. High amounts of not identified saprophytic fungi were detected on dead insects after exposure of N3 to substrates from the second (44.7%) and fourth (38.1%) farms.

Mortality of *T. infestans* N3, 15 days after exposure to substrates, which had been collected and tested one month after application of CG 14 in treated hen houses of the farms, varied between 0% in the first farm and 52% in the fourth farm. Both, *B. bassiana* and *M. anisopliae* were isolated from cadavers (Table II). Mortality of N3 decreased when insects were exposed to substrates collected in the farms two or three months after treatment and varied between 0% and 8%, two months, and between 0 and 10%, three months after application. *M. anisopliae*, but no *B. bassiana* was detected on cadavers after exposure of N3 on two and three months old substrates.

Percent of CFU identified as *B. bassiana* originating from substrates collected after application of CG 14 in the hen houses was highest, 24 h after treatment, with 67.7 (standard error of the mean 15.8%) and decreased significantly from numbers found one month, 7.4 (5.6%) two months, 1.5 (1.1%), and three months, 0.9 (0.5%) ($H = 8.6$, $P = 0.036$, $DF = 15; 3$) (Fig. 2). No difference in the number of CFU identified as *M. anisopliae*, which varied between 8.9 (7.7%), 24 h after application and 19.3 (8.8%), one month after treatment, was observed up to three months after application of CG 14 ($H = 1.6$, $P = 0.65$, $DF = 15; 3$) (Table II).

Characterization of B. bassiana isolates - Analysis by RAPD showed high similarity (> 97%) of most tested isolates (82.4%) with CG 14, including all isolates detected in the four farms in substrates or *T. sordida* after application of CG 14. One isolate detected in substrate in the third farm before fungal treatment was 100% similar to CG 14. Another isolate in the second and two isolates in the fourth farm detected in substrates collected before treatment, differed at > 82%, > 85% and 39%, respectively from CG 14 (Fig. 3). These isolates were not observed within the tested group of *B. bassiana* isolated from substrates or *T. sordida* after treatment of hen houses.

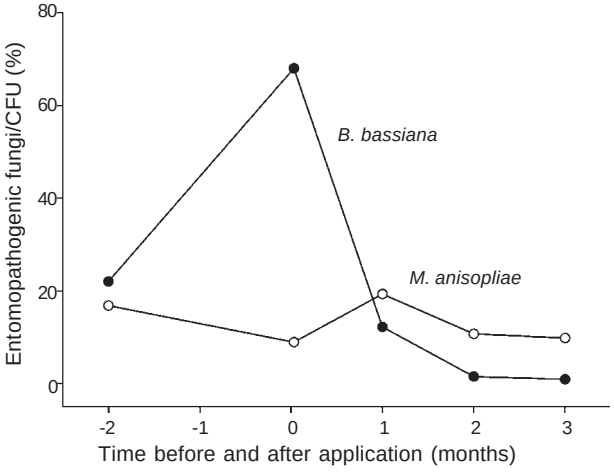

Fig. 2: relative number of colony forming units (CFU) identified as *Beauveria bassiana* and *Metarhizium anisopliae*, and detected within two months before and three months after application of *B. bassiana*, CG 14, in substrates from fungus-treated areas.

Semi-field test - Initial mortality was observed during 10 days after exposure of *T. sordida* to fungus-treated bricks inside the bags which had been installed in each farm (Fig. 4). Median cumulative mortality of insects, 25 days after treatment, reached 45 (2.9%) compared to 22.5 (6.3%) for the control insects with no significant difference between values obtained for the test and control mortalities (t = 25.0, P = 0.057). No fungal development was observed on cadavers which had been exposed in the bags under field conditions. Twenty five days after exposure of insects on the bricks, 80 live *T. sordida* nymphs (N1 - N5) and adults found on fungus-treated bricks and 56 individuals from the control bricks were transferred to the laboratory. Mortality of insects, 10 and 25 days after incubation at RH > 98% was 33.9 (7.8%) and 61.5 (12%), respectively. Control mortality 25 days after incubation was 12 (6%). Except one N5 from fungus-treated bricks of the fourth farm and dead control insects, all cadavers showed development of *B. bassiana* after exposure in a humid chamber.

TABLE II

Detection of *Beauveria bassiana* and *Metarhizium anisopliae* in the hen houses, after application of *B. bassiana* CG 14 [a]

Time after application	Mortality (%) (SEM), of *Triatoma infestans* [b]	Fungi (%) (SEM), on cadavers [c]				Number (SEM) of CFU [d]	*M. anisopliae* and *B. bassiana*/CFU (%) (SEM) [e]	
		B. b.	*M. a.*	*B. b.* and *M. a.*	S		*B. b.*	*M. a.*
24 h	53.5 (18.5)	60.6 (6.6)	18.7 (11.3)	1.4 (1.4)	20.7 (12.0)	28.1 (11.4)	67.7 (15.8)	8.9 (7.7)
1 month	20.0 (12.5)	18.7 (4.4)	52.6 (25.9)	0	9.3 (2.2)	18.9 (4.9)	7.4 (5.6)	19.3 (8.8)
2 months	4.0 (2.3)	0	87.5 (12.5)	0	12.5 (12.5)	38.6 (5.2)	1.5 (1.1)	10.7 (6.8)
3 months	3.5 (2.2)	0	53.3 (29.1)	0	46.7 (29.1)	41.8 (14.7)	0.9 (0.5)	9.8 (6.0)

a: mean values (standard error of the mean) found in four hen houses of four different farms each, located in the rural proximity of São Luís de Montes Belos, Goiás, Brazil. Oil-formulated conidia of *B. bassiana* CG 14, Embrapa, had been applied before at a final 10^6 conidia/cm^2. Five samples of substrate each hen house were tested; *b*: 10 *T. infestans* third instar nymphs were exposed to each substrate and incubated at 25°C and relative humidity close to saturation; *c*: cadavers were incubated at 25°C and relative humidity close to saturation; *d*: 1 g of substrate was suspended in 10 ml 0.1% Tween 80 and 100 ml of a 10^{-2} dilution inoculated on the medium and incubated at 25°C; *e*: other fungi were not identified; *Bb*: *B. bassiana*; *Ma*: *M. anisopliae*; S: saprophytic fungi

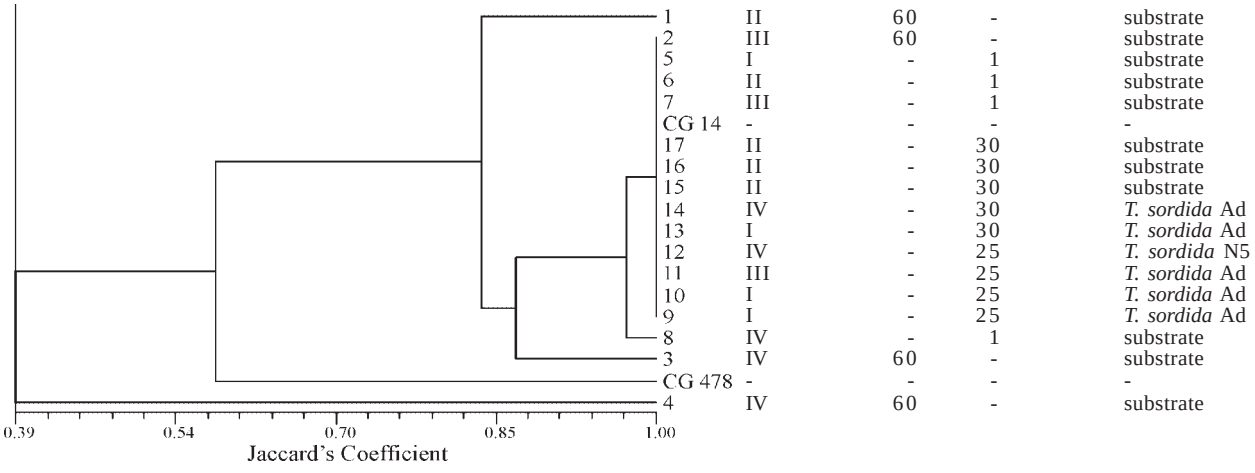

Fig. 3: dendrogram constructed from RAPD data, indicating the relationships among *Beauveria bassiana*, CG 14, and *B. bassiana* isolates detected in substrates or dead *Triatoma sordida* adults (Ad) or fifth instar nymph (N5) originating from hen houses located in four farms in Central Brazil within two months before and one month after application of CG 14 and an outgroup isolate, CG 478. A similarity matrix was calculated using the Jaccard coefficient, and the tree was generated from this matrix by unweighted pair group method, arithmetic mean (UPGMA).

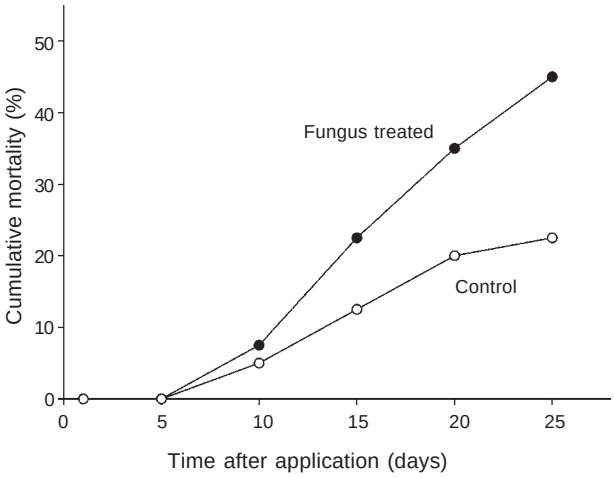

Fig. 4: percent cumulative mortality of *Triatoma sordida* after exposure to bricks containing conidia of *Beauveria bassiana*, CG 14, in peridomestic areas in Central Brazil.

Laboratory assays - Cumulative mortality of *T. sordida* was ≥ 95%, 15 days after direct spraying of conidia on the insects and exposure at 75% RH and RH > 98%. No significant difference was found between mortalities obtained at different humidities tested, 5 ($t = 1.4$, $P = 0.21$), 10 ($t = 0.8$, $P = 0.48$) and 15 days ($t = 0.7$, $P = 0.54$) after treatment. However, no mortality was observed after exposure of *T. sordida* on a fungus-treated brick and exposure at 75% RH during 15 days. At relative humidity close to saturation, 90% of insects had died, 10 days and all insects were found dead, 15 days after exposure on the brick. *B. bassiana* developed on all cadavers in a humid chamber, independently of the application method and humidity during the infection tested.

All *T. infestans* N3 survived after exposure on fungus-treated filter papers from different farms and incubation at 75% RH and 25°C during 15 days, independently of the period of exposure of the filter papers under field conditions. When insects were incubated on filter papers which had been transferred 24 h after fungal application to the laboratory at RH > 98%, 52.5 (16%) of N3 had died within 10 days and total mortality was observed 15 days after incubation. Mortality reached 12.5 (12.5%), 15 days after exposure, when tested on one month old filter papers and no more mortality was observed, testing filter papers which had been exposed two and three months under field conditions. Colored spots and growth of saprophytic fungi were observed on the filter paper, one month after treatment and exposure in the farms. *B. bassiana* developed on all dead insects after transfer to a humid chamber.

DISCUSSION

Our results clearly showed that peridomestic populations of *T. sordida* were reduced after application of *B. bassiana* strain CG 14 in their habitats. Insects were killed and *B. bassiana* was found recycling on cadavers. However, triatomine populations could not be eliminated permanently in the fungus-treated areas. Investigations on the activity of entomopathogenic fungi on triatomine bugs under field conditions are important in order to evaluate their potential for biological control of those vectors. Frequently, results obtained in the laboratory are not reproduced in field tests, where interaction among pathogens, vectors and environment are complex. In the laboratory, triatomines were highly susceptible to *B. bassiana* and *M. anisopliae* (Romaña & Fargues 1987, Luz et al. 1998a, b, Lecuona et al. 2001). In another study, the strain CG 14 induced total mortality of *T. infestans* N3 in the laboratory at RH > 98% and 85% mortality at 50% RH, 15 days after inoculation (Luz et al. 1998a). Reduced activity of this and other *B. bassiana* strains against *T. infestans* found in that study and in preliminary field tests by Luz et al. (1999) may be due to sub-optimal conditions of humidity. Temperature and moisture in the triatomine habitats are not constant and may vary according to daily or seasonal cycles. Simulation of humidity under laboratory conditions showed that constant or minimal periods of 12 h 97% RH induced rapid and total mortality of *R. prolixus* treated with *B. bassiana* and permitted recycling of the fungus on cadavers. At lower moistures more insects survived and *B. bassiana* did not emerge from dead insects (Luz & Fargues 1998, 1999, Fargues & Luz 1998, 2000). Only at 43% RH an increasing susceptibility of *R. prolixus* and *T. infestans* to fungal infection was reported by Luz (1990) and Romaña (1992), respectively, and can be expected also for other triatomine species. Many triatomine species, such as *T. sordida* occur in regions with arid or semi-arid climate and humidity during the dry season may then be unfavorable for external fungal development. It is important to consider microclimatic conditions in triatomine's habitat which may differ significantly from conditions prevailing in the area. A dumping effect of fluctuating temperature and humidity, which delays conditions of temperature and humidity as shown by Lorenzo and Lazzari (1999) and Vazquez-Prokopec et al. (2002) in domestic and peridomestic ecotopes of *T. infestans*, compared to ambient conditions, can interfere with fungal development on insects. During the experiment moderate precipitations were observed, but RH monitored in one of the shelters did not reach 97% during at least 12 h a day. However, in the fungus-treated hen house of the first farm, where most dead triatomines were detected after application of CG 14, RH was obviously favorable for high rates of infection and development of *B. bassiana* on cadavers. Similar observations in peridomestic habitats with prolonged periods of elevated moisture were also reported in simulated field trials with *B. bassiana* and *R. prolixus* in Colombia (Luz 1994).

Interestingly high mortalities were observed in the laboratory after direct application of the oil-based formulation, independently of humidity tested. This was not observed after indirect treatment by contact to treated bricks under semi-field conditions, where ambient humidity was unfavorable for fungal development due to the permanent ventilation of the bags or in the laboratory at 75% RH. Direct treatment provided a distinct higher dose of conidia on each insect than indirect application after exposure to treated bricks. However, direct treatment of triatomine insects, which are active during the night seems difficult. Formulations and application techniques which

increase quantitative inoculation of fungal propagules on target insects will probably enhance fungal activity against triatomines under field conditions.

Quantitative evaluation of the effect of CG 14 on free-living peridomestic triatomine bugs proved to be difficult. *T. sordida* such as most other species are active at night and hide in their resting places during the day. Triatomine nymphs and adults often remain immobile even after exposure to day light and younger instars are more difficult to detect due to their small size. Moreover, *T. sordida* as other species (Zeledón et al. 1973) have a distinct camouflaging activity, covering their body with fine particles. The time-limited day capture was considered as the most adequate method to detect routinely live and dead triatomines during a prolonged period in their habitats without affecting live individuals. However, numerous agonizing or dead insects, even adults, were probably not detected and consequently not considered. Infected individuals can modify their behavior and leave their hiding places during the day. Many of these insects were presumably preyed by fowl and other vertebrates or arthropods and numerous dead insects disappeared without delay by the activity of ants. The high heterogeneity of live insects found in fungus-treated and control hen houses throughout the field tests was probably related to the small experimental areas and the initial reduced population size in three of the tested farms. Insects could move freely between treated, control and untreated areas. Behavior of the triatomines in the treated areas may have been altered at the beginning of the experiment and during controls of mortality. Many triatomine species including *Triatoma* spp. avoid wet habitats. Application of aqueous oil formulation, which caused a temporary moistening of the habitats, induced probably a time limited evasion in both, fungus-treated and control hen houses of a part of the *T. sordida* population to untreated areas. A part of contaminated individuals remained and died there and consequently disappeared undetected. Wiesinger (1956) did not find an influence of humidity on hiding activity of *T. infestans* and insects were not repelled by a wet surface. However, other studies showed that the same species preferred to stay in refuges with 20% RH or even at dryer conditions (Roca & Lazzari 1994, Lorenzo & Lazzari 1999). Infected or healthy insects from control or untreated areas moved later to fungus-treated areas, which explains why populations in the treated areas varied and were not completely eliminated. Fungal application in larger areas would certainly reduce the interfering migration and permit a better quantitative evaluation during field tests.

Stage frequency of triatomine populations in peridomestic habitats varies according to the season and may interfere with the dynamic of the mycosis. Remarkably more elder nymphs and adults than younger instars were observed in the hen houses during the tests performed in January. This corresponds to observations of Forattini et al. (1979) who reported a dispersal activity of *T. sordida* adults starting between March and April and a clear increase of first instar nymphs between July and August. The low number of individuals of all different stages tested in our study did not allow speculations about a stage-dependent susceptibility of *T. sordida* to *B. bassiana* in the field. In the laboratory, susceptibility of *R. prolixus* to infection with *B. bassiana* declined with higher nymphal instars (Luz 1994). However, Romaña and Fargues (1992) found an elevated susceptibility of *R. prolixus* N3 and N5 compared to N1 to *B. bassiana*.

It is likely that most insects succumbed due to CG 14 inoculation with high doses of conidia and not due to indigenous *B. bassiana* or *M. anisopliae* strains. Nevertheless, an interaction between CG 14 and other local strains and their action cannot be excluded, as one isolate found before the treatment showed high similarity to CG 14. Previous tests showed that *B. bassiana* and *M. anisopliae* occurred in most of the vector habitats used in this study before application of CG 14 (C Luz, LFN Rocha, and GV Nery unpublished observations).

An elevated number of *B. bassiana*, all with high similarity to CG 14, when scored by RAPD, was detected 24 h up to one month after fungal application in the substrates or on fungal killed insects. However, not all *B. bassiana* isolates were analyzed by RAPD. The number of *B. bassiana* in the substrates collected in the fungus-treated hen houses declined quickly to values found before application. This indicates that persistence of high amounts of CG 14 conidia in the substrates did not exceed one month. Results showed that multiplication of *B. bassiana* on cadavers in the habitats may occur. However, development and survival of entomopathogenic fungi may be impaired by other saprophytic microorganisms such as fungi and bacteria. *B. bassiana* showed survival rates of at least three months in dead *R. prolixus* under laboratory conditions (Luz 1994), and may persist for long periods in cadavers and produce new conidia and propagate fungal infection to healthy insects at favorable conditions. In our study, high fungal inoculum decreased in a few weeks after application to natural levels and must be renewed if necessary. A rapid decrease in conidial populations within 20 days after application of *B. bassiana* in soils was also reported by Inglis et al. (1997). Integrated control strategies alternating inundative application of fungi in the rainy season and synthetic insecticides in the dry season or a combined application of fungi and insecticides in the rainy season could promote an efficient and permanent reduction of triatomine vectors in peridomestic areas. *B. bassiana* could contribute to reduce triatomine populations in peridomestic areas and interrupt domestic invasion by secondary vector species in Central Brazil or other regions with prolonged precipitations during the rainy season. Further testing of improved oil-water formulations, application techniques and fungal persistence under field conditions will be useful to consolidate biological control of triatomine vectors.

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