PATHOGENICITY OF MORPHOLOGICAL MUTANTS AND WILD-TYPES OF *Metarhizium anisopliae* (METSCH.) VAR. *majus* AND *minus* AGAINST *Anthonomus grandis* BOHEMAN

Clidenor D. Oliveira[1], José P. Siqueira Jr[1], Paulo H. Nakamura[2] e José C. Silva[1]

RESUMO

Patogenicidade de Mutantes Morfológicos e Tipos Selvagens de *Metarhizium anisopliae* (Metsch.) var. *majus* e *minus* ao *Anthonomus grandis* Boheman

Mutantes morfológicos de *Metarhizium anisopliae* (Metsch.) var. *majus* e *minus* foram obtidos após tratamento com 8-metoxipsoraleina associada à luz ultravioleta longa. A patogenicidade desses isolados foi ensaiada em adultos de *Anthonomus grandis* Boheman (bicudo do algodoeiro) em laboratório. Os resultados mostraram que os insetos são susceptíveis às variedades *majus* e *minus* de *M. anisopliae*, bem como a seus mutantes morfológicos. Os valores de LT_{50} mostram menor patogenicidade dos mutantes morfológicos em comparação com as linhagens parentais.

PALAVRAS-CHAVE: Insecta, fungo entomopatogênico, bioensaio, LT_{50}, controle biológico.

ABSTRACT

Morphological mutants of *Metarhizium anisopliae* (Metsch.) var. *majus* and *minus* were obtained after treatment with 8-methoxypsoralen plus near ultraviolet light. These isolates were tested for pathogenicity toward adults of *Anthonomus grandis* Boheman (boll weevil) in laboratory bioassays. Results showed the susceptibility of the insects to *M. anisiopliae* strains of the *majus* and *minus* types as well as to their morphological mutants. LT_{50} values showed a lower pathogenicity of the morphological mutants, when compared to the wild-type parents.

KEY WORDS: Insecta, entomopathogenic fungus, bioassay, LT_{50}, biological control.

INTRODUCTION

The boll weevil, *Anthonomus grandis* Boheman, is a major insect pest of upland and perennial cotton in Brazil (Ramalho & Jesus 1988). Problems caused by insecticides used for

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[1] Departamento de Biologia Molecular, CCEN, Universidade Federal da Paraíba, 55059-970, João Pessoa, PB.

[2] Departamento de Estatística, CCEN, Universidade Federal da Paraíba, 58059-970, João Pessoa, PB.

controlling this pest have led to begin the application of alternative methods, as for example the use of entomopathogens. The entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. has been reported as a potential agent for this purpose, with promissing results (McLauglin 1962, Camargo *et al.* 1984, Batista Filho *et al.* 1985, Coutinho & Cavalcanti 1988, Coutinho & Oliveira 1991, Wright & Chandler 1992).

Metarhizium anisopliae (Metsch.) is a common fungal pathogen of insects from several orders and it has been widely used in Brazil for the control of spittlebugs on sugar cane and other gramineous crops. The pathogenicity of various *M. anisopliae* mutants to different hosts has been reported (Al-Aidroos & Roberts 1978, 1980, Garcia *et al.* 1985, Riba *et al.* 1985, Messias *et al.* 1986, Silva & Messias 1986, Bagalhi 1987, Silva *et al.* 1989). The ability of wild type strain of *M. anisopliae* to infect the *A. grandis* has also been reported (Jamarillo & Alves 1986). The present study represents the first attempt to examine the susceptibility of *A. grandis* adults to wild type strains and morphological mutants of *M. anisopliae*, under laboratory conditions.

MATERIAL AND METHODS

Fungi. Two wild types isolates of *M. anisopliae* were used in these experiments. The strain 133-C was isolated from adult spittlebug *Aeneolamia selecta* (Stal) collected on a pasture, and the strain 153-B from adult spittlebug *Mahanarva posticata* (Stal) collected on a sugar cane field, both in the State of Pernambuco, Brazil. Both strains were kindly supplied by M.L.N. Aquino (IPA-PE, Brazil). The strain 133-C was characterized as being of the *anisopliae* (= *minus*) variety and the strain 153-C as *majus* based on the spore measurement (Oliveira 1991).

Mutants isolation. Suspension of conidia (ca. 1 x 10^6/ml) in 0.85% NaCl solution (saline) were treated with 8-methoxypsoralen in combination with near ultraviolet light (8MOP-NUV) as described by Silva *et al.* (1989), in order to allow ca. 5% survival. Mutagenized conidia were plated at 30-50 survivors/plate and morphological variants were isolated after visual selection. The stability of the character was confirmed following sucessive subculture (every 10 days for three months). The morphological variants and their parental strains showed normal growth on complete solid medium (CM) of Pontecorvo *et al.* (1953), as well on minimal medium (Czapec-Dox Agar, Difco).

Bioassays. In each bioassay, conidia were collected from seven days culture grown on CM at 28°C, and were suspended in 0.1% Tween 80; after shaking and couting in a hemocytometer, dilutions in saline were carried out in order to obtain the desired concentration. Pathogenicity of the wild type strains and morphological variants of *M. anisopliae* toward adults *A. grandis*, was tested. The insects were collected on a cotton field in Paraíba, Brazil (CNPA/EMBRAPA). The conidia viabilities of each isolate were determined prior to bioassay in CM, according to the methods of Silva & Messias (1986). The arrangement of the experiments was similar to that described earlier (Silva *et al.* 1989). Groups of five newly emerged adult insects were placed in sterilized polypropylene cylinders (5 x 10 cm) lined with filter paper and sprayed with 0.2 ml of a conidial suspension (ca. 1 x 10^7/ml). Insects were allowed on this surface for 60 min to obtain infection and then transferred to new flasks with square bolls of cotton as food source. The flasks were placed in a cylindrical glass container (15 x 20 cm) stoppered with gauze to allow ventilation, and maintained under controlled conditions at 28°C, 90% R.H., and photoperiod of 16 h. In each bioassay, 50 insects were employed and the mortality was determined daily. Control insects were treated in the same manner, except that 0.2 ml saline

was spread on the filter paper. Probit analysis (Finney 1971) was performed on the mortality data in order to determine the median lethal time (LT_{50}) for each bioassay.

Reisolation of the fungus. Dead hosts were desinfected with 2.5% sodium hypochlorite for five min and then washed repeatedly with sterilized water. After this procedure the insects were transferred to plates with CM. The plates were incubated for 10 days at 28°C to permit the development of the mycelium and conidia, for fungus identification.

RESULTS AND DISCUSSION

Several spore-colour variants were obtained from the parent strains 133-C and 153-B, following treatment with 8MOP in combination with near ultraviolet light. However, except for five of them, these variants mostly reverted to the parental form after only two-three

Table 1. Proportion of boll weevil adults dead after four, eight, 12 and 16 days exposed with two wild-type and morphological variants of *Metarhizium anisopliae* applied at 10^6 conidia/ml.

Strains[1]	Spore Colour	Time (days)			
		4	8	12	16
133-C (Wild Type)	green	0.20	0.52	0.82	0.92
133-C.1 (mutant)	yellow	0.02	0.08	0.26	0.54
133-C.2 (mutant)	pale vinaceous	0.02	0.22	0.48	0.70
133-C.3 (mutant)	brown	0.02	0.22	0.44	0.72
153-B (Wild Type)	green	0.08	0.40	0.70	0.84
153-B.1 (mutant)	yellow	0.02	0.18	0.36	0.64
153-B.2 (mutant)	yellow tan	0.02	0.16	0.34	0.64

[1] The sample size for each strain was 50 boll weevil adults and the experience was repeated twice.

subcultures, due perhaps to either non-genic changes or incomplete genetic damages induced by mutagenic treatment (Hannan 1972). Several spore-colour mutants of *M. anisopliae* have been previously induced utilizing germicidal ultraviolet light as mutagen (Tinline & Noviello 1971, Messias & Azevedo 1980, Frigo 1983, Silveira & Azevedo 1984, Lugli 1988, Bagalhi *et al.* 1991) or even ethyl methane sulfonate (Bergeron & Messing-Al-Aidroos 1982, Magoon & Messing-Al-Aidroos 1986). Our results show that the 8MOP-NUV combination was effective on inducing mutation for this character. 8MOP-NUV was also effective on inducing genetic quantitative variation for exoenzymes production in *M. anisopliae* (Silva *et al.* 1989) as well for penicillin production in *Aspergillus nidulans* (Simpson & Caten 1979).

The results of the pathogenicity of the five morphological variants of *M. anisopliae* and their parental wild strains are summarized (Table 1). Mortality did not occur in the control. Viable spore counts of each isolate were performed 24 h prior to each bioassay and had 90-99% germination. All dead insects were anaysed and the fungus was reisolated from all of them, indicating that the mortality was due to *M. anisopliae*. Table 2 provides the summary

Table 2. Lethal time (LT_{50}) and relative potency of two wild-type and morphological variants of *Metarhizium anisopliae* to adults of *Anthonomus grandis*, calculated with basis on data described in Table 1.

Strains	LT_{50} Fiducial Limits			Regression Line		
	Lower	LT_{50} (days)	Upper	Intercept	Slope	Relative Potency (95% I.C.)
133-C	6.10	7.08	8.02	1.8209	3.7398	1.00
133-C.1	14.07	16.06	20.52	0.0545	4.0923	0.43 (0.30-0.56)
133-C.2	10.90	12.17	13.87	0.3629	4.2721	0.58 (0.45-0.71)
133-C.3	11.00	12.26	13.96	0.2827	4.3330	0.58 (0.44-0.70)
153-B	8.08	9.13	10.58	1.3642	3.7859	1.00
153-B.1	12.15	13.72	16.23	0.4388	4.0105	0.66 (0.52-0.79)
153-B.2	12.37	13.95	16.52	0.2960	4.1100	0.65 (0.51-0.78)

results of the bioassays. It was possible to calculate the relative potency of LT_{50} values for the morphological variants and parental strains because hypothesis of parallelism of the regression lines was accepted with $P>0.05$ (Bliss 1935). The observed LT_{50} values led us to conclude that the morphological variants were less effective than the parental wild types perhaps due to a pleiotropic effect as already observed for other auxotrophic mutants (Paris & Ferron 1979, Riba *et al.* 1985, Messias *et al.* 1986, Bagalhi 1987). The results do not exclude the possibility of obtaining other spore-colour mutants with the same or more virulent parental strains which could be utilized as markers of recongniton pattern when reisolated from field.

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