Selection of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorok. Strains for Control of *Cornitermes cumulans* (Kollar)

Pedro J. Neves[1*] **and Sérgio Batista Alves**[2]
[1]*Departamento de Agronomia, Universidade Estadual de Londrina, Caixa Postal 6001, C.E.P. 86051-990, Londrina-PR, Brazil.* [2]*Departamento de Entomologia, Fitopatologia e Zoologia Agrícola, Universidade de São Paulo, Escola Superior de Agricultura Luiz de Queiroz. Caixa Postal 9, C.E.P. 13418-900, Piracicaba - SP, Brazil*

ABSTRACT

Fifty strains of the entomopathogenic fungi Beauveria bassiana *(Bals.) Vuill. and* Metarhizium anisopliae *(Metsch.) Sorok. were tested against the termite* Cornitermes cumulans. *In the first phase of the experiments, several bioassays were conducted and the five best strains were selected. The criterion for strain selection during this phase was the confirmed mortality above 50% five days after application of the fungus. Three* M. anisopliae *and two* B. bassiana *strains were the most virulent. The second phase of the experiments consisted of a bioassay and a conidial production test using a rice medium. The best* M. anisopliae *and* B. bassiana *strains were selected using both the confirmed insect mortality nine days after application of the fungus and the yield of conidia. Considering results from the bioassay,* M. anisopliae *1037 showed the highest confirmed (57.8%) and total (89.2%) mortalities after nine days. Among the* B. bassiana *strains, 447 was the most virulent with confirmed and total mortalities of 45.9% and 89.8%, respectively.* M. anisopliae *1037 had the highest conidial yield with mean of* 3.37×10^{12} *conidia/kg of rice, followed by* B. bassiana *strain 447 with* 2.66×10^{12} *conidia/kg of rice. The* M. anisopliae *strain 1037 was the highest virulent followed by* B. bassiana *strain 447. This strains showed the greatest potential as agents to be used in biological control programs against* C. cumulans.

Key words: Entomophatogenic fungi, termits, biological control

INTRODUCTION

The mount termite *Cornitermes cumulans* (Kollar, 1832), is one of the most common insects in Brazilian tropical pastures. Their nests reduce useful pasture area and hinder mechanized treatments. Their occurrence is also associated with pasture degradation (Valério *et al.*, 1995).

There are mechanical and chemical control techniques for this insect. The mechanical control destroy the nests with an implement such as a plow (Ávilla & Goulart, 1992; Ávila & Rumiatto 1995a) or a “termite drill” with variable results (Ávila & Rumiatto, 1995b). The chemical control methods are more widely used, and are highly efficient (Furquim *et al.*, 1968; Mariconi *et al.*, 1971; Wilcken, 1992; Mariconi *et al.*, 1994). However, these products contaminate the environment, food, cattle and farmers, so alternative less ecologically aggressive controls should be studied.

The use of entomopathogenic fungi, applied in high concentration in inundative (flooding) strategy, is an biological alternative for control of this pest. They are effective because the termites are social insects which live in nests where the temperature and humidity vary little and are suitable for this type of pathogen (Fernandes & Alves, 1991; Fernandes & Alves, 1992a; Alves *et al.*, 1995).

The first studies on *C. cumulans* control using fungi were carried out by Fernandes & Alves (1992a). However they only compared eight strains of the entomopathogenic fungi *B. bassiana* and *M. anisopliae*. The strains selected were *B. bassiana* 868 and *M. anisopliae* 865. The control levels for small nests (30 to 40 cm base diameter) were quite high. However, for large nests (50 to 150 cm base diameter) the results were very variable, with control efficiency ranging from 0 to 100% (Alves *et al.*, 1995).

The present study was carried out as part of a

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[*] Author for correspondence

program to increase the efficiency of large nest control (50 to 150 cm base diameter).

The objective was to test the virulence of 50 strains of *B. bassiana* (26) and *M. anisopliae* (24) for the control of *Cornitermes cumulans* colonies.

MATERIAL AND METHODS

First phase: The virulence of fifty *Beauveria bassiana* (Bals.)Vuill and *Metarhizium anisopliae* (Metsch.). All strains belong to the pathogen bank at the Insect Pathology Laboratory at the Department of Entomology at ESALQ/USP.

Second phase: The five previously selected strains were again studied for virulence in bioassays and for conidial production.

Biossays for virulence comparison: Conidia were produced in complete culture medium (CM) (0.36g potassium phosphate, 1.05g sodium phosphate, 0.6g magnesium sulfate, 1g potassium chloride, 1.58g sodium nitrate, 5 g yeast extract, 20g agar, 1000ml distilled water (s.q.f.) (Alves *et al*., 1998). Eight to 12 strains and a control were used as treatments in each bioassay. The insects were collected from nests in the field, and sprayed with 0.5 ml (10^8 conidia ml^{-1} with 100% viability) in sterile water plus Tween 80 (1 ml l^{-1}) in a hood, using a micro sprayer ("PAASCH" Airbusch type with 15 pounds/sq. in). A randomized complete block design with five replications was used, each with 20 workers (at least one soldier). Only sterile water and Tween 80 were applied to the control. The insects were sprayed with a single application per treatment and then transferred to translucent 4.5 cm diameter, 6 cm high plastic containers (20 per container), containing a piece of the carton portion of the nest and a strip of filter paper attached to the lid and kept moist. They were then transferred to an incubator (B.O.D.) at 25±0.5°C. Mortality was evaluated daily and the dead individuals were washed in 70% alcohol and distilled water (3 times) for 30 seconds and transferred to a moist chamber. Total mortality was considered to be the number of dead individuals in the treatment regardless of the cause. Confirmed mortality was obtained by registering the number of dead insects on which the fungus sporulated, after superficial decontamination. The corrected mortality was calculated by the difference between total death and the control. The insects which died on the first day in all the bioassays were replaced in the respective treatments, eliminating possible variations from death caused by handling during the experiment setting.

The selection parameter was an accumulated confirmed mortality over 50% up to the fifth day after inoculation (first phase) and the accumulated confirmed mortality up to the ninth day (second phase).

Conidia production: Conidia production was also compared in the second phase using the production methodology developed by Alves & Pereira, (1989) where 200g of pre-cooked rice, sterilized in plastic bags were inoculated with 5 ml of fungal suspension (5 x 10^8 conidia ml^{-1}) and them transferred to an incubator (B.O.D.) at 25±0.5°C. After micelial growed, rice were transferred to trays for fungi sporolation. When the fungus was completely sporulated on trays, a gram of rice with fungus was removed from each tray. After that 20 ml of sterile water plus tween was applied. The number of conidia was evaluated using a Neubauer chamber. A randomized complete block design with four replications (200 gram/bag) was used. The data obtained was log transformed (x + 0.5). The analysis of variance was made and the means were compared by the Tukey test at a level of 5% probability. The bioassays and the production tests were carried out at 25±0.5°C, 70±5% relative humidity and 24 hour photophase.

RESULTS AND DISCUSSION

Table 1 shows the results of mortality test. The mortality data did not fit the Probit model because there was a highly significant χ^2 and high heterogeneity for the majority of strains. Thus graphic analysis of confirmed mortality was chosen. Confirmed mortality as a selection parameter was chosen because: total mortality equal or greater than 80% for 35 strains on the fifth day made it impossible to differentiate them (Table 1 and Fig. 1); there was no confirmed mortality in the control in any of the bioassays. Twenty-four (48%) of all strains, had accumulated total mortality rates greater than 90%, 17 *M. Anisopliae* and 7 *B. bassiana*, up to the fifth day after application (Figure 1). On the other hand, there was an inverse performance for accumulated confirmed mortality on the fifth day where 38 strains (76%), 19 *M. anisopliae* and 19 *B. bassiana*, caused 0 to 3% mortality and only five strains (10%), 3 *M. anisopliae* and 2 *B. bassiana*, caused a mortality greater than 50% (Figure 1). Total accumulated mortality means for the control at the first stage varied from 4.4% to 19.5% for the 2nd and 7th days after application, respectively, for the 6 bioassays.

Total mortality represents the death caused directly (confirmed mortality) and indirectly by the pathogen (e.g. septicemia) besides mortality caused by other factors such as stress and handling. Mortality by septicemia (rot) where there was no sporulation of the pathogen, may have been caused by a bacterial invasion during the fungus penetration, through the integument or digestive system. Under laboratory conditions manipulation of the insects and abiotic conditions during the setting up and running of the bioassays; it is therefore fairly variable and may not represent the real virulence of the pathogen. However, direct mortality (confirmed) shows the colonizing capacity of the pathogen overcoming all the competitive microbial agents present in the insect, confirming its virulence. This is also important because sporulation increases the dissemination of the pathogen in the environment.

Table 1. Total mortality (%) (accumulated), corrected and confirmed, of *Cornitermes cumulans* 3, 4 and 5 days after inoculation.

	Mortality (%)								
	Third day			Fourth day			Fifth day		
Strain	Total[1]	Corr.[2]	Confir.[3]	Total	Corr.	Confir.	Total	Corr.	Confir
447[4]	50.5	42.3	31.7	78.2	69.0	55.4	86.1	75.9	63.4
760[4]	48.5	40.3	35.0	75.7	66.5	60.2	91.3	81.1	74.8
E9[5]	53.1	44.9	43.8	81.3	72.1	69.8	96.9	86.7	84.4
1097[5]	49.0	40.8	34.0	82.0	72.8	64.0	94.0	83.8	75.0
1037[5]	80.2	56.4	39.6	97.2	65.9	53.8	99.0	65.3	55.7

[1] Total mortality; [2] Corrected mortality; [3] Confirmed mortality;
[4] *Beauveria bassiana*; [5] *Metarhizium anisopliae*

When selecting *M. anospliae* and *B. bassiana* strains to control *C. cumulans*, Fernandes & Alves (1992a) found that total mortality varied from 20 to 100% on the 2nd day and from 50 to 100% on the 4th day. However, they used another inoculation methodology, where the carton portion surface was treated. The insects were thus in permanent contact with the conidia deposited on this surface decreasing the effect of the superficial cleaning behavior carried out by the workers which is considered to be one of the main resistance or defense mechanisms against entomopathogenic fungi. This may have caused a decrease in the lethal time and an increase in mortality. Fernandes & Alves (1992a) only collected confirmed mortality data in the second bioassay and found a variation of 95 to 100% and 9 to 42% on the 5th day after inoculation for *M. anisopliae* and *B. bassiana* strains respectively. The accumulated total mortality for the control on the 9th day was 8.3% and no confirmed mortality was observed in the bioassay of the second phase (Table 2). On the second phase data did not fit the Probit model for the previously mentioned reasons. The accumulated confirmed mortality on 9th day was greater for strains 1037 and 447. However, strain 1037 reached confirmed mortality levels greater than 50% more rapidly than the other strains, being 51% on the fifth day (Table 2). Also, mortality caused by strain 1037 on the 3rd day after inoculation was significantly greater and on the fifth day strains 1037 and 447 had greater mortality than the others. Thus strain 1037 (*M. anisopliae*) was more virulent and caused high confirmed death levels. Strain 447 was the most virulent of *B. bassiana* strains.

A possible cause for the differences in strain virulence may be germination speed and consequent penetration (Moorhouse *et al.*, 1993). Two most virulent strains were obtained from *Solenopsis* spp (Hymenoptera: Formicidae) which were phylogenetically very different from termites, but were also sociable and have the behavior of superficial cleaning for decontamination (Pereira *et al.*, 1993). Thus, rapid germination and penetration in the insect may have been one of the factors which enabled the infection of these ants by these pathogens.

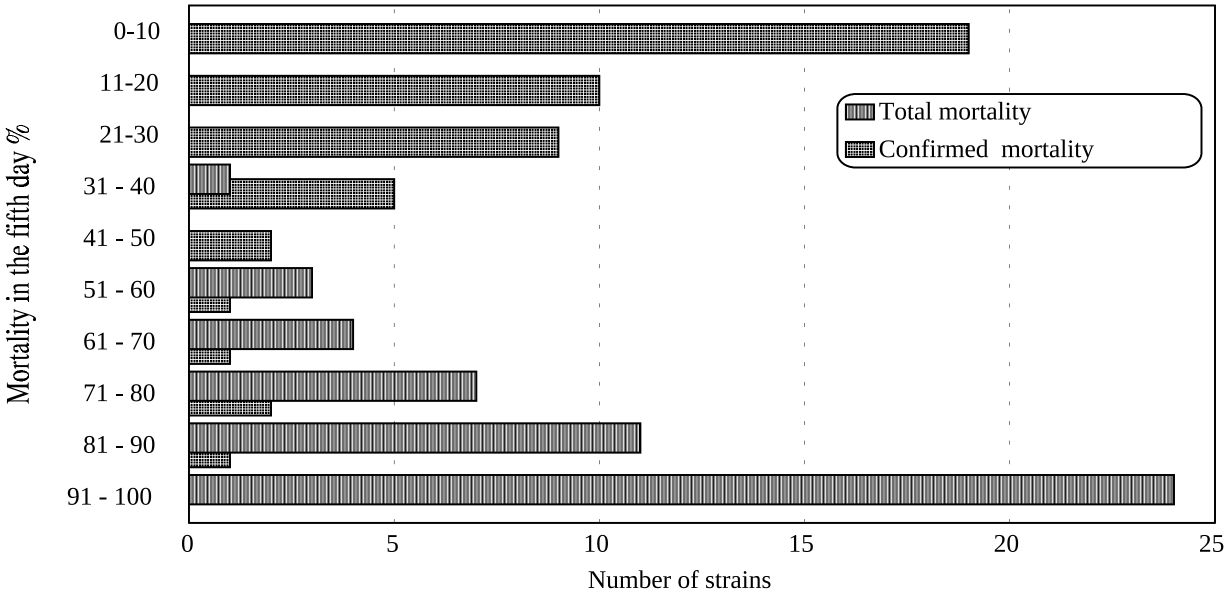

Figure 1. Strain distribution frequency of *Metarhizium anisopliae* and *Beauveria bassiana* in relation to total and confirmed mortality of *Cornitermes cumulans*, in the fifth day after inoculation in the first phase of strains selection.

Table 2. Total and confirmed mortality (%) (accumulated), of *Cornitermes cumulans* in the second phase of strains selection.

	Strains and % of mortality (accumulated)									
	1037 [1]		E9 [1]		1097 [2]		447 [2]		760 [2]	
Days	Tot.[3]	Conf.[4]	Tot.	Conf.	Tot.	Conf.	Tot.	Conf.	Tot.	Conf.
2	4.9	1.96 a[5]	8.8	0.0 a	3.7	0.0 a	6.12	0.0 a	4.1	0.0 a
3	28.4	13.7 a	24.5	0.0 b	13.0	0.0 b	16.3	1.0 b	15.3	0.0 b
4	57.4	33.3 a	44.1	4.9 b	29.6	5.6 b	40.8	13.2 ab	42.9	5.1 b
5	77.5	51.0 a	65.7	21.6 b	53.7	23.1 b	69.4	34.7 ab	61.2	12.2 b
6	82.3	55.8 a	75.5	30.4 ab	62.0	29.6 ab	75.5	37.8 ab	72.4	14.3 b
7	82.3	55.8 a	80.4	34.3 ab	71.3	36.1 ab	80.6	40.8 ab	77.5	14.3 b
8	87.3	56.9 a	85.3	37.3 ab	74.1	37.0 ab	83.7	43.9 a	77.5	14.3 b
9	89.2	57.8 a	87.3	37.3 ab	75.9	37.0 ab	89.8	45.9 a	78.6	14.3 b

[1] *Metarhizium anisopliae*; [2] *Beauveria bassiana*;
[3] Total mortality; [4] Confirmed mortality;
[5] Means following by the same letters are not significantly different by Tukey at 5% of probability, in the same day.

This characteristic may also have been decisive in the colonization success and in the expression of greater virulence for *C. cumulans*. However, this may not be the only important characteristic in these two strains, which makes them highly virulent for different insect species. Alves *et al*., (1997) showed that strain 1037 was highly virulent by the speed of its lethal action for *Culex quinquefasciatus, Blatella germanica, Diatraea saccharalis, Solenopsis saevissima* and *Heterotermes tenuis*. Other factors such as differences in ability to overcome barriers in the immune system are probably also acting in the differentiation of the virulence among the strains. This ability may be associated with the production of toxins or secondary metabolites such as dextruxins (Vey *et al*., 1982). Thus virulence may be determined by different intrinsic characteristics in the strain, which act in a additive manner in its total expression. The manifestation of these characteristics is also closely related with biotic and abiotic variations. Thus a series of important characteristics throughout the infection, colonization and reproduction process make strains 1037 and 447 be considered more virulent for *C. cumulans.*

The most virulent strains 1037 and 447 showed no differences in relation to conidial production (Table 3). The conidial production of *B. bassiana* 760 was the only one statistically different (smaller) from the others. Alves & Pereira (1989) showed that the production in large scale was superior to the one obtained in this study for the best *B. bassiana* strain 447 and smaller them the best *M. anisopliae* strain 1037. The authors obtained an average productions of 6,2 x 10^{12} and 8,13 x 10^{11} conidia per kilogram of rice for *B. bassiana* and *M. anisopliae*, respectively. Fernandes (1991) observed productions of 3,3 x 10^{12} conidia per kilogram of rice, for *B. bassiana* strain 868; 3,23 x 10^{12} for strain 865; 2,35 x 10^{12} for strain 866 and 2,45 x 10^{12} for strain 259, all of them *M. anisopliae* strains. The productivity obtained by Fernandes (1991) was very close to the one obtained in this study. So, except for strain 760, in relation to productivity in rice, all the others strains studied can be used.

The comparison of these results with those obtained by other authors have two main limitations. The first one is related to the variation in rice hardness, which is impossible to be the same on two different experiments. The second limitation is related to the type of rice used in different experiments.

The present study showed that of the tested strains, 1037 (*M. anisopliae*) was the most virulent. Strain 447 was the most virulent *B. bassiana* strain and the second of the 50 tested strains. Thus strain 1037 (*M. anisopliae*) may be used in biological control programs for *C. cumulans* colonies in the field.

Table 3. Conidia production average for the different strains.

Strains	Means[1]	Means[2]
1037 [3]	337.15 a [5]	3.37
447 [4]	265.57 a	2.66
E9 [3]	153.62 a	1.54
1097 [3]	104.03 a	1.04
760 [4]	12.28 b	0.12

[1] Original means (x 10^7 conidia per rice gram);
[2] Original means (x 10^{12} conidia per rice kilogram);
[3] *Metarhizium anisopliae*; [4] *Beauveria bassiana*;
[5] Means following by the same letters are not significantly different by Tukey at 5% of probability.Transformed data by log (x + 0.5)

RESUMO

Nesta pesquisa foram testados cinqüenta isolados dos fungos entomopatogênicos *Beauveria bassiana* (Bals.) Vuill. e *Metarhizium anisopliae* (Metsch.) Sorok. sobre *Cornitermes cumulans*. Na primeira fase foram realizados vários bioensaios, selecionando-se os 5 melhores isolados. O critério usado para a seleção foi a mortalidade confirmada acima de 50%, no quinto dia após a aplicação. Três isolados de *M. anisopliae* e dois de *B. bassiana* foram os mais virulentos. A segunda fase de seleção constou de um bioensaio e um teste de produção de conídios em meio de arroz, selecionando-se o melhor isolado de *M. anisopliae* e de *B. bassiana*. Os critérios adotados foram: mortalidade confirmada até ao nono dia após a inoculação e quantidade de conídios produzidos. Considerando os resultados do bioensaio, o isolado 1037 *M. anisopliae* foi o que apresentou maior média de mortalidade confirmada (57,8%) e mortalidade total (89,2%), decorridos nove dias da inoculação. Dos isolados de *B. bassiana*, o 447 foi o mais virulento, causando mortalidade confirmada média de 50,5% e total de 89,8%. O isolado 1037 de *M. anisopliae* foi o mais produtivo, com produções médias de 3,37 x 10^{12} conídios/kg de arroz, seguido do 447 com 2,66 x 10^{12} conídios/kg de arroz. Estes isolados possuem grande potencial para utilização em programas de controle biológico de *C. cumulans*.

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Received: June 06, 1999;
Revised: September 02, 1999;
Accepted: October 05, 1999.