Pathogenicity of *Beauveria bassiana* and *Metarhizium flavoviride* (Deuteromycotina) to *Schistocerca americana* (Orthoptera: Acrididae)

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ABSTRACT The entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium flavoviride* Gams and Rozspal have been widely tested for the suppression of grasshoppers. However, with the exception of *Melanoplus sanguinipes* (F.), there has been little research on Nearctic species. We examined the potential of these 2 microbial agents for control of the American grasshopper, *Schistocerca americana* (Drury), and the migratory grasshopper, *M. sanguinipes*. *M. flavoviride* was much more virulent than *B. bassiana* to both grasshopper species. At the conidial dosage of 1.2×10^5, *M. flavoviride* produced 69.2 and 74.2% mean mortality 7 d after treatment in *S. americana* and *M. sanguinipes*, respectively. In contrast, *B. bassiana* produced 1.7 and 11.7% mean mortality 7 d after treatment in *S. americana* and *M. sanguinipes*, respectively. Treatment with *M. flavoviride* also caused a significant reduction in feeding beginning 48 h after treatment of 6th-instar *S. americana*. Furthermore, *M. flavoviride*-treated *S. americana* showed an average reduction of 36.6% mean cumulative consumption, 5–8 d after treatment, when compared with untreated grasshoppers.

KEY WORDS biological control, grasshopper, microbial control

THE ENTOMOPATHOGENIC DEUTEROMYCETE fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium flavoviride* Gams and Rozspal have been tested against grasshoppers in both the laboratory and field (Mason and Erlandson 1994, Goettel et al. 1995). Numerous isolates of *B. bassiana* have been tested against *Melanoplus sanguinipes* (F.) in laboratory studies, most resulting in relatively high mortality (Marcandier and Khachatourians 1987, Moore and Erlandson 1988, Khachatourians 1992, Foster et al. 1994b, Inglis et al. 1995). Field trials conducted with *B. bassiana* against various grasshopper species have resulted in both successes and failures (Johnson et al. 1992; Lobo Lima et al. 1992, 1994; Nasseh et al. 1992; Johnson and Goettel 1993; Foster et al. 1994a; Inglis et al. 1997). All of the research conducted with *M. flavoviride* is limited to African grasshopper species. Many isolates of *M. flavoviride* have been tested in laboratory bioassays against *Schistocerca gregaria* Forskal with variable virulence (Bateman et al. 1992, 1993; Prior 1992; Goettel et al. 1995; Prior et al. 1995). *M. flavoviride* has also been tested against the acridids *Locusta migratoria* L. (Welling et al. 1994), *Chortoicetes terminifera* (Walker), and *Phaulacridium vittatum* (Sjostedt) (Milner and Prior 1994), causing high levels of mortality. Published field trials conducted with *M. flavoviride* against various grasshoppers species resulted in high levels of mortality (Bateman et al. 1992; Lomer et al. 1992, 1993a, b, 1994; Douro-Kpindou et al. 1995).

Schistocerca americana (Drury) is commonly the most economically important grasshopper in Florida, sometimes causing severe losses to the citrus and ornamental industries (Griffiths and Thompson 1952, Capinera 1993). Neither *B. bassiana* nor *M. flavoviride* have been evaluated against *S. americana*. *M. sanguinipes* is a common pest grasshopper on the western rangelands of North America (Capinera and Sechrist 1982, Pfadt and Hardy 1987). Many reports on the susceptibility of *M. sanguinipes* to *B. bassiana* exist in the literature, but *M. flavoviride* has not been tested on this insect.

In this study, relative susceptibility of *S. americana* to *B. bassiana* and *M. flavoviride* was determined by measuring mortality and estimating LT_{50} values under 2 conidial dosages. The relative susceptibility of adult *M. sanguinipes* to 1 conidial dosage of *B. bassiana* and *M. flavoviride* was also determined as a positive control, because the susceptibility of this species to *B. bassiana* has been published previously.

Reduction in food consumption is a behavioral change frequently observed in insects infected with entomopathogenic fungi (Hajek and St. Leger 1994). A reduction in feeding rate is beneficial from the perspective of plant protection because entomopathogenic fungi may require a long incubation period to kill its host. Extensive crop damage could result during this long incubation period. Therefore, a feeding assay was conducted to examine a reduc-

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tion in feeding in *M. flavoviride*-treated 6th-instar *S. americana* before host death.

Materials and Methods

Grasshopper Culture. The *S. americana* colony was obtained from individuals collected from the field in northern Florida and maintained in laboratory culture since 1991. First through 5th instars were kept in aluminum screen cages (30.5 by 30.5 by 30.5 cm), and 6th instars and adults in aluminum screen cages (54.6 by 38.1 by 38.1 cm). The *M. sanguinipes* colony is a nondiapausing strain (Pickford and Randell 1969), with all instars kept in aluminum screen cages (30.5 by 30.5 by 30.5 cm). The culture conditions for all grasshoppers were 32 ± 2°C, 40 ± 10% RH, and a photoperiod of 14:10 (L:D) h. Grasshoppers received Romaine lettuce daily and were also provided with a dry diet consisting of wheat bran, whole wheat flour, soy flour, and dry fish food at the ratio of 2:1:1:0.01. Cages containing individuals that were not to be used for experiments received Romaine lettuce that had been lightly misted with triple sulfa antibiotic solution made up of 3 g sulfamethazine, 6 g sulfathiozole, and 4 g sulfapyridine (Sigma, St Louis, MO) in 987 ml of deionized water. Cages containing individuals that were to be used in an experiment were removed from the triple sulfa antibiotic treatment at least 2 wk before their use.

Fungal Culture. *M. flavoviride* conidia (isolate IMI 330189), originally isolated from *Ornithacris cavroisi* (Finot) in Niger, was received from the International Institute of Biological Control, Silwood Park, U.K., and maintained in peanut oil (Sigma, p-2144) at 4°C. *B. bassiana* conidia (isolate GHA), originally isolated from *M. sanguinipes* near Three Forks, MT, was received from Mycotech (Butte, MT) and maintained at 15°C. Fungal cultures of both species were grown from their original stock conidia on SDAY (Sabouraud dextrose agar + 5% yeast extract [Difco, Detroit, MI]), and maintained at a photoperiod of 14:10 (L:D) h and 25°C. Conidia of *B. bassiana* and *M. flavoviride* were harvested at either 10 or 14 d after inoculation by gently scraping the culture surface with a plastic sterile loop (i.e., experimental preparations of both fungi were the same age at time of use) and stored immediately at 15°C until use. *M. flavoviride* had been cultured once on SDAY plates and *B. bassiana* had been cultured 3 times on SDAY plates from their respective source conidia.

Experimental preparations of *B. bassiana* and *M. flavoviride* were prepared by suspending conidia in peanut oil and lower concentrations were prepared from this material. The conidial concentrations were estimated by using a hemacytometer. To determine viability of the conidia used in the experiments, the preparations were spread onto SDAY plates and incubated for 24 h at 25°C. The *B. bassiana* and *M. flavoviride* conidia both produced >95% germination.

Comparative Pathogenicity Assay. Sixth-instar *S. americana* and adult *M. sanguinipes* were treated with fungal preparations applied topically to the pronotum using a 10-μl micropipette (Hamilton, Reno, NV). Both grasshopper species were treated with *B. bassiana* and *M. flavoviride* at the dosage of 1.2×10^5 conidia per insect, *S. americana* received the additional dosage of 1.2×10^3 conidia per insect of both *B. bassiana* and *M. flavoviride*. *M. sanguinipes* received their conidial dosage in 0.5 μl of peanut oil and *S. americana* in 2 μl of peanut oil. These volumes of peanut oil were determined to minimally adversely affect the respective grasshopper species. Controls received pure peanut oil. Four repetitions, over time, of 30 grasshoppers were used per fungal species and dosage level.

Following treatment, grasshoppers were maintained individually in 500-ml plastic containers covered by a lid with an aluminum mesh opening. They were kept in a rearing room at 30 ± 2°C, 45 ± 10% RH, and a photoperiod of 14:10 (L:D) h. Starting the day of inoculation, Romaine lettuce was provided every 3rd d and the dry diet every 6th d. Daily mortality was assessed for 21 d. Grasshoppers that died during the bioassay were placed individually on moistened filter paper in sealed 250-ml plastic containers and maintained at 25°C to allow sporulation of fungus from the cadavers. This was observed >3 d after death. Only individuals with apparent *B. bassiana* and *M. flavoviride* hyphal growth and conidial sporulation arising from intersegmental membranes were considered to be sporulating cadavers. Grasshoppers that died within 3 d after treatment with apparent bacterial infection (e.g., *Pseudomonas* spp.), which is sometimes associated with our laboratory colonies, were discarded from analysis.

Feeding Assay. Sixth-instar *S. americana* were collected within 24 h of molting to obtain approximately same-aged individuals. Grasshoppers in the feeding assay were treated with 1.2×10^5 conidia of *M. flavoviride* in 2 μl peanut oil, applied topically to the pronotum using a 10-μl micropipette. The controls received 2 μl of pure peanut oil. Three repetitions, over time, of 15 grasshoppers were used per *M. flavoviride*-treatment and control. After treatment, individual grasshoppers were placed in sealed 2-liter plastic containers with a moistened paper towel on the floor of the container and were fed daily a single lima bean 'Camgreen Bush Lima' leaf trimmed into a rectangle measuring 30–32 cm^2. The use of the moistened paper towel has been shown to maintain the turgidity of lima bean leaves for the duration of their use. Every 24 h, the leaf material remaining in each replicate was measured using a portable leaf area meter (Model LI-3000A, LI-COR, Lincoln, NE) to determine the leaf area consumed by the individual grasshoppers. Lima bean plants were maintained under greenhouse conditions, with leaves harvested daily. All grasshoppers were maintained in a rearing room at 30 ± 2°C and a photoperiod of 14:10 (L:D) h.

Table 1. Mean percentage of cumulative mortality (± SE) and mean percentage of sporulation ± SE of *S. americana* and *M. sanguinipes* treated with *M. flavoviride* and *B. bassiana* at 7, 14, and 21 d after treatment

Treatment	Species	Mean % mortality ± SE			% mean sporulation ± SE
		7 d	14 d	21 d	
Control	*S. americana*	0 ± 0	11.7 ± 3.5	14.2 ± 4.2	
	M. sanguinipes	6.7 ± 5.6	12.5 ± 9.2	16.7 ± 12.2	
1.2×10^5	*S. americana*				
	M. flavoviride	69.2 ± 0.1	99.2 ± 0.01	100 ± 0	94.2 ± 2.1
	B. bassiana	1.7 ± 1.1	7.5 ± 1.6	21.7 ± 4.2	7.1 ± 7.1
	M. sanguinipes				
	M. flavoviride	74.2 ± 6.4	97.5 ± 0.8	99.2 ± 0.8	93.1 ± 5.8
	B. bassiana	11.7 ± 7.4	30.0 ± 13.0	39.2 ± 15.7	75.6 ± 11.7
1.2×10^3	*S. americana*				
	M. flavoviride	8.3 ± 5.5	75.8 ± 4.8	88.3 ± 2.9	91.6 ± 2.2
	B. bassiana	1.0 ± 1.0	10.0 ± 3.6	15.0 ± 6.2	0 ± 0

$n = 120$.

Data Analyses. The effect of treatment combination (i.e., grasshopper and fungal species) on percentage of mortality at 7, 14, and 21 d after treatment and percentage of sporulation, was determined by factorial analysis of arcsine transformed corrected mortality (Abbott 1925), using PROC general linear model (GLM) (SAS Institute 1989). Means were separated by the Fisher least square difference (LSD) procedure (SAS Institute 1989). However, when there was a significant interaction between the grasshopper and fungal species, both factors were analyzed at fixed levels of the other factor.

The LT_{50} estimates and their confidence limits were calculated by survival analysis, using LIFEREG (SAS Institute 1989). Data corresponding to surviving individuals are termed censored data and are also included in survival analysis. LT_{50} estimates were determined to not differ significantly if their 95% CIs overlapped. The Weibull model (1951) was used in the survival analysis. The survival function of the Weibull model is defined as $\exp[-(x/b)^c]$, where the x parameter is day after treatment, b is a shape parameter that varies among treatments (equal to exp[intercept]), and c is a scale parameter (equal to 1 per scale). Both the intercept and scale are estimated in survival analysis using the LIFEREG procedure (SAS Institute 1989). The median time to death, or LT_{50}, is equal to $b(\ln 2)^{1/c}$. To evaluate the fit of the Weibull model to the actual data, Goodman and Kruskal's gamma (1979) was calculated to assess the relationship between the predicted and observed mortalities at 3.5-d intervals starting at 0–3.5 d after treatment and continuing on to 21+ d after treatment (i.e., censored data).

In the feeding assay, a 2-sample t-test was performed to determine the differences between control and treated grasshoppers in leaf area (cm^2) consumed daily per individual grasshopper. The cumulative consumption per individual grasshopper as a function of time was analyzed by simple linear regression, using PROC REG (SAS Institute 1989), for the control and treated grasshoppers and were compared by calculating their 95% CIs.

Results and Discussion

Comparative Pathogenicity. *M. flavoviride* was much more virulent than *B. bassiana* to both *S. americana* and *M. sanguinipes*. By the 14th d after treatment, the *M. flavoviride* conidial dosage of 1.2×10^5 produced 99.2 and 97.5% mortality in *S. americana* and *M. sanguinipes*, respectively (Table 1). In contrast, by the 14th d after treatment, the *B. bassiana* of the same conidial dosage produced only 7.5 and 30% mortality in *S. americana* and *M. sanguinipes*, respectively.

At the conidial dosage of 1.2×10^5, the mean percentage of grasshopper mortality caused by *M. flavoviride* was greater than *B. bassiana* at 7 d ($F = 148.58$; df = 1, 12; $P < 0.001$), 14 d ($F = 317.10$; df = 1, 12; $P < 0.001$), and 21 d after treatment ($F = 138.77$; df = 1, 12; $P < 0.001$). There was no significant interaction between grasshopper and fungal species at 7 d ($F = 0.4121$; df = 1, 12; $P = 0.72$), and 21 d after treatment ($F = 3.27$; df = 1, 12; $P = 0.0956$). However, there was a significant interaction 14 d after treatment ($F = 14.99$; df = 1, 12; $P = 0.0022$), which was caused by a greater percentage of mortality of *M. sanguinipes* compared with that of *S. americana* in the *B. bassiana* treatment ($F = 19.29$; df = 1, 12; $P < 0.001$).

The percentage of sporulation of *M. flavoviride* from cadavers of *S. americana* and *M. sanguinipes* was not significantly different ($F = 0.039$; df = 1, 12; $P > 0.25$) (Table 1). There was a higher level of sporulation by *M. flavoviride* than by *B. bassiana* from cadavers of *S. americana* ($F = 42.38$; df = 1, 12; $P < 0.001$). However, a statistically significant difference between *M. flavoviride* and *B. bassiana* sporulation was not observed in *M. sanguinipes* cadavers ($F = 2.128$; df = 1, 12; $P > 0.10$). Furthermore, there was a greater percentage of sporulation of *B. bassiana* from *M. sanguinipes* cadavers than from *S. americana* cadavers ($F = 27.53$; df = 1, 12; $P < 0.001$). Whether fungal sporulation on an insect cadaver indicates that it was the cause of death, or is simply a facultative saprophyte is somewhat debatable (Tanada and Kaya 1993), although such

Table 2. Time of death (LT_{50} estimate) of *S. americana* and *M. sanguinipes* treated with *M. flavoviride* and *B. bassiana*

Treatment	Species	LT50 (95% CI)[a]	Intercept ± SE[b]	Scale ± SE[b]
1.2×10^5	*S. americana*			
	M. flavoviride	7.2 (6.8- 7.7)	2.09 ± 0.03	0.31 ± 0.02
	B. bassiana	29.1 (24.0-34.3)	3.50 ± 0.11	0.35 ± 0.06
	M. sanguinipes			
	M. flavoviride	6.6 (6.1- 7.2)	2.04 ± 0.04	0.41 ± 0.03
	B. bassiana	25.2 (19.9-30.5)	3.48 ± 0.12	0.69 ± 0.09
1.2×10^3	*S. americana*			
	M. flavoviride	12.3 (11.3-13.2)	2.64 ± 0.04	0.37 ± 0.03
	B. bassiana	42.0 (25.7-58.3)	3.92 ± 0.23	0.50 ± 0.11

Gamma values determined for the pooled repetitions of *S. americana* and *M. sanguinipes* are 0.925 ± 0.015 and 0.883 ± 0.032 (gamma estimate ± ASE), respectively. The gamma values being >0.8 describes a good fit between the observed and predicted values, and thus the use of the Weibull model is appropriate. $n = 120$.

[a] LT_{50} estimates are given in days. Survival analysis (SAS Institute 1989) on cumulative mortality of pooled repetitions.

[b] Survival function of the Weibull model is described as $\exp[-(x/b)^c]$: where x is day after treatment, b is exp[intercept], and c is 1/scale. LT_{50} estimate is described as $b(\ln 2)^{1/c}$.

confirmation of pathogenicity has been made by many authors examining grasshoppers infected with *M. flavoviride* and *B. bassiana* (Moore and Erlandson 1988, Bateman et al. 1993, Johnson and Goettel 1993, Foster et al. 1994b, Shah et al. 1994, Prior et al. 1995). Therefore, the sporulation of *B. bassiana* from the cadavers of *M. sanguinipes* confirms its pathogenicity, although with low virulence.

Metarhizium flavoviride produced significantly shorter LT_{50} estimates than those produced by *B. bassiana* for both *S. americana* and *M. sanguinipes* (Table 2). The LT_{50} estimate for *M. flavoviride*, at the conidial dosage of 1.2×10^5, was not significantly different between *S. americana* and *M. sanguinipes*. The conidial dosage of *M. flavoviride* had a significant influence on the observed LT_{50} estimates of *S. americana*, with an ≈5 d difference in LT_{50} estimate between the 2 dosages tested. A correlation between an increase in conidial dosage and a decrease in LT_{50} estimate of entomopathogenic Deuteromycete fungi has also been observed by other authors (Carruthers et al. 1985, McDowell et al. 1990, Milner and Prior 1994, Prior et al. 1995). In contrast with the response observed in *M. flavoviride*-treated *S. americana*, the conidial dosage of *B. bassiana* did not have such an influence on LT_{50} estimates. This further demonstrates the lack of efficacy of *B. bassiana* toward *S. americana*.

The LT_{50} estimates of all 3 *B. bassiana* treatments (i.e., the 2 conidial dosages for *S. americana* and the 1 dosage for *M. sanguinipes*) were not significantly different, and the 95% CIs were large. The lack of virulence of *B. bassiana* toward *S. americana* was not completely surprising because it reportedly failed in field trials conducted against *S. gregaria* (Nasseh et al. 1992). However, the lack of high virulence of *B. bassiana* toward *M. sanguinipes* was not expected because it is reported to cause high levels of mortality at the same or lower conidial dosages than those of the current study (Marcandier and Khachatourians 1987, Moore and Erlandson 1988, Khachatourians 1992). Furthermore, the same isolate used in the current study, GHA, has been reported to cause high levels of mortality in adult *M. sanguinipes* (Inglis et al. 1995). However, a lack of high virulence of *B. bassiana* toward *M. sanguinipes* has been reported before, and may have been the result of a change in production technique or in the fungus itself (Foster et al. 1994b).

Feeding Assay. In the feeding assay, the treated grasshoppers began dying 6 d after treatment, and all were dead by the 11th d. The LT_{50} estimate produced was 8.29 ± 0.45 d (mean ± SE).

The cumulative consumption per control and treated grasshopper as a function of time was linear (Fig. 1). However, the slope of the control grasshoppers (29.86 ± 0.26, slope ± SE) was much larger than that of the treated grasshoppers (16.74 ± 0.72) ($P < 0.05$). The variation in consumption among treated grasshoppers was much greater than that among control grasshoppers, as suggested by the R^2 values of 0.64 and 0.97 for the treated and control grasshoppers, respectively. The large variation among the treated individuals may be the result of differences in disease development among individual grasshoppers.

Starting 2 d after treatment, daily consumption per individual became significantly different between the control and treated groups ($P = 0.004$), with the difference remaining statistically significant for the duration of the experiment (Table 3). Furthermore, between 5 and 8 d after treatment there was an average decrease of 36.6% mean cumulative consumption in *M. flavoviride*-treated grasshoppers compared with untreated grasshoppers.

Treatment with *M. flavoviride* has been observed to cause a decrease in food consumption as early as 1-4 d after treatment in *S. gregaria* (Moore et al. 1992, Seyoum et al. 1994). Treatment with *Nomuraea rileyi* (Farlow) Samson has been observed to cause a decrease in consumption 2 d after treatment in *Heliothis zea* (Boddie) (Mohamed et al. 1982) and 5 d after treatment in *H. virescens* (F.) (Mohamed 1982). Treatment with *B. bassiana* has been observed to cause a decrease in consumption 3 d after treatment in *Leptinotarsa decemlineata* (Say) (Fargues et al. 1994). Some authors have associated the

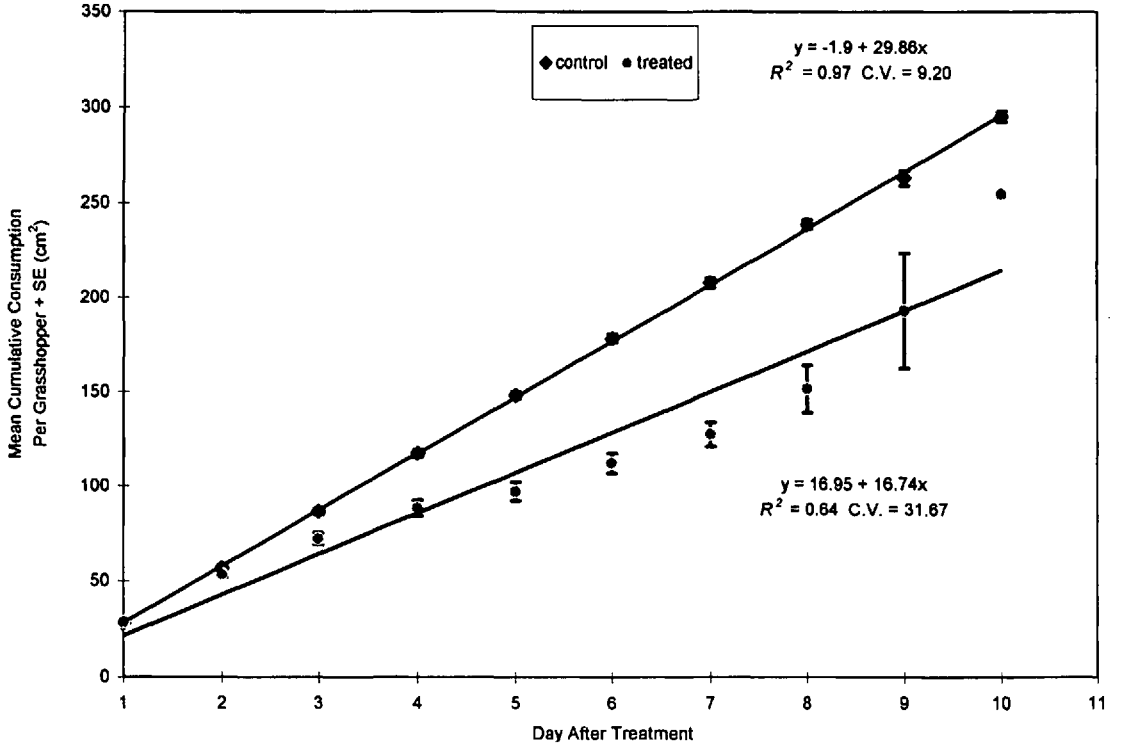

Fig. 1. Effect of M. flavoviride on cumulative consumption of lima bean leaves per 6th-instar *S. americana*.

decrease in food consumption with the proximity of host mortality (Thorvilson et al. 1985, Hajek 1989). The decrease in consumption in the current study began at least 3 d before host death and may have been more a function of physiological disturbance in *S. americana* (Zacharuk 1973, Cheung and Grula 1982, Mohamed et al. 1982). Hyphal bodies of *M. flavoviride* were observed in *S. americana* hemolymph beginning 72 h after treatment, with an increase in hyphal body number over time (Sieglaff 1996). This correlates with the more apparent disparities in consumption between the control and treated grasshoppers beginning 72 h after treatment. The presence of hyphal bodies of *Metarhizium anisopliae* (Metschnikoff) Sorokin in the hemolymph of the Elateridae have been associated with partial paralysis (Zacharuk 1973). Determination of the precise reason for the reduction in food consumption in *S. americana* treated with *M. flavoviride* will require further study.

Table 3. Mean daily consumption of lima bean leaves (cm^2 ± SE) per individual 6th instar *S. americana* treated with 1.2×10^5 conidia of *M. flavoviride*

Days after treatment	Control (*n*)[a]	Treated (*n*)[a]	*t*	*P*
1	27.9 ± 0.8 (45)	28.4 ± 0.8 (45)	−0.48	0.32
2	29.0 ± 0.6 (45)	25.2 ± 1.3 (45)	2.70	0.004
3	30.0 ± 0.7 (45)	18.8 ± 1.6 (45)	6.45	<0.001
4	30.7 ± 0.7 (45)	16.1 ± 1.5 (45)	8.76	<0.001
5	29.8 ± 0.7 (44)	8.9 ± 1.3 (45)	14.16	<0.001
6	30.1 ± 0.9 (44)	7.5 ± 1.2 (38)	15.21	<0.001
7	29.3 ± 0.9 (44)	4.6 ± 1.4 (26)	14.66	<0.001
8	28.6 ± 1.0 (40)	7.9 ± 2.8 (10)	6.91	<0.001
9	26.2 ± 1.6 (32)	7.0 ± 7.0 (3)	2.71	0.06
10	29.4 ± 0.7 (15)	5.4 (1)[b]	NC	NC

NC, not calculated.

[a] Numbers used for analysis. The decrease in sample sizes is attributed to death (in treated and untreated group) or molting (in untreated group).

[b] One grasshopper survived for 10 d and thus represents the only consumption for that period.

Decrease in consumption has been shown to be dosage dependent, with the modification on feeding behavior being more rapid with higher dosages (Moore et al. 1992, Fargues et al. 1994). This suggests that in field applications the use of a higher conidial dosage than that used in the current study may provide a faster response and less foliar damage.

This study demonstrates that *M. flavoviride* is much more virulent than *B. bassiana* to *S. americana* and *M. sanguinipes* under laboratory conditions. *M. flavoviride* was shown to be effective at moderate conidial dosages and to produce mortality relatively quickly. In contrast, *B. bassiana* was shown to be ineffective at the conidial dosages tested. The current work demonstrates that *M. flavoviride* is a better candidate than *B. bassiana* for field trials conducted against either *S. americana* or *M. sanguinipes*. Furthermore, this study demonstrates that there is a decrease in food consumption by 6th-instar *S. americana* beginning 2 d after treatment with *M. flavoviride*. The relatively long incubation period required by *M. flavoviride* is a negative attribute

affecting its potential use for the control of grasshoppers. However, the behavioral modification of a decrease in food consumption by *M. flavoviride*-treated *S. americana* 2 d after treatment partially compensates for the delayed mortality. This strengthens the argument for the use of *M. flavoviride* for the control of field populations of *S. americana*.

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