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Evaluation of *Beauveria bassiana* (Hyphomycetes) Strains as Potential Agents for Control of *Triatoma infestans* (Hemiptera: Reduviidae)

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J. Med. Entomol. 38(2): 172–179 (2001)

ABSTRACT Chagas disease constitutes a major human health problem in most Latin American countries. This endemic disease is transmitted by several species of triatomine bugs, the most important being *Triatoma infestans* (Klug). In this article, we report on the selection of strains of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. virulent to *T. infestans* for possible use as bioinsecticides. Four strains of *B. bassiana* isolated from Argentina (Bb 1, 10, 25, and 65) were evaluated. To calculate mortality and mean lethal time, nymphs and adults of *T. infestans* were treated with conidia produced on complete agar medium and wheat brain and rice husk medium (WH). The LD_{50} for nymphs and adults of *T. infestans* was calculated. The effect of different temperatures (18, 22, 26, 30, and 34°C) and relative humidities (35 and 90% RH) on mortality of nymphs were studied. We evaluated the compatibility of strains with Deltamethrin and Beta-Cypermethrin. Although the strain Bb 25 failed to grow on WH, the other three strains showed similar mortality values independent of the culture medium used to grow the microorganisms. The lowest LD_{50} values for nymphs were obtained with the strains Bb 10 and 65 and for adults were Bb 1, 10, and 65. The highest mean mortality was obtained with strain Bb 10, and among temperatures the highest mean mortality was observed at 26°C. Relative humidity did not affect the mortality of *T. infestans* nymphs with all strains and temperatures assayed. Deltamethrin did not affect any of the three strains for the four studied doses, and Beta-Cypermethrin could be used in combination with the fungus only at low doses. The strain Bb 10 was selected for future assays under natural climatic conditions.

KEY WORDS *Triatoma infestans*, *Beauveria bassiana*, biological control, entomopathogenic fungus, temperature, pyrethroids

CHAGAS DISEASE CONSTITUTES a major human health problem in most Latin American countries. Currently, 24.7 million people are infected in the region, 6.2 million suffering severe heart disorders (WHO 1991). This endemic disease is transmitted by many species of insect vectors; the blood-sucking bug *Triatoma infestans* (Klug) is the most important in several countries of that region (Schofield 1985). Although it has different degrees of incidence, the disease is spread over 86% of the geographic area of Argentina, affecting ≈2.3 million inhabitants (Segura et al. 1994).

Transmission of *Trypanosoma cruzi* Chagas usually takes place indoors and the number of triatomines in the houses depends on ecological and socio-cultural factors (Zeledon and Rabinovich 1981). The degree of transmission of Chagas disease is directly proportional to the *T. infestans* population size. Control of these vectors is usually carried out with pyrethroid insecticides (Gürtler et al. 1998), some of which affect the growth of *Beauveria bassiana* (Bals.) Vuill. (Anderson and Roberts 1983, Lecuona and Diaz 1996).

Triatomine control depends on coordinated and integrated actions aimed to improve the cultural and socio-economical conditions, as well as on the use of more efficient and less toxic control methods. In this regard, fungal species pathogenic to triatomines, potentially useful for the biocontrol of the pest, were studied by different authors (Sherlock and Guitton 1982, Romaña and Fargues 1987, Luz et al. 1998). These results encouraged attemps to develop a bioinsecticide using entomopathogenic fungi. However, environmental factors affect the efficiency of these fungi. Temperature affects the vegetative growth of *B. bassiana* (Fargues et al. 1997). Likewise, Luz and Fargues (1997) observed that conidia of *B. bassiana* are able to germinate in vitro at temperatures between 15 and 35°C and the germination is largely reduced by relative humidity lower than 95.5%. Nevertheless, in vitro results do not always agree with those observed in vivo (Inglis et al. 1996). Temperature and relative humidity affected the sporulation of *B. bassiana* on cadavers of *Rhodnius prolixus* Stal and *T. infestans* under laboratory conditions (Fargues and Luz 1998, Luz and Fargues 1998, Luz et al. 1998), showing the

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importance of the humidity for the transmission of the fungus to other insects.

In Argentina, previous research with *B. bassiana* and *Metarhizium anisopliae* (Metsch.) Sorok. confirmed the pathogenicity of these fungi on *T. infestans* and led to the selection of virulent native strains for the first time (Lecuona 1999). The results of experiments to select *B. bassiana* strains virulent to *T. infestans*, with the purpose of developing a bioinsecticide, are presented in this paper.

Materials and Methods

***Triatoma infestans* Populations.** *T. infestans* were collected from rural areas of Santiago del Estero province by staff members of the Servicio Nacional de Chagas (Córdoba, Argentina). Insects were reared in laboratory at 27 ± 1°C, 80 ± 10% humidity and fed on hens. Third stage nymphs from the laboratory colony were sent to the Laboratorio de Hongos Entomopatógenos (IMYZA-INTA Castelar, Argentina) where they were used for experiments. When adults were treated, the age could not be accurately assessed because they were collected from experimental chicken houses of the Servicio Nacional de Chagas.

***Beauveria bassiana* Cultures.** Four strains of *B. bassiana* (Bb) of the INTA culture collection were used. Bb 1, 10, and 25 were isolated from *Diatraea saccharalis* (F.) (Lepidoptera: Pyralidae), and Bb 65 was isolated from *Nezara viridula* (L.) (Hemiptera: Pentatomidae). To be used in this work, the strains were reisolated through a passage on *T. infestans* nymphs. These strains were maintained in plates on complete agar medium (CAM) composed of (g/liter): KH_2PO_4, 0.4; Na_2HPO_4, 1.4; SO_4Mg, 0.6; KCl, 1; NH_4NO_3, 0.7; glucose, 10; agar, 15; and yeast extract, 5. Conidia were harvested by scraping 2-wk-old plates incubated at 26 ± 0.5°C. Viability was assessed by counting four replicates of 100 conidia incubated on CAM for 24 h. Conidia were recorded as germinated when the germ tube was at least as long as the width of the conidium.

Fungal production by solid-state fermentation used the wheat brain and rice husk medium (WH) described by Arcas et al. (1999). The culture medium was prepared in 500-ml Erlenmeyer flasks, and sterilized for 20 min at 120°C. The conidial suspensions used as inocula were obtained from 2-wk-old cultures, grown at 26 ± 0.5°C on Sabouraud dextrose agar supplemented with 0.5% yeast extract. The conidia were harvested by shaking vigorously the plates floaded with a sterile solution of distilled water with Tween 80 (0.01%). Inoculum concentration was standardized to obtain initial counts of 10^6 conidia per gram of initial dry matter of WH medium. After inoculation, the flasks were placed for 7–10 d in a growth chamber at 26 ± 0.5°C and relative humidity over 90%. After this period of culture, no changes in conidia yields were observed.

***Triatoma infestans* Mortality and Mean Lethal Time of Strains.** The mean lethal time (MLT) defined as the number of days needed to achieve an accumulated 50% mortality, was estimated using linear interpolation of corrected, cumulative daily mortalities (Moore et al. 1995). MLT of the four Bb strains were estimated with inoculum obtained from CAM and WH media. However, only three strains (Bb 1, 10, and 65) grew on WH medium. Forty third-stage nymphs of *T. infestans* fed on hens for 1 wk, and 60 adults fed on hens for 2 wk were used in these assays. Inoculation was carried out by immersion of each replicate of insects in a suspension of 1×10^8 conidia/ml in 0.01% sterile aqueous Tween 80 for 6 s. For immersion, the insects were placed in a plastic cylindrical strainer (4 by 4.5 cm). A completely randomized design with four replicates of 10 nymphs and 15 adults each was used. After being immersed in the fungal suspension, the nymphs and adults were individually maintained at 27 ± 0.5°C in plastic flasks (4.5 by 2.5 cm), in darkness and without feeding for 18 d. Control insects were immersed in 0.01% sterile aqueous Tween 80. The mortality of insects was recorded daily and cadavers were held in humid chambers (saturated atmosphere) to allow fungal sporulation. As a preliminary test of the effect of the fungus on eggs and the hatching nymphs, four replicates of 15 eggs each within 5 d to hatching were inoculated by immersion in a suspension of 1×10^8 conidia/ml of the strain Bb10 using the same methodology. Hatched nymphs were maintained during 15 d with the remaining chorion at 27 ± 0.5°C, in darkness and without feeding. The mortality of nymphs was recorded daily and cadavers were held in humid chambers.

LD_{50} Assessment. To estimate the LD_{50} of the four strains grown on CAM, six dilutions were used at concentrations ranging from 1×10^5 to 5×10^7 conidia/ml, plus a control. Forty nymphs and 40 adults were tested with each concentration and individually maintained at 27 ± 0.5°C, in darkness and without feeding for 15 d as described previously. Control insects were immersed in 0.01% sterile aqueous Tween 80. The mortality of insects was recorded daily and the cadavers were held in humid chambers to allow fungal sporulation. LD_{50} estimation was performed by probit analysis (G. A. Milliken 1989, Kansas State University, free distribution program). The criterion used to determine significant differences between strains was lack of overlap among 95% confidence intervals (Zhao et al. 1995).

Strains Compatibility with Pyrethroid Insecticides. The in vitro compatibility of *B. bassiana* strains with two pyrethroids commonly used for *T. infestans* control: Deltamethrin (K-Othrina FW 0.75%, Aventis, Argentina; field rate: 25 mg [AI]/m^2) and Beta-Cypermethrin (Sipertrin FW 5%, Chemotecnica, Argentina; field rate: 50 mg [AI]/m^2) were examined. The following procedure was modified from Anderson and Roberts (1983). Final concentrations of the pyrethroids suspensions were adjusted to 10, 50, 100, and 200% of the recommended field application rate. Strains of *B. bassiana* were incorporated as 0.2 ml of 5×10^8 conidia/ml suspensions, in 0.01% Tween 80, to a mix final volume of 10 ml. All treatments were compared with a control of conidia added to water without pyrethroids.

Table 1. Effect of *B. bassiana* strains grown on different media on nymphs and adults of *T. infestans*

Stage	Strain	Conidia grown on CAM medium		Conidia grown on WH medium	
		Mortality (%)[a]	MLT (days)[b]	Mortality (%)[a]	MLT (days)[b]
Nymph	Bb 1	100	6.6 (0.09)	97.5 (2.5)	8.0 (0.41)
	Bb 10	100	7.1 (0.04)	97.5 (2.5)	7.9 (0.42)
	Bb 25	100	7.3 (0.20)	NG[c]	NG
	Bb 65	100	6.5 (0.40)	97.5 (2.5)	7.8 (0.25)
Adult	Bb 1	98.3 (1.7)	5.4 (0.05)	93.3 (2.7)	8.8 (0.44)
	Bb 10	100	4.8 (0.13)	95.0 (3.2)	7.2 (0.20)
	Bb 25	75.0 (5.7)	5.4 (0.29)	NG	NG
	Bb 65	97.5 (2.5)	7.5 (0.6)	93.3 (4.7)	7.3 (0.68)

[a] Percentage of infected insects (±SE) after 18 d. 40 nymphs or 60 adults were treated with a suspension of 1×10^8 conidia/ml. For the control, no dead insects were observed.
[b] Mean lethal time (±SE) for 50% mortality.
[c] NG, This strain did not grow on WH medium.

B. bassiana–pyrethroid mixtures were shaken at 200 rpm for 10 h at room temperature. Then, each mixture was diluted 10,000 times and 0.1 ml was spread onto CAM plates held at 26°C. Colony-forming units (CFU) were counted after 3 d. Three plates were made for each treatment and four replicates of each treatment were done.

Effect of Temperature and Relative Humidity on the Mortality of *T. infestans* Nymphs. The study of the influence of temperature and relative humidity was carried out only with nymphs and with the strains that grew on WH medium. The method used was modified from Marcandier and Khachatourians (1987a). Hermetic plastic boxes (34.5 by 23.5 by 20.0 cm) were used as test chambers. Trials were performed at 18, 22, 26, 30, and 34 ± 0.5°C. To obtain relative humidity values of 35 ± 10% and 90 ± 10%, H_2SO_4 (Teixera Alves 1986) and $K_2Cr_2O_7$ (Winston and Bates 1960) saturated solutions, respectively, were placed in Erlenmeyer flasks located into the test chambers. Relative humidity was monitored inside each test chamber with a HumiPro Sensor (accuracy: ± 4%) attached to a data logger (LogIT SL 200 with PC software, DCP Microdevelopments and SCC Research, UK). The measurements were taken 9 cm above the chamber bottom. The temperature was monitored with a Minitemp Sensor (accuracy: ± 1°C) attached to a similar data logger. The test chambers were kept in temperature-controlled incubators in darkness.

A completely randomized design for three main factors (temperature, relative humidity, and strains) was used. Four replicates of 25 nymphs were assayed per treatment. Four treatments were tested: the strains Bb 1, 10, and 65 at a concentration of 1×10^8 conidia/ml and a control with 0.01% sterile aqueous Tween 80. After the inoculation, each nymph was individually placed in a small plastic tube (4.5 by 2.5 cm) covered by fine mesh voile to assure a good air exchange between the plastic tubes and the test chamber. Experiments lasted no more than 18 d and the dead insects were separated daily and placed into a humid chamber at 26 ± 0.5°C to confirm mycosis. The angular transformation was used on mortality percentage values that were analyzed by analysis of variance (ANOVA) and Tukey test ($\alpha = 0.05$) (SAS Institute 1989). The survival functions were estimated by the Kaplan-Meier method and they were graphed for each strain and temperature in their respective relative humidity levels, using SAS version 6.12 (Allison 1995).

Results and Discussion

***Triatoma infestans* Mortality and Mean Lethal Time of Strains.** *T. infestans* nymphal mortality treated with conidia grown on CAM medium was 100% and MLT ranged between 6.5 and 7.3 d. Adult mortality was between 75 and 100% with MLT between 4.8 and 7.5 d. The results obtained with the strains Bb 1, 10, and 65 grown on WH medium were similar to those obtained with the conidia grown on CAM medium. Mortality of *T. infestans* nymphs treated with conidia grown on WH medium was 97.5% and MLT values ranged from 7.8 to 8.0 d. Adults had 93–95% mortality and MLT between 7.2 and 8.8 d (Table 1). No dead control insects were observed.

The four *B. bassiana* strains used in the experiments were virulent to *T. infestans* nymphs as observed previously by Lecuona (1999). The results showed that *T. infestans* is susceptible to these fungal strains, however three of them were isolated from Lepidoptera and only one from Hemiptera. According to Moorhouse et al. (1993), this fact suggests that the host–pathogen specificity was not as strict as that reported by Romaña and Fargues (1987), who observed that only those strains isolated from Hemiptera were highly pathogenic for *R. prolixus.* Strain Bb 65, isolated from the hemipteran *N. viridula* was more pathogenic to *T. infestans* than to its original host, and so were the strains isolated from the lepidopteran *D. saccharalis* (Bb 1, 10 and 25) (Lecuona 1999).

Unlike the results observed with *T. infestans* nymphs, to which all strains showed similar performance, data from treated adults showed that Bb 25 caused only 75% mortality. Because strain Bb 25 could not be used against adults and could not be produced on WH medium, it was eliminated from subsequent studies. The results obtained with conidia produced on WH medium demonstrated that the efficiency of the strains Bb 1, 10, and 65 was not affected by the mass-production process, suggesting that this meth-

Table 2. LD_{50} (conidia/ml) of *B. bassiana* strains to *T. infestans* nymphs and adults

Stage	Strain	LD_{50} (CI)	Intercept	Slope	Chi-square	Significance lack of fit (5%)
Nymph	Bb 1	21.7×10^6 (12–53)b	3.07	0.825	6.29	NS
	Bb 10	2.1×10^6 (1.3–3.2)a	3.76	0.941	6.02	NS
	Bb 25	10.1×10^6 (5.7–22.0)b	3.48	0.776	3.05	NS
	Bb 65	1.8×10^6 (0.5–5.7)a	3.84	0.919	10.13	S[a]
Adult	Bb 1	5.3×10^6 (3.2–9.5)AB	3.59	0.819	3.46	NS
	Bb 10	1.6×10^6 (0.4–5.2)A	3.96	0.858	9.51	S
	Bb 25	11.5×10^6 (5.9–30.7)B	3.71	0.627	5.44	NS
	Bb 65	2.5×10^6 (0.5–14.1)AB	3.84	0.828	13.18	S

CI, 95% confidence intervals for LD_{50} values. LD_{50} values followed by the same letter are not significantly different based on failure of 95% CI to overlap (Zhao et al. 1995). Lower case letter refers to LD_{50} for nymphs, and upper case letter to LD_{50} for adults.

[a] Because of significant lack of fit, confidence intervals were computed using T = 2.776 instead of Z = 1.96 for $\alpha = 0.05$.

odology can be used to obtain the primary inoculum for future bioinsecticide formulation.

When strain Bb 10 was applied to eggs of *T. infestans* in the preliminary assay, all nymphs successfully eclosed from eggs but were killed soon afterward by the fungus. These results suggest that this fungus has the ability to infect different stages of this pest.

LD_{50} Assessment. Although the significant lack of fit found in probit analysis could throw doubt about the accuracy of the LD_{50} in some treatments (Table 2), wider confidence intervals estimated on the value of T = 2.776 instead of that Z = 1.96 (α = 0,05), allowed compatibility among strains.

The intercept showed similarity, all slopes were lower than one and they did not show significant differences despite their distinct absolute values. The confidence intervals indicated dissimilarity of virulence among the strains in both *T. infestans* stage. On nymphs, Bb 10 and 65 strains were more efficient than Bb 1 and 25, although the confidence interval upper limit of the Bb 65 strain was similar to the confidence interval lower limit of Bb 25 strains (Table 2). Bb 10 also showed a smaller confidence interval being more precise and predictable. On adults, the differences among strains were lower than those observed on nymphs, being the strain Bb 25 the less efficient and Bb 10 the most virulent (Table 2).

Strains Compatibility with Pyrethroid Insecticides. Differences in compatibility due to insecticides at the doses tested were remarkable. Beta-Cypermethrin (Sipertrin Fw 5%) at 200 and 100% of the recommended field application rate caused total inhibition of all strains because no CFU was obtained at the dilution plated. The 50% dose caused inhibition of >90% of CFU counts, in relation to the control ($P <$ 0,05) (Table 3). Only the 10% concentration of this insecticide did not affect adversely any of the three strains with CFU counts similar to the control ($P <$ 0,05) (Table 3). However, Deltamethrin (K-Othrina Fw 0.75%) did not inhibit any of the three strains at any concentrations tested (Table 3). It is worth of mention that some treatments produced higher CFU counts than the control so, apparently, would cause a stimulation of conidia germination. This fact has been previously observed by Anderson and Roberts (1983), and may be due to the disruption of conidia aggregations favored by carriers and adjuvants present in the insecticide formulations. These clumps would tend to persist and behave as single CFUs in the water treatment.

It is known from different test methods that pyrethroids can inhibit entomopathogenic fungi, depending on the doses as well as on the species and strains analyzed (Anderson and Roberts 1983, Lecuona and Díaz 1996). Although we have found no differences among strains, it was not the case for the two pyrethroids tested; the formulation based on Deltamethrin (K-Othrina Fw 0.75%) was compatible with the three strains and could be used in integrated pest management (IPM) programs without negative effects on *B. bassiana.* However, Beta-Cypermethrin (Sipertrin Fw 5%) could be used in combination with the fungus at low doses.

Effect of Temperature and Relative Humidity on the Mortality of *T. infestans* Nymphs. The mortality percentages calculated for each combination of temperature and relative humidity are given on Table 4. Significant differences for strains (F = 25.001; df = 2, 7; $P < 0.0001$) and temperature (F = 265.223; df = 4, 7; $P < 0.0001$), but not for relative humidity (F = 0.023;

Table 3. Percent CFU of *B. bassiana* strains treated at different concentrations of pyrethroids

Strain	Deltamethrin (K-Othrina)		Beta-Cypermethrin (Sipertrin)	
	Concentration[a]	CFU (%)[b]	Concentration	CFU (%)
Bb 1	200	92 (7)a	200	0[c]
	100	95 (9)a	100	0[c]
	50	103 (4)a	50	7 (0.7)a
	10	96 (2)a	10	124 (17)b
Bb 10	200	123 (7)a	200	0[c]
	100	128 (4)a	100	0[c]
	50	113 (5)a	50	6 (0.5)a
	10	107 (5)a	10	103 (6)b
Bb 65	200	104 (16)a	200	0[c]
	100	139 (23)a	100	0[c]
	50	112 (21)a	50	5 (0.7)a
	10	96 (12)a	10	104 (6)b

[a] Final concentrations of the pyrethroids suspensions were adjusted to 10, 50, 100, and 200% of the recommended field application rate.

[b] Percent CFU was calculated as follows: [(CFU in experimental teatment ÷ CFU in control) × 100]. Values are means ± SE based on four replicates. Means followed by the same letter in each column are not significantly different using Tukey test (α = 0.05).

[c] No CFU were counted at the dilution plated.

Table 4. Mortality (%) of *T. infestans* nymphs treated with *B. bassiana* strains and maintained at different temperature and relative humidity (RH) levels

Strain	RH (%)	Mortality (%)[a]					
		18°C	22°C	26°C	30°C	34°C	Mstrain[b]
Bb 1	35	50 (6.6)	27 (3.0)	96 (1.6)	74 (1.1)	22 (3.8)	
	90	31 (4.4)	30 (2.6)	94 (1.1)	79 (1.9)	36 (1.6)	
							54.2 (4.5)b
Bb 10	35	62 (8.8)	59 (6.6)	97 (1.0)	79 (1.0)	43 (3.0)	
	90	56 (3.3)	73 (7.2)	97 (1.9)	76 (1.6)	32 (2.3)	
							67.4 (3.4)a
Bb 65	35	32 (5.9)	44 (3.6)	97 (1.0)	74 (2.0)	42 (2.0)	
	90	34 (6.8)	45 (4.1)	98 (1.1)	78 (2.6)	34 (1.1)	
							57.8 (4.0)b
Mtemp[c]		44.2 (3.4)C	46.3 (3.7)C	96.5 (0.3)A	77.1 (0.9)B	34.8 (1.7)D	

[a] Percentage of infected nymphs (± SE) after 18 d 100 nymphs were treated with a suspension of 1×10^8 conidia/ml.
[b] Mstrain, Mean mortality (% ± SE) of strains over all temperatures and relative humidities. Means followed by the same lower case letter are not significantly different using Tukey test ($\alpha = 0.05$).
[c] Mtemp, Mean mortality (% ± SE) for all strains at each temperature and relative humidity. Means followed by the same upper case letter are not significantly different using Tukey test ($\alpha = 0.05$).

df = 1, 7; $P = 0.882$) were detected. The highest strain mean mortality (67.4%, statistically different from the other means with Tukey, $\alpha = 0{,}05$) was obtained with strain Bb 10. Among temperatures the highest mean mortality (96.5%) was observed at 26°C (Table 4). Both temperatures 26 and 30°C were statistically different from 18, 22, and 34°C (Tukey, $\alpha = 0.05$) and from each other. Interaction between strains and temperature was detected ($F = 9.156$; df = 8, 14; $P < 0.0001$). Fig. 1 shows survival distribution function graphs for strains and temperatures at both humidity conditions. Bb 1 and 65 reach a mortality maximum of 50% at 18, 22, and 34°C. Both strains need 26 or 30°C to be effective. Bb 10 survival functions, however, have the last observation (18 d) under 40% survival in all cases except 34°C. For all strains, the temperature at which survival function declines faster is 26°C, followed by 30°C. Bb 10 is not as temperature-dependent as Bb 1 and 65, a fact that caused the interaction between strain and temperature. The strain-temperature combination of Bb 10–26°C had declined fastest in survivorship (Table 4; Fig. 1).

Using in vitro studies, Luz and Fargues (1997) demonstrated that temperature and humidity affect the germination of *B. bassiana* in vitro. At extreme temperatures or beyond the optimal range (20–30°C), germination reaches values of 99% only after 48 h. Relative humidity below 90% at 25°C prevents conidia germination. These results, like those of Walstad et al. (1970), cannot be extended to in vivo situations (Hajek et al. 1996, Inglis et al. 1996), although they are useful in strain selection (Vidal et al. 1997). Relative humidity did not affect the mortality of nymphs with all strains and temperatures assayed. The relative humidity is more important for fungal sporulation on insect cadavers (Luz and Fargues 1998) than for fungal penetration and infection. Our results are consistent with those of Marcandier and Khachatourians (1987b) who showed that the infection with *B. bassiana* on *Melanoplus sanguinipes* (F.) occurred independently of the relative humidity. Thus, environments with low relative humidity hinder the development of epizootics by diminishing the sporulation and spread of the fungus (Fargues and Luz 1998, Luz and Fargues 1998). However, these aspects may not be critical for the use of fungi as bioinsecticides because an adequate microclimate to promote infections can be found on the intersegmental membranes of the insects cuticle (McCauley et al. 1968, Doberski 1981, Ramoska 1984, Marcandier and Khachatourians 1987b, James et al. 1998), or the genital pore and anus in ticks (Samish and Rehacek 1999).

Temperature may be a limiting factor for using entomopathogenic fungi (Carruthers et al. 1985). Our results showed that the highest mortalities occurred at 26°C (mean = 96.5%), this temperature corresponds to the optimal temperature for in vitro growth of this fungus (Fargues et al. 1997). Higher temperatures (30°C) are more efficient (mean = 77.1%) than lower ones (22°C, mean = 46.3%). The lowest mortality values were observed at 34°C, these results are consistent with those of Inglis et al. (1996) for *M. sanguinipes* (F.) nymphs infected with *B. bassiana.* Thus, it may be important to develop formulations protected from the detrimental effects of high and low temperatures.

Triatoma infestans is highly active in Argentine's Chaco region during the warmer season (means from 25 to 32°C) (Gorla and Schofield 1985). The observed performance of the *B. bassiana* strains under a similar temperature range has a practical importance for applying this fungus as bioinsectide in the endemic area. According to our results, for the *T. infestans–B. bassiana* system, the temperature is the most important factor involved on the expression of pathogenicity regardless of the environmental relative humidity. These results support the testing of *B. bassiana* as a bioinsecticide against pests in semiarid areas, as suggested by Marcandier and Khachatourians (1987b) and Luz et al. (1998).

Strains Bb 10 and 65 appear to be the most promising among the studied strains. However, strain Bb 10 produced the highest mortalities under all experimental temperatures and had significant differences when statistically tested against the other strains. Therefore,

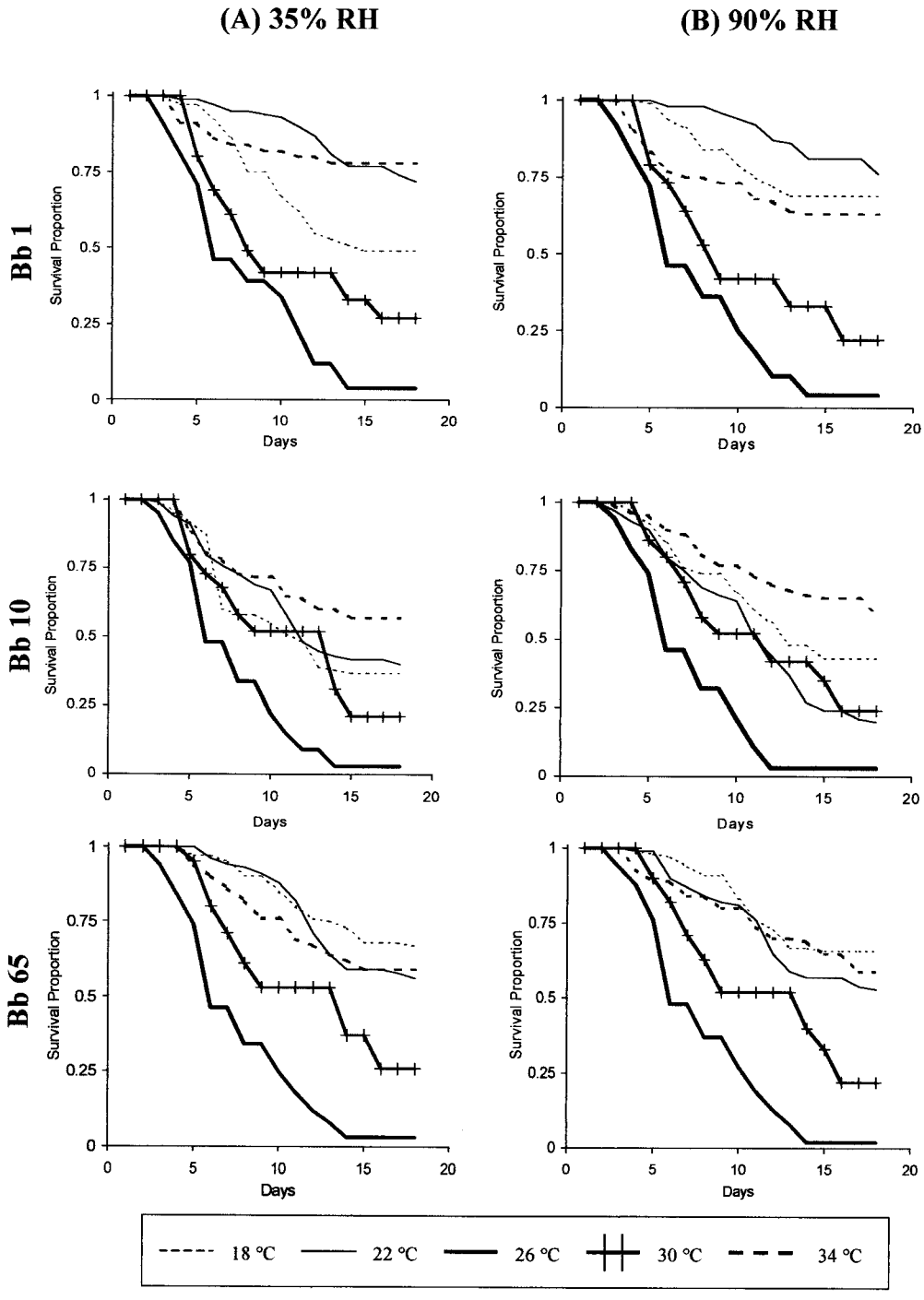

Fig. 1. Survival curves of *T. infestans* with strains of *B. bassiana* (Bb 1, 10, and 65) at different temperatures and relative humidities. (A) 35% RH. (B) 90% RH. No dead control insects were observed.

B. bassiana strain Bb 10 has been selected to be formulated as a bioinsecticide. This biopesticide will be used in future field assays in experimental chicken houses against the most important and widespread vector of Chagas disease in Argentina.

Acknowledgments

We thank Estela R. Favret (IMYZA-CCVyA-CNIA-INTA Castelar, Argentina) for her useful suggestions on writing this paper; Alicia Cristina González, and Marcelo Caldeo for

expert technical assistance; Delmi M. Canale, Servicio Nacional de Chagas (Córdoba, Argentina) for kindly providing the triatomine bugs; Ricardo Gürtler for the critical review, and Nancy Kahn and Julio Di Rienzo for statistical assistance. This study was supported with funds from the Centro Argentino-Brasileño de Biotecnología (CABBIO) and Secretaría de Ciencia y Técnica (SECyT-PICT 08–04526). J.D.E. and M.F.B. were granted with a fellowship from CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina).

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Received for publication 27 June 2000; accepted 14 September 2000.