Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *87* (3): 363-368, jul./sep. 1992

RELATIVE SUSCEPTIBILITY OF DIFFERENT STAGES OF *RHODNIUS PROLIXUS* TO THE ENTOMOPATHOGENIC HYPHOMYCETE *BEAUVERIA BASSIANA*

CHRISTINE A. ROMAÑA & JACQUES F. FARGUES*

CHU Necker, Laboratoire de Parasitologie, 156 rue de Vaugirard, 75015 Paris, France
*INRA Station de Recherches de Lutte Biologique, La Minière, 78285 Guyancourt Cedex, France

Laboratory bioassays were conducted to determine the relative susceptibility of eggs, 1st-, 3rd-, 5th-instar nymphs and adults of Rhodnius prolixus *to one isolate of the entomopathogenic hyphomycete,* Beauveria bassiana. *Treatments consisted of directly spraying on insects of increasing doses of inoculum (3 x 10^2 to 3 x 10^5 conidia per cm^2). Mortality due to all doses of conidia was very high in the five tested stages of the target insect. Experiments on eggs demonstrated that the fungal isolate was able to kill eggs before they hatched. Both time-mortality and dose-mortality responses showed that the susceptibility of* R. prolixus *varied according to its stage of development and increased with age. As a matter of fact, at the dose of 3 x 10^3 conidia per cm^2, LD 50 varied between 11.2 days in 1st-instar nymphs and 6.4 days in both 5th-instar nymphs and adults. Comparison of LD50 permitted to estimate that 1st-instar nymphs were about 700-fold less susceptible than the two oldest stages.*

Key words: *Rhodnius prolixus* – stages – *Beauveria bassiana* – entomopathogenic hyphomycete

Despite the potentiality for chemical insecticide control of the triatomine vectors, Chagas' disease emerges as a major public health problem in Latin America (Schofield et al., 1987). Previous attempts to select effective entomogenous pathogens candidates for an alternative control of vectors have been unsuccessful (Dias & Leão, 1967; Roberts & Strand, 1977; Romaña & Romaña, 1981). More recently, some authors reported the susceptibility of different *Triatominae* species to the hyphomycetes, *Beauveria bassiana* and *Metarhizium anisopliae* (Moura Costa, 1978; Sherlock & Guitton, 1982; Silva & Messias, 1985; Messias et al., 1986; Romaña & Fargues, 1987; Romaña et al., 1987). Most safety tests to vertebrates conducted on *B. bassiana* revealed no pathogenic lesions or infection (Saik et al., 1990; Siegel & Shadduck, 1990). Nevertheless, allergic manifestations (Israljet et al., 1975) and opportunistic infections (i.e. mild keratitis, Ishibashi et al., 1987) have been reported. These reports should be carefully and prudently evaluated, because this fungus is widely distributed in all terrestrial environments (Glare & Milner, 1991). Anyway no tangible risks have yet been detected by safety tests (including infection, toxicity, allergenicity and mutagenicity tests) with registered entomopathogenic hyphomycetes (Burges, 1981; Payne, 1988; Ferron et al., 1991).There is very little published information on the influence of factors affecting the host susceptibility of the assassin bugs to entomopathogenic fungi. All stages of insect development, eggs, larvae, pupae and adults, are generally susceptible to fungal infections (Ferron, 1985). However, changes of susceptibility according to the contaminated host stage have been reported frequently (Ferron, 1985). Therefore, the objective of this study was to evaluate the relative susceptibility of different stages of development of *Rhodnius prolixus* to *B. bassiana*.

MATERIALS AND METHODS

Fungus – The fungal isolate, *B. bassiana* INRA 297 was selected because of its high virulence for first-instar larvae of *R. prolixus* (Romaña & Fargues, 1987; Romaña et al., 1987). Conidia were obtained from two week-old sporulating cultures grown at 25 ± 1 °C on agar slants of semisynthetic medium (Romaña

Research supported by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases and by the INRA and Scientific Committee of Necker-Enfants Malades School of Medicine, Paris, France.

Received 31 July 1991.
Accepted 28 April 1992.

& Fargues, 1987). Conidia were harvested from these surface cultures directly by scraping. Inocula were suspended in sterile distilled water by shaking in 10 ml flasks containing five to six dozen glass beads (3 mm diameter). No surfactant was added, since 5 min agitation at 700 oscillations per minute (10 cm of vertical travel) on a mechanical shaker was found to produce homogeneous suspensions of viable single conidia. Suspensions were then adjusted to defined concentrations according to the hemacytometer counts.

Insects – Experiments were carried out on a trypanosome-free strain of *R. prolixus* obtained from the National Health Institute, Bogota, Colombia. Insects were reared by using guinea pigs as a blood meal source. They were held at 28° ± 1°C and 85 ± 5% RH in darkness (Romaña & Fargues, 1987).

Standard bioassay procedure – The relative susceptibility of eggs, 1st-, 3rd- and 5th-instar nymphs and adults, was evaluated by directly spraying titrated conidia suspensions onto insects by using a spray tower apparatus (Romaña & Fargues, 1987; Rodriguez-Rueda & Fargues, 1980). Seven concentrations of conidia were tested (from 10^5 to 10^8 conidia per ml) resulting in surface coverage rates of 3×10^2, 10^3, 3×10^3, 10^4, 3×10^4, 10^5 and 3×10^5 conidia per cm^2, respectively. Four replicates of 20 test insects were used for each inoculum dose (including control lots sprayed with sterile distilled water).

Tested insects consisted of either newly deposited eggs, 24-48h old, starved nymphs or starved adults, held for 30 days without a blood meal. Ten ml of dosage were sprayed onto batches of 20 individuals placed in glass Petri dishes (9 cm diameter) for 2 min. After 20 min drying, treated insects were transferred to 12 cm^3 glass pill-boxes, which already contained a fan-folded filter paper. The boxes containing their batch of 20 individuals were closed by a cap of perforated parafilm. Then, they were placed in an airtight parallelipipedic plastic container (12 cm long, 6 cm wide and 12 cm high) divided into two compartments of 432 cm^3 with a nylon frame. The space below this frame was filled with 200 ml of sterile water. Thus, up to eight pill-boxes could be placed into the upper chamber, where humidity was maintained near saturation. Containers were held during the experiments in the darkness at 25° ± 1°C.

Mortalities were checked daily for one month. The insects still alive were counted and the dead ones removed daily and held in moist chambers for seven days for fungal proliferation at the test temperature. The sporulation of the fungus on the surface of dead insects was assumed to indicate that the fungus was the cause of death (Romaña & Romaña, 1981).

Mortality data were analyzed by the two-way analysis of variance (***0.1, **1, *5% levels of significance) with the angular (arcsine) transformation of mortality rates; comparison of means were made by the Duncan's test (at 1 and 5%). Regression analysis was used to calculate time mortality-responses (LT 50 and LT 90) and dose-mortality (LD 50 and LD 90) using log-probit transformations of data.

RESULTS

Susceptibility of R. prolixus *eggs to* B. bassiana *INRA 297* – When treated with *B. bassiana* INRA 297, *R. prolixus* eggs showed a high mortality before hatching (Table I). These data suggested that this fungal isolate was able to invade the eggs of its host. Moreover, the mortality caused by the fungus depended on the concentration of conidia, and both LD 50 (4.6×10^3 conidia per cm^2) and LD 90 (5.3×10^4 conidia per cm^2) were relatively low (Table I).

Susceptibility of nymphs and adults of R. prolixus *to* B. bassiana *INRA 297* – Mortality due to causes other than infection by *B. bassiana* averaged less than 10% in individuals sprayed with conidia suspension. Moreover, this natural mortality did not differ significantly between contaminated insects and controls. In contrast, mortality induced by all doses of conidia was very high in the four tested stages of development of *R. prolixus*.

Comparison of time-mortality responses showed that the duration of the mycosis incubation period varied according to the dose of fungal inoculum and to the stage of the host development (Table II). It clearly appeared that the susceptibility of *R. prolixus* to *B. bassiana* INRA 297 increased with age. For example, at the dose of 3×10^3 conidia per cm^2, LT 50 varied between 11.2 days (for individuals treated in 1st-instar nymphs) and 6.4 days (for individuals treated either in 5th-instar nymphs

TABLE I

Susceptibility of eggs of *Rhodnius prolixus* to *Beauveria bassiana* INRA 297: mortality rates and dose-mortality response during the egg stage (before hatching)

Mortality[a] induced by the following fungal concentrations (conidia per cm^2)				Lethal doses[b] from data recorded 20 days postspraying	
10^3	10^4	10^5	3×10^5	LD50	LD90
26.7 ± 4.6	57.8 ± 5.1	71.9 ± 7.2	90.0 ± 0.0	4.6×10^3	5.3×10^4

Four replicated experiments of 40 eggs per dosis and per replicate (control lots, less than 10% natural mortality).
a: average of angularly transformed percentages of egg mortality: mean ± S. D., analysis of variance: dose effect $F = 48.47^{***}$.
b: estimated both LD50 and LD90 values: regression analysis of probit-mortality and log-time.

TABLE II

Relative susceptibility of nymphs and adults of *Rhodnius prolixus* to *Beauveria bassiana* INRA 297: time-mortality response[a]

Contamined host stage	Lethal times	Time-mortality responses (days) to the following fungal concentrations (conidia per cm^2)						
		3×10^2	10^3	3×10^3	10^4	3×10^4	10^5	3×10^5
1st-instar nymphs	LT50	–	10.5	11.2	10.4	9.2	–	5.5
	LT90	–	18.2	18.8	12.1	12.0	–	9.9
3rd-instar nymphs	LT50	30[b]	13.0	8.0	–	6.2	6.0	5.6
	LT90	*c*	24.0	10.7	–	8.4	7.3	7.0
5th-instar nymphs	LT50	9.9[b]	7.9	6.4	4.8	5.3	–	4.6
	LT90	*c*	10.1	8.0	6.3	7.9	–	5.6
Adults	LT50	7.0	–	5.9	–	5.1	–	4.4
	LT90	9.0	–	7.0	–	7.0	–	6.0

a: estimated both LT50 and LT90 values: regression analysis of probit-mortality and log-time.
b: observed values.
c: 90% mortality non observed at 30 days postspraying.
–: not done.

or in adults). Although this high degree of variability of the host susceptibility must be kept in mind, it can be underlined that LT 50 at the highest concentrations of conidia remained relatively low under the conditions of the reported bioassays. Thus, LT 50 varied between 5.5 and 4.1 days when tested at the dose of 3×10^5 conidia per cm^2 and between 8.9 and 6.9 days at 3×10^4 conidia per cm^2.

These results were confirmed by the analysis of dose-mortality responses recorded at six or ten days postspraying (Table III). According to observed mortality rates and to both LD 50 and LD 90 values, the 1st-instar nymphs appeared less susceptible than the other tested stages of *R. prolixus*. Thus, in 1st-instar nymphs, observed mortality rates recorded at six days postspraying were very low. In contrast, in both 5th-instar nymphs and adults, at ten days postspraying, they were too high to establish a dose-mortality relation. Comparison of LD 50 showed that 1st-instar nymphs were 15-fold less susceptible than 3rd-instar nymphs (from data recorded at ten days postspraying) and that these last ones were about 50-fold less susceptible than both 5th-instar nymphs and adults at six days postspraying (Table III).

TABLE III

Relative susceptibility of nymphs and adults of *Rhodnius prolixus* to *Beauveria bassiana* INRA 297: dose-mortality response[a]

Contaminate host-stage	Lethal doses	Dose-mortality responses (conidia per cm^2) at the following times postspraying (days) 6	10
1st-instar	LD50	*b*	2.57×10^4 (1.72-3.84)
nymphs	LD90	*b*	1.10×10^5 (0.52-2.16)
3rd-instar	LD50	1.40×10^4 (0.68-2.30)	1.76×10^3 (1.23-2.53)
nymphs	LD90	1.70×10^5 (0.51-4.53)	5.63×10^4 (3.14-10.11)
5th-instar	LD50	3.51×10^3 (2.36-5.21)	*c*
nymphs	LD90	1.50×10^4 (0.76-2.96)	*c*
Adults	LD50	1.67×10^3 (0.82-3.39)	*c*
	LD90	1.83×10^4 (0.63-5.29)	*c*

a: estimated both LD50 and LD90 values with confidence limits (upper-lower): regression analysis of probit-mortality and log-dose.
b: cumulative mortality observed at 6 days postspraying less than 50%.
c: cumulative mortality observed at 10 days postspraying too high for establishing a linear dose-mortality relation.

DISCUSSION

Experimental infections, induced under controlled conditions, allow the testing of the entomopathogenic potential of fungal isolates and the susceptibility of the different stages of development of the target insect (Hall & Papierok, 1982). Diverse modes of infecting insects have been carried out in bioassays, including spraying titrated water suspensions of conidia directly on the test organisms (Fargues, 1972; Ferron & Robert, 1975; Rodriguez-Rueda & Fargues, 1980) and exposing insects to treated substrates (Ignoffo et al., 1976; Maniania & Fargues, 1986).

It is generally postulated that the mortality responses is directly dependent on the inoculum dosage (Burges & Thompson, 1971; Pinnock & Brand, 1981; Ferron, 1985). Bioassays carried out with *B. bassiana* INRA 297 on eggs of *R. prolixus* showed a good correlation between probit mortality and log dose. This dose-response relationship really exists for numerous fungi and insect hosts (Ferron, 1978, 1985; Hall & Papierok, 1982), although there are some exceptions. In the latter case, the fungus-induced mortality due to various doses of spores is very high after a certain period of time (Ferron, 1967; Romaña & Fargues, 1987). Thus, in bioassays carried out on *Rhodnius* nymphs and adults, mortality data recorded at six or ten days postspraying permitted to estimate LD 50, but after 20 days mortality rates reached 100%. Because of the high susceptibility of nymphs and adults to *B. bassiana* INRA 297, the non lethal threshold of inoculum is very low (3×10^2 conidia per cm^2) and the effect of the inoculum dosage mainly concerns the speed of the infection process.

Because fungal infection of insects depends on both temperature and moisture (Ferron, 1978, 1985; Hall & Papierok, 1982) the bioassays reported in this paper have been carried out under favourable conditions, i. e. 25°C and near-saturated air. In fact, *B. bassiana* INRA 297 remains highly pathogenic to *Rhodnius* nymphs at temperatures ranging from 15°C to 30°C (Fargues & Romaña, unpublished). Its pathogenic activity at 93% R. H. does not differ from that at 100% R. H., but at lower R. H. levels, ranging from 89 to 64%, it decreases significantly (although the fungus is still able to provoke around 50% mortality at 64% R. H.) (Fargues & Romaña, unpublished).

There are relatively few studies on the susceptibility of insect eggs to mycoses (Ferron, 1985). Fungi have been reported in association with eggs, but most of them were not true pathogens (Undeen & Nolan, 1977; Martin, 1977; Lynch & Lewis, 1978). Most bio-assays conducted with the entomopathogenic hyphomycetes, *B. bassiana, M. anisopliae* or *Nomuraea rileyi* showed only a low mortality during the egg stage of their host species (Bajan & Kmitowa, 1969; Lappa et al., 1972; Lappa & Goral, 1974; Rodriguez-Rueda & Fargues, 1980; Riba et al., 1983; Poprawski et al., 1985). However, several experiments carried out on eggs demonstrated the ability of some isolates of *Paecilomyces fumosoreus* or *M. anisopliae* to penetrate the chorion and to kill the eggs of their respective hosts, *Mamestra brassicae* (Rodriguez-Rueda & Fargues, 1980) and *Ostrinia nubilalis* (Riba et al., 1983). The high susceptibility of *R. prolixus* eggs to *B. bassiana* INRA 297 might be an interesting area of investigation for biological control.

According to the different larval stages, the host susceptibility depends on the host species and on the fungal isolate (Ferron, 1985). For example, the susceptibility of *Leptinotarsa decemlineata* larvae to *B. bassiana* decreased with age (Fargues, 1972). In contrast, *Melolontha melolontha* white grubs were clearly more susceptible to *Beauveria brongniartii* in the oldest larval instar (Ferron, 1967). The susceptibility of adults to mycoses has been little studied (Ferron, 1985). On the one hand, adults of *M. melolontha, Acanthoscelides obtectus* or *Oryctes rhinoceros* were highly susceptible to their specific hyphomycetous isolates of *B. brongniartii* (Ferron, 1985), *B. bassiana* (Ferron & Robert, 1975) and *M. anisopliae* (Ferron et al., 1975). On the other hand, adults of the Colorado beetle, *L. decemlineata,* appeared non susceptible to *Beauveria* isolates pathogenic to larvae (Bajan & Kmitowa, 1969).

Thus, the differences in both time-mortality and dose-mortality responses found between the different tested stages of *R. prolixus* demonstrate that *B. bassiana* INRA 297, previously selected according to its aggressiveness towards 1st-instar nymphs (Romaña & Fargues, 1987; Romaña et al., 1987), is definitely a good candidate for microbial control of this vector in environments favourable to mycoses (Ferron, 1985). Laboratory experiments are carried out to investigate the infectivity of *B. bassiana* against *R. prolixus* under different microclimatic conditions and to estimate their host range among the major vector species of *Triatominae.* Field trials have been planned in Venezuela and in Argentina against domestic populations of *R. prolixus* and *Triatoma infestans,* respectively. However this microbial control method must be improved in the peridomestic environment, since this particular biotope is considered the most critical point in the control of Chagas' disease vectors.

ACKNOWLEDGEMENTS

To Jean-François Pays for his encouragement and support. To Annette Rossignol, Anne Nadaud and Hervé Le Guillouzic for the technical assistance. To Dr A. -M. Moulin and Miss K. Pelis for their help in reviewing the english manuscript.

REFERENCES

BAJAN, C. & KMITOWA, K., 1969. Pathogenicity of entomogenous fungi isolated from hibernating imagines of the Colorado beetle (*Leptinotarsa decemlineata*) Say. *Ekol. Pol., 17:* 409-420.

BURGES, H. D., 1981. *Microbial Control of Pests and Plants Diseases.* Acad. Press, New York.

BURGES, H. D. & THOMPSON, E. M., 1971. Standardization and assay of microbial insecticides, p. 591-622. In H. D. Burges & N. W. Hussey (eds). *Microbial Control of Insects and Mites.* Acad. Press, New York.

DIAS, J. C. P. & LEÃO, A. E. de A., 1967. Parasitismo de fungos (*Beauveria bassiana*) sobre triatomíneos brasileiros criados em laboratório. *Atas. Soc. Biol., 2:* 85-87.

FARGUES, J., 1972. Etude des conditions d'infection des larves de doryphore, *Leptinotarsa decemlineata* Say, par *Beauveria bassiana* (Bals.) Vuill. (*Fungi imperfecti*). *Entomophaga, 17:* 319-337.

FERRON, P., 1967. Etude au laboratoire des conditions écologiques favorisant le développement de la mycose à *Beauveria tenella* du ver blanc. *Entomophaga, 12:* 257-293.

FERRON, P., 1978. Biological control of insect pests by entomogenous fungi. *Ann. Rev. Entomol., 23:* 409-442.

FERRON, P., 1985. Fungal Control, vol. 12, p. 313-346. In G. A. Kerkut & L. I. Gilbert (eds). *Comprehensive Insect Physiology, Biochemistry and Pharmacology,* Pergamon Press, Oxford.

FERRON, P. & ROBERT, P. H., 1975. Virulence of entomopathogenic fungi (*Fungi imperfecti*) for the adults of *Acanthoscelides obtectus (Coleoptera: Bruchidae). J. Invertebr. Pathol., 25:* 379-388.

FERRON, P.; ROBERT, P. H. & DEOTTE, A., 1975. Susceptibility of *Orycetes rhinoceros* adults to *Metarhizium anisopliae. J. Invertebr. Pathol., 25:* 313-319.

FERRON, P.; FARGUES, J. & RIBA, G., 1991. Fungi as microbial insecticides against pests, p. 665-706. In D. K. Arora, L. Ajello & K. G. Mukersi (eds), *Handbook of Applied Mycology.* Marcel Dekker Inc., New York.

GLARE, T. R. & MILNER, R. J., 1991. Ecology of entomopathogenic fungi, p. 547-612. In D. K. Arora, L. Ajello & K. G. Mukersi (eds). *Handbook of Applied Mycology.* Marcel Dekker Inc., New York.

HALL, R. A. & PAPIEROK, B., 1982. Fungi as biological control agents of arthropods of agricultural and medical importance. *Parasitology, 84:* 205-240.

IGNOFFO, C. M.; MARSTON, N. L.; HOSTETTER, D. L.; PUTTLER, B. & DICKERSON, W. A., 1976. Susceptibility of the cabbage looper *Trichoplusia ni* and velvet bean caterpillar, *Anticarsia gemmatalis* to several isolates of the entomopathogenic fungus *Nomuraea rileyi. J. Invertebr. Pathol., 28:* 259-262.

ISHIBASHI, Y.; KAUFMAM, H. E.; ICHINOE, M. & KAGAWA, S., 1987. The pathogenicity of *Beauveria bassiana* in the rabbit cornea. *Mykosen, 30:* 115.

IZRAJLET, L. I.; DROZDOVA, L. V. & EGLITE, M. E., 1975. Hygienic evaluation of beauverin and some prospects of using microbiological means of plant protection. *Gig. Sanit., 11:* 91-94 (in russian).

LAPPA, N. V. & GORAL, V. M., 1974. The influence of *Metarrhizone* and *Beauverin* on Colorado beetle

eggs. *Zakh. Rosl. Kiev, 19:* 68-71 (in ukrainian).
LAPPA, N. V.; GORAL, V. M. & MARKYS, V. G., 1972. Action of Beauverin on eggs of *Eurygaster integriceps. Zakh. Rosl. Kiev., 16:* 23-24 (in ukrainian).
LYNCH, R. E. & LEWIS, L. C., 1978. Fungi associated with eggs and first-instar larvae of the european corn borer. *J. Invertebr. Pathol., 32:* 6-11.
MANIANIA, N. K. & FARGUES, J., 1986. Spécificité des hyphomycètes entomopathogènes pour les larves de lépidoptères *Noctuidae. Entomophaga, 29:* 451-464.
MARTIN, W. W., 1977. The development and possible relationships of a new *Atkinsiella* parasitic in insect eggs. *Amer. J. Bot., 64:* 760-769.
MESSIAS, C. L.; DAOUST, R. A. & ROBERTS, D. W., 1986. Virulence of a natural isolate, auxotrophic mutants, and a diploid of *Metarhizium anisopliae* var. *anisopliae* to *Rhodnius prolixus. J. Invertebr. Pathol., 47:* 231-233.
MOURA-COSTA, M. D., 1978. Susceptibilidade do triatomineo *Rhodnius prolixus* ao fungo entomopatogénico *Metarhizium anisopliae. An. Acad. Med., Bahia, 1:* 35-44.
PAYNE, C. C., 1988. Pathogens for the control of insects: where next? *Phil. Trans. R. Soc. Lond, 318,* 225-248.
PINNOCK, D. E. & BRAND, R. S., 1981. A quantitative approach to the ecology and the use of pathogens for insect control, p. 655-665. In H. D. Burges, *Microbial Control of Pests and Plants Diseases.* Acad. Press, New York.
POPRAWSKI, T. J.; MARCHAL, M. & ROBERT, P. H., 1985. Comparative susceptibility of *Otiorrhynchus sulcatus* and *Sitona lineatus (Coleoptera: Curculionidae)* early stages to five entomopathogenic Hyphomycetes. *Environ. Entomol., 12:* 247-253.
RIBA, G.; MARCANDIER, S.; RICHARD, G. & LARGET, I., 1983. Sensibilité de la pyrale du mais (*Ostrinia nubilalis*) (Lep.: Pyralidae) aux hyphomycètes entomopathogènes. *Entomophaga, 28:* 55-64.
ROBERTS, D. W. & STRAND, M. A., 1977. Pathogens of medically important arthropods. *Bull. WHO,* Geneva, 55, 419 p.
RODRIGUEZ-RUEDA, D. & FARGUES, J., 1980. Pathogenicity of entomopathogenic hyphomycetes, *Paecilomyces fumosoroseus* and *Nomuraea rileyi,* to eggs of Noctuids, *Mamestra brassicae* and *Spodoptera littoralis. J. Invertebr. Pathol., 36:* 399-408.
ROMAÑA, C. & ROMAÑA, C. A., 1981. Experimental infection of *Triatoma infestans* with the fungus *Beauveria tenella,* p. 215-217. In E. V. Canning *Parasitological topics.* Allen Press Inc., Kansas, USA.
ROMAÑA, C. A. & FARGUES, J., 1987. Sensibilité des larves de l'hémiptère hématophage *Rhodnius prolixus (Triatominae)* aux hyphomycètes entomopathogènes. *Entomophaga, 32:* 167-179.
ROMAÑA, C. A.; FARGUES, J. & PAYS, J. F., 1987. Mise au point d'une méthode biologique de lutte contre les *Triatominae,* vecteurs de la maladie de Chagas, avec des hyphomycètes entomopathogènes. *Bull. Soc. Path. Ex., 80:* 105-111.
SAIK, J. E.; LACEY, L. A. & LACEY, C. M., 1990. Safety of microbial insecticides to vertebrates-Domestic animals and wildlife, p. 115-134. In Laird, Lacey & Davidson (eds). *Safety of Microbial Insecticides.* CRC Press Inc., Boca Raton, Florida.
SCHOFIELD, C. J.; MINTER, D. M. & TONN, R. J., 1987. The Triatomine Bugs. Biology and Control. Adv. Ser. Vector Control Training and Information Guide, WHO, Geneva, 41 p.
SHERLOCK, I. & GUITTON, N., 1982. Observações sobre a ação do fungo *Metarhizium anisopliae* Metschnikoff sobre algumas espécies de *Triatominae (Hemiptera, Reduviidae). Rev. Inst. Med. Trop. Sao Paulo, 24:* 234-239.
SIEGEL, J. P. & SHADDUCK, J. A., 1990. Safety of microbial insecticides to vertebrates- Humans, p. 101-115. In Laird, Lacey & Davidson (eds). *Safety of Microbial Insecticides.* CRC Press Inc., Boca Raton, Florida.
SILVA, J. C. & MESSIAS, C. L., 1985. Viruléncia de *Metarhizium anisopliae* var. *anisopliae* a *Rhodnius prolixus. Ciênc. Cult., 7:* 37-40.
UNDEEN, H. & NOLAN, R. A., 1977. Ovarian infection and fungal spore oviposition in the blackfly *Prosimulium mixtum. J. Invertebr. Pathol., 30:* 97-98.