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Susceptibility of *Rhodnius pallescens* (Hemiptera: Reduviidae) of fifth instar nymph to the action of *Beauveria* spp.

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Abstract

Pineda Gutiérrez F, Saldarriega Osorio Y, Calle Osorno J, Uribe Soto S. 2003. Susceptibility of *Rhodnius pallescens* (Hemiptera: Reduviidae) of fifth instar nymph to the action of *Beauveria* spp. Entomotropica 18(3):163-168.

Laboratory bioassays were performed to determine the susceptibility of 5^{th} instar nymphs of the insect *Rhodnius pallescens* Barber to the entomopathogenic fungi, *Beauveria* spp. The insects were bred and kept at the Mycology Laboratory, Instituto de Biología, Universidad de Antioquia. Two strains of the entomopathogenic fungi *Beauveria bassiana were* isolated from the triatomine bug *R. pallescens* and two of *B. brongniartii* from the weevil *Homalinotous validus* (Olivier), both of which inhabit *Attalea butyracea* palms in the San Onofre region of the Colombian department of Sucre. The pathogenicity of *Beauveria* isolates to *R. pallescens* was studied by exposing laboratory-reared insects to fungal inocula consisting of 1×10^7 spores/ml smeared on pieces of canvas. The different isolates produced variable degrees of mortality in the bugs, ranging from 77.5% *B. bassiana* (Bb $UdeA_{14}$) to 100% *B. brongniartii* (Bt $UdeA_{16}$) at 22 days. LT_{50} values of 10,11 and 12 days were estimated for the isolates *B. bassiana* (Bb $UdeA_{14}$), *B. brongniartii* (Bt $UdeA_{15}$) and *B. bassiana* (Bb $UdeA_{13}$) respectively. The lowest recorded LT_{50} value was 7 days for *B. brongniartii* (Bt $UdeA_{16}$), this isolate being significantly more effective than the others and the control ($X^2 = 200.84$, $p< 0.0001$). Fifth instar nymphs of *R. pallescens* were more susceptible to *B. brongniartii* (Bt $UdeA_{16}$) than the other strains tested, and 50% of the insects showed mycosis 10 days after being exposed to this strain ($X^2 = 47.08$, $p < 0.0001$).

Additional key words: *Beauveria bassiana*, *Beauveria brongniartii*, Entomopathogenic fungus, *Homalinotus validus.*

Resumen

Pineda Gutiérrez F, Saldarriega Osorio Y, Calle Osorno J, Uribe Soto S. 2003. Susceptibilidad de *Rhodnius pallescens* (Hemiptera: Reduviidae) de V estadío de desarrollo a la acción de *Beauveria* spp. Entomotropica 18(3):163-168.

Se realizaron ensayos de laboratorio para determinar la susceptibilidad del V estadio de desarrollo del insecto *Rhodnius pallescens* Barber al hongo entomopatógeno *Beauveria* spp. Los insectos se criaron y mantuvieron en el laboratorio de Micología del Instituto de Biología de la Universidad de Antioquia. Se aislaron dos cepas del hongo entomopatógeno *B. bassiana* del insecto triatomino *R. pallescens* y dos de *B. brongniartii* del curculionido *Homalinotous validus* (Olivier), habitantes de las palmeras de *Attalea butyracea* en la región de San Onofre, Departamento de Sucre, Colombia. La patogenicidad de *Beauveria bassiana* a *R. pallescens* fue estudiada exponiendo los insectos a concentraciones del hongo de 1 x 10^7 esporas/ml impregnadas en pedazos de lona. Los diferentes aislamientos produjeron varios grados de mortalidad en los insectos, desde 77,5% *B. bassiana* (Bb UdeA$_{14}$) a 100% *B. brongniartii* (Bt UdeA$_{16}$) a los 22 days. Los valores de TL_{50} de 10,11 y 12 días fue estimado para los aislamientos *B. bassiana* (Bb UdeA$_{14}$), *B. brongniartii* (Bt UdeA$_{15}$) and *B. bassiana* (Bb UdeA$_{13}$) respectivamente. El valor TL_{50} más bajo fue de 7 días para *B. brongniartii* (Bb UdeA$_{16}$), siendo este aislamiento más efectivo que los otros y el control (X^2 = 200.84, p< 0.0001). Ninfas de quinto estadio de *R. pallescens* fueron más susceptibles a *B. brongniartii* (Bt UdeA$_{16}$) que a las otras cepas probadas, y 50% de los insectos mostraron micosis a los 10 días después de haber sido expuestos a esta cepa (X^2 = 47.08, p < 0.0001).

Palabras clave adicionales: *Beauveria bassiana*, *Beauveria brongniartii*, hongos entomopatógenos, *Homalinotus validus.*

Introducción

Chagas' disease caused by *Trypanosoma cruzi* and transmitted by triatomine bugs of the subfamily Reduviidae is a serious public health problem in Latin America. It affects 3.4% of the population of Colombia, where 3.6 million people inhabit high-risk areas (Moncayo & Guhl 1997) in the center, south and Caribbean coast of the country (D' Alessandro et al. 1981; Corredor et al. 1990).

Table 1. Isolates and species of *Beauveria*, evaluated during the present study, toghether with
insects hosts collected in San Onofre (Sucre).

Host species	*Beauveria* sp.	Isolate
Rhodnius pallescens (Hemiptera: Reduviidae)	*B. bassiana*	Bb UdeA$_{13}$
Rhodnius pallescens (Hemiptera: Reduviidae)	*B. bassiana*	Bb UdeA$_{14}$
Homalinotus validus (Coleoptera: Curculionidae)	*B. brongniartii*	Bt UdeA$_{15}$
Homalinotus validus (Coleoptera: Curculionidae)	*B. brongniartii*	Bt UdeA$_{16}$

The entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. Criv. is a natural pathogen of many insect species (Couteaudier & Viaud 1997) which has been used for many years as a biological pest control agent (Ferron et al. 1991).

The mycelium of the fungus penetrates the insect cuticle, invading the tissues and causing death (Hajek & St Leger 1994). Pathogenecity is also due to mycotoxins that block the insect inmune system (Kachatourians 1991; Uribe et al. 1997).

Romaña & Romaña (1981); Romaña et al. (1987); Romaña & Fargues (1987); Romaña (1992), reported the susceptibility of triatomines to entomopathogenic fungi in nature. A total of 23 species of This insects are known from Colombia, including *Rhodnius prolixus* Stål.; *R. robustus* Larrouse; *R. pallescens* Barber; *R. brethesi* Matta; *Triatoma dimidiata* Latreille*; T. maculata* Erichson; and *T. venosa* Stål.; all of which have been found in houses (Molina et al. 2000). The principal vector of Chagas' disease in Colombia is *R. prolixus* which is anthropophilic, occurs a high population densities, has a short life cycle, and is highly susceptible to infection with *T. cruzi*. This species has a high dispersal capacity, which together with is ability to adapt to a variety of ecological conditions explains its wide geographical distribution in Colombia (D'Alessandro et al. 1984; Schofield & Dujardin 1997).

Other triatomine species occur in forested areas, such as *R. pallescens* which lives in *Attalea butyracea* (Muttis ex l. f.) Wess. Boer (Arecaceae) palms in the San Onofre region of the Colombian department of Sucre (Romaña et al. 1999) and is the main vector of *T. cruzi* in Panama. This species also occurs in Belize where it lives in chicken houses, as well as being associated with opossums and sloths (Lent & Wygodzinsky 1979, Christensen & De Vasquez 1981). *Homalinotus validus* (Coleoptera: Curculionidae) is an inhabitant of the *Attalea butyracea* palms in the San Onofre region of the Colombian department of Sucre.

The objective of the present study were to isolate, cultivate, identify *Beauveria* spp. and to determine the susceptibility of *R. pallescens* to these fungi.

Materials and Methods

Origin of insects and fungal isolates: The insects isolates were obtained from the palm trees *A. butyracea* and the fungus from the triatomine bug *Rhodnius pallescens* and the weevil *homalinotus validus* in the municipality of San Onofre, which has an area of 30 km^2 and includes the rural sectors of Las Brisas and Vista Hermosa (lat 09°60'0" and 09°80'11"N, long 75°28'05" and 75°42'00" W respectively), between Higuerón, Plan Parejo and La Libertad (Calle 2000).

Specimens of *R. pallescens* and *H. validus* were captured in the field by dismantling the crowns of 30 *A. butyracea*. The triatomines were sorted by instars and nutritional status. The live insects were placed in plastic tubes sealed with muslin and stored unfed for forty days. Specimens that died during this period were placed in plastic Petri dishes with a diameter of 5.5 cm, together with a small piece of dampened cotton to induce fungal sporulation.

Isolates of *Beauveria* spp.: The isolates obtained and their origins are listed in Table 1.

Isolating fungi: Dead insects invaded by the fungus were observed under the stereomicroscope and mycelium removed for inoculation on Petri dishes of Sabouraud dextrose and malt extract agar. The Petri dishes were incubated for one week at 25 °C. Slides preparations of the cultures were stained with lactophenol blue and mounted for detailed examination. Fungi were identified according to De Hoog (1972), Hugues (1973) and Mangenot & Reisenger (1976). All fungi isolated from weevils were found on dead specimens within the crowns of palm trees, so that no laboratory rearing of *H. validus* was carried out. The isolates of *B. bassiana* and *B. brongniartii* used in this study were preserved in a cold chamber at 4 °C at the Mycology Laboratory at the Biology Institute of the Unversity of Antioquia.

Culture media: The fungi were kept in malt extract agar (MEA) and Sabouraud dextrose agar (SDA) (Oxoid LTD., Basingstoke, Hampshire, UK). The technique of Romaña & Fargues 1987 was used to obtained the spores. The spores were inoculated in an inclined semi-synthetic medium (Romaña & Fargues 1987) for two weeks at 25 °C. Spores concentrations were determined with a Neubauer haemocytometer, counting the central square of 16 cells in two drops of suspension for each replicate. A spore concentration of 1 x 10^7 spores/ml was used in the bioassays.

Table 2. Proportions of *Rodnius pallescens* of diferent developmental stages invaded by fungi during quarantine

Developmental stage	Showing mycosis (%)	Not showing mycosis (%)	Total (%)
Nymphal instar 1	10 (7.94)	5 (5.38)	15 (6.85)
Nymphal instar 2	22 (17.46)	14 (15.05)	36 (16.43)
Nymphal instar 3	26 (20.63)	16 (17.20)	42 (19.18)
Nymphal instar 4	11 (8.73)	13 (13.98)	24 (10.96)
Nymphal instar 5	33 (26.19)	26 (27.96)	59 (26.94)
Adult	24 (19.05)	19 (20.43)	43 (19.63)
Totals	126 (100.00)	93 (100.00)	219 (100.00)

Table 3. Proportions of adult male and female *Rodnius pallescens* invaded by fungi during quarantine

Sex	With mycosis (%)	Without mycosis (%)	Total
Females	10 (41.67)	8 (42.11)	18 (41.86)
Males	14 (58.33)	11 (57.89)	25 (58.14)
Total	24 (100.00)	19 (100.00)	43 (100.00)

Insect rearing: Live specimens of *R. pallescens* were maintained in darkness in an air conditioned chamber (WTBbinder 78532 (Tuttlingen/Germany), at 26.5 °C and 80% relative humidity (Romaña & Fargues 1987). The insects were kept in plastic containers of dimensions 16x12 cm containing a central opening covered with muslin. Cross-shaped cardboard ladders were placed inside the containers as refuges for the bugs, which were allowed to take blood from mice (*Mus musculus* Linneaus) for 2-3 h twice per week.

Direct contamination of insects: Four replicates of 20 fith instar nymphs of *R. pallescens* and a control proup were exposed to 1 x 10^7 spores/ml. They were kept in complete darkness in an air conditioned chamber (WTBbinder 78532 (Tuttlingen/Germany), at 26.5°C and 80% relative humidity. Live insects were counted and dead ones placed in sterile containers with a small piece of moist cotton, then stored in an incubator at "25 ºC" for 7 days. The insects were observed daily. All dead insects were assumed to have succumbed to the fungi that had developed on their cadavers (Romaña & Romaña 1981). Samples of the fungal mycelium were stained in lactophenol blue and mounted on slides for examination under the microscope to confirm that the fungi responsible were *Beauveria* spp.

Statistical analysis: Data on survival rate of *R. pallescens* and mycosis were analysed using Freeman Halton's Test on the Statxact-4 stastistical programme.

Results

Of the four autochthonous isolates of *Beauveria* from San Onofre (Sucre), two were *B. bassiana* and two (2) *B. brongniartii* (Sacc.) Petch, obtained respectively from *R. pallescens* and *H. validus* (Table 1). A total of 376 specimens of *R. pallescens* were collected in palm trees, of which 271 were unfed and 105 fed. In all 210 unfed and nine fed triatomines died during quarantine, of which 126 (124 unfed) presented some type of mycosis while 93 showed no signs of fungus (Table 2).

Among the adult bugs, 14 males and 10 females showed some sort of mycosis while 11 males and eight females were fungus-free (Table 3). There was no significant association between mycosis and the stage of nymphal development (X^2 = 2.44, p = 0.79) or sex (X^2 = 0.32, p = 0.064) but an association was observed between mycosis and unfed insects (X^2 = 4.79, p = 0.028).

With respect to insect mortality due to *Beauveria* (strains Bb $UdeA_{13}$, Bb $UdeA_{14}$, Bt $UdeA_{15}$, Bt $UdeA_{16}$), significant differences were found ($X^2 = 200.84$, $p < 0.0001$) between treated and control groups for fifth instar nymphs of *R. pallescens* (Figure. 1). The LT_{50} value for this group was seven days. Mortality of 100% was obtained with the Bt $UdeA_{16}$ isolate 21 days after exposure (Figure 1).

Fith instar nymphs of *R. pallescens* were more susceptible to *B. brongniartii* ($UdeA_{16}$) than the other strains tested and 50% of the insects showed mycosis 10 days after being exposed to this strain ($X^2 = 47.08$, $p < 0.0001$) (Figure 2).

Discussion

The isolates obtained during the present study belonged to two species of *B. bassiana* originating from triatomine bugs and two of *B. brongniartii* from weevils. Even if the isolates of *B. bassiana* did indeed originate exclusively from triatomines and those of *B. brongniartii* only from weevils in the present study, it cannot be concluded that these fungi are pathogenic only to these insects. Workers in previous studies carried out in the San Onofre region have isolated *B. bassiana* both from weevils and triatomines (Arroyave 1995; Calle 2.000).

Figure 1: Mortality due to invasion with *Beauveria* strains Bb $UdeA_{13}$, Bb $UdeA_{14}$, Bt $UdeA_{15}$ and Bt $UdeA_{16}$ among fifth instar nymphs of *Rhodnius pallescens:* differences significant between treatment and control groups ($X^2 = 200.84$, $p < 0.0001$).

Figure 2: Percentage of mycosis of *Rhodnius pallescens* over time, showing that after 10 days 50% of the insects showed mycosis with the strain Bt $UdeA_{16}$ ($X^2 = 47.08$, $p < 0.0001$).

These insects are often naturally infected by the fungi, irrespective of sex or developmental stage, as confirmed by the laboratory quarantine data (Table 2 and Table 3).

A higher infection rate by some type of fungus was seen in the triatomines classified as unfed, suggesting that these were more susceptible to mycosis.

All the *Beauveria* isolates used in the laboratory assays produced a high mortality and fungal esporulation in fifth instar nymphs of *R. pallescens.*

Infection was produced by invasión across the integument, a feature that distinguishes entomomycosis from viral, bacterial or protozoal infections (Romaña & Romaña 1981).

Mortality in adults of *R. pallescens* was attributed to mycosis caused by *B. bassiana* or *B. brongniartii,* since these were recuperated from the dead insects.

The susceptibility of adult triatomines to mycosis has been little studied (Ferron 1985). In susceptibility tests carried out with first instar nymphs of *R. prolixus, R. pallescens, R. robustus, R. negletus and Eratyrus cuspidatus,* concentrations of 3x 10^8 spores/ml of the *B. bassiana* strains INRA 297 and $UdeA_1$, induced 100% mortality in all the insects. The LT_{50} values varied according to species, including 7.5 days for *R. pallescens* infected with INRA 297 and only 4.6 days for the same species esposed to the isolate $UdeA_1$ (Arroyave 1995). The LT_{50} value for fifth instar nymphs of *R. pallescens* obtained during the present study (7 days) was some what lower than that obtained by these authors with the strain INRA 297. When exposed to the $UdeA_1$ strain, fifth instar nymphs showed LT_{50} values a little greater than those obtained for first instar nymphs exposed to a higher concentration of the fungus. These results show that the susceptibility may be affected by the developmental stage, the concentration of the spores and the species of fungus, as well as factors such as the geographical origin of the fungus, and the physiological state of the insects (Romaña & Fargues 1992; Arroyave 1995; Romaña 1995).

The pathogenecity of fungi of the genus *Beauveria* to triatomines was demonstrated in previous studies (Romaña & Romaña 1981; Romaña & Fargues 1987; Romaña 1992). The results of the present study are very similar to those obtained by these researchers and provide further information on the potential of entomopathogenic fungi for the microbiological control of triatomines in their own habitat.

At present three major programmes are directed towards the control of Chagas' diseases, *i.e*., the Southern Cone Initiative and similar multinational efforts involving countries of the Andean region and Central America. These have produced excellent results in the control of triatomines at the domiciliary level.

However wild foci of these insects remain, presenting the threat of reinfestation. This might best be countered by means of microbiological control using entomopathogenic fungi such as *Beauveria* spp.

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References

Arroyave ME. 1995. Susceptibilidad de algunas especies de Triatominae (Hemiptera: Reduviidae) al hongo entomopatógeno *Beauveria bassiana* (Fungi Imperfecti). [Tesis de grado] Medellín (Colombia): Universidad de Antioquia, Facultad de Ciencias Exactas y Naturales. 36p.

Calle JJ. 2000. *Vers* un contrôle microbiologique des populations colombiennes de Triatominae, insectes vecteurs de la maladie de Chagas. [Thèse]. France: Universitè de Paris. 140p.

Couteaudier Y, Viaud M. 1997. New insights into population structure of *Beauveria bassiana* with regard to vegetative compatibility groups and telomeric restriction of fragment length polymorphism. FEMS Microbiol Ecol 22:175-182.

Corredor A, Santa Cruz MM, Páez S, Guatame AT. 1990. Distribución de los triatominos domiciliarios en Colombia. Bogotá (Colombia): Ministerio de Salud. 144p.

Christensen HA, De Vasquez AM. 1981. Host Feeding Profiles of *Rhodnius pallescens* (Hemiptera: Reduviidae) in Rural Villages of Central Panama. Am J Trop Med Hyg 30(1): 278-83.

D'alessandro A, Barreto P, Thomas M. 1981. Nuevos registros de triatominos domiciliarios y extradomiciliarios en Colombia. Colombia. Medica 12:75-85.

D'alessandro A, Barreto P, Saravia N, Barreto M. 1984. Epidemiology of *Trypanosoma cruzi* in the Oriental Plains of Colombia. Am J Trop Med Hyg 33(6):1.084-1.095.

De Hoog GS. 1972. The genera *Beauveria*, *Isaria*, *Tritirachium* and *Acrodontium* gen. Nov. Stud Mycol 1:1-41.

Ferron P. 1985. Fungal control. In: GA, Gilbert LI, editors. Comprehensive Insect Physiology, Biochemistry and Pharmacology, Pergamon. Vol 12, p. 313-346.

Ferron P, Fargues J, Riba G. 1991. Fungi as microbial insecticides against pests. ln: Arora DK, Ajello L, Mukerji Kq, Drouchet E, editors. Handbook of Applied Mycology, human, animals and insects. New York: Marcel Dekker. Vol. 2, p. 665-706.

Hajek AE, Leger RJ. 1994. lnteractions between fungal pathogens and insect hosts. Ann Rev Entomol 39:293-322.

Hugues SI. 1973. Conidiospores, conidia and classification, Can J Bot 31:577-659.

Khachatourians GG. 1991. Physiology and genetics of entomopathogenic fungi. ln: Arora DK, Ajello L, Mukerji Kq, Drouchet E, editors. Handbook of Applied Mycology, human, animals and insects. New York: Marcel Dekker.Vol 2 p. 613-663.

Lent H, Wygodzinsky P. 1979. Revision of the Triatominae (Hemiptera, Reduviidae) and their significance as vectors of Chagas'disease, Bull Am Mus Nat Hist (New York) 163:520p.

Mangenot F, Reisenger O. 1976. Form and function of conidia as related to their development, In: Weber DJ, Hess WM, editors. The fungal spore form and function. New York: John Wiley. p. 789-847.

Molina JA, Gualdrón LE, Brochero HL, Olano VA, Barrios D, Guhl F. 2000. Distribución actual e importancia epidemiológica de las especies de triatominos (Reduviidae: Triatominae) en Colombia. Biomedica 20:344-60.

Moncayo A, Guhl F. 1997. Programas en la eliminación de la transmisiòn de la enfermedad de Chagas en los paises suramericanos. In: Vectores de enfermedad de Chagas en los paises andinos. UNDP/WORLD BANK/WHO/Special Programme for Research and Training in Tropical disease (TDR). Uni-Andes. Centro de Investigación en Microbiología y Parasitología Tropical-CIMPAT-Santafé de Bogotá.

Romaña CA, Romaña CH. 1981. Experimental infection of *Triatoma infestans* with the fungus *Beauveria tenella.* In: Canning EV, editor. Parasitological topics. A presentation of volume to PCC GARNHAM FRF on the occasion of his 80th birthday. Kansas: Allen Press. p. 215-217.

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

Romaña C, Fargues J, Pays JF. 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 Pathol Exp 80:105-11.

Romaña CA. 1992. Recherches sur les potentialités des Hyphomycètes entomopathogènes (Fungi Imperfecti) dans la lutte microbiologique contre les Triatominae (Heteroptera). [Thèse]. Montpellier: Université de Montpellier 134p.

Romaña CA, Fargues J. 1992. Relative susceptibility of different stages of *Rhodnius prolixus* to the entomopathogenic hyphomycetes *Beauveria bassiana.* Mem Inst Oswaldo Cruz 87:363-368.

Romaña CA. 1995. Potencial for triatoma biological control. In: Proceeding of the International Workshop on Population Genetics and Control of Triatominae. Santo Domingo de los Colorados, Ecuador. Schofield CJ editor. México: INDRE. p 87-88.

Romaña CA, Pizarro JC, Rodas R, Guilbert E. 1999. Palm trees as ecological indicators of risk areas for Chagas disease. Trans Roy Soc Trop Med Hyg 93:594-595.

Schofield CJ, Dujardin JP 1997. Chagas disease vector control in Central America. Parasitol Today 13(4):141-144.

Uribe S, Saldarriaga Y, Pineda F, Arango GJ, Vélez ID. 1997. Producción de beauvericina por *Beauveria bassiana* 9401 aislado sobre *Lutzomyia* sp. (Diptera: Psychodidae) vectores de leishmaniosis. Rev Col Entomol 3:137-141.