BIOLOGICAL CONTROL

Populational Parameters of *Spalangia endius* Walker (Hymenoptera: Pteromalidae) on Pupae of *Musca domestica* L. (Diptera: Muscidae) Treated with Two Strains of *Beauveria bassiana* (Bals.) Vuil. (Deuteromycetes)

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Neotropical Entomology 36(4):537-541 (2007)

Parámetros Poblacionales de *Spalangia endius* Walker (Hymenoptera: Pteromalidae) sobre Pupas de *Musca domestica* L. (Diptera: Muscidae) Tratadas con Dos Cepas de *Beauveria bassiana* (Bals.) Vuil. (Deuteromycetes)

RESUMEN - El parasitoide *Spalangia endius* Walker es un reconocido controlador de pupas de dípteros, tal como la *Musca domestica* L. El hongo entomopatógeno *Beauveria bassiana* (Bals.) Vuil. es un regulador de poblaciones de insectos, incluidas estas plagas sinantrópicas. El objetivo del trabajo fue explorar las posibilidades de utilización conjunta de ambos agentes para el control de la mosca. Se trataron pupas recién formadas de esta plaga con una suspensión de conidios (10^8 conidios/ml). Pupas inoculadas con dos cepas (Bb6 y Bb10) del hongo fueron ofrecidas a los parasitoides hembra. En un ensayo se les ofreció pupas inoculadas el primer día y en otro, inoculadas también al día siguiente; en los dos ensayos se les colocaron pupas normales hasta la muerte del parasitoide. Se usaron 30 hembras de *S. endius* para cada cepa y ensayo. A través del estudio de la progenie del parasitoide se construyeron tablas de vida, obteniéndose las tasas netas de reproducción (R_0) e intrínsecas de incremento natural (r_m) entre otros parámetros demográficos, así como el porcentaje de parasitoidismo y la proporción de sexos. *B. bassiana* no afectó significativamente la biodemografía del parasitoide cuando se lo expuso una sola vez, en cambio la R_0 y la rm fueron más bajas que en el testigo sin el hongo cuando fueron expuestos dos veces, aunque no se observó esporulación en los cadáveres de *S. endius*.

PALABRAS-CLAVE: Control biológico, mosca doméstica, hongo entomopatógeno

ABSTRACT - The parasitoid *Spalangia endius* Walker is an efficient controller of Dipteran pupae, such as *Musca domestica* L. The entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuil. is a regulator of insect populations, including these synanthropic pests. The aim of this work was to explore the possibilities of utilizing both agents in a combined form for the biocontrol of the domestic fly. Recently formed *M. domestica* pupae were inoculated by immersion in conidia suspension (10^8 conidia/ml) with two strains of *B. bassiana* (Bb6 and Bb10). The inoculated pupae were offered to the female parasitoid. In one bioassay they were offered pupae inoculated a single day and in other, pupae inoculated the following day as well. In both bioassays non inoculated (control) pupae were offered to the parasitoids until their death. Thirty females of *S. endius* were used for each strain and bioassay. From the study of the parasitoid offspring, life tables were built and the reproduction net rate (R_0) and intrinsic natural increase (r_m) were obtained among other demographic parameters; the parasitism percentages and sex ratios were also analyzed. *B. bassiana* did not affect significantly the biodemography of the parasitoid when pupae were inoculated a single time. On the other hand the R_0 and the rm were smaller than that of the control without the fungus when pupae were inoculated twice, although sporulation was not observed in the cadavers of *S. endius.*

KEY WORDS: Entomopathogenic fungus, biological control

The microhymenopteran *Spalangia endius* Walker is a well-known pupal parasitoid of *Musca domestica* L. (Morgan *et al*. 1976) and it is used for the control of this fly in confined animal production systems (Crespo *et al*.1998). The estimation of the populational parameters has been used to study the population dynamics of a species in particular (Southwood 1995), to select natural enemies (Janssen & Sabelis 1992), to evaluate the impact of a beneficial insect on

a pest (Bellows *et al*. 1992) and to analyze the possibilities of success in a parasitoid release, in rural facilities, for the control of synanthropic dipterans (Morgan *et al*. 1989). On the other hand, the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuil. is an efficient regulator agent of populations of several orders and families of insects (Ferron *et al*. 1991), among them Diptera Muscidae as the domestic fly (Watson *et al.* 1995, 1996; Darwish & Zayed (2002) that has been investigated with the purpose of developing a mycoinsecticide to be used in the Integrated Pest Management (IPM) (Lecuona *et al.* 2005b). The objective of the present work consisted on analyzing the possibilities of using both agents (parasitoid and entomopathogenic fungus) in a combined form for the biocontrol of the domestic fly.

Materials and Methods

***M. domestica* rearing.** In the Domestic Fly Insectary (DFI) of the Instituto de Microbología y Zoología Agrícola (IMYZA) – Instituto Nacional de Tecnología Agropecuaria (INTA), Castelar, Buenos Aires, Argentina), this species is massively reared since 1992. The larvae are maintained for five days at 28 ± 1ºC and 50 ± 10% RH on a diet that contains 40% of wheat bran, 50% beer yeast and 10% water. At the third instar they are separated by sieve and placed on dry corn flour in plastic trays until reaching the pupal stage.

***S. endius* rearing.** Massive rearing of the parasitoid is carried out, in the DFI of the IMYZA, on pupae of domestic fly, following the methodology proposed by Morgan (1986). The fly pupae are exposed to the parasitoids when the presence of the cuticle in third larval instar, present in the pupary formation, is evidenced. These pupae are selected by their white color that indicates the beginning of pupation, and they are placed in chambers at a temperature of 29 ± 1°C and 60-70% RH; the adults of *S. endius* emerge after 21 days, approximately.

***B. bassiana* culture.** The strains Bb6 and Bb10 of the fungi collection of the Laboratory of Entomopathogenic Fungi (LEF) of IMYZA were used. These strains were isolated from *Diatraea saccharalis* (F.) (Lepidoptera: Pyralidae) in Argentina. To be used in this work, the strains were reisolated through a passage in adults of *M. domestica* and they were maintained in dishes with complete agar media (CAM) containing (g/l): KH2PO4, 0,4; Na2HPO4, 1,4; SO4Mg, 0,6; KCl, 1; NH4NO3,0,7; glucose, 10; agar, 15; and yeast extract, 5. Conidia were collected from fungal colonies of 14 days old and incubated in dishes at 26 ± 0,5ºC. The viability was checked at 18, 22, 26, 30 and 34 ± 0.5ºC by counting the number of germinated conidia, incubated in CAM with 1 x 10^7 conidia/ml, in 10 microscope fields at 8, 12, 16, 20 and 24h after inoculation. Conidia are considered germinated when the germinative tube reaches the same or longer length as the width of the conidial body.

Bioassays. Cohorts of 30 females (< 12h old) each of *S. endius* coming from the massive rearing of the DFI were used. Each female was placed individually in glass tubes of 15 x 1.5 cm, covered with cotton. Two bioassays were carried out with pupae of *M. domestica* (< 24h old); in the first one, they were inoculated a single time with the fungal strains and in the second, the treatment was repeated the following day. The pupae were inoculated independently with the strains Bb6 and Bb10 by immersion, during three seconds for three consecutive times in a conidia suspension at a concentration of 10^8 conidia/ml in sterile water added with Tween 80 (0.05%). The immersion was carried out placing the pupae in a cylindrical plastic basket (Ø = 41 mm; h = 40 mm) and this one well as well placed in a 120 ml plastic cup containing the suspension.. Then, the pupae were placed on paper towel to drain the spare liquid, finishing its drying in a laminar flow cabinet. Once dried out, 15 pupae were placed in contact with the females of the parasitoid of each tube. In both bioassays, control treatment was carried out using immersed pupae as previously described, in sterile water with Tween 80 (0,05%) but without the fungus. After the first one and / or second day, according to the bioassay pupae were offered to the parasitoids. The bioassays were maintained in darkness at 26 ± 0.5°C and 50 ± 10% RH. The pupae were replaced daily until the death of all the females of the parasitoids.

Previous sexing, the parasitoids offspring and the emerged flies from non parasitoidized pupae were scored. For each female parasitoid, the following parameters were obtained: longevity, preoviposition period and the number of male and females descendants per day. Due to the difficulty of obtaining data about the parasitoid development inside the pupae of the host fly, the number of dead parasitoids in the preimaginal stage was estimated subtracting to the total of offered pupae, the number of emerged flies, the number of born parasitoids and the number of pupae dead naturally. This last number was obtained making observations on 50 pupae taken daily of the same massive rearing previously used for the two described bioassays.

The estimated populational parameters were: net reproductive rate (R_0) that represents the average total number of females per female produced in a generation; intrinsic rate of populational increase (r_m) that expresses the innate capability of increase in number per time unit, mean generational time (T), that is the mean longevity of a generation; finite rate of increase (λ), that is the number of times the population increases per time unit and duplication time (D) that represents the time that a population needs to be duplicated in number. The Standard Errors of Mean (SEM) of each studied populational parameters with α = 0.05, indispensable for the comparison among cohorts, were calculated by the "jackknife" method (Thomazini & Berti Filho 2000, La Rossa & Kahn 2003). The analysis of the demographic parameters, as well as the longevity of mature females of *S. endius* was carried out through ANOVA and Newman-Keuls test with α = 0.05, using the statistical software STATISTICA' 99 ed., Statsoft Inc., 1999.

The parasitoidism percentage was calculated for each strain and the control in both bioassays, assuming that the total number of parasitized pupae is the sum among the number of dead parasitoids in the preimaginal stage and the total of emerged parasitoids, and then this sum is divided by the total number of offered pupae. The analysis of the parasitism

percentages and emerged female parasitoids was carried out through common procedures used for the comparison among proportions (Pagano & Gauvreau 2001).

Results and Discussion

The demographic parameters of *S. endius* were not affected by the strains of *B. bassiana* when they were inoculated a single time, since differences with the control were not found (Table 1).On the other hand, when they were inoculated twice (Table 2) both strains influenced on the demography of the parasitoid, reducing the capability of populational increase. Although the generational time (T) was significantly smaller in the pupae treated with Bb6 strain, there were no differences in the intrinsic rate of natural increase (r_m) regarding to the treatment with the Bb10. The parameters (λ and D) showed the same tendency as r_m because they are mathematically related to this rate. The proportion of emerged females of *S. endius* was not affected by the fungus in both bioassays since significant differences with their respective controls were not observed ($\alpha = 0.05$) varying from 61% to 64% with one immersion and from 66% to 67% with two immersions.

In a previous study (La Rossa *et al.* 2002), the populational parameters of *S. endius* were obtained on pupae of *M. domestica*, being the r_m and R_0 0.119 and 24.54, respectively, smaller than those of the controls of the present assay. Thus, the remaining humidity caused by the immersions would be able to increase those rates. The parasitoidism percentage in the pupae treated with the fungus was significantly lower than that of the control with one or two immersions. In the first bioassay differences were observed among the two strains, showing the treatment with the strain Bb10, the lowest percentage (Fig. 1A). In the second bioassay both strains acted reducing the proportion of parasitoidized pupae with regard to the control (Fig. 1 B).The discrepancy between the parasitoidism percentages and the populational parameters, evidenced mostly in the bioassay with one immersion, is due to that in the first ones both sexes are computed in the observations while in the seconds only are involved the females and the descendant of the same sex. Thus, while the parasitoidism percentage showed differences among the treatments, the populational parameters were similar in the first bioassay.

Although *B. bassiana* would not directly affect *M. domestica* pupae the insertion of this fungus in confined animal production systems would help to control the adult flies (Lecuona *et al*. 2005b). The presence of *B. bassiana* conidia on the pupae of flies could produce significant reductions in the parasitoidism percentage without altering the populational parameters of *S. endius* with a single application (Bioassay 1).

Table 1. Demographic parameters and longevity of females of *S. endius* on pupae of *M. domestica* treated with two strains of *B. bassiana* with a single immersion (Bioassay 1).

Treatment	rm [1]	R0 [2]	T [3]	λ [4]	D [5]	Longevity [6]
Bb6	0.144 a (0.0028)	45.425 a (3.47)	26.38 a (0.101)	1.156 a (0.003)	4.792 a (0.095)	14.60 a (0.48)
Bb10	0.141 a (0.0029)	41.139 a (3.135)	26.34 a (0.145)	1.152 a (0.004)	4.914 a (0.096)	12.93 a (0.40)
Control	0.142 a (0.0028)	40.218 a (2.934)	26.08 a (0.177)	1.152 a (0.003)	4.894 a (0.095)	13.87 a (0.60)

[1]Intrinsic rate of natural increase; [2]Net reproductive rate; [3]Generational time (days); [4]Finite rate of increase (1/day); [5]Duplication time (days); [6]Adult longevity (days); [7]Standard error of mean. Values followed by the same letter in each column do not differ significantly (Newman-Keuls Test, $\alpha = 0.05$), n = 30.

Table 2. Demographic parameters and longevity of females of *S. endius* on pupae of *M. domestica* treated with two strains of *B. bassiana* with two successive immersions in a 24h interval (Bioassay 2).

Treatment	rm [1]	R0 [2]	T [3]	λ [4]	D [5]	Longevity [6]
Bb6	0.109 b (0.0044)	20.54 b (2.75)	27.86 b (0.415)	1.115 b (0.005)	6.389 b (0.257)	8.13 b (0.80)
Bb10	0.113 b (0.0044)	27.44 b (3.87)	30.41 a (0.437)	1.121 b (0.005)	6.086 b (0.233)	11.83 a (0.99)
Control	0.125 a (0.0033)	38.71 a (3.83)	29.15 a (0.176)	1.134 a (0.004)	5.527 a (0.146)	13.80 a (0.61)

[1]Intrinsic rate of natural increase; [2]Net reproductive rate; [3]Generational time (days); [4]Finite rate of increase (1/day); [5]Duplication time (days); [6]Adult longevity (days); [7]Standard error of mean. Values followed by the same letter in each column do not differ significantly (Newman-Keuls Test, $\alpha = 0.05$), n = 30.

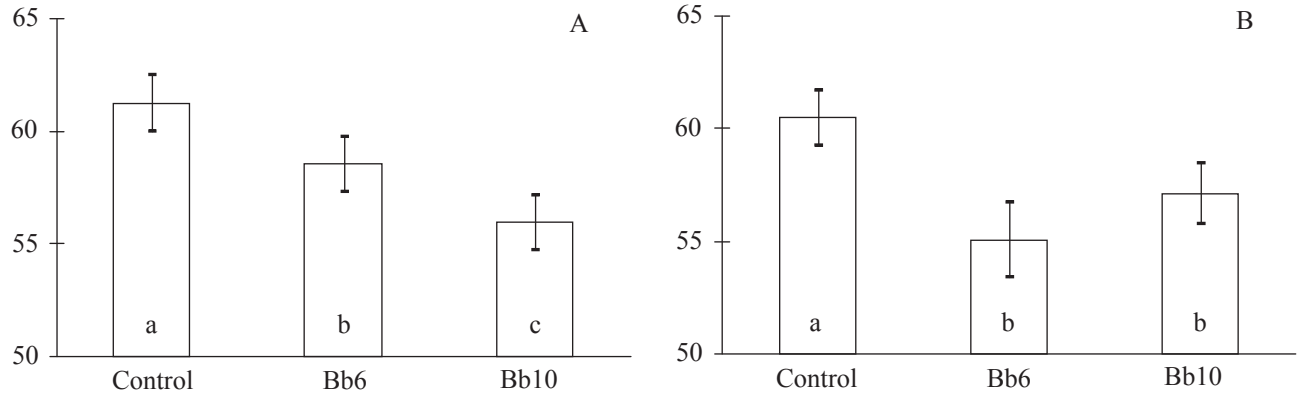

Fig. 1. Parasitoidism percentage of *S. endius* on pupae of *M. domestica* treated with two strains of *B. bassiana*, with a single immersion (A) and two immersions (B). Same letters on the columns indicate that there are not significant statistical differences among percentages. The outline at the top of columns indicates the 95% Confidence Interval range.

Geden *et al*. (1995) working with 13 strains of *B. bassiana* found that only two of them were slightly more virulent in adult flies that on the parasitoid *Muscidifurax abductor* Girault & Sanders. In the present study sporulation of *B. bassiana* was not observed in cadavers of *S. endius*, but the longevity of the adult parasitoids was significantly smaller with the strain Bb6 with two immersion treatments (Table 2). This could be indicating the presence of some factor that would affect the parasitoid-host relationship when the exposition is frequent. For the above mentioned and in accordance with Skovgård & Steenberg (2002), it would not be convenient to apply the fungus on the parasitoid rearing places. Anyway, the bioassays performed in the present work are considered under an extreme condition, since in future mycoinsecticide applications the aspersions would not be directed toward the places where there are pupae of flies (manure). Also, surely not liquid but solid formulations would be used in form of baits, minimizing the impact on the populations of *S. endius* and other pteromalids species used in biological control (Lecuona *et al*. 2005a).

Acknowledgments

To Luis Díaz and Mariana Turica for their technical assistance in the development of the present work and to Estela Favret for the bibliographical search and the help in its translation into English.

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Received 11/VIII/06. Accepted 07/II/07.