

## Microbial Control of *Musca domestica* (Diptera: Muscidae) with Selected Strains of *Beauveria bassiana*

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**ABSTRACT** Nineteen strains and species of entomopathogenic fungi were examined for their potential as biological control agents of the house fly, *Musca domestica* L. Bioassays against larvae, pupae, and adult males and females in small containers indicated five strains of *Beauveria bassiana* (Bals.) Vuill. were relatively more virulent. Three of the virulent strains, chosen for efficiency of production in a rice-based, diphasic fungal production system, were further evaluated by mixing spore-contaminated rice residue with sugar and tested as a food bait for adult flies in a 33-m<sup>3</sup> walk-in chamber. Up to 90% mortality was obtained within 15 d of exposure. The results indicate bait formulations of the most virulent and aggressive strain should be further developed and studied.

**KEY WORDS** *Musca domestica*, *Beauveria bassiana*, entomopathogenic fungi, biological control, fungus production

HOUSE FLIES, *Musca domestica* L., are well known poultry and livestock pests and are widespread vectors of human pathogens. Modern integrated pest management (IPM) programs combine biological, cultural, and/or chemical control to control populations of this pest (Morgan et al. 1975a, b; Rutz and Axtell 1981; Axtell 1986; Crespo and Lecuona 1996a, b; Crespo et al. 1998).

Although biological control of house fly is currently focused mostly on pupal parasitoids, entomopathogenic fungi are ubiquitous in nature and could be considered for manipulation in IPM programs. In this regard, Deuteromycetes (mitosporic fungi), which are frequently found attacking different pests in several ecosystems, may offer promise for development. Notably, *Beauveria bassiana* (Bals.) Vuill. and *Metarrhizium anisopliae* (Metch.) Sorok were reported as potentially useful control agents for house fly (Rizzo 1977; Steinkraus et al. 1990; Kuramoto and Shimazu 1992; Barson et al. 1994; Bywater et al. 1994; Watson et al. 1995, 1996; Renn et al. 1999).

Despite recent interest in mass-producing these entomopathogens for field application (Thomas et al. 1987; Jenkins et al. 1998), a mycoinsecticide for the biocontrol of house fly has not been developed, mostly due to lack of a reliable, efficient, and cost-effective way to produce candidate fungi (Nelson et al. 1996). Methods used thus far to produce these fungi try to maximize conidial yield. Compared with hyphae or blastospores, conidia are probably the most appropriate propagule for field release, due to their greater

stability under dry conditions (Soper and Ward 1981, Feng et al. 1994). Jenkins et al. (1998) described a diphasic fungus production system that used rice as a solid substrate. Conidia of the desired fungus are sieved from the rice substrate, but the solid rice residue also contains appreciable amounts of residual conidia.

The aims of the present experimental study were to select highly virulent strains of entomopathogenic fungi that may be used in the development of a mycoinsecticide against *M. domestica* and to evaluate the solid diphasic production residue as a bait formulation to kill house fly adults under simulated indoor conditions.

### Materials and Methods

***M. domestica* Culture.** Larvae of a strain maintained at Instituto de Microbiología y Zoología Agrícola-Instituto Nacional de Tecnología Agropecuaria (IMYZA-INTA) Castelar insectary since 1992 were selected from 5-d-old cohorts reared at 28 ± 1°C and 50 ± 10% RH on a diet of 40% wheat bran, 50% brewer's yeast, and 10% water. Third instars were separated by sieving and placed on dry maize flour in plastic trays held at 26 ± 1°C for two more days to allow pupation. Pupae weighing ≈22 mg were selected and housed in glass tubes sealed with cotton plugs until adults emerged, ≈5 d later. Adults were sexed by shape of compound eyes and genitalia. Only adults <12 h old were used in assays.

**Fungal Cultures.** Different strains of *B. bassiana*, *Paecilomyces fumoso-roseus* (Wize) Brown & Smith, and *Sporothrix* sp. Hektoen & Perkins ex Nicot & Mariot, were used in the experiment. Isolates were obtained from the INTA culture collection (Labora-

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Table 1. Strains of *B. bassiana* (Bb), *P. fumoso-roseus* (Pfr), and *Sporothrix* sp. (Sp) assays on adults of *M. domestica*

Strain	Origin	Site and year
Bb 1	<i>Diatraea saccharalis</i> (Lepidoptera: Pyralidae)	Pergamino, Argentina, 1990
Bb 4	<i>Phyrdenus muriceus</i> (Coleoptera: Curculionidae)	Chacras de Coria, Argentina, 1996
Bb 5	<i>D. saccharalis</i>	Lincoln, Argentina, 1990
Bb 6	<i>D. saccharalis</i>	9 de Julio, Argentina, 1990
Bb 10	<i>D. saccharalis</i>	Elortondo, Argentina, 1990
Bb 14	<i>Spilosoma virginica</i> (Lepidoptera: Lymantriidae)	Pergamino, Argentina, 1990
Bb 16	<i>S. virginica</i>	Pergamino, Argentina, 1990
Bb 20	<i>Doru lineare</i> (Dermaptera: Forficulidae)	Pergamino, Argentina, 1990
Bb 22	<i>D. lineare</i>	Pergamino, Argentina, 1990
Bb 29	<i>D. saccharalis</i>	Murphy, Argentina, 1990
Bb 38	<i>Nezara viridula</i> (Hemiptera: Pentatomidae)	Chapecó, Brazil, 1984
Bb 65	<i>N. viridula</i>	Oliveros, Argentina, 1992
Bb 67	<i>N. viridula</i>	Oliveros, Argentina, 1992
Bb 72	Soil	Oliveros, Argentina, 1994
Bb 80	Soil	Pergamino, Argentina, 1993
Bb 81	<i>Phyrdenus muriceus</i> (Coleoptera: Curculionidae)	Córdoba, Argentina, 1993
Bb 96	<i>Cyclocephala signaticolis</i> (Coleoptera: Scarabaeidae)	Balcarce, Argentina, 1996
Pfr 4	<i>M. domestica</i>	Brittany, France, 1984
Sp 1	<i>Dysdercus chaquensis</i> (Hemiptera: Pyrrhocoridae)	Anta Talavera, Argentina, 1988

torio de Hongos Entomopatógenos, IMYZA-INTA Castelar) and were originally isolated from soil or a variety of insect hosts, including *M. domestica* (Table 1).

Each fungal strain was cultured for one generation on larvae of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) and maintained in plates of complete agar medium (CAM), consisting of 0.4 g of  $\text{KH}_2\text{PO}_4$ , 1.4 g of  $\text{NaHPO}_4$ , 0.6 g of  $\text{SO}_4\text{Mg}$ , 1.0 g of KCl, 0.7 g of  $\text{NH}_4\text{NO}_3$ , 10 g of glucose, 15 g of agar, 5 g of yeast extract, and 1 liter of distilled water. Conidia were scraped from 2-wk-old plates incubated at  $26 \pm 0.5^\circ\text{C}$ . Viability was assessed by counting four replicates of 100 conidia incubated on CAM for 24 h. Spores were scored as viable if their germ tubes were at least as long as the width of the conidia. Only strains that showed viability higher than 98% were used in subsequent experiments.

**Bioassays against House Fly Adults and Immatures.** Assays with adults were conducted on cohorts of 20 males or 20 females housed separately in small, cylindrical cages (13 cm in diameter by 23 cm in height) made of plastic mesh and kept at a constant temperature of  $26 \pm 1^\circ\text{C}$ . Each cage contained a 10-cm petri dish lined with a piece of Whatman No. 1 filter paper that had been treated with 3 ml of a aqueous suspension of conidia of a chosen fungal culture ( $1 \times 10^8$  conidia/ml), and 10 g of sugar. Adults were transferred directly from the glass tubes to the cages to minimize manipulation. The flies were left in each cage for at least 2 d with water ad libitum, whereupon the water and treated papers were removed and replaced with cotton soaked with a solution of 80 g of sugar, 195 g of powdered milk, and 900 ml of water. The experiments were run in a completely randomized design with four replicates per treatment, including controls. Dead flies were removed, sorted by sex, and counted daily during 15 d of incubation.

Fungal virulence was indexed by the percentage of each sex that had died at 15 d, as estimated by the Kaplan-Meier method (Kaplan and Meier 1958). Mor-

tality data were arcsine square-root transformed before analysis. Mean 15-d mortality levels among treatments were analyzed with one-way analysis of variance (ANOVA), and compared with Tukey's test ( $\alpha = 0.05$ ). Fungal aggressiveness was indexed by mean life span (days to 50% survival) and was analyzed by one-way ANOVA and Tukey's test. Differences between sexes were compared by Student's *t*-test.

Assays with third instars were conducted on groups of 72-h-old larvae, 25 per group, arranged in a completely randomized design with five replicates of each chosen fungal strain. Each group was housed in a petri dish with lid lined with Whatman No. 1 filter paper that had been moistened with 1 ml of distilled water. Larvae were immersed for 1 s in an aqueous suspension of conidia of a chosen strain, adjusted to  $1 \times 10^8$  conidia/ml + Tween 80 (0.01%), and then placed on filter papers. Control larvae were dipped in 0.01% Tween 80 solution. Petri dish bases were then placed on their lids and sealed with Parafilm to retain moisture (Barson et al. 1994). Larvae were not provided with food. Dishes with larvae were incubated in the dark at  $26 \pm 0.5^\circ\text{C}$ , and mortality was monitored daily during 15 d. Subsequent adult emergence was recorded after 2 wk. Assays with pupae used the same methods as used with larvae.

**LC<sub>50</sub> Assessment with Selected Strains.** Five doses of conidia from the three most promising fungal strains grown on CAM were presented to sets of 25 adult flies of mixed sex and housed in small cages as described above. Doses were  $3$  and  $4.7 \times 10^5$ ,  $3$  and  $4.7 \times 10^6$ , and  $3 \times 10^7$  conidia/cm<sup>2</sup> and were applied to filter papers in aqueous solutions as described above. There were four replicates of each dose. Lethal concentrations required to kill one-half the adults (LC<sub>50</sub>) was estimated using probit analysis according to the procedure of Finney (1971) by using a probit analysis program written and donated to us by G. A. Milliken (Kansas State University, Manhattan, KS, 1989), and differences in LD<sub>50</sub> values between strains were

Table 2. Effects of selected strains of *B. bassiana* on mortality and longevity of female and male *M. domestica*

Strain	Mortality (%) <sup>a</sup>		Mean survival (d) <sup>b</sup>	
	Female	Male	Female	Male
Bb 72	97.2 (80.3 ± 3.7)a	97.9 (81.6 ± 3.9)ab	7.6 (0.8)ab	6.6 (0.7)a
Bb 10	92.9 (74.5 ± 2.8)ab	96.2 (78.8 ± 6.0)ab	7.9 (0.7)ab	6.5 (0.5)a
Bb 6	90.0 (71.5 ± 4.4)ab	98.0 (81.9 ± 3.2)a	6.9 (0.4)a	6.1 (0.2)a
Bb 96	89.0 (70.6 ± 4.7)b	90.1 (71.6 ± 4.1)b	9.0 (0.3)b	7.2 (0.8)a
Bb 4	87.7 (69.5 ± 4.5)b	96.4 (79.1 ± 5.6)ab	8.0 (0.8)ab	7.4 (0.9)a

No infections were observed in matching spore-free control cages. Means in columns followed by the same letter not significantly different (Tukey's test;  $\alpha = 0.05$ ).

<sup>a</sup> Backtransformed percentage mortality (and mean  $\pm$  SE on arcsine square-root scale). ANOVAs among strains:  $F = 4.54$ ;  $df = 1, 19$ ;  $P < 0.01$  (females) and  $F = 3.17$ ;  $df = 1, 19$ ;  $P < 0.05$  (males).

<sup>b</sup> Mean days ( $\pm$  SE) to 50% mortality (Kaplan–Meier method). ANOVAs among strains:  $F = 5.37$ ;  $df = 1, 19$ ;  $P < 0.01$  (females) and  $F = 2.54$ ;  $df = 1, 19$ ;  $P \approx 0.08$  (males).

judged to be significant if their respective 95% confidence intervals (CIs) did not overlap (Zhao et al. 1995).

**Productivity of Diphasic System for *B. bassiana*.** A diphasic, liquid-solid fungus production system (Jenkins et al. 1998) was developed to produce spores of three selected strains of *B. bassiana*. Briefly, 35 ml of a liquid medium (20 g of glucose, 20 g of yeast extract, 1 liter of distilled water) was poured into a 125-ml Erlenmeyer flask. The flask was plugged with cotton, covered with aluminum foil, and autoclaved for 30 min at 121°C. Once cool, the flask was inoculated with 1 ml of a spore suspension ( $1 \times 10^8$  conidia/ml) in 0.05% Tween 80. The liquid culture was then incubated on a rotary shaker at 200 rpm for 3 d at  $26 \pm 2^\circ\text{C}$ . This first phase of this procedure provided an inoculum of suspended mycelial fragments in active growth phase for transfer onto the solid substrate. The second, solid production phase was performed in autoclavable 30- by 60-cm bags containing 300 g of parboiled rice and 75 g of distilled water. After being autoclaved for 20 min at 121°C, they were cooled to room temperature and inoculated with 35 ml of the liquid inoculum, diluted into 40 ml of sterile distilled water. Once inoculated, the bags were incubated at  $26^\circ\text{C}$  for 2 wk.

For each strain, sporulated mycelia obtained from the solid substrate was dried for 7 d at  $26 \pm 1^\circ\text{C}$  in a 30% RH (DRYCLIM-27 dehumidifier, Carel, Padova, Italy) and then further dried for 5 d to  $\approx 12\%$  H<sub>2</sub>O in a desiccator with silica gel. Relative humidity was monitored with a HumiPro sensor ( $\pm 4\%$ ) attached to a data logger (LogIT SL 200 with PC software, DCP Microdevelopments and SCC Research, Norfolk, United Kingdom). Conidia were sieved from the solid substrate by shaking on 300- $\mu\text{m}$  mesh screen for 20 min. The separated conidial powder and remaining, spore-contaminated rice residue were stored separately at  $4^\circ\text{C}$  in sealed polypropylene bags.

A completely randomized design with three replicates of the three strains was used to compare conidial yields of the different strains. Yields of conidial powder of each strain were weighed and expressed as grams per kilogram of rice substrate. Conidia in the harvested powders were counted in an improved Neubauer chamber, and density was expressed as counts of conidia per gram of powder. Yields and

densities of the different strains were analyzed with one-way ANOVA, and means were compared by Tukey's test ( $\alpha = 0.05$ ).

Conidia viability was evaluated after 3-mo storage at  $4^\circ\text{C}$ . To measure viability, one mg of conidia per replicate was added to 10 ml of 0.01% sterile aqueous Tween 80. Viability was assessed by counting four replicates of 100 conidia incubated on CAM for 24 h at  $26 \pm 0.5^\circ\text{C}$ .

**Bioassays of Rice Residues from Production of Selected Strains.** An initial series of experiments tested the control potential of baits formulated with spore-contaminated rice residue used for mass production of three selected *B. bassiana* strains. Assays were conducted in cylindrical, plastic mesh cages (1.5 by 0.5 by 0.5 m), each containing 200 adults <12 h old. Baits were presented in two plastic trays (16 by 11 cm), and each tray contained 4 g of rice residue and 5 g of sugar. Doses of all strains were  $1.2 \times 10^9$  conidia/g of finished bait. A completely randomized design with three replicates per strain was used, for a total of 12 cages. During the 15 d of the experiment, temperature was  $26 \pm 1^\circ\text{C}$ , relative humidity was 80%, and dead adults were removed and counted daily.

A final series of larger scale experiments was performed in a 33-m<sup>3</sup> airtight room. For each assay, mixed sex cohorts of 2,000 adults <12 h old were released, and then a 0.24-m<sup>2</sup> plastic tray with food bait as described above was placed inside. Doses were equivalent to  $5 \times 10^{11}$  conidia/m<sup>2</sup>, temperature was  $26 \pm 2^\circ\text{C}$ , relative humidity was 80%, and water was provided ad libitum. Three replicates per fungal strain and spore-free controls were used, and mortality was recorded daily during 15 d.

## Results and Discussion

**Bioassays against House Fly Adults and Immatures.** Five of the original 19 fungal species and strains produced mortality rates that exceeded 85% (Table 2). In females, significant differences in virulence (percentage of mortality at 15 d) occurred among Bb 72 (97.2%), Bb 96 (89.0%), and Bb 4 (87.7%). In males, levels of virulence of Bb 6 (98%) and Bb 96 (90.1%) were significantly different from the remaining strains. Strain Bb 6 was most aggressive in females, yielding a

**Table 3. Probit analysis of virulence of three strains of *B. bassiana* for adult *M. domestica***

Strain	LC <sub>50</sub> (CI) <sup>a</sup>	Intercept	Slope	χ <sup>2</sup>	P <sup>b</sup>
Bb 72	5.2 by 10 <sup>9</sup> (3.7–6.8)a	2.944	1.197	5.37	0.147
Bb 10	122 by 10 <sup>9</sup> (67–314)b	4.444	0.511	1.84	0.606
Bb 6	174 × 10 <sup>9</sup> (100–373)b	4.169	0.669	5.09	0.165

Values followed by the same letter not different as judged by overlapping 95% CIs.

<sup>a</sup> LC<sub>50</sub>, number of conidia per square meter required to kill 50% of flies.

<sup>b</sup> Probability that observed mortality levels departed from linear response on probit scale, based on calculated χ<sup>2</sup>, df = 3.

mean life span significantly less than with strain Bb 96 (6.9 versus 9 d, respectively). Mean life spans of males with all five strains were statistically indistinguishable.

Significant differences in virulence against females and males were observed with Bb 4 (87.7 and 96.4% mortality, respectively, *P* < 0.04), and with Bb 6 (90.0 and 98.0%, respectively; *P* ≈ 0.01). Mean life spans also were different between females and males with Bb 6 (6.9 and 6.1 d, respectively; *P* ≈ 0.02), Bb 10 (7.9 and 6.5 d, respectively; *P* ≈ 0.02), and Bb 96 (9.0 and 7.2 d, respectively; *P* ≈ 0.01).

Bioassays with third instars and pupae indicated none of the five chosen fungal strains caused mortality greater than in untreated controls (data not shown). Steinkraus et al. (1990) reported 52–73% mortality in third instars infected by *B. bassiana*. Likewise, Watson et al. (1995) obtained mortality rates between 48 and 56% in second instars with high doses (10<sup>10</sup> conidia/ml); rates were negligible with lower doses. In contrast, we were unable to infect larvae and pupae with any of the five *B. bassiana* strains, and similar results were obtained by Geden et al. (1995). These contradictory outcomes may be the result of differences in strain virulence (Roberts and Yendol 1971, Lecuona et al. 1996), in assay methods, in conidial doses, in culture methods, or refractoriness to infection in the IMYZA-INTA house fly culture.

**LC<sub>50</sub> Assessment with Selected Strains.** Three of the five previously assayed strains were selected for further bioassay in small cages. These strains were Bb 6, 10, and 72 and caused the greatest levels of mortality among females. Quantitative bioassays indicated that strain Bb 72 had the lowest LC<sub>50</sub> and the greatest slope (Table 3). This finding was consistent with the earlier small cage assays (Table 2) that indicated strain Bb 72 was more virulent than strains Bb 10 and Bb 6.

**Productivity of Diphasic System for *B. bassiana*.** Yields of conidial powder of strain Bb 72 in the diphasic system averaged 104.3 g/kg rice and greatly exceeded yields of the other two strains (Table 4). Furthermore, the powder contained 6.8 × 10<sup>12</sup> conidia/g. The observed level of productivity with this strain was of the same order of magnitude as reported by Bartlett and Jaronski (1988) and Feng et al. (1994). In subsequent experiments, food baits made with the spore-contaminated rice residue contained 0.56 ± 0.04 × 10<sup>9</sup> conidia/g rice of Bb 6, 1.34 ± 0.07 × 10<sup>9</sup> conidia/g rice of Bb 10, and 2.74 ± 0.01 × 10<sup>9</sup> conidia/g rice of Bb 72.

**Table 4. Productivity of three strains of *B. bassiana* in biphasic production system with rice substrate, and bioassays of baits formulated with spore-contaminated rice residues**

Strain	Production quantities		Bioassay	
	Powder yield g/kg rice	Conidial density (no./g powder)	Mortality <sup>a</sup>	Longevity (d) <sup>b</sup>
Bb 72	104.3 (9.9)a	6.63 (0.70) × 10 <sup>10</sup> a	97.7 (0.4)a	8 (0.07)
Bb 10	30.3 (1.8)b	2.59 (0.14) × 10 <sup>10</sup> b	64.5 (8.5)b	11 (0.15)
Bb 6	35.0 (2.9)b	5.33 (1.15) × 10 <sup>10</sup> b	97.0 (1.3)a	9 (0.08)

<sup>a</sup> Mean ± SE (%) males and females dead after 15-d incubation with rice residue bait.

<sup>b</sup> Mean number of days lived ± SE, by Kaplan–Meier method.

**Bioassays of Rice Residues from Production of Selected Strains.** The strains Bb 6 and Bb 72 caused mortality rates >97% and mean longevities were 8 and 9 d, respectively (Table 3). In contrast, Bb10 caused only 64.5% mortality and the flies lived substantially longer. Geden et al. (1995) obtained a mortality rate of 94% with *B. bassiana* by the ninth day after infection during a 2-h confinement time of flies on plywood boards (0.1 m<sup>2</sup>) treated with a liquid formulation of 10<sup>7</sup> conidia/cm<sup>2</sup>. Using food baits (sugar + milk), the same author achieved a mortality rate of 96% on the ninth day after infection with a dose of 10<sup>8</sup> conidia/g bait, and 90% on the seventh day after infection with a higher dose (10<sup>9</sup> conidia/g). The doses of the present three strains were equivalent to the highest dose used in Geden et al. (1995), although our fly/volume ratio was lower (1 fly/1,875 cm<sup>3</sup> and 1 fly/40 cm<sup>3</sup>, respectively). The latter difference could explain the discrepancies between the two studies. Rizzo (1977) reported a shorter mean longevity of 4.7 d in small test tubes with much greater fly/volume ratio. Similarly, Watson et al. (1995) reported a 99% mortality rate, which may have been enhanced by crowding of flies on treated surfaces in treated petri dishes.

In view of the results in both the bioassays and diphasic production, strain Bb 72 was selected for the larger scale experiment in the airtight room. Although bioassays with this strain indicated its virulence was equivalent to that of Bb 6, yields of strain Bb 72 conidia were much greater in the biphasic production system, which may be a clear advantage for future mycoinsecticide production.

Assays in the airtight room produced an average mortality rate of 92.4 ± 3.8% and a mean longevity of 9 d. These levels of virulence and aggressiveness were similar to those seen earlier, despite the great difference in density of flies per square meter. Notwithstanding the higher survival rates compared with the study of Rizzo (1977), mortality rates in our study were similar to those obtained by Kuramoto and Shimazu (1992), Barson et al. (1994), and Renn et al. (1999).

All in all, the virulence of Bb 72 against adult *M. domestica*, together with its effectiveness in conidial production, indicates this strain has promise in future mycoinsecticidal development. Furthermore, it is evident that both the conidial powder and the coarser rice residue from the biphasic production sys-

tem are infectious. Both products have roughly equal production costs. The present results indicate that it may be feasible to control house fly in confined spaces that lack fly breeding media. It remains to be seen whether similar levels of infection can be created in larger, more realistic spaces such as warehouses and poultry farms.

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