Pathogenicity of *Metarhizium anisopliae* and *Beauveria bassiana* against *Phyllophaga crinita* and *Anomala flavipennis* (Coleoptera: Scarabaeidae)[1]

L. A. Rodríguez-del-Bosque,[2] F. Silvestre,[3] V. M. Hernández,[4] H. Quiroz[3] and J. E. Throne[5]

Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias Campo Experimental Río Bravo. Apartado Postal 172, Río Bravo, Tam., México 88900

J. Entomol. Sci. 40(1): 67-73 (January 2005)

Abstract Five isolates of *Beauveria bassiana* (Balsamo) Vuillemin and 3 isolates of *Metarhizium anisopliae* (Metschnikoff) Sorokin were tested against third-instar larvae of *Phyllophaga crinita* (Burmeister) and *Anomala flavipennis* Burmeister under laboratory conditions using the "maximum challenge test" protocol. The *M. anisopliae* strains were more virulent than the *B. bassiana* strains, with the isolates MAGL3N and MAGL4N of *M. anisopliae* causing the highest mortality in both white grub species. Regardless of scarab species, mortality caused by MAGL3N was >63% after 4 days and >96% after 10 days. The other strain of *M. anisopliae,* MAGC2N, also caused high mortality in *A. flavipennis,* but at a slower rate than MAGL3N. Median lethal time (LT_{50}) for MAGL3N was 2.9 days for *P. crinita* and 3.0 d for *A. flavipennis.* The LT_{50} for MAGL4N was 5.3 d for *P. crinita* and 7.6 d for *A. flavipennis* while the LT_{50} for MAGC2N was 4.4 d for *A. flavipennis. Metarhizium anisopliae* is a potential biological control agent for *P. crinita* and *A. flavipennis* and should be further investigated for possible development.

Key Words Biological control, entomopathogens, white grubs, *Metarhizium anisopliae, Beauveria bassiana, Phyllophaga crinita, Anomala flavipennis,* entomogenous fungi, laboratory bioassays

The white grubs *Phyllophaga crinita* (Burmeister) and *Anomala flavipennis* Burmeister (Coleoptera: Scarabaeidae) are the most important soil insect pests of maize and grain sorghum in northeastern Mexico (Rodríguez-del-Bosque et al. 1995). *Phyllophaga crinita* also causes severe damage to agricultural crops, forage grasses and turfgrass in Texas, U.S.A. (Teetes 1973, Huffman et al. 1976, Merchant and Crocker 1995). *Anomala flavipennis* causes economic damage to several crops, including maize, wheat, potato and grasses in Kansas (Hayes and McColloch 1924).

Historically, control measures for white grubs have depended mainly on conventional chemical insecticides. Microbial control of scarab larvae with the bacterium *Bacillus popillae* Dutky has been commercially available for decades, and entomog-

[1]Received 20 February 2004; accepted for publication 05 July 2004.
[2]Address inquiries (email: rodriguez.luis@inifap.gob.mx).
[3]Facultad de Ciencias Biológicas, U.A.N.L. Ciudad Universitaria, San Nicolás de los Garza, N.L., México.
[4]Centro Nacional de Referencia de Control Biológico, DGSV-SAGARPA, Apartado Postal 133, Tecomán, Col., México.
[5]USDA-ARS Grain Marketing and Production Research Center, 1515 College Avenue, Manhattan, KS 66502.

enous nematodes have become commercially available recently (Tanada and Kaya 1993). However, use of these alternative control technologies is limited (Jackson 2003). The entomopathogenic fungi *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) are potentially important microbial control agents of soil insects (Poprawski and Yule 1991, Glare 1992, Villani et al. 1994, Schwarz 1995, Flores et al. 2002, Hernández et al. 2003, Jackson 2003). Currently, *M. anisopliae* and *B. brongniartii* (Saccardo) Petch are commercially labeled for white grubs in Australia and Europe, respectively (Keller 1992, Milner 1992, Jackson 1993, 2003). The objective of this study was to determine the pathogenicity of *M. anisopliae* and *B. bassiana* strains against *P. crinita* and *A. flavipennis* larvae under laboratory conditions.

Materials and Methods

Five isolates of *B. bassiana* and 3 of *M. anisopliae* (Table 1) from the entomopathogenic fungi collection of the Centro Nacional de Referencia de Control Biológico, DGSV-SAGARPA (Tecomán, Colima, México) were propagated on Sabouraud's dextrose agar (Goettel and Inglis 1997) in Petri dishes (90 × 15 mm) for 16 d at 27 ± 1°C. After incubation, conidia were obtained by scraping the fungal mass from the surface of the culture medium and mixed with 50 g of Celite 289® (diatomaceous earth, Mexico, D.F.). Conidia concentration in the fungus-Celite mixture was determined for each isolate with a Neubauer chamber (Goettel and Inglis 1997). Then, Celite was added as needed in order to standardize mixtures at 1.7×10^9 conidia/g in all isolates. Conidia-Celite mixtures were stored in paper bags for 6 d until the experiment began.

A total of 300 third-instar larvae each of *P. crinita* and *A. flavipennis* was field-collected (Campo Experimental Río Bravo [INIFAP] near Río Bravo, Tamaulipas, Mexico), placed individually in 30-ml plastic cups containing soil and covered with

Table 1. Origin of isolates of the entomopathogenic fungi tested against *Phyllophaga crinita* and *Anomala flavipennis*

Fungus	Isolate code	Host	Origin
Beauveria bassiana	BBGC1	*Phyllophaga* sp.	State of Colima*
	BBGC4	*Phyllophaga* sp.	Ocotlán, Jalisco
	BBGC6	*Phyllophaga* sp.	Ocotlán, Jalisco
	BBGC7	*Phyllophaga* sp.	Ocotlán, Jalisco
	BBGN3	*Galleria mellonella***	Tepic, Nayarit
Metarhizium anisopliae	MAGC2N	*Phyllophaga lalanza*	Tepic, Nayarit
	MAGL3N	*Galleria mellonella***	Tepic, Nayarit
	MAGL4N	*Galleria mellonella***	Tepic, Nayarit

* Unknown locality.

** Not a natural host, but used as a bait host for detection of entomopathogenic fungi in soil (Zimmerman 1986).

plastic lids, transported to the laboratory, and maintained at 25 ± 1°C for 3 d before the bioassay. *Phyllophaga crinita* and *A. flavipennis* larvae were differentiated by raster characteristics and anal opening shape (Ritcher 1966).

A laboratory bioassay was conducted to determine the virulence of the fungi on *P. crinita* and *A. flavipennis*. For each white grub species, 9 treatments (8 isolates and a check) with 30 larvae each were arranged in a completely random design. Field-collected larvae were surface-sterilized with a 2% NaCl solution, washed with distilled water, and placed on paper towels to eliminate excess water. The bioassay followed the "maximum challenge test" methodology, useful for separating virulent from non-virulent strains at the early stages of entomopathogen screening programs (Milner 1992, Shannon et al. 1993). The fungus-Celite mixtures of each isolate were placed separately in 150-ml plastic cups. Using sterile forceps, each larva was placed in the cup and rolled over the fungus-Celite mixture for maximal exposure for 5 s. After treatment, larvae were placed individually in 30-ml cups with a 1-cm^3 piece of carrot as a food source. Distilled water (1-ml) was sprayed into the cup before covering with a plastic lid. The check treatment (no fungus application) only included the carrot piece and was sprayed with water. Larvae were maintained at 25 ± 1°C, and mortality was determined every 48 h for 18 d. Larvae were considered dead when no response was observed after touching the grub on the thoracic segments with a probe.

Linear regression models ($y = a + bx$, where y = cumulative number of dead larvae; a = intercept; b = slope; and x = days after treatment) and median lethal time (LT_{50}) were estimated by probit analysis, using log-probit transformation (Throne et al.

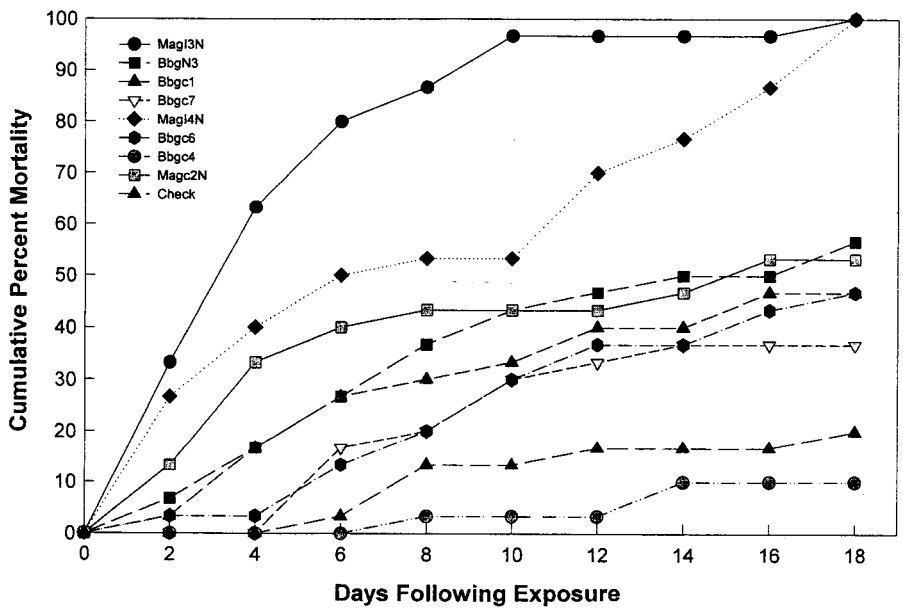

Fig. 1. Percent cumulative percent mortality of *Phyllophaga crinita* in response to strains of *Metarhizium anisopliae* and *Beauveria bassiana*.

1995). Intercepts, slopes and LT_{50}'s were compared ($P < 0.05$) among only those isolates killing at least 75% of the grubs as recommended by Robertson and Preisler (1992).

Results and Discussion

The fungal isolates showed differential pathogenicity against both scarab species (Figs. 1, 2). Regardless of isolates, mortality of *A. flavipennis* in response to the fungal treatments was generally greater than that of *P. crinita.* This suggests a differential susceptibility to the fungi between the 2 scarab species. In general, the *M. anisopliae* strains were more virulent than those of *B. bassiana,* corroborating other studies with *Phyllophaga* spp. (Poprawski and Yule 1991, Shannon et al. 1993, Flores et al. 2002). In both white grub species, the strains MAGL3N and MAGL4N of *M. anisopliae* caused the highest mortality levels, resulting in the greatest slopes (*b*) in the regression models (Table 2). Mortality caused by MAGL3N after 4 d postexposure was 63% for *P. crinita* and 73% for *A. flavipennis.* Regardless of white grub species, mortality by MAGL3N was >96% at 10 days following exposure.

The strain MAGL4N also caused high mortality in both white grubs, but the level was lower after 4 and 10 days than that caused by MAGL3N (Figs. 1, 2). The LT_{50} for MAGL4N was twice that of MAGL3N for *A. flavipennis.* The isolate MAGC2N was also effective, but only against *A. flavipennis.* The *B. bassiana* strains showed poor-to-medium pathogenic activity against both scarab species. The response to *B. bassi-*

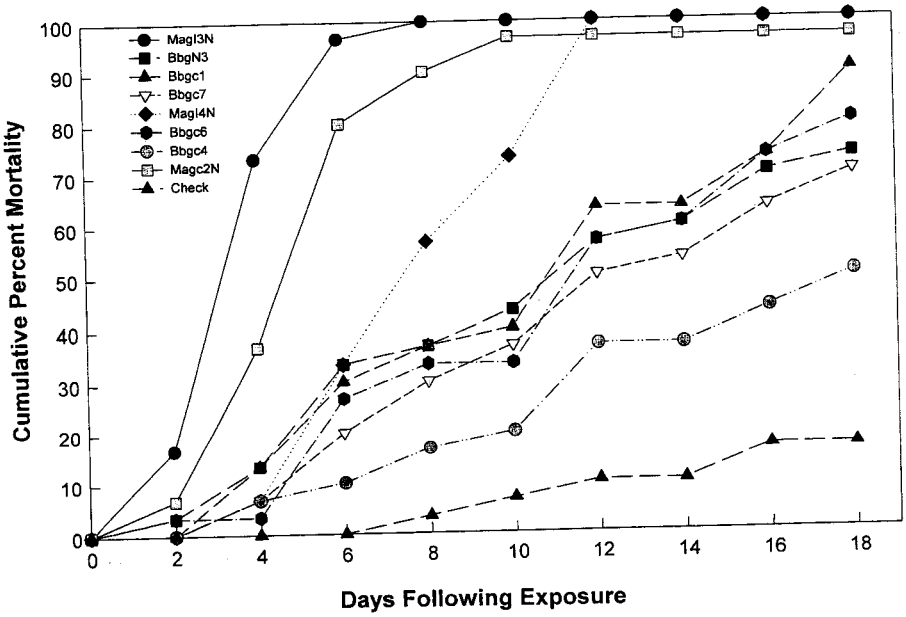

Fig. 2. Percent cumulative percent mortality of *Anomala flavipennis* in response to strains of *Metarhizium anisopliae* and *Beauveria bassiana.*

Table 2. Comparison of intercepts, slopes and mean lethal times (LT_{50}) among isolates killing at least 75% of *P. crinita* and *A. flavipennis* larvae

	P. crinita			*A. flavipennis*		
Isolate	Intercept (*a*) ± SE	Slope (*b*) ± SE	LT_{50} (LL-UL)	Intercept (*a*) ± SE	Slope (*b*) ± SE	LT_{50} (LL-UL)
MAGL3N	−1.10 ± 0.33 a	2.38 ± 0.48 a	2.9 (1.8-4.2) a	−2.67 ± 0.50 a	5.58 ± 0.91 a	3.0 (2.5-3.6) a
MAGL4N	−0.98 ± 0.30 a	1.35 ± 0.31 a	5.3 (2.5-11.2) a	−3.61 ± 0.65 a	4.10 ± 0.75 a	7.6 (6.1-9.8) c
MAGC2N				−2.78 ± 0.48 a	4.30 ± 0.66 a	4.4 (3.6-5.5) b
BBGC6				−2.23 ± 0.44 a	2.02 ± 0.41 b	12.6 (8.1-22.2) c

Intercepts, slopes and LT_{50}'s were compared ($P < 0.05$) among only those isolates killing at least 75% of the grubs as recommended by Robertson and Preisler (1992). Although the isolate BBGC1 killed >75% of *A. flavipennis* larvae, the log-probit model could not fit to the data.
Values within a column followed by the same letter are not significantly different ($P < 0.05$).

ana in both species tended to be linear; whereas, response to *M. ansisopliae* was sigmoid. Thus, the probit model fit some of the *B. bassiana* data poorly, especially for strain BBGC1 (Table 3).

The lowest LT_{50} calculated from the log-probit models was for MAGL3N with 2.9 d for *P. crinita* and 3.0 d for *A. flavipennis* (Table 2). LT_{50} values for MAGL4N were about 2X that of those for MAGL3N, although the LT_{50} values were not statistically different for these 2 strains on *P. crinita.* The LT_{50} values for BBGC6 were about 4X that for MAGL3N on *A. flavipennis.* The LT_{50} values for MAGL3N in the present study are lower than those reported for other strains tested against white grubs (Berlanga and Hernández 1999, Flores et al. 2002).

The most virulent strains in this study, MAGL3N and MAGL4N, also were highly active against other species of *Phyllophaga* and *Anomala* in Southwestern Mexico (Hernández et al. 2003), showing their potential against a range of white grub species. Although pathogenicity is one of the prerequisites of a potential microbial control agent, results obtained under laboratory conditions do not necessarily guarantee success under field conditions (Villani et al. 1994, Vidal et al. 1997). Thus, additional studies must be conducted to further evaluate these isolates against white grubs under greenhouse and/or field conditions (Milner 1992, Shannon et al. 1993).

Acknowledgment

We thank Rosalio Navarro for technical assistance, and Susie Legaspi (USDA, ARS), Robert L. Crocker (Texas A&M University), and Sergio Sánchez (University of Texas) for helpful comments on an early draft.

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