EFFECTS OF THE FUNGUS *Beauveria bassiana* (BAL.) VUILL BEHAVIOR, OVIPOSITION, AND SUSCEPTIBILITY TO SECUNDARY INFECTIONS OF ADULT *Cerotoma arcuata* (OLIVIER, 1791) (COLEOPTERA: CHRYSOMELIDAE)

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RESUMO

Efeito de *Beauveria bassiana* no comportamento alimentar, oviposição e susceptibilidade a infecções secundárias de adultos de *Cerotoma arcuata*

O controle de *Cerotoma arcuata* (Olivier, 1791), através do fungo *Beauveria bassiana*, está sendo investigado no Centro Nacional de Pesquisa de Arroz e Feijão. Como parte deste estudo, adultos de *C. arcuata* foram inoculados em laboratório com o isolado CP 5, para observar os seus efeitos durante o intervalo entre exposição e morte do inseto. O dano causado pelos insetos tratados às folhas não diferiu significativamente ($p > 0,05$) dos insetos não tratados durante sete dias após tratamento. Entretanto, a área média consumida por inseto tratado no dia anterior à morte foi 38,3 mm^2. Este valor, comparado com 43,1 mm^2 nos outros dias, sugere apenas um leve declinio na atividade alimentar imediatamente antes da morte causada pelo fungo. Similarmente, a oviposição e a viabilidade dos ovos não diferiram entre insetos tratados com *B. bassiana* e a testemunha. A exposição de *C. arcuata* aos patógenos oportunistas ou secundários *Serratia marcescens* e *Aspergillus* sp., aos três dias após tratamento com *B. bassiana*, não afetou a oviposição e a viabilidade dos ovos. No entanto, houve indicações de redução de esporulação de *B. bassiana* em insetos aparentemente estressados pelos patógenos oportunistas.

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INTRODUCTION

One of the most serious disadvantages of the use of entomopathogenic fungi for insect control is the relatively long time period between exposure and death of the insect. During this interval, there may be substantial damage to host plants and proliferation of the pest.

Although concern over this problem is often expressed, quantitative evaluations are rare. There are contradictory reports of the effects of *Beauveria bassiana* on the fecundity of Coleoptera. MULLER-KOGLER & STEIN (1970) reported a reduction of oviposition by the curculionid *Sitona lineatus* proportional to the dose of conidia applied. BAJAN & KMITOWA (1972), however, reported an increase in the fecundity of the chrysomelid beetle *Leptinotarsa decimilineata* Say associated with *bassiana* infection. Reports of other sublethal behavioral effects of *B. bassiana* were not encountered in the literature.

Interaction of *B. bassiana* with other microbes, particularly secundary or potential pathogens, has also received little attention. Combinations of the fungus and the primary pathogen *Bacillus thuringiensis* have been tested against several insects, generally without interaction (KRIEG, 1971). Masera (1934, cited in KRIEG, 1971), however, reporeted a reduction in *B. bassiana* induced mortality of *Tenębrio molitor L.*, 1758 when the fungus was used in combination with *Serratia marcescens*.

The purpose of this study was to evaluate the effects of *B. bassiana* infection on the fecundity, feeding behavior, and susceptibility to secondary infections of *Cerotoma arcuata* (Olivier, 1791), a pest of grain legumes.

MATERIAL AND METHODS

INSECTS AND FUNGUS

Cerotoma arcuata were taken from a laboratory colony maintained at the Brazilian National Rice and Bean Research Center (CNPAF) and initiated with field insects from the same locale. Adults of 3-7 days postemergence were used in all experiments.

B. bassiana CP5 was isolated from *Cerotoma* sp. in a cowpea field near Manaus, Brazil and is maintained in the culture collections at CNPAF and Boyce Thompson Institute (as ARSEF 789). The fungus was grown on potato dextrose agar supplemen-

ted with yeast extract at 26 ± 1°C. Viability of the conidia was above 90% for all experiments.

APPLICATION OF *B. bassiana*

Beetles were sprayed in groups of five in a laboratory spray apparatus at a concentration of 14,300 conidia/mm^2 for feeding and oviposition experiments and 560 conidia/mm^2 for tests of interactions with opportunistic pathogens.

FEEDING ASSAYS

Postexposure feeding was evaluated by placing 79 beetles individually in 10-cm Petri dishes with a single cowpea leaf and moist filter paper lining. The insects were incubated at 26 ± 1°C for 9 days. The leaves were replaced daily and measured by means of an electronic leaf area meter to determine the area consumed. For both the feeding and fecundity assays, data were collected only for those beetles for which infection was confirmed by sporulations of the fungus on the integument after death. Data for the day of death of a given insect were not included in the analyses. The experiment was carried out three times.

OVIPOSITION AND EGG HATCH ASSAYS

To quantify oviposition and egg hatch, female beetles were sprayed and incubated as for the fecundity tests, but eggs were removed and counted daily and placed in 3.5-cm Petri dishes with moist filter paper. Cowpea sprouts, which appear to stimulate eclosion, were added to each dish. Hatched and inviable eggs were counted 10 days after being deposited. This experiment too was conducted three times.

EFFECTS OF SECUNDARY PATHOGENS

Three days after application with *B. bassiana*, *C. arcuata* was exposed to either of two common opportunistic pathogens that were isolated from cadavers of *C. arcuata* and often cause secundary infections. One half ml of a suspension of either 2 x 10^9 cells/ml of *S. marcescens* or 10^7 x 10^8 conidia/ml of *Aspergillus* sp. in 1% NaCl and 0.05% Tween 80 was applied to cowpea leaves which were left in the dishes for 48 hr to ensure adequate exposure by feeding or contact. Mortality data were taken every day through 8 days. Cadavers were held under high humidity for at least one week to allow sporulation. Treatment with only the opportunistic pathogens, as a control were included. This experiment was performed twice.

DATA ANALYSIS

Analysis of variance (ANOVA) was used for all of the data presented except the percent of females that oviposited. The latter data were not taken in a manner that permitted analysis. Data from the replications of the fecundity experiments and the feeding experiments were pooled, there being no effect of repetition detected in the analysis. Data from experiments with secundary pathogens, however, were maintained separately because of detectible differences between experiments.

RESULTS AND DISCUSSION

LEAF CONSUMPTION

Leaf consumption per day did not differ significantly between treatment and control beetles for any day after exposure to *B. bassiana* ($p > 0.05$) (Table 1). The grand mean of areas consumed over all days prior to the death was 41.1 mm^2 (SE = 2.64) for infected beetles and 43.1 mm^2 (SE = 2.27) for control beetles. Mean consumption by infected insects for days immediately before their individual deaths was 38.3 mm^2, indicating only a slight decline in feedig activity even within 48 hr of death.

OVIPOSITION AND EGG VIABILITY

Neither the number of eggs nor the percent hatch was significantly reduced by *B. bassiana* infection (Table 2). Similarly, the percent of females that layed eggs on a given day showed little difference between treatment and untreated insects (Table 3). The variance in the oviposition data were very high due to tendency of the insects to deposit their eggs in batches. Nevertheless, it is clear that *B. bassiana* lacks any effect on number and viability of eggs produced by infected *C. arcuata* that would enhance its value as a biological control agent.

These results contrast with those of BAJAN & KMITOWA (1972), who reported more than two-fold increase in oviposition by another chrysomelid, *L. decemlineata* when infected by *B. bassiana*. However, Fargues (personal communication) working with the same species, did not detect such an increase.

INTERACTION WITH OPPORTUNISTIC PATHOGENS

Aspergillus sp. and *S. marcescens* are most often described as secondary or opportunistic pathogens, but in some ca-

ses primary pathogenicity has been indicated (see BURGES & HUSSEY, 1971 and CANTWELL, 1974 for reviews). In the first experiment (Table 4), there was 37% mortality in control insects and 54 and 64% respectively for *S. marcescens* and *Aspergillus* applied without *B. bassiana*. On the other hand, control mortality in the second experiment was much lower and was not associated with elevated mortality in the treatments of opportunistic pathogens alone. This suggests that the high mortality associated with these agents in the first experiment was due to the apparently weakened condition of the test insects and supports the hypothesis that *S. marcescens* and the *Aspergillus* species used in these experiments are indeed opportunistic pathogens.

There was a small amount of contamination by *B. bassiana* among insects not treated with the fungus in both experiments. However, the number of insects was too low to draw conclusions regarding the relationship between this contamination and the opportunistic pathogens.

There appears to be little or no enhancement of suceptibility of *C. arcuata* to secondary infections associated with *B. bassiana* infection (Table 4). The mortality in the *B. bassiana* - *S. marcescens* treatment in experiment 2 was the only case of mortality associated with an opportunistic pathogen that was significantly higher than that for *B. bassiana* alone ($p < 0.05$). It was not, however, significantly higher than the combined mortality attributed to *B. bassiana* and *S. marcescens* individual treatments. In the first experiment, the mortalities associated with the combined treatments were actually lower than for *B. bassiana* alone, although not significantly so. If there is a real biological interaction between these opportunistic pathogens and *B. bassiana* in terms of pathogenicity, it is probably an antagonistic one, as was indicated by Masera (1934, cited in KRIEG, 1971).

In the first experiment, where the effect of the opportunists on mortality was the greater, there was significantly less sporulation of *B. bassiana* on cadavers of insects treated with that fungus and treated with opportunistic pathogens than on those sprayed with *B. bassiana* alone ($p < 0.05$). It is likely that this is due to inhibition of *B. bassiana* development by these fast-growing microbes when their development was favored by host stress. Presumably this inhibition would be less pronounced in unstressed insects, as in the second experiment. Such an inhibition could have a depressant effect on the development of *B. bassiana* epizootics under natural conditions that are stressfull to *C. arcuata*.

All of the above results indicate the need for rapidly acting isolates of *B. bassiana*, both in terms of inducing mortality in the insect host and completion of its life cycle.

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ABSTRACT

Beauveria bassiana (Bals.) Vuill. is currently being investigated for control of leaf-feeding chrysomelid beetles in Brazil. As part of this evaluation adult *Cerotoma arcuata* (Olivier, 1791) were sprayed in the laboratory with isolate CP 5 and tested for its effects during the interval between exposure and death. Feeding damage to cowpea leaves did not differ statisfically ($p < 0.05$) between treated and untreated beetles through seven days posttreatment, although the mean area consumed per treated insect per day was 38.3 mm^2 for the days prior to their individual deaths. This value compared to 43.1 mm^2 over all days suggests only a slight decline in feeding activity shortly before death. Similarly, neither ovi

position nor percentage of eggs that hatched differed between *Beauveria*-infected and control insects. Mortality of C. *arcuata* was not increased by exposure to the secondary or opportunistic pathogens, *Serratia marcescens* or *Aspergillus* sp. 3 days after treatment with *B. bassiana*. However, a reduction of sporulation of *B. bassiana* was indicated on apparently stressed beetles that were exposed to the opportunistic pathogens.

TABLE 1 - Cowpea leaf consumption by *Cerotoma arcuata* (Olivier, 1791) infected with *Beauveria bassiana* (CP 5).

Days posttreatment	No. of infected insects	AREA CONSUMED PER INSECT IN mm (SE)	
		B. bassiana INFECTED	Control (n = 71)
1	79	52 (7.1)	26 (4.7)
2	79	41 (6.7)	38 (5.6)
3	79	38 (6.7)	43 (6.0)
4	71	41 (5.3)	50 (6.1)
5	62	29 (4.1)	43 (6.0)
6	30	43 (9.6)	49 (8.1)
7	10	45 (11.8)	40 (5.1)

TABLE 2-Effect of *Beauveria bassiana* (CP 5) on oviposition and egg hatch by *Cerotoma arcuata* (Olivier, 1791).

Days posttreatment	*B. bassiana*				Control			
	Mean eggs/♀	(SE)	Percent hatch	(SE)	Mean eggs/♀	(SE)	Percent hatch	(SE)
1	0.03	(0.02)	75	(25.0)	0.13	(0.08)	79	(21.4)
2	1.33	(0.51)	55	(11.8)	0.54	(0.17)	83	(7.5)
3	3.82	(0.97)	79	(3.7)	2.79	(0.73)	76	(6.3)
4	2.56	(0.73)	80	(6.5)	2.38	(0.79)	80	(6.5)
5	5.68	(1.21)	74	(6.3)	4.49	(1.03)	68	(7.1)
6	3.54	(0.85)	66	(8.1)	4.62	(1.11)	82	(4.9)
7	1.50	(0.57)	76	(4.8)	3.38	(0.93)	76	(9.2)
8	2.58	(1.31)	88	(3.7)	5.12	(1.08)	72	(7.1)

TABLE 3 - Effect of *Beauveria bassiana* (CP 5) on percentage of *Cerotoma arcuata* (Olivier, 1791) that oviposited.

Days posttreatment	Females that laid eggs (%)	
	Infected	Control
1	2	4
2	10	15
3	27	26
4	23	23
5	35	33
6	31	33
7	14	18
8	16	32

TABLE 4 - Effect of *Beauveria bassiana* (CP 5) exposure on the susceptibility of *Cerotoma arcuata* (Olivier, 1791) to secondary infection by *Serratia marcescens* and *Aspergillus* sp.

Treatment[1]	Experiment 1			Experiment 2		
	n	mortality (%)[2]	Cadavers with sporulation of *B. bassiana*(%)[2]	n	mortality (%)[2]	Cadavers with sporulation of *B. bassiana*(%)[2]
B. bassiana + *S. marcescens*	67	64.9 ab	16.7 bc	51	86.2 a	25.3 a
B. bassiana + *Aspergillus*	68	69.4 ab	21.6 b	49	73.1 ab	34.2 a
B. bassiana	67	77.7 a	42.5 a	49	65.3 b	28.7 a
S. marcescens	79	53.7 ab	0.0 c	59	16.8 c	1.7 b
Aspergillus	70	64.3 ab	4.3 bc	60	5.0 c	0.0 b
Control	59	37.2 b	0.0 c	60	15.0 c	1.7 b

[1] *B. bassiana* was sprayed onto insects at 560 conidia/mm^2. Secondary pathogens were applied via cowpea leaves 3 days later - 0.5 ml of either 2 x 10^9 conidia/ml *S. marcescens* or 10^7 (exp. 1) or 10^8 (exp. 2) *Aspergillus*.

[2] Values within columns followed by the same letter are not significantly different by Tukey's test (P=0.05).

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