

The pathogenicity of *Metarhizium anisopliae* and *Beauveria bassiana* for adults of the Japanese beetle, *Popillia japonica* (Coleoptera: Scarabaeidae)

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Abstract. Conidia of *Metarhizium anisopliae* and *Beauveria bassiana* were evaluated for activity against adults of the Japanese beetle, *Popillia japonica*, under laboratory conditions. The LC₅₀ values 7 days after exposure to *M. anisopliae* and *B. bassiana* were 0.7 and 0.026 mg of conidia/100 adults, respectively at 22–24°C. A sharp increase in mortality was observed 3 and 4 days after treatment with either 10 mg of *B. bassiana*/100 adults or 10 mg of *M. anisopliae*/100 adults, respectively. The LT₅₀ values at 10 mg/100 beetles for *M. anisopliae* and *B. bassiana* were 4.2 and 3.1 days, respectively. Onset of mortality was further delayed at lower dosages of both fungi. Mortality of adults that were exposed to beetles treated with 10 mg of conidia of *M. anisopliae*/100 adults immediately following application of conidia or 24 hrs posttreatment was an average of 60 and 43% cumulative mortality, respectively, within 9 days of exposure. Similar exposures of healthy adults to beetles that had been treated with 10 mg of *B. bassiana* conidia/100 adults resulted in an average of 79 and 43% cumulative mortality, respectively. Beetles killed either by *M. anisopliae* or *B. bassiana* and subsequently placed on damp soil manifested signs of patent infection with the fungi, with subsequent production of conidia. The infectivity of *M. anisopliae* and *B. bassiana* for adult Japanese beetles and the delay in mortality following treatment provide potential for dispersal of these entomopathogens within populations of *P. japonica*.

INTRODUCTION

Epizootics of the entomopathogenic fungi *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria* spp. are reported from several scarab species and other soil inhabiting Coleoptera (Fleming, 1968; Hurpin & Robert, 1972; Gruner, 1973; Young, 1974; Glare, 1992). The most successful use of fungi against scarabs is exemplified in field trials and control programs using *B. brongniartii* (Sacc.) Petch against the European cockchafer, *Melolontha melolontha* L. (Ferron, 1967; Hurpin & Robert, 1972; Keller et al., 1986, 1989; Zimmermann, 1992). Control in certain localities in Europe has been achieved using aerial application of blastospores to aggregations of the adult beetles (Keller et al., 1989; Zimmermann, 1992). Besides direct mortality of the adults, the larval habitat was contaminated by the diseased beetles, principally ovipositing females, that died in the soil. Subsequent transmission to and mortality of larvae have resulted in reduction of *M. melolontha* populations.

Investigations on the effects of certain Hyphomycetes on the Japanese beetle, *Popillia japonica* Newman, also reveal potential use of these fungi for the control of this species. Preliminary studies on the activity of *Beauveria bassiana* (Bals.) Vuill. against adult beetles demonstrated a high level of mortality following topical application of conidia

(Rex, 1940). Quantitative assessment of the efficacy of the fungus, however, was not performed. The efficacy of *M. anisopliae* used as a microbial larvicide of the beetle is summarized by Fleming (1968). More recent evaluation of this fungus reveals fair potential as a pathogen of Japanese beetle larvae (Martins, 1988; Krueger et al., 1991, 1992).

Since the introduction of Japanese beetle onto Terceira Island (Azores, Portugal) in the early 1970s it has spread into the majority of available habitats on the island and, in the absence of a complex of natural enemies, has steadily increased in numbers (Martins et al., 1988). One of the few native entomopathogens found in association with *P. japonica* on the island is *M. anisopliae* (Martins, 1988). Though ubiquitous, the natural incidence of mycosis due to *M. anisopliae*, under most conditions, is not sufficiently high to reduce the population of *P. japonica* on a large scale. Incidence ranges from an average of <1% to higher than 8% in warmer months (A. Martins, personal communication). Research on the use of *M. anisopliae* for control of *P. japonica* thus far conducted on Terceira has focused on augmentation of the fungus through application of mass produced conidia to larval habitats (Martins, 1988). The objective of our studies was to provide background on the pathogenic effects of *M. anisopliae* and *B. bassiana* on adult beetles.

MATERIALS AND METHODS

The strain of *M. anisopliae* used in our bioassays was isolated from infected larvae of *P. japonica* on Terceira Island (isolate ARSEF 3329, Entomopathogenic Fungus Culture Collection, USDA-ARS, Plant Soil and Nutrition Laboratory, Ithaca, NY). Conidia were produced at the Department of Biology, University of the Azores in São Miguel using the methods of Goettel (1984). The strain of *B. bassiana* was originally isolated from *Cydia pomonella* (L.) and furnished by Dr. Emélia Frazão, Centro Nacional de Protecção da Produção Agrícola, Oeiras, Portugal. Conidia of this strain of *B. bassiana* were produced at the Japanese Beetle Control Project Lab on Terceira Island on Sabouraud dextrose agar, harvested with a rubber spatula, placed in an incubator at 30°C overnight to remove excess moisture and then passed through a 250 µm sieve. Spore counts of both conidial preparations were made by direct counts of a 10⁻⁶ dilution in a hemacytometer. Spore viability was determined by plating 100 µl of the conidial suspensions on Sabouraud dextrose agar and counting colonies after 48 hrs. The spore counts of the *M. anisopliae* and *B. bassiana* preparations were ca 2 × 10⁷ and 3.6 × 10⁷ conidia/mg, respectively. Both preparations were ca 70% viable at the time of testing.

Adult *P. japonica* were collected using Japanese beetle traps (Trécé, Inc., Salinas, CA) (Klein & Edwards, 1989) baited with the floral attractant, phenethyl propionate and eugenol (3:7) (Ladd et al., 1976). Traps were placed in the field at mid-morning and the beetles removed before 4 P.M. Prior to bioassays of the fungi the adults were briefly held under laboratory conditions in 50 × 50 × 50 cm screen cages and provided fresh blackberry and/or grape leaves.

Conidia of *M. anisopliae* were bioassayed against adult beetles by placing 100 individuals in each of six 140 ml polystyrene specimen cups. The screw caps on the cups were perforated to provide ventilation. Five dosages of conidia ranging from 0.5 to 10 mg/conidia/100 beetles were used for bioassay. Conidia for each dosage were weighed out on a Mettler balance and added on top of the beetles in each of 5 cups. The sixth cup was used as a control. The cups were then periodically rotated end-over-end during the following hour to help distribute the inoculum. Constant movement of the beetles in the cups also aided in the even distribution of the conidia. One hour after application of conidia to the cups, 30 beetles from each dosage were removed and divided among 3 holding cages. The cages consisted of 950 ml plastic containers, and the lids were perforated with air holes. Dental wick and 100 ml water reservoirs under each cage were used to provide free water and to maintain high humidity. The wicks protruded ca. 5 cm into the cages through holes cut in the bottom of each cage. Approximately 10 blackberry (*Rubus* sp.) leaves were added to each cage following exposure. The beetles were held at 22–24°C and leaves were changed and mortality was assessed daily for up to 8 days. Four replicate tests were conducted on each of 4 separate dates.

Exposures to conidia of *B. bassiana* and post exposure handling of beetles were carried out in the same manner as that of the *M. anisopliae* bioassays except that three replicate tests were conducted on each of 3 separate dates and mortality was assessed daily for up to 9 days. Initially the same dosages of conidia as for *M. anisopliae* were used for evaluation of adulticidal activity of *B. bassiana*. These and all dosages above 0.1 mg/100 adults resulted in 100% mortality within 7 days. Eventually 3 lower dosages were used for bioassays and calculation of LC_{50} (0.01, 0.05 and 0.1 mg/100 adults). In order to weigh inoculum more precisely for the 3 lower dosages, conidia were mixed with talc at a ratio of 990 mg of talc to 10 mg of conidia. Adults in the controls received the same amount of talc as the maximum received by the treated beetles (10 mg/100 beetles).

Beetles that died following exposure to either *M. anisopliae* or *B. bassiana* were transferred to 0.95 l plastic tubs containing 500 g of moistened sterilized soil (8.5 kg soil: 1.5 l water) and observed after 1 week at 22–24°C for signs of conidial production.

An experiment was designed to study the effects of contamination of untreated beetles by beetles that had received 10 mg of conidia of either fungus/100 beetles. Prior to treatment with a given fungus, 2 lots of 60 beetles each were marked with different colors of nail enamel by placing a small dot at the distal end of the right elytron. Unmarked beetles were treated with 10 mg of *M. anisopliae* per 100 individuals. Immediately following the 1 hr exposure period, 30 beetles were removed and divided into 3 groups of 10 beetles each and placed in the holding cages described above. A control group of 30 untreated beetles was also placed in 3 holding cages with *Rubus* leaves. Immediately afterward, 10 marked and untreated beetles were placed in each of the six cages. The cages were kept at 22–24°C. Twenty-four hrs following application of fungus, 10 beetles from the second color group of marked and untreated beetles were placed in each of the cages. Leaves were changed daily and mortality was assessed after 1 week and again 9 days after the experiment was started. Excessively high mortality in the control beetles after 9 days, particularly in those that had been painted, precluded further observations. Four replicate tests were conducted on each of 4 separate dates. The same protocol was followed for studying the effects of contamination with *B. bassiana*, except that 3 such assays were conducted on 3 separate dates.

Probit analyses were conducted on the bioassay data (total mortality 7 days after initial exposure) after correction for control mortality using Abbott's formula followed by log transformation. Data from the experiments on contamination of untreated beetles with fungi from treated beetles were analyzed using ANOVA and Tukey's studentized multiple range test after arcsine-square root transformations on the percentage mortality.

RESULTS

Daily observation of the beetles treated with either fungus revealed a dosage related delay in onset of mortality (Figs 1 and 2). At the highest dosage for both species, 10 mg of

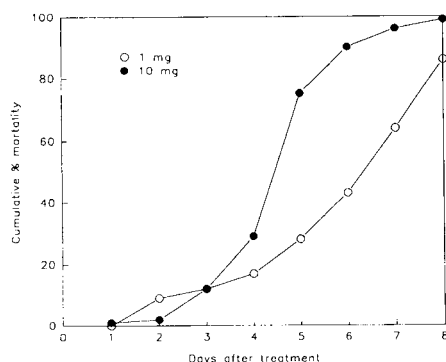


Fig. 1. The effect of dosage (in mg conidia per 100 adults) of *Metarhizium anisopliae* on the mortality of adults of *Popillia japonica* over time.

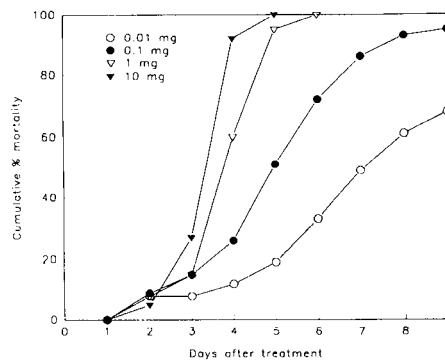


Fig. 2. The effect of dosage (in mg conidia per 100 adults) of *Beauveria bassiana* on the mortality of adults of *Popillia japonica* over time.

conidia/100 adults, there was a sharp increase in mortality 4 and 3 days after treatment with *M. anisopliae* and *B. bassiana*, respectively. The LT_{50} values at 10 mg/100 beetles for *M. anisopliae* and *B. bassiana* were 4.2 and 3.1 days, respectively. Onset of the majority of mortality was further delayed at lower dosages (Figs 1 and 2).

The effect of dosage on mortality 1 week following application of fungi is presented in Tables 1 and 2 for *M. anisopliae* and *B. bassiana*, respectively. The LC_{50} values after 7 days for *M. anisopliae* and *B. bassiana* were 0.7 and 0.026 mg of conidia/100 adults, respectively. Additional mortality was observed after 1 week for the lower dosages of both fungi (Figs 1 and 2).

TABLE 1. Effect of dosage on pathogenicity of *Metarhizium anisopliae* for *Popillia japonica* (22–24°C).

dosage (mg/100 adults)	% mortality \pm s. e.
control	8 \pm 1.11
0.5	43 \pm 12.21
1.0	64 \pm 3.31
2.5	84 \pm 6.24
5.0	90 \pm 4.07
10	96 \pm 2.84

LC_{50} = 0.7 mg conidia/100 adults (95% C. I. 0.5–0.9)

R value = 0.996

Probit analysis was conducted on the bioassay data after correction for control mortality using Abbott's formula followed by log transformation. Four replicate tests conducted on each of 4 separate dates. Total mortality was assessed 7 days after initial exposure.

TABLE 2. Effect of dosage on pathogenicity of *Beauveria bassiana* for *Popillia japonica* (22–24°C).

dosage (mg/100 adults)	% mortality \pm s. e.
control	17 \pm 2.03
0.01	49 \pm 16.17
0.05	62 \pm 12.99
0.1	76 \pm 13.21
1.0	100

LC_{50} = 0.026 mg conidia/100 adults (95% C. I. 0.014–0.040)

R value = 0.96

Probit analysis was conducted on the bioassay data after correction for control mortality using Abbott's formula followed by log transformation. Three replicate tests conducted on each of 3 separate dates. Total mortality was assessed 7 days after initial exposure.

All of the beetles that were killed with either *M. anisopliae* or *B. bassiana* and subsequently transferred to tubs containing damp soil manifested signs of patent infection with the fungi with subsequent production of conidia.

The mortality of adults that were exposed to beetles that had been treated with 10 mg of conidia/100 adults of *M. anisopliae* or *B. bassiana* immediately following application of conidia or 24 hrs posttreatment is presented in Tables 3 and 4, respectively. The untreated beetles that were exposed to fungus-treated beetles immediately after application of the fungus responded with significantly higher mortality than the control groups. There was

significantly less mortality in the groups that were exposed to *Beauveria*-treated beetles 24 hrs posttreatment. When the beetles were held for an additional 2 days the mortality of the beetles that had been contaminated increased considerably.

TABLE 3. Effect of contamination on healthy adult Japanese beetles placed in contact with individuals treated with *Metarhizium anisopliae* (10 mg/100 beetles) shortly after or 24 hrs after treatment.

Treatment group	% mortality \pm s.e.	
	1 week	9 days
Control group 1	9 \pm 3.08 a	13 \pm 5.24 a
control group 2 (painted)	12 \pm 4.15 a	20 \pm 7.51 ab
control group 3 (painted)	18 \pm 2.84 ab	20 \pm 4.04 ab
contaminated 24 hrs later	20 \pm 5.82 ab	43 \pm 7.13 bc
contaminated 1 hr after treatment	45 \pm 6.18 b	60 \pm 5.13 c
<i>Metarhizium</i> treated	96 \pm 2.53 c	100 d

Means followed by the same letter in the same column are not significantly different at the 0.05 level as determined by Tukey's studentized multiple range test. Four replicate tests conducted on each of 4 separate dates.

TABLE 4. Effect of contamination on healthy adult Japanese beetles placed in contact with individuals treated with *Beauveria bassiana* (10 mg/100 beetles) immediately following treatment or 24 hrs after treatment.

Treatment group	% mortality \pm s.e.	
	1 week	9 days
Control group 1	9 \pm 4.02 a	12 \pm 3.82 a
control group 2 (painted)	10 \pm 1.73 a	13 \pm 2.78 a
control group 3 (painted)	15 \pm 1.15 a	19 \pm 1.50 ab
contaminated 24 hrs later	25 \pm 3.17 a	43 \pm 8.29 b
contaminated 1 hr after treatment	56 \pm 10.05 b	79 \pm 11.92 c
<i>Beauveria</i> treated	96 \pm 2.10 c	100 d

Means followed by the same letter in the same column are not significantly different at the 0.05 level as determined by Tukey's studentized multiple range test. Three replicate tests conducted on each of 3 separate dates.

DISCUSSION

Most of the efficacy studies on entomopathogenic fungi for the microbial control of the Japanese beetle have been conducted on larval stages. Research by Martins (1988) and Krueger et al. (1991, 1992) revealed the potential for augmentative or inundative treatments with *M. anisopliae* for the control of *P. japonica* larvae. Despite the adulticidal activity of *B. bassiana*, research on the larvicidal activity for *P. japonica*, however, revealed negligible potential as a microbial control agent of the Japanese beetle (Rex, 1940).

Despite occasional epizootics of *M. anisopliae* in larval populations, the capacity of this fungus to disperse appears to be limited. The infectivity of *M. anisopliae* and *B. bassiana* for adult Japanese beetles and the delay in mortality following treatment indicate good potential for dispersal of these entomopathogens within *P. japonica* populations. The sexual and aggregative behavior of the beetle (Fleming, 1972; Iwabuchi & Takahashi, 1983), and

the transfer of conidia from treated beetles to other adults observed in our studies, would further enhance dissemination as well as augmenting natural infection levels. The subsequent production of conidia in adult beetle cadavers indicates the potential of autodissemination by augmenting the number of fungal spores in the soil or for the introduction of exotic efficacious strains into larval habitats. This optimal combination of infectivity for both adults and larvae of *M. melolontha* by the same fungus is seen with *B. brongniartii* (Keller et al., 1989; Zimmermann, 1992).

Trapping techniques for the Japanese beetle have become more efficient due to improvements in trap design and development of pheromone and floral lures with increased attractancy (Klein, 1981). Effective trapping technology could provide a means of infecting large numbers of adult beetles with a limited supply of inoculum. Beetles could be collected en masse with subsequent application of conidia before they are released or they could be automatically treated with the naturally adhering conidia as they pass through modified Japanese beetle traps. Both of these methods and the possibility for augmenting natural levels of *M. anisopliae* through treatment of and dispersal via adult *P. japonica* warrant further investigation.

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