Microbial Control of *Schistocerca americana* (Orthoptera: Acrididae) by *Metarhizium flavoviride* (Deuteromycotina): Instar Dependent Mortality and Efficacy of Ultra Low Volume Application Under Greenhouse Conditions

D. H. SIEGLAFF, R. M. PEREIRA, AND J. L. CAPINERA

Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611–0620

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ABSTRACT The entomopathogenic fungus *Metarhizium flavoviride* Gams & Rozsypal has been widely tested for the suppression of African grasshoppers, but there has been no research on Nearctic species. We examined the potential of *M. flavoviride* for the control of the American grasshopper, *Schistocerca americana* (Drury). When tested under conidial dosages of 1.2×10^3, 1.2×10^4, and 1.2×10^5, 4th-instar *S. americana* were significantly more susceptible to *M. flavoviride* than 6th-instar and adult *S. americana*. At the conidial dosage of 1.2×10^5, *M. flavoviride* produced 89.7, 12.8, and 17.6% mean mortality 7 d after treatment in 4th-instar, 6th-instar, and adult *S. americana*, respectively. By the 14th d after treatment, the *M. flavoviride* conidial dosage of 1.2×10^5 produced 99.4, 86.1, and 80.8% mean mortality in 4th-instar, 6th-instar, and adult *S. americana*, respectively. The LD_{50} estimates for *M. flavoviride* of 1.7×10^4 conidia for the 4th-instar, 1.3×10^4 conidia for the 6th-instar, and 3.1×10^4 conidia for the adult *S. americana* are generally greater than those used for other acridids. Furthermore, mortality in *M. flavoviride*-treated *S. americana* was positively dosage dependent, as described by mean percentage of cumulative mortality and LT_{50} estimates. Greenhouse trials evaluating ultra low volume application to grasshoppers, foliage, or grasshoppers and foliage produced from 78.1 to 92.0% mean mortality 14 d after treatment. In this simulated field trial, grasshoppers and *M. flavoviride* were exposed to temperatures ranging from 10 to 40°C, which demonstrated the potential of *M. flavoviride* to be effective under large temperature fluctuations, as may occur in the field.

KEY WORDS *Schistocera americana*, *Metarhizium flavoviride*, grasshoppers, microbial control, biological control

Schistocerca americana (Drury) is one of the more important grasshoppers in Florida and has caused severe losses to Florida's citrus and ornamental industries (Griffiths and Thompson 1952, Capinera 1993). The most effective control of *S. americana* in Florida has been through use of ultra low volume application of malathion (FDACS 1992), but outbreaks of *S. americana* in citrus-growing regions of Florida in 1991 prompted a search for alternatives for their control. Entomopathogenic fungi are considered to have great potential as microbial control agents of grasshoppers (Greathead 1992, Prior et al. 1992, Goettel et al. 1995); the deuteromycete fungi hold the most promise (Prior and Greathead 1989). Many field trials have been conducted against African grasshoppers using the deuteromycete fungus *Metarhizium flavoviride* Gams & Rozsypal (Bateman et al. 1992; Lomer et al. 1992, 1993a, b, 1994; Kooyman and Godonou 1994; Douro-Kpindou et al. 1995). Overall, these field trials have reported successful suppression of grasshopper populations.

Susceptibility to entomopathogenic deuteromycete fungi can be influenced by host species, age, and life stage. The relationship between mortality and host age is important both in determining the optimal time for field application of entomopathogenic fungi for pest suppression and in predicting the development of mycosis in the field (Carruthers et al. 1985). Susceptibility generally has been observed to decrease with age (Zacharuk and Tinline 1968, Boucias et al. 1984, Carruthers et al. 1985, Feng and Carruthers 1985, McDowell et al. 1990, Prior et al. 1995), possibly as a result of maturation of the host's immune response (McCoy et al. 1988). However, relative susceptibility has also been shown to increase with host age (Glare 1994), or life stage (Zacharuk and Tinline 1968, Rath et al. 1995) in some insect species.

The entomopathogenic deuteromycete fungi can be applied by various methods for the control of grasshoppers (Auld 1992, Goettel and Roberts 1992). Grasshoppers have been exposed to fungi by using the following 3 methods: (1) direct spray with conidial suspensions, (2) exposure to foliage treated with conidial suspensions, and (3) exposure to bran bait formulations containing fungal mycelium or

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conidia (Johnson et al. 1988; Delgado et al. 1990, 1991; Bateman et al. 1992; Bradley and Wood 1992; Johnson et al. 1992; Lobo Lima et al. 1992; Nasseh et al. 1992; Johnson and Goettel 1993; Lomer et al. 1993a, b, 1994; Kooyman and Godonou 1994; Douro-Kpindou et al. 1995). All 3 application methods showed both successes and failures. Potential reasons for the differences in success are inherently different susceptibilities among grasshopper species, different environmental conditions, or behavioral avoidance of treated materials (Johnson et al. 1992, Nasseh et al. 1992).

In this study, the relative susceptibility of 3 stages of *S. americana* to *M. flavoviride* was determined by measuring mortality and estimating LD_{50} and LT_{50} values. As a comparison, the relative susceptibility of the 3 stages of *S. americana* toward an ultra low volume formulation of malathion was determined. Also, we sought to determine if *M. flavoviride* could cause mortality in *S. americana* under greenhouse conditions simulating those occurring in the field. The restriction of trials to a greenhouse rather than the field is necessary because *M. flavoviride* is not known to occur in the United States (Humber 1992), and it is not cleared for field release. Three different application methods were tested to evaluate different modes of contact between host and fungal inoculum, and to compare these results to those obtained indoors under more controlled conditions.

Materials and Methods

Grasshopper Culture. The *S. americana* colony was obtained from individuals field collected in North Florida, and maintained in laboratory culture since 1991. First through 5th instars were kept in aluminum screen cages measuring 30.5 by 30.5 by 30.5 cm, and 6th instars in aluminum screen cages measuring 54.6 by 38.1 by 38.1 cm. The culture conditions for all grasshoppers were 32°C, 40 ± 10% RH, and a photoperiod of 14:10 (L:D) h. All cages 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 a triple sulfa solution made up of 3 g of sulfamethazine, 6 g of sulfathiozole, and 4 g of 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. A stock material of *M. flavoviride* conidia (isolate IMI 330189), originally isolated from *Ornithacris cavroisi* (Finot) in Niger, was received from the International Institute of Biological Control (IIBC), Silwood Park, United Kingdom, and maintained in peanut oil (p-2144, Sigma) at 4°C. Approximately 14 mo after receiving the original stock material from the IIBC, fungal cultures were grown from the 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 *M. flavoviride* were harvested 7–12 d after inoculation by gently scraping the culture surface with a plastic sterile loop. Harvested conidia were stored immediately at −15°C until use (<2 wk after harvest). We found it necessary to culture our own *M. flavoviride* because the original stock material received from IIBC began to lose its viability, as measured by percentage of germination on SDAY plates, after ≈6 mo of storage. Our bioassays were conducted over a 2-yr period, and originally we planned to use only the original stock conidia. However, the decline in viability after 6 mo of storage circumscribed the culturing of *M. flavoviride* by our laboratory, and thus either the original stock conidia (original stock) or conidia from fungus cultured once on SDAY (1SDAY) were used in the experiments.

Experimental preparations of *M. flavoviride* were processed by suspending conidia in peanut oil, and lower concentrations were prepared from this material. The conidial concentrations were estimated with 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 1SDAY material produced >95% germination, and the original stock material >85% germination.

Fungal Bioassay. *S. americana* were treated with *M. flavoviride* (1SDAY) preparations applied topically to the pronotum using a 10-μl micropipette (Hamilton, Reno, NV). Grasshoppers were treated with 1.2×10^3, 1.2×10^4, and 1.2×10^5 conidia per insect. Fourth instars received their conidial dosages in 1 μl of peanut oil and 6th instars and adults in 2 μl of peanut oil. Controls received pure peanut oil. Four repetitions of 22–30 grasshoppers were used per dosage level. The total number of grasshoppers treated in the 4th and 6th instars and adult stages were 450, 355, and 505, respectively.

The same procedures were used with the original stock conidia, except that the treatment dosages ranged from 6×10^2 to 7×10^5 conidia per insect. The 4th instars were treated with 8 conidial dosages in 3 repetitions for a total of 276 grasshoppers. The 6th instars were treated with 10 conidial dosages in 5 repetitions for a total of 516 grasshoppers. The adults were treated with 6 conidial dosages in 3 repetitions for a total of 306 grasshoppers.

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 a photoperiod of 14:10 (L:D) h, 30 ± 2°C, and 45 ± 10% RH. Starting on 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 sporu-

Table 1. The 6 treatments defined by application method of *M. flavoviride* (treatments 1–4) or blank peanut oil (treatment 5)

Treatment no.	Foliage	Grasshoppers	Treatment
1	Not sprayed	Sprayed in cage	Grasshoppers alone
2	Sprayed in cage	Not sprayed	Foliage alone
3	Sprayed in cage	Sprayed in cage	Grasshoppers and foliage
4	Not sprayed	Topical	Topical treatment
5	Sprayed in cage	Sprayed in cage	Oil control
6	Not sprayed	Not sprayed	No treatment control

Treatments that did not include spray of grasshoppers or foliage received it immediately after treatment.

lation of *M. flavoviride* from the cadavers. This was observed 3–10 d after death. Only individuals with apparent *M. flavoviride* growth and sporulation arising from intersegmental membranes were considered as sporulating cadavers. Grasshoppers that died within 3 d after treatment with apparent bacterial infection, which is sometimes associated with our laboratory colonies (e.g., *Pseudomonas* spp.), were discarded from analysis.

Malathion Bioassay. A stock solution of 95% Cythion ultra low volume malathion was received from the American Cyanamid Company (EPA registration no. 241-208, Princeton, NJ). Experimental preparations were prepared by mixing a known quantity of the stock solution with acetone (A18-500, Fisher, Fair Lawn, NJ), and lower concentrations were prepared from this material. Malathion preparations were applied topically to the pronotum as described for conidial suspensions. Fourth instars received dosages ranging from 0.25 to 20 μg in 1 μl of acetone, 6th instars and adults received dosages ranging from 10 to 500 μg in 2 μl of acetone. Controls received pure acetone. Three repetitions of 18–30 grasshoppers were used per dosage level. The total number of grasshoppers treated in the 4th and 6th instars and adult stages were 374, 450, and 450, respectively. Grasshoppers were maintained as described previously, and mortality was assessed for 72 h at 12-h intervals.

Greenhouse Trial. Simulated field trials were conducted within a tightly sealed, air conditioned greenhouse covered by a double-wall polycarbonate material (Lexan-Thermoclear, Kansas City, MO). Concurrent laboratory trials were conducted within the above-mentioned indoor rearing room. Four repetitions were conducted between June 1994 and December 1995. Temperature and relative humidity conditions in the greenhouse varied between repetitions. The average ± variation of the temperature and relative humidity during the greenhouse trial was 30 ± 8°C and 75 ± 25% RH in June 1995, 32 ± 8°C and 75 ± 25% RH in July 1995, 25 ± 10°C and 70 ± 30% RH in October 1995, and 20 ± 10°C and 50 ± 25% RH in December 1995. All treated grasshoppers were kept within steel mesh cages measuring 58 by 58 by 58 cm.

The 6 treatments in the experiments involved various methods of application of either *M. flavoviride* (1SDAY) formulated in peanut oil (treatments 1–4) or blank peanut oil (treatment 5) (Table 1). An electric ultra low volume sprayer (model no. 1035, ARL, Lowell, MI) was used to deliver treatments 1, 2, 3, and 5 ≈1 m from the cage. Experimental units (i.e., cages) in treatments 1–3 received a total of 1.45×10^8 conidia of *M. flavoviride* (1SDAY) in 5 ml of peanut oil. Each individual grasshopper in treatment 4 received 5.8×10^4 conidia of *M. flavoviride* (1SDAY) in 2 μl peanut oil (i.e., same overall conidial concentration as applied to cages) applied topically as described above. Cages in treatment 5 (oil control) received 5 ml of blank peanut oil. Application of treatments was conducted at night to limit the exposure of *M. flavoviride* conidia to harmful UV radiation (Moore et al. 1993, Hunt et al. 1994). Each cage was supplied with 3 potted lima bean plants either at the time of treatment (treatments 2, 3, 5, and 6) or directly following treatment (treatment 1 and 4). One cage of each treatment was maintained in the greenhouse and 1 cage in the rearing room (except treatment 6, which was maintained only in the greenhouse). Romaine lettuce was provided when needed.

Twenty to 35 prereproductive adult *S. americana* were used per treatment, in each of 4 repetitions (except treatment 6, which was missing in the October 1995 repetition). The total numbers of grasshoppers treated were 216, 216, 204, 202, 209, and 87, for treatments 1–6, respectively. Daily mortality was assessed for 21 d. Sporulation of *M. flavoviride* from cadavers was observed as previously described.

Data Analyses. For the experiments involving *M. flavoviride* (original stock) and malathion, LD_{50} estimates and their 95% CI for the 3 stages of *S. americana* were determined by probit analysis (Finney 1971) on corrected (Abbott 1925) cumulative mortality of pooled repetitions. In the experiments involving the original stock conidia of *M. flavoviride*, analysis was done with data for the time after treatment that displayed the best dosage response (i.e., 5 d for the 4th instar, and 14 d for the 6th instar and adult). LD_{50} estimates were not calculated for the experiments involving the 1SDAY conidia because the use of only 3 dosages is not recommended in dose response analysis (Robertson and Preisler 1992). In the experiments involving malathion, analysis was done on the cumulative mortality after 72 h.

The effects of dosage level of *M. flavoviride* (1SDAY) and host stage on percent mortality 7, 14, and 21 d after treatment was determined by factorial analysis of arcsine transformed corrected (Abbott

Table 2. Mean percentage of cumulative mortality ± SE and mean percentage of sporulation ± of 3 stages of *S. americana* treated with 3 dosages of *M. flavoviride* (1SDAY) at 7, 14, and 21 d after treatment

Stage/treatment[a]	*n*	Mean % mortality SE			Mean % sporulating cadavers ± SE
		7 d	14 d	21 d	
4th instar					
10^5	135	89.7 ± 5.4	99.4 ± 0.6	99.4 ± 0.6	74.1 ± 3.2
10^4	135	59.5 ± 14.6	92.4 ± 5.1	97.2 ± 1.8	76.4 ± 0.7
10^3	135	29.5 ± 11.5	55.1 ± 10.1	58.1 ± 10.7	70.8 ± 0.6
Control	135	10.0 ± 3.3	14.4 ± 2.4	20.3 ± 4.7	
6th instar					
10^5	117	12.8 ± 10.5	86.1 ± 6.0	90.9 ± 7.6	84.0 ± 0.7
10^4	114	2.0 ± 2.0	46.2 ± 15.2	60.3 ± 16.2	82.7 ± 1.6
10^3	114	0 ± 0	19.1 ± 8.1	41.3 ± 10.0	59.8 ± 4.2
Control	114	5.1 ± 3.3	10.5 ± 1.8	14.6 ± 1.9	
Adult					
10^5	110	17.6 ± 8.1	80.8 ± 2.8	91.4 ± 3.9	91.7 ± 0.4
10^4	160	0 ± 0	35.1 ± 3.2	52.6 ± 4.3	92.7 ± 0.1
10^3	155	2.5 ± 1.6	23.1 ± 6.4	35.6 ± 6.9	84.7 ± 1.8
Control	208	4.0 ± 1.4	5.5 ± 2.3	6.0 ± 2.1	

[a] All dosages in all treatments (×1.2).

1925) mortality. Likewise, the effects of dosage level of *M. flavoviride* (1SDAY) and host stage on percentage of sporulating grasshopper cadavers was determined by factorial analysis of arcsine transformed data. Means were separated by the Fisher least square difference procedure ($P = 0.05$, SAS Institute 1989).

LT_{50} estimates and their confidence limits were determined by survival analysis for the experiments involving *M. flavoviride* (1SDAY) and malathion, using PROC LIFEREG (SAS Institute 1989). All 3 conidial dosages of *M. flavoviride* (1SDAY) and different application methods in the greenhouse trial were analyzed. The malathion dosages of 10 μg for the 4th instar, and 500 μg for the 6th instar and adult stages were analyzed. Data corresponding to surviving individuals are termed censored data and are also included in survival analysis (SAS Institute 1989). LT_{50} estimates were determined not to be significantly different if their 95% CI 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 which varies among treatments (equal to exp[intercept]), and c is a scale parameter [equal to 1/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 fit of the Weibull model to the data, the Goodman and Kruskal gamma (1979) was calculated to assess the relationship between the predicted and observed mortalities within 3.5 d intervals after treatment. A value of gamma equal to 1 indicates the maximum positive association between predicted and observed times of death. More familiar measures of association for model-fitting, such as R^2, were not appropriate because of the censored lifetimes in the study.

Results and Discussion

Fungal Bioassay. All 3 stages of *S. americana* were susceptible to *M. flavoviride*. By 14 d after treatment, the highest dosage of 1.2×10^5 conidia of *M. flavoviride* produced 99.4, 86.1, and 80.8% mortality in the 4th and 6th instar and adult *S. americana*, respectively (Table 2). Moreover, by 14 d after treatment the lowest dosage of 1.2×10^3 conidia of *M. flavoviride* produced 55.1, 19.1, and 23.1% mortality in the 4th and 6th instar and adult *S. americana*, respectively (Table 2).

The LD_{50} estimates for *M. flavoviride* (original stock) are 1.7×10^4 conidia for the 4th instar 5 d after treatment, 1.3×10^4 conidia for the 6th-instar 14 d after treatment, and 3.1×10^4 conidia for the adult *S. americana* 14 d after treatment (Table 3). These LD_{50} estimates for the 3 stages of *S. americana* are ≈2 logs larger than that estimated for 5th-instar *Phaulacridium vittatum* (Sjostedt) 7 d after treatment, and ≈1 log larger than that estimated for 5th-instar *Chortoisetes terminifera* (Walker) 6 d after treatment (Milner and Prior 1994). The LD_{50} estimates for the 3 stages of *S. americana* are in the same range as that of adult *S. gregaria* (Bateman 1992, Bateman et al. 1993). However, the studies conducted against adult *S. gregaria* calculated LD_{50} estimates 5 d after treatment, whereas the current study calculated LD_{50} estimates for 6th-instar and adult *S. americana* 14 d after treatment. These results would suggest that *S. americana* is less susceptible to *M. flavoviride* than other acridids tested, including *S. gregaria*.

Both instar of *S. americana* and conidial dosage of *M. flavoviride* (1SDAY) had a significant influence on percent mortality at 7 d ($F = 51.46$; df = 2, 29; $P = 0.0001$, for age; $F = 11.69$; df = 2, 29; $P = 0.0002$, for dosage), 14 d ($F = 26.43$; df = 2, 29; $P = 0.0001$, for age; $F = 40.99$; df = 2, 29; $P = 0.0001$, for dosage) and 21 d after treatment ($F = 13.24$; df = 2, 29; $P =$

Table 3. Effect of stage on the susceptibility (LD_{50} estimate) of *S. americana* to *M. flavoviride* (original stock) and malathion

Treatment/stage	n	df	Slope ± SE	LD_{50} (95% CI)	χ^2
M. flavoviride					
4th instar	276	6	1.08 ± 0.10	1.7×10^4 ($0.4–77.7 \times 10^4$)	45.70*
6th instar	516	8	1.26 ± 0.05	1.3×10^4 ($0.9–2.9 \times 10^4$)	19.04*
Adult	306	4	1.52 ± 0.05	3.1×10^4 ($2.3–4.8 \times 10^4$)	9.39**
Malathion					
4th instar	374	8	2.45 ± 0.05	3.24 (2.68–3.82)	14.20**
6th instar	450	3	1.29 ± 0.02	135.69 (108.02–172.72)	2.27
Adult	450	3	1.69 ± 0.02	159.03 (133.48–191.23)	7.41**

LD_{50} estimates are given in conidia (*M. flavoviride*) and micrograms (malathion) per insect. Profit analysis performed on Abbott's (1925) corrected cumulative mortality of pooled repetitions. Malathion using cumulative mortality received 72 h after application, and *M. flavoviride* using cumulative mortality received 5 d after application for the 4th instar and 14 d after application in the 6th instar and adult stages. *, $P < 0.05$; **, $P < 0.10$.

0.0001, for age; $F = 32.42$; df = 2, 29; $P = 0.0001$, for dosage) (Table 2). There was no significant interaction between host instar and conidial dosage at 7 d ($F = 2.07$; df = 4, 29; $P = 0.1101$), 14 d ($F = 1.37$, df = 4, 29; $P = 0.2689$), and 21 d after treatment ($F = 1.68$; df = 4, 29; $P = 0.1813$). The 4th instar had greater percent mortality than the 6th instar and adult stages at all 3 sample times. These results agree with many other studies that have reported a decrease in susceptibility to the entomopathogenic deuteromycete fungi as host stage increased (Zacharuk and Tinline 1968, Boucias et al. 1984, Carruthers et al. 1985, Feng and Carruthers 1985, McDowell et al. 1990, Prior et al. 1995).

Seven days after treatment, the highest conidial dosage of 1.2×10^5 produced greater mortality than the other 2 conidial dosages of 1.2×10^4 and 1.2×10^3. The percent mortality produced by *M. flavoviride* also displayed a positive dosage response at 14 and 21 d after treatment, when a more clear dose response was produced. A correlation between an increase in dosage of entomopathogenic fungi and an increase in mortality has been observed by numerous authors (Ferron 1977). Such a time-dependent effect on LD_{50} estimates and their confidence intervals should be considered when calculating dose response values such as LD_{50} or LC_{50} estimates.

The difference in susceptibility among the 3 stages of *S. americana* to malathion was similar to that from *M. flavoviride* treatment. The LD_{50} estimates, given in micrograms per grasshopper, are 3.2, 135, and 159.0 μg per grasshopper for 4th and 6th instars and adults, respectively (Table 3). These results demonstrate a decrease in susceptibility to malathion with an increase in insect stage. More importantly, the LD_{50} estimates display the greater susceptibility of the 4th instar, and essentially equal susceptibility between the 6th-instar and adult stages. The LT_{50} estimates (95% CI) for the 3 stages of *S. americana*, given in hours, are 23.1 (18.3–27.9), 47.9 (39.8–56.1), and 39.1 (30.3–47.9) for 4th and 6th instars and adults, respectively. The 4th instar is much more susceptible to malathion treatment than the other 2 stages ($P < 0.05$), as occurred with *M. flavoviride* treatment.

Both age of *S. americana* ($F = 7.30$; df = 2, 28; $P = 0.0028$) and treatment level ($F = 3.45$; df = 2, 28; $P = 0.0458$) had an influence on *M. flavoviride* sporulation on cadavers (Table 3). However, the interaction between host age and treatment level was not significant ($F = 0.53$; df = 4, 28; $P = 0.7133$). The adults had greater percentage of sporulating cadavers than 4th- and 6th-instar cadavers. Furthermore, 1.2×10^3 conidia produced a lower mean percentage of sporulating cadavers than 1.2×10^4 conidia. However, mean percentage of sporulating cadavers was not significantly different between the dosages of 1.2×10^3 and 1.2×10^5 conidia. The production of conidia after host death is directly responsible for secondary cycling of a microbial control agents, potentially making them more efficient than nonpersistent insecticides currently used against grasshoppers (Thomas et al. 1995). These results demonstrate that the age of *S. americana*, and possibly conidial dosage, may affect secondary cycling of *M. flavoviride* in the field.

At all 3 conidial dosages of *M. flavoviride*, the 4th instar had lower LT_{50} estimates than that of the 6th instar and adult (Table 4). The 6th instar and adult LT_{50} estimates were not significantly different from each other at all 3 conidial dosages. However, a positive relationship between an increase in the LT_{50} estimate and an increase in host age was observed at all 3 conidial dosages. This agrees with other studies that have shown an increase in LT_{50} estimates of entomopathogenic deuteromycete fungi with an increase in host age (Boucias et al. 1984, Carruthers et al. 1985, McDowell et al. 1990, Prior et al. 1995).

As the conidial dosages of *M. flavoviride* (1SDAY) increased, the LT_{50} estimate decreased in all 3 *S. americana* stages. A relationship between an increase in conidial dosage and a decrease in the LT_{50} estimate of entomopathogenic deuteromycete fungi has also been observed by others (Carruthers et al. 1985, McDowell et al. 1990, Milner and Prior 1994, Prior et al. 1995).

The LT_{50} estimate for 4th-instar *S. americana* treated with *M. flavoviride*, at comparable conidial dosages, is ≈2–3 times larger than that of 5th-instar *C. terminifera* and *P. vittatum* (Milner and Prior 1994) and about twice as large as that of 4th- and 5th-instar *S. gregaria* (Prior et al. 1995). However, the LT_{50} estimate for 4th-instar *S. americana* is sim-

Table 4. Effect of stage and conidial dosage on the time of death (LT_{50} estimate) of *S. americana* treated with *M. flavoviride*

Stage/treatment[a]	*n*	LT_{50} (95% CI)	Intercept ± SE	Scale ± SE	Gamma ± ASE
4th instar					0.714 ± 0.040
10^5	135	5.3 (4.9–5.7)	1.80 ± 0.04	0.39 ± 0.02	
10^4	135	7.8 (7.1–8.5)	2.22 ± 0.04	0.46 ± 0.03	
10^3	135	14.6 (12.3–16.9)	2.96 ± 0.08	0.75 ± 0.07	
6th instar					0.617 ± 0.042
10^5	117	10.4 (9.7–11.1)	2.46 ± 0.03	0.32 ± 0.02	
10^4	114	15.8 (14.3–17.3)	2.92 ± 0.05	0.42 ± 0.04	
10^3	114	20.7 (18.6–22.9)	3.18 ± 0.06	0.40 ± 0.05	
Adult					0.521 ± 0.044
10^5	110	10.5 (9.5–11.5)	2.50 ± 0.04	0.42 ± 0.03	
10^4	160	18.6 (16.9–20.3)	3.09 ± 0.05	0.45 ± 0.04	
10^3	155	22.4 (19.7–25.2)	3.30 ± 0.07	0.51 ± 0.06	

ASE, asymptotic standard error.

[a] All dosages in all treatments (×1.2). LT_{50} estimates are given in days. Survival analysis (SAS Institute 1989) on cumulative mortality of pooled repetitions. The survival function of the Weibull model is described as $\exp[-(x/b)^c]$, where x is the day after treatment, b is exp[intercept], and c is 1/scale. LT_{50} estimate is described as $b(\ln 2)^{1/c}$. Gamma values received for all 3 stages of grasshopper were >0.5, which validates the use of the Weibull model.

ilar to that of 3rd- and 4th-instar *Locusta migratoria* at a comparable conidial dosage (Welling et al. 1994). The LT_{50} estimates for the 6th-instar and adult *S. americana* treated with *M. flavoviride* are ≈3–4 times larger than that of 5th-instar *C. terminifera* and *P. vittatum* (Milner and Prior 1994), ≈3–5 times larger than that of 4th- and 5th-instar *S. gregaria* (Prior et al. 1995), ≈2–3 times larger than that of adult *S. gregaria* (Bateman et al. 1993, Prior et al. 1995), and about twice as long as that of 3rd- and 4th-instar *L. migratoria* at comparable conidial dosages (Welling et al. 1994).

The observed disparities among LT_{50} estimates of the age groups of *S. americana* and other acridids may be the result of inherently different susceptibility among acridids, differences among isolates of *M. flavoviride* (Prior 1992), differences in the site of inoculation (Prior et al. 1995), or differences among experimental methods or conditions. Interestingly, the study reporting a similar LT_{50} estimate to that of 4th-instar *S. americana* used a blastospore suspension of a different isolate of *M. flavoviride* (Welling et al. 1994) and not the same isolates as in the other studies (Bateman et al. 1993, Milner and Prior 1994, Prior et al. 1995).

Greenhouse Trial. All application methods of *M. flavoviride* resulted in significant mortality of *S. americana* adults under both greenhouse and laboratory conditions (Figs. 1 and 2). By the 14th d after treatment, 78.1, 87.1, 92.0, and 62.7% mortality occurred in the greenhouse trials by the application methods of grasshoppers alone, foliage alone, grass-

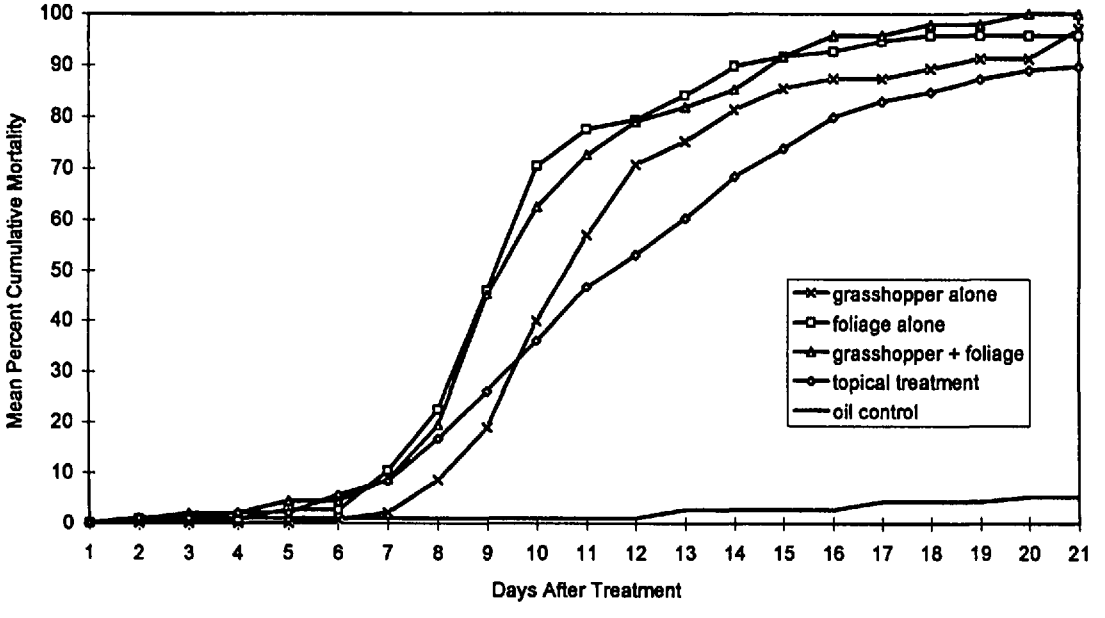

Fig. 1. Mean percentage of cumulative mortality of *S. americana* adults treated with an ultra low volume suspension of *M. flavoviride* under laboratory conditions.

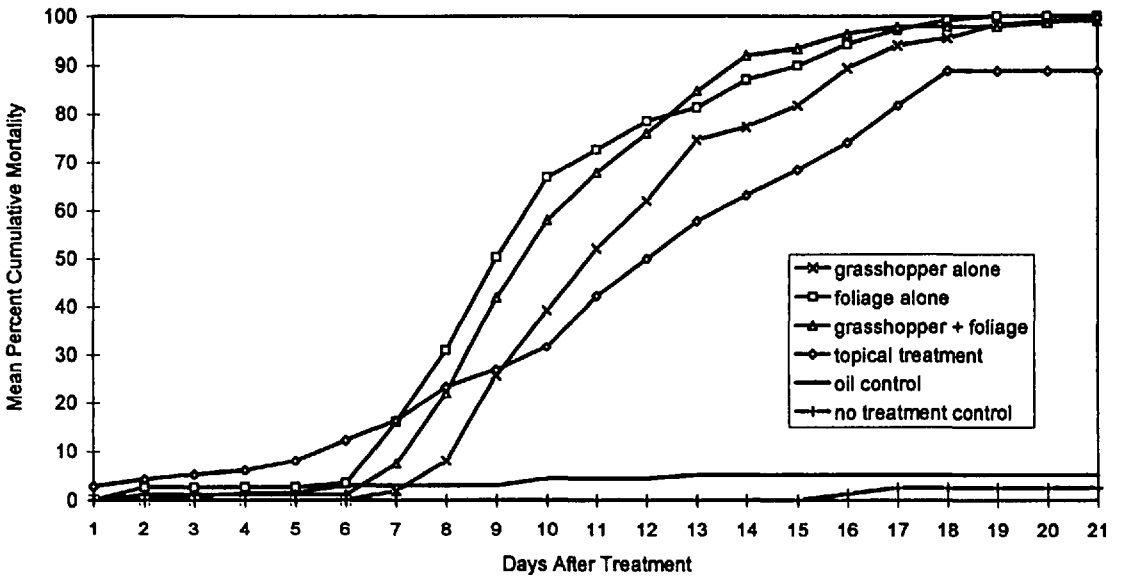

Fig. 2. Mean percentage of cumulative mortality of *S. americana* adults treated with an ultra low suspension of *M. flavoviride* under greenhouse conditions.

hoppers and foliage, and topical treatment, respectively (Fig. 2). By the 14th d after treatment, 83.8, 90.3, 85.4, and 66.9% mortality occurred in the laboratory trials by the same application methods, respectively (Fig. 1). No application method was obviously better in producing rapid mortality (Table 5). However, the application methods involving treated foliage (i.e., foliage alone, and grasshoppers and foliage) produced slightly smaller LT_{50} estimates than those not involving treated foliage (i.e., grasshoppers alone, and topical treatment). No field trials have been conducted against grasshoppers with a foliar application of *M. flavoviride*. However, the results obtained from foliar application of *Beauveria bassiana* (Balsamo) Vuillemin, another deuteromycete fungi, have been variable (Johnson et al. 1988; Delgado et al. 1990, 1991; Bradley and Wood 1992; Johnson et al. 1992; Lobo-Lima et al. 1992). Environmental differences may be responsible for the observed disparities. For example, successful trials were conducted in the laboratory and North America, whereas failures were conducted in Mali and Cape Verde.

Successful infection of *S. americana* in the current study may be caused by both immediate contact of grasshoppers with fungal formulations and lack of extremely adverse environmental conditions. Whether contact with the inoculum had to be immediate to cause mortality or if *M. flavoviride* could have kept its virulence for a slightly longer period of time would have to be determined by further study. Foliar application probably increased the contact with inoculum, augmenting dosage received, and thus decreased the LT_{50} estimate produced.

Trial location did not have a significant influence on time to death (i.e., LT_{50} estimates). This suggests

Table 5. Effect of application method and experimental location on the time of death (LT_{50} estimate) of *S. americana* treated with *M. flavoviride*

Location/ application method	*n*	LT_{50} (95% CI)	Intercept ± SE	Scale ± SE
Greenhouse				
Grasshopper alone	110	12.5 (11.8–13.1)	2.61 ± 0.03	0.25 ± 0.02
Foliage alone	109	10.5 (9.8–11.2)	2.47 ± 0.03	0.31 ± 0.02
Grasshopper and foliage	104	11.0 (10.3–11.7)	2.50 ± 0.03	0.27 ± 0.02
Topical treatment	100	13.0 (11.7–14.3)	2.72 ± 0.05	0.43 ± 0.04
Rearing room				
Grasshopper alone	106	11.9 (11.1–12.8)	2.59 ± 0.03	0.30 ± 0.02
Foliage alone	107	10.3 (9.5–11.0)	2.45 ± 0.03	0.33 ± 0.02
Grasshopper and foliage	100	9.8 (9.2–10.4)	2.38 ± 0.03	0.27 ± 0.02
Topical treatment	102	13.0 (11.9–14.1)	2.70 ± 0.04	0.36 ± 0.03

LT_{50} estimates are given in days. Survival analysis (SAS Institute 1989) on cumulative mortality of pooled repetitions. The survival function of the Weibull model is described as $\exp[-(x/b)^c]$, where x is the day after treatment, b is exp[intercept], and c is 1/scale. LT_{50} estimate is described as $b(\ln 2)^{1/c}$. Gamma value determined for the pooled repetitions of application methods and trial location is 0.458 ± 0.06 (gamma estimate ± asymptotic standard error), which validates the use of the Weibull model for these calculations.

Table 6. Effect of application method and trial location on sporulation of *M. flavoviride* on cadavers of *S. americana*

Location/application method	*n*	Mean % sporulating cadavers ± SE
Greenhouse		
Grasshopper alone	110	88.4 ± 8.3
Foliage alone	109	86.1 ± 8.1
Grasshopper and foliage	104	87.2 ± 9.7
Topical treatment	100	69.2 ± 14.9
Rearing room		
Grasshopper alone	106	94.3 ± 3.0
Foliage alone	107	91.8 ± 2.2
Grasshopper and foliage	100	90.4 ± 4.9
Topical treatment	102	93.6 ± 3.6

that great fluctuations in temperature and humidity, as experienced under greenhouse conditions, did not adversely affect the virulence of *M. flavoviride* against *S. americana* adults. However, in the current study, *M. flavoviride* was applied at night, and grasshoppers were placed in their respective treatments immediately after application. Furthermore, the greenhouse in which the trial was conducted reduces levels of harmful UV radiation, which has been shown to affect adversely *M. flavoviride* conidia (Moore et al. 1993, Hunt et al. 1994). A field trial is necessary to demonstrate the effects of field level solar radiation on the virulence of *M. flavoviride* toward *S. americana*.

Neither application method ($F = 0.38$; df = 3, 24; $P = 0.77$) nor experimental location ($F = 1.79$; df = 1, 24; $P = 0.19$) had an influence on *M. flavoviride* sporulation on cadavers (Table 6). Furthermore, the interaction between application method and experimental location was not significant ($F = 0.54$; df = 3, 24; $P = 0.66$). However, the greenhouse trials did produce a slightly lower percentage of sporulating cadavers than the laboratory trials; this was most pronounced in the topical treatment application method. Large variation occurred among experimental repetitions and may be responsible for the lack of statistical significance. These results indicate that application method and large temperature fluctuations do not affect the potential for secondary cycling. However, cadavers were immediately placed into ideal conditions after death (i.e., 25°C and 100% RH), possibly obscuring any effect trial conditions had on sporulation. Sporulation of *B. bassiana*, another deuteromycete fungi, from cadavers has been shown to occur only when the relative humidity is close to, or at, 100% RH (Ferron 1977, Marcandier and Khachatourians 1987). This may also occur in *M. flavoviride*.

This study demonstrates that *M. flavoviride* can cause significant mortality of *S. americana*. *M. flavoviride* was shown to be effective at moderate conidial dosages and to produce mortality relatively quickly. However, *S. americana* requires higher levels of inoculum and a longer incubation period when compared with some African grasshopper species. Because the 4th instar was most susceptible to *M. flavoviride*, younger instars should be targeted in the field. The use of a lower conidial dosage coupled with a more rapid mortality when treating younger instars supports this tactic. However, the 6th-instar and adult *S. americana* may also potentially be controlled in the field. Moreover, the capacity of secondary cycling of *M. flavoviride* may be increased when adults are the target stage.

Various methods of ultra low volume application of conidial suspensions of *M. flavoviride* can cause significant mortality of caged *S. americana* adults under both laboratory and greenhouse conditions. These results warrant further testing in the field where the potential of both direct and foliar spray of *M. flavoviride* as application methods for the control of *S. americana* must be determined.

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