PUBLIC HEALTH

Detection of Entomopathogenic Fungi in Peridomestic Triatomine-Infested Areas in Central Brazil and Fungal Activity Against *Triatoma infestans* (Klug) (Hemiptera: Reduviidae)

CHRISTIAN LUZ, LUIZ F.N. ROCHA AND GUSTAVO V. NERY

Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, C. postal 131 74001-970 - Goiânia, GO

Neotropical Entomology 33(6):783-791 (2004)

Detecção de Fungos Entomopatogênicos em Áreas Peridomiciliares Infestadas por Triatomíneos no Brasil Central e Atividade dos Fungos Contra *Triatoma infestans* (Klug) (Hemiptera: Reduviidae)

RESUMO - Foram detectados 31 isolados de *Metarhizium anisopliae* (Metsch.) Sorok. e 15 isolados de *Beauveria bassiana* (Bals.) Vuill. em 148 amostras de substratos coletadas em habitats peridomiciliares infestados com triatomíneos de 24 fazendas localizadas no Centro do Brasil. A maioria dos isolados foi encontrada em solos misturados com fezes de animais ou solos de áreas de poleiros e chiqueiros. Os fungos foram detectados com um método de captura utilizando *Triatoma infestans* (Klug) como isca, e um método combinado *in vitro* e *in vivo*, com meio Chase modificado e *T. infestans*. Os primeiros estudos sobre a atividade dos fungos indicaram que todos os isolados foram patogênicos para *T. infestans* quando testados em umidade relativa acima de 98% e 25 ± 0,5°C. Porém, a atividade foi reduzida em umidade relativa de 75%. Os resultados ressaltam o potencial de *B. bassiana* e *M. anisopliae* como agentes de controle para os vetores da doença de Chagas. As duas espécies ocorrem naturalmente em habitats peridomiciliares de triatomíneos no Centro do Brasil e podem contribuir para o controle desses vetores e reduzir o risco de reinfestação das casas após eliminação de espécies domiciliares.

PALAVRAS-CHAVE: Triatominae, *Beauveria bassiana*, *Metarhizium anisopliae*, peridomiciliar, ocorrência

ABSTRACT - From 148 substrate samples collected in peridomestic triatomine-infested habitats of 24 farms in Central Brazil, 31 isolates of *Metarhizium anisopliae* (Metsch.) Sorok. and 15 isolates of *Beauveria bassiana* (Bals.) Vuill. were obtained. Most of the isolates were found in substrates that consisted of soil mixed with animal feces or soil only in areas of trees where poultry roost and pig houses. Fungi were detected with an insect bait method using *Triatoma infestans* (Klug), and combined *in vitro* and *in vivo* techniques with modified Chase medium and *T. infestans*. All isolates were highly virulent to *T. infestans* third instar nymphs at a relative humidity > 98% and temperature of 25 ± 0.5°C. However, activity against nymphs was reduced at 75% relative humidity. Results underline the potential of *B. bassiana* and *M. anisopliae* as agents for biological control of vectors of Chagas disease. Both species occur naturally in peridomestic habitats of triatomines in Central Brazil and may contribute to control these vectors and to reduce the risk of reinfestation of houses after eliminating domestic vector species.

KEY WORDS: Triatominae, *Beauveria bassiana*, *Metarhizium anisopliae*, peridomestic, occurrence

Despite the national and international efforts and successes in eliminating domestic triatomine vectors, the risk of vectorial transmission of Chagas disease has not been eliminated. *Triatoma infestans* (Klug), formerly one of the most important domestic vectors of *Trypanosoma cruzi* (Chagas), the agent of Chagas disease, has almost disappeared in most of Latin America (Dias *et al.* 2002). However, synthetic insecticides that showed satisfactory results inside houses were less effective against vector species in peridomestic areas. This is mainly due to degradation of these chemicals by abiotic factors, especially ultraviolet light, rain or wind (Bos 1988, Dias 1991). Peridomestic triatomine populations may persist and invade insect-free houses. Natural enemies such as predators, parasitoids and pathogens, which may contribute to control sylvatic or peridomestic vector populations, were summarized

by Ryckman & Blankenship (1984). Entomopathogenic fungi, *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorok., were highly active against Triatominae under laboratory conditions (Romaña & Fargues 1987; Luz *et al.* 1998 a, b; Lecuona *et al*. 2001). However, there are only few reports about natural infection of triatomines with these or other fungi. In India, *B. bassiana* was isolated from *Linshcosteus* sp. Distant (Parameswaran & Sankaran 1979), and the isolate was highly virulent for *Linshcosteus* sp., *Triatoma rubrofasciata* (De Geer) (Parameswaran & Sankaran 1979) and *Rhodnius prolixus* Stål (Luz 1994). Recently, Luz *et al*. (2003) detected a new species of *Evlachovaea* Borisov & Tarasov on a *Triatoma sordida* Stål fourth instar nymph cadaver originating from a farm in Central Brazil. This fungus was highly active against *T. infestans* and other triatomine species (Luz *et al*. 2003; Luz *et al*. 2004 b). Low humidity in the vector's habitat, which affects insect infection and mummification (Luz & Fargues 1999, Fargues & Luz 1998), and a rapid disintegration of fungus-killed insects under natural conditions may be the reason that there is little information published about the natural occurrence of entomopathogenic fungi on triatomine bugs. Here we report the natural occurrence of pathogenic fungi in substrates from peridomestic triatomine habitats in Central Brazil, and the activity of detected isolates against *T. infestans* under laboratory conditions.

Materials and Methods

Tests were carried out during 2001 on farms with peridomestic infestations of triatomine bugs located near to the cities Formosa, Goiatuba and São Luís de Montes Belos, in the Central Brazilian state of Goiás.

Sampling of Substrates. Samples of soil and organic substrates such as animal feces, litter and bark were collected from different triatomine habitats such as in poultry houses (39.2%), under trees in which these poultry roost (22.3%), in pig houses (12.8%), barns (19.6%), corrals (1.4%) and other deposits (4.7%). In Formosa and Goiatuba, five samples taken at 10 farms and 12 samples from each of four farms in São Luís de Montes Belos were examined. About 25 g substrate were scraped at randomly selected locations to a depth to 2-3 cm, transferred to plastic bags and stored in a polystyrene cooler at 20°C. The composition of the samples was analyzed macroscopically, and substrates were classified into five groups: soil with no visible organic material, soil mixed with animal feces, soil mixed with litter, litter mixed with animal feces, and bark.

***In Vivo* Detection Technique.** Laboratory reared newly emerged and unfed third instar nymphs (N3) of *T. infestans* were used for the detection of entomopathogenic Hyphomycetes and for assays on fungal activity. The *T. infestans* colony was originally from Paraná state, and has been maintained in the laboratory since 1981. Insects were fed on chickens every month and maintained at 25 ± 0.5°C, 75 ± 5% relative humidity (RH), with 12h photophase (Silva 1985).

Substrate of each sample was homogenized and about 3 g of this homogenate were transferred to petri dishes (90 x 15 mm). Ten N3 were exposed on the substrate, and the dishes moved around carefully in order to intensify the contact of insects with the substrate. Dishes were then incubated for 15 days at 25 ± 0.5°C and relative humidity close to saturation (RH > 98%). Mortality was monitored daily. Dead insects were dipped in 93% alcohol, surface-sterilized in 2.5% sodium hypochlorite for 3 min, and then washed three times for 1 min in sterile water. Cadavers were then incubated for 15 days at 25 ± 0.5°C and RH > 98%. Fungal development on the cadavers was evaluated daily, and the emergent fungi were inoculated onto complete medium (CM) – 0.001 g $FeSO_4$, 0.5 g KCl, 1.5 g KH_2PO_4, 0.5 g $MgSO_4 \times 7H_2O$, 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 – to which chloramphenicol (1 g/1000 ml) was routinely added.

Combined *in Vitro* and *in Vivo* Detection Techniques. Hyphomycetes were isolated from the substrates using modified Chase medium (MCM) (Chase *et al*. 1986) – 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. Samples of 1 g substrate were suspended in 10 ml sterile 0.1% Tween 80 and vortexed for 3 min. Each suspension was then diluted (1:99) in distilled sterile water, spread onto MCM, and incubated for 20 days at 25 ± 0.5°C and 12h photophase. Developing colony forming units (CFU) were examined daily, and macroscopically different fungi inoculated separately on CM added with chloramphenicol. Twenty days after development of CFU, 10 *T. infestans* N3 were exposed for five min directly on the fungal cultures and then transferred to RH > 98% at 25 ± 0.5°C for 15 days. Dead insects were examined for fungi as mentioned above.

Tests About Fungal Activity. Regardless of how many times any single fungous species might be detected in a sample, only one isolate of any species from a single sample was tested against insect hosts. Fungal isolates found on MCM or dead insects after exposure of N3 to substrates or CFU were cultured on CM for 15 days at 25 ± 0.5°C. Ten *T. infestans* N3 were exposed for 5 min directly on the sporulated cultures. Insects were then incubated for 15 days at 25 ± 0.5°C and RH > 98%. Fungi were isolated from dead insects as mentioned above. Their macroscopic appearance was compared with the inoculated cultures and they were identified by microscopic examination. Fungi that showed no pathogenicity to *T. infestans* were not identified. All isolates that proved to be pathogenic to *T. infestans* were stored by the fungal culture collection of the Institute of Tropical Pathology and Public Health, Federal University of Goiás, Goiânia, Brazil.

Fungal activities were tested at two different relative humidities: Ten *T. infestans* N3 were directly sprayed with 5 ml suspended 10^8 conidia/ml at a final $5.3x10^6$ CFU/cm^2 using a Potter spray tower (Burkard, Hertfordshire, UK), dried for one hour at environmental temperature and relative humidity and then incubated for 15 days in containers (33 x 37 x 22 cm) at 25 ± 0.5°C, RH of 75 ± 5% and RH > 98%. Relative humidity

of 75% inside containers was maintained by a saturated aqueous solution of NaCl, and RH > 98% was maintained with distilled water only (Winston & Bates 1960).

The activity of six *M. anisopliae* isolates that induced mortality ≥ 80% at RH 75% 20 days after treatment was tested by applying five different concentrations of conidia – 10^6, $3.3x10^6$, 10^7, $3.3x10^7$ and 10^8 conidia/ml – corresponding to $5.3x10^4$, $1.6x10^5$, $5.3x10^5$, $1.6x10^6$ and $5.3x10^6$ CFU/cm^2 on the test surface, as mentioned before on *T. infestans* N3. The final deposit of CFU/cm^2 treated surface was determined by spraying suspended conidia at $3.3x10^7$ and 10^8 conidia/ml as mentioned before on sterile coverslips (18 x 18 mm). Coverslips were then transferred in 10 ml sterile 0.1% Tween 80 and 100 ml inoculated on CM added with chloramphenicol. The number of CFU was checked daily for five days after inoculation. Control insects were treated with 0.1% Tween 80 only. Treated nymphs were dried as mentioned before and incubated in containers at 25 ± 0.5°C, RH of 75 ± 5% and RH > 98%. For all assays, mortality of insects was recorded daily for 20 days after treatment. Development of *M. anisopliae* on dead insects was examined as mentioned above.

Data Analysis. Isolates detected in different habitats and substrates were analyzed by the genmod procedure which fits generalized models (McCullagh & Nelder 1989) and the Fisher's exact test, both at 5% level for significance. Methods of detection were tested by McNemar's test (Fleiss 1981). Lethal concentrations to kill 50% (LC_{50}) were calculated by Probit analysis (SAS Institute Inc. 2000).

Results

A total of 148 substrate samples were analyzed for entomopathogenic fungi. Substrates consisted on soil containing animal feces (30.4% of total samples), soil with no visible organic material (29.1%), litter mixed with animal feces (22.3%), soil and litter without visible animal feces (14.9%) and pure bark (3.3%). A total of 31 *M. anisopliae* and 15 *B. bassiana* isolates were detected in different samples independently of the kind of substrate, habitat, locality and method of detection. The highest number of entomopathogenic fungi (45.7%), with 68.8% of all detected *B. bassiana* and 32.3% of the *M. anisopliae* isolates, was found in substrates collected in Formosa. In São Luís de Montes Belos was observed 34.7% and in Goiatuba 19.6% of all pathogenic fungal isolates. A total of 66.7% of the farms was positive for entomopathogenic fungi with 80.0% positive farms in Formosa, 40.0% in Goiatuba and 100% in São Luís de Montes Belos. In 25.0% of all farms both *B. bassiana* and *M. anisopliae* were detected. The greatest number of fungi was isolated from substrates collected under roosting trees (48.3%) followed by samples originating from pig houses (28.3%), poultry houses (11.7%), barns (8.3%), other deposits (3.4%) and corrals (0%) (Fig. 1). The highest numbers of *B. bassiana* and *M. anisopliae* isolates were detected in samples of soil mixed with animal feces (46.6%) or pure soil (40.0%). The number of entomopathogenic fungi detected in substrates that consisted of soil and litter (6.7%) or litter and feces (6.7%) was distinctly reduced, and no fungi were detected in bark (Fig. 2). High mortality (≥ 90%) of laboratory-reared N3 of *T. infestans* with subsequent development of saprobic fungi on dead insects was observed after exposure to 70.6% of the field-collected samples from Goiatuba but to only 15.4% of the substrates from Formosa and 45.4% from São Luís de Montes Belos. The saprobic fungi isolated from the cadavers showed no activity against *T. infestans* in subsequent tests.

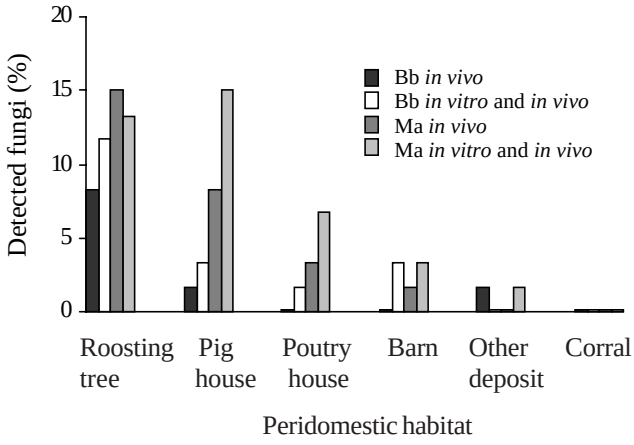

Figure 1. Occurrence (%) of *B. bassiana* (*Bb*) and *M. anisopliae* (*Ma*) sampled in peridomestic triatomine habitats in Central Brazil using *in vivo* and combined *in vitro* and *in vivo* detection techniques.

A significant association was detected between the percent of fungal isolates (*B. bassiana*, P = 0.012; *M. anisopliae*, P = 0.024) and the habitat but not in relation to the substrate (*B. bassiana*, P = 0.365; *M. anisopliae*, P = 0.393). Fisher's exact test showed a significant association between habitats but no association of the substrate with the percent incidence of *B. bassiana* (P = 0.007; P = 0.373) when considering all kinds of habitats and substrates. The association between habitat, substrate and the percent incidence of fungi was significant for *M. anisopliae* according to the same statistical test (P < 0.001; P = 0.023). *Beauveria bassiana* and *Metarhizium anisopliae* were recovered by the *in vivo* or the two-step *in vitro/in vivo* techniques to be present in a collective percentage of 33.3% and 29.0% of samples, respectively. McNemar's test showed no significant difference between the number of *B. bassiana* (P = 0.109) and *M. anisopliae* (P = 0.286) detected with the *in vivo* or two-step *in vitro/in vivo* techniques, respectively.

All *B. bassiana* and *M. anisopliae* isolates induced mortality of *T. infestans* N3 after exposure to the sporulating fungal cultures and incubation at 25 ± 0.5°C and RH > 98%. They were isolated from cadavers, and the identification of the fungal species was confirmed for all isolates. All 15 isolates of *B. bassiana* (except for IP 166, IP 170 and IP 231) and all 27 isolates of *M. anisopliae* induced 100% of mortality of N3, 15 days after treatment and incubation at 25 ± 0.5°C and RH > 98% (Table 1). At 75% RH, the *M. anisopliae* isolates IP 177 and IP 232 induced 100% mortality; eight isolates – IP 174, IP

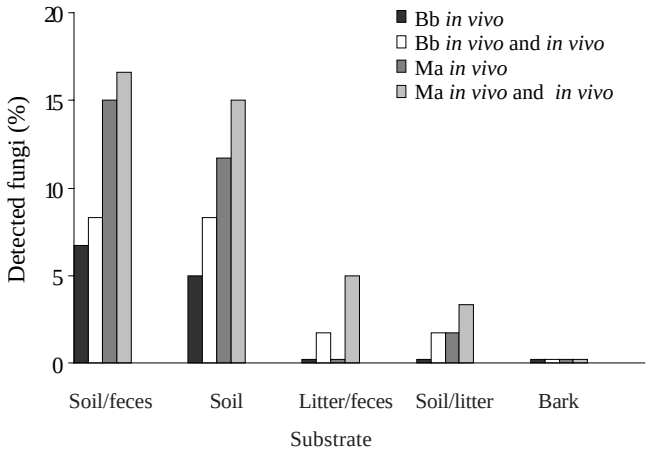

Figure 2. Occurrence (%) of *B. bassiana* (*Bb*) and *M. anisopliae* (*Ma*) sampled in substrates in peridomestic triatomine infested areas in Central Brazil using *in vivo* and combined *in vitro* and *in vivo* detection techniques.

176, IP 178, IP 179, IP 220, IP 222, IP 223, IP 226 – from Goiatuba or São Luís de Montes Belos caused 80% or 90% mortality 20 days after treatment. The cumulative mortality of N3 inoculated by *B. bassiana* varied from 10% (IP 161 and IP 229) to 60% (IP 171) 20 days after exposure at RH 75% and 25 ± 0.5°C (Table 1).

Values of LC_{50} 10 days after treatment of *T. infestans* with *M. anisopliae* isolates from Goiatuba (IP 176, IP 177) and São Luís de Montes Belos (IP 223, IP 226, IP 232) and exposure to RH > 98% varied from $3.76x10^4$ CFU/cm^2 (for IP 226) to $3.49x10^5$ CFU/cm^2 (for IP 223); there were no significant differences among LC_{50} results for the tested isolates. Mortality of N3 treated with IP 222 (São Luís de Montes Belos) and incubated at high humidity was too elevated to calculate the LC_{50}. At RH 75% mortality was too low to calculate values of LC_{50} 20 days after treatment, for all isolates tested (Table 2).

Discussion

Results clearly showed that *B. bassiana* and, even more commonly, *M. anisopliae* are present in peridomestic triatomine-infested areas in Central Brazil. Both species are cosmopolitan and commonly found in soils or from infected soil-dwelling insects (Glare & Milner 1991, Vänninen 1995, Tarasco *et al*. 1997). Both species were isolated from substrates collected in typical vector habitats, and all isolates proved to be highly active against *T. infestans* at favorable conditions of moisture. *M. anisopliae* is considered a mildly thermophilic species in comparison to *B. bassiana* (Fargues *et al*. 1992, Ouedraogo *et al*. 1997), and the higher incidence of this pathogen in this study may be related to the tropical climate in Central Brazil.

Fungi were detected in peridomestic habitats of triatomine bugs that are generally located close to their domestic or wild animal hosts. The number of fungi detected depended on the habitat. The highest fungal incidence was found in areas below poultry roosts. The habitat-dependent distribution of fungi may reflect differences in the relative population sizes of insects found in these habitats. In studies in Argentina most *T. infestans* were found in poultry houses or close to fowl refuges (Cecere *et al*. 1996, Gajate *et al*. 2001). Natural habitats of *Rhodnius* spp. Stål are trees, especially palms, and these insects are frequently observed close to peridomestic perches. *T. sordida* is also common in palm trees in Argentina (Bar & Colli 2001).

B. bassiana and, especially, *M. anisopliae* were more frequently detected in soil mixed with feces or soil alone than in litter with or without added feces or on bark. In a study about the mycoflora in dusts and litter from poultry houses, Vissiennon (1999) found such dust to include many fungi; while fungi potentially hazardous for human or animal health were found in these dusts, no entomopathogens were detected. Biotic and abiotic factors may be more important for fungal occurrence and survival than any specific habitat or substrate. Composition of the substrate or the diversity and number of microorganisms may affect survival and development of entomopathogenic fungi. Extreme temperature conditions, especially those exceeding 35ºC, may injure or kill fungi (Fargues *et al.* 1992, Ouedraogo *et al*. 1997, Luz & Fargues 1997). Such elevated temperatures can easily be achieved in closed rooms such as poultry houses, barns or other unventilated spaces after prolonged exposure to sunlight. In areas covered by vegetation such as arboreal roosting sites for poultry or other protected habitats, sunlight is generally reduced, and a combination of moderate temperatures and elevated moisture, especially during the rainy season, can be expected. In field tests in Colombia with *B. bassiana* against *R. prolixus*, the highest humidities and moderate temperatures were measured in a poultry house covered by vegetation and in a palm tree. In both habitats, all fungus-treated insects succumbed quickly to infection, and the fungus sporulated well on diseased cadavers (Luz 1994).

Entomopathogenic fungi are disseminated in peridomestic areas by infected insects, human or animal activity, or wind. Most triatomine species are active during the night and generally hide during the day in shelters near their hosts. When they leave their resting places at night to feed on hosts, they may be contaminated by fungi. Most adults move without flying, and both adults and nymphs get into close contact with substrates while resting in their daytime hiding places and during nocturnal activity. Nymphs of several species exhibited camouflaging activities by covering their bodies with fine dust particles (Zeledón *et al.* 1973). Other peridomestic insects may also interfere to dissemination of fungi. *B. bassiana* was isolated from the darking beetle, *Alphitobius diaperinus* (Panzer), in poultry houses in USA (Castrillo & Brooks 1998) and has been tested as control agent against this important pest (Geden *et al.* 1998, Crawford *et al.* 1998, Perez *et al.* 1999). Domestic animals may disperse entomopathogenic fungi as shown by Mitra *et al*. (1998) who detected *B. bassiana* in a skin scraping from ruminants in India.

In our study two methods to detect entomopathogenic fungi in substrates were applied: an *in vivo* technique using *T. infestans* as live selective bait; the other combined *in vitro* and *in vivo* techniques with MCM, a selective medium for

Table 1. Cumulative mortality of *T. infestans* after application of *M. anisopliae* and *B. bassiana* isolates originating from peridomestic rural areas in Central Brazil and exposure at different humidities.

		Time after treatment (days)					
		5	10	20	5	10	15
Species	Isolate	Relative humidity 75%			Relative humidity > 98%		
M. anisopliae	IP 156[1]	0	10	10	0	70	100
	IP 159[1]	0	0	0	10	80	100
	IP 162[1]	0	10	10	0	50	100
	IP 164[1]	10	40	40	10	100	100
	IP 167[1]	0	20	20	0	100	100
	IP 168[1]	0	0	0	10	100	100
	IP 169[1]	0	0	0	20	100	100
	IP 173[2]	10	40	40	10	100	100
	IP 174[2]	20	70	80	40	100	100
	IP 175[2]	20	60	70	30	100	100
	IP 176[2]	20	80	80	10	60	100
	IP 177[2]	20	90	100	10	100	100
	IP 178[2]	20	50	80	0	70	100
	IP 179[2]	20	70	80	10	70	100
	IP 181[2]	0	10	20	30	100	100
	IP 220[3]	20	70	80	20	100	100
	IP 221[3]	10	40	40	10	90	100
	IP 222[3]	20	70	90	30	100	100
	IP 223[3]	20	80	90	40	100	100
	IP 225[3]	10	50	60	20	100	100
	IP 226[3]	30	90	90	30	100	100
	IP 227[3]	10	70	70	10	100	100
	IP 230[3]	0	10	20	20	100	100
	IP 232[3]	20	80	100	30	100	100
	IP 233[3]	30	60	70	20	100	100
	IP 234[3]	0	30	40	40	100	100
	IP 235[3]	0	40	50	10	100	100
B. bassiana	IP 155[1]	0	10	20	0	30	100
	IP 157[1]	0	10	50	0	50	100
	IP 158[1]	0	20	20	0	90	100
	IP 160[1]	0	20	30	0	40	100
	IP 161[1]	0	0	10	0	100	100
	IP 165[1]	0	20	40	0	50	100
	IP 166[1]	0	10	20	0	10	80
	IP 170[1]	0	10	20	0	10	50
	IP 171[1]	0	30	60	10	70	100
	IP 172[1]	0	30	30	0	70	100
	IP 180[2]	0	50	50	0	30	100
	IP 224[3]	0	0	30	0	30	100
	IP 228[3]	0	20	40	0	10	100
	IP 229[3]	0	10	10	10	100	100
	IP 231[3]	0	0	30	0	30	90

Third instar nymphs were treated directly with suspended conidia (10^8 conidia/ml, corresponding to $5.31x10^6$ CFU/cm^2 tested surface) and then incubated at 25 ± 0.5°C. All isolates cited originated from Formosa[1], Goiatuba[2] and São Luís de Montes Belos[3] and are being stored by the fungal culture collection of the Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, GO.

Table 2. Lethal concentration 50% (LC_{50}) (CFU/cm^2) and respective 95% confidence intervals (95% C.I.) calculated for *T. infestans* third instar nymphs treated with *M. anisopliae* isolates detected in peridomestic areas of Central Brazil 10–20 days after exposure at relative humidity (RH) of 75% and > 98%.

Isolate	RH > 98%		RH 75%
	LC_{50} at 10 days (C.I.)	LC_{50} at 15 days (C.I.)	LC_{50} at 20 days (C.I.)
IP 176	$1.14x10^5$ ($1.10x10^3$-$3.79x10^5$)	a	b
IP 177	$8.03x10^4$ ($4.68x10^3$-$2.64x10^5$)	$2.85x10^4$ (0.08-$7.20x10^4$)	b
IP 222	a	a	b
IP 223	$3.49x10^5$ ($7.68x10^4$-$1.64x10^6$)	$2.10x10^4$ (0.01-$7.63x10^4$)	b
IP 226	$3.76x10^4$ ($4.29x10^2$-$1.28x10^5$)	a	b
IP 232	$6.13x10^4$ ($3.40x10^3$-$1.71x10^5$)	a	b

Third instar nymphs were treated directly with five concentrations of conidia, 10^6, $3.3x10^6$, 10^7, $3.3x10^7$ and 10^8 conidia/ml, corresponding to $5.3x10^4$, $1.6x10^5$, $5.3x10^5$, $1.6x10^6$ and $5.31x10^6$ CFU/cm^2 surface. Insects were then incubated at 25 ± 0.5°C. a: mortality of nymphs too high to calculate LC_{50}, b: mortality insufficient to calculate LC_{50}. Fungal culture collection, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, GO.

hyphomycete fungi in a first step and trapping pathogenic fungi from colonies on the medium with *T. infestans* as the second step. *T. infestans* proved to be highly effective as *in vivo* bait for *B. bassiana* and *M. anisopliae*. Satisfactory results with insect bait methods using other insects such as *Galleria mellonella* L. (Zimmermann 1986, Tarasco *et al*. 1997) and species of *Tenebrio* L. (Vänninen *et al*. 1989) were reported by other authors. Fungal detection in the present study did not depend on the method used. However, MCM based on the fungicide dodine (and including antibiotics such as chlortetracycline, penicillin or streptomycin) inhibit the development of other entomopathogenic fungi. An *Evlachovaea* sp. which was detected on a dead *T. sordida* specimen in Formosa (Luz *et al.* 2003) was not isolated from substrates using either technique discussed here.

Detection of fungi from any substrate sample was qualitative rather than quantitative since there was no way within this study to determine the titer of pathogenic fungal propagules in the substrate or whether all propagules of any pathogenic fungus detected were of the same genotype. It is important to note that insects exposed to the same sample may have become infected with more than one strain of the same species of fungus. Isolates of different samples collected in the same area may have been the same strain. Moreover, isolates detected in substrates with both methods may have been different strains.

The high mortality of nymphs after exposure to substrates collected in Goiatuba but without development of entomopathogenic fungi on many cadavers was probably not due to fungi or other pathogenic microorganisms but to residues of synthetic insecticides in the substrates. Houses and peridomestic installations are sprayed occasionally with pyrethroids by agents of the Brazilian National Health Foundation. Those insecticides currently being used for triatomine vector control have a residual activity of several months (Nayak *et al*. 2002). Moreover, inert dusts or small particles in the substrates may have mechanically abraided the cuticle during exposure of nymphs, thus leading to desiccation and insect death as related by Beament (1946) and Wigglesworth (1958).

Virulence of all fungal isolates was promising at RH > 98%, but activity against nymphs was distinctly reduced and varied between isolates at RH 75%. Previous studies showed that fungal activity of most isolates of *B. bassiana* and *M. anisopliae* when tested against triatomine vectors under laboratory conditions was highest at RH > 98% (Luz *et al*. 1998 a, b; Luz & Fargues 1999). A significant increase of natural control by fungi may be expected especially during the rainy season when high humidity is prevalent during prolonged periods in vector habitats. By simulating the variation of temperature and humidity regimes in the laboratory, Fargues & Luz (2000) demonstrated that *B. bassiana* induced high and rapid mortality of fungus-treated *R. prolixus* after exposure to 12/12h alternating cycles of RH 97% and RH 75%. A significant reduction in mortality was observed when daily RH 97% exposure time declined from 12h to 8h per day. The ability of *B. bassiana* to sporulate on *R. prolixus* cadavers is also closely related to temperature and moisture (Luz & Fargues 1998, Fargues & Luz 1998). Exposure to RH ≥ 97% for at least

12-16h per day is necessary for conidiogenesis by *B. bassiana* on *R. prolixus*, and the intensity of conidial production was enhanced by high diurnal temperatures between 28°C and 35°C (Fargues & Luz 1998).

Microclimatic investigations in peridomestic habitats of R. prolixus in Colombia showed that prolonged periods of humidity close to saturation can occur in such triatomine habitats as palm trees and poultry houses (Luz 1994).

Our results showed that entomopathogenic fungi can be important for natural control or as agents of biological control of peridomestic triatomine populations. More investigations about fungi that occur naturally in peridomestic areas and their function as control agents against triatomine vectors could contribute to a better understanding of fungal population dynamics. Initial studies in field conditions were promising. These fungi can reduce invasion of houses by peridomestic triatomines and the consequent levels of Chagas disease vectored by these insects (Luz *et al.* 1999, Luz *et al*. 2004a). More field tests with artificially released fungi may confirm the capacity of these microbes for the biological control of triatomine vectors of Chagas disease.

Acknowledgments

This study was supported by the National Council of Scientific and Technological Development (CNPq Centro Oeste). The authors thank Nicanor R. Silva for the technical support, Célia M.T. Cordeiro for advice and support on statistical analysis, Richard A. Humber for the critical review, and the National Foundation of Health (Funasa) of Brazil for its collaboration during the field work.

Literature Cited

Bar, M.E. & C.W. Colli. 2001. *Triatoma sordida* Stål 1859 (Hemiptera, Reduviidae: Triatominae) in palms of northeastern Argentina. Mem. Inst. Oswaldo Cruz 96: 895-899.

Beament, J.W.L. 1946. The cuticular lipoids of insects. J. Exp. Biol. 22: 115-131.

Bos, R. 1988. The importance of peridomestic environmental management for the control of the vectors of Chagas' disease. Rev. Arg. Microbiol. 20 (supl.): 58-62.

Cecere, M.C., R.E. Gürtler, D. Canale, R. Chuit & J.E. Cohen. 1996. El papel del peridomicilio en la eliminación de *Triatoma infestans* de comunidades rurales argentinas. Bol. Oficina Sanit. Panam. 121: 1-9.

Chase, A.R., L.S. Osborne & V.M. Ferguson. 1986. Selective isolation of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* from an artificial potting medium. Fla. Entomol. 69: 285-292.

Crawford, P.J., W.M. Brooks, J.J. Arends. 1998. Efficacy of field-isolated strains of *Beauveria bassiana* (Moniliales: Moniliaceae) as microbial control agents of the lesser mealworm (Coleoptera: Tenebrionidae). J. Econ. Entomol. 91: 1295-1301.

Dias, J.C.P. 1991. Control of Chagas disease in Brazil: Which strategy after the attack phase? Ann. Soc. Belgique Med. Trop. 71 (supl.): 75-86.

Dias, J.C.P., A.C. Silveira & C.J. Schofield. 2002. The impact of Chagas disease control in Latin America - a review. Mem. Inst. Oswaldo Cruz 97: 603-612.

Fargues, J. & C. Luz. 1998. Effects of fluctuating moisture and temperature regimes on sporulation of *Beauveria bassiana* on cadavers of *Rhodnius prolixus*. Biocontrol Sci. Tech. 8: 323-334.

Fargues, J. & C. Luz. 2000. Effects of fluctuating moisture and temperature regimes on the infection potential of *Beauveria bassiana* for *Rhodnius prolixus*. J. Invertebr. Pathol. 75: 202-211.

Fargues, J., N.K. Maniania, J.C. Delmas & N. Smits. 1992. Influence de la température sur la croissance *in vitro* d'hyphomycètes entomopathogènes. Agronomie 12: 557-564.

Fleiss, J.L. 1981. Statistical methods for rates and proportions. 2. ed. New York, J. Wiley , 321.

Gajate, P., S. Pietrokovsky, L.A. Orrego, O. Pérez, A. Monte, J. Belmonte & C.W. Colli. 2001. *Triatoma infestans* in greater Buenos Aires, Argentina. Mem. Inst. Oswaldo Cruz 96: 473-477.

Geden, C.J., J.J. Arends, D.A. Rutz & D.C. Steinkraus. 1998. Laboratory evaluation of *Beauveria bassiana* (Moniliales: Moniliaceae) against the lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae), in poultry litter, soil, and a pupal trap. Biol. Control. 13: 71-77.

Glare, T.R. & R.J. Milner. 1991. Ecology of entomopathogenic fungi, p. 547-612. In D.K. Arora, L. Ajello & K.G. Mukerji (eds.). Handbook of applied mycology. Humans, animals and insects, vol. 2. New York, Marcel Dekker Inc., 783p.

Lecuona, R.E., J.D. Edelstein, M.F. Berretta, F.R. Rossa & J.A. Argas. 2001. Evaluation of *Beauveria bassiana* (Hyphomycetes) strains as potential agents for control of *Triatoma infestans* (Hemiptera: Reduviidae). J. Med. Entomol. 38: 172-179.

Luz, C. 1994. Biologische Bekämpfung der Überträger der Chagaskrankheit (Triatominae). Einfluss von Temperatur und Luftfeuchtigkeit auf die larvale Entwicklung von *Rhodnius prolixus* sowie die Infektion mit *Beauveria bassiana* (Deuteromycetes) und

Sporulation des Pilzes auf den Kadavern. Ph. D., Eberhard-Karls-Univ. Tübingen, 175p.

Luz, C., I.G. Silva, B.P. Magalhães, C.M.T. Cordeiro & M.S. Tigano. 1999. Control of *Triatoma infestans* (Reduviidae, Triatominae) with *Beauveria bassiana*: Preliminary assays on formulation and application in the field. An. Soc. Entomol. Bras. 28: 101-110.

Luz, C., I.G. Silva, C.M.T. Cordeiro & M.S. Tigano. 1998 a. *Beauveria bassiana* (Hyphomycetes) as a possible control agent for the vectors of Chagas disease. J. Med. Entomol. 35: 977-979.

Luz, C. & J. Fargues. 1997. Temperature and moisture requirements for conidial germination of an isolate of *Beauveria bassiana*, pathogenic to *Rhodnius prolixus*. Mycopathologia 138: 117-125.

Luz, C. & J. Fargues. 1998. Factors affecting conidial production of *Beauveria bassiana* from fungus-killed cadavers of *Rhodnius prolixus*. J. Invertebr. Pathol. 72: 97-103.

Luz, C. & J. Fargues. 1999. Dependence of the entomopathogenic fungus, *Beauveria bassiana*, on high humidity for infection of *Rhodnius prolixus*. Mycopathologia 146: 33-41.

Luz, C., L.F.N. Rocha, G.V. Nery, B.P. Magalhães & M.S. Tigano. 2004a. Activity of oil-formulated *Beauveria bassiana* against *Triatoma sordida* in peridomestic areas in Central Brazil. Mem. Inst. Oswaldo Cruz 99: 211-218.

Luz, C., L.F.N. Rocha & I.G. Silva. 2004b. Pathogenicity of *Evlachovaea* sp. (Hyphomycetes), a new species isolated from *Triatoma sordida*, in Chagas disease vectors under laboratory conditions. Rev. Soc. Bras. Med. Trop. 37: 189-191.

Luz, C., L.F.N. Rocha & R.A. Humber. 2003. Record of *Evlachovaea* sp. (Hyphomycetes) on *Triatoma sordida* in the State of Goiás, Brazil and its activity against *Triatoma infestans* (Reduviidae, Triatominae). J. Med. Entomol. 40: 451-454.

Luz, C., M.S. Tigano, I.G. Silva, C.M.T. Cordeiro & S.M. Aljanabi. 1998 b. Selection of *Beauveria bassiana* and *Metarhizium anisopliae* to control *Triatoma infestans*. Mem. Inst. Oswaldo Cruz 93: 839-846.

McCullagh, P. & J.A. Nelder. 1989. Generalized linear models. 2. ed. Chapman and Hall, New York, 511p.

Mitra, S.K., A. Sikdar & P. Das. 1998. Dermatophytes isolated from selected ruminants in India. Mycopathologia 142: 13-16.

Nayak, M.K., P.J. Collins & R.A. Kopittke. 2002. Comparative residual toxicities of carbaryl, deltamethrin and permethrin as structural treatments against three liposcelidid psocid species (Psocoptera: Liposcelididae) infesting stored commodities. J. Stored Prod. Res. 38: 247-258.

Ouedraogo, A., J. Fargues, M.S. Goettel & C.J. Lomer. 1997. Effect of temperature on vegetative growth among isolates of *Metarhizium anisopliae* and *M. flavoviride*. Mycopathologia 137: 37-43.

Parameswaran, G. & T. Sankaran. 1979. Record of *Beauveria bassiana* (Bals.) Vuill. on *Linshcosteus* sp. (Hemiptera: Reduviidae: Triatominae) in India. J. Entomol. Res. 1: 113-114.

Perez A., B. Szczypel, R. Larramendy, N. Acosta, A. Gonzalez. 1999. Biopreparado entomopatogeno contra el coleoptero *Alphitobius diaperinus*. Rev. Cubana Cienc. Avicola 23: 45-52.

Romaña, C.A. & J. Fargues. 1987. Sensibilité des larves de l' hémiptère hématophage, *Rhodnius prolixus* (Triatominae) aux hyphomycètes entomopathogènes. Entomophaga 32: 167-179.

Ryckman, R.E. & C.M. Blankenship. 1984. The parasites, predators and symbionts of the Triatominae (Hemiptera, Reduviidae, Triatominae). Bull. Soc. Vector Ecol. 9: 85-111.

SAS Institute Inc. 2000. What's New in SAS ® Software for Release 8.1, Cary, N. C., SAS Institute Inc.

Silva, I.G. 1985. Influência da temperatura na biologia de triatomíneos. I *Triatoma rubrovaria* (Blanchard, 1834) (Hemiptera, Reduviidae). Rev. Goiana Med. 34: 29-34.

Tarasco, E., C. Bievre, B. Papierok, M. Poliseno & O. Triggiani. 1997. Occurrence of entomopathogenic fungi in soils in Southern Italy. Entomologica Bari 31: 157-166.

Vänninen, I. 1995. Distribution and occurrence of four entomopathogenic fungi in Finland: Effect of geographical location, habitat type and soil type. Mycol. Res. 100: 93-101.

Vänninen, I., G.B. Husberg & H.M.T. Hokkanen. 1989. Occurrence of entomopathogenic fungi and entomoparasitic nematodes in cultivated soils in Finland. Acta Entomol Fenn 53: 65-71.

Vissiennon, T. 1999. Fungal flora in chicken stalls and its etiopathogenic importance for humans and animals. Berl. Münch. Tierärztl. Wochenschr. 112: 104-107.

Wigglesworth, V.B. 1958. Abraison of the insect cuticle by aqueous suspensions of small particles. Nature 15: 779-780.

Winston, P.W. & D.H. Bates. 1960. Saturated solutions for the control of humidity in biological research. Ecology 41: 232-237.

Zeledón, R., C.E. Valeiro & J.E. Valeiro. 1973. The camouflage phenomenon in several species of Triatominae Hemiptera Reduviidae. J. Med. Entomol. 10: 209-211.

Zimmermann, G. 1986. *Galleria* bait method for detection of entomopathogenic fungi in soil. J. Appl. Entomol. 2: 213-215.

Received 08/07/03. Accepted 10/05/04.