

Laboratory and Field Evaluation of *Beauveria bassiana* Against Sugarcane Stalkborers (Lepidoptera: Pyralidae) in the Lower Rio Grande Valley of Texas

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ABSTRACT Of $\approx 18,200$ ha planted to sugarcane in south Texas, only ≈ 80 ha ($<0.5\%$) are treated with insecticides because this type of control is widely regarded as ineffective against stalkboring pyralids, the key pests of sugarcane. Therefore, nonchemical control measures, such as resistant varieties and biological controls, must be evaluated to mitigate the losses caused by stalkborers. We performed laboratory and field evaluations on the use of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) (strain GHA) against the Mexican rice borer, *Eoreuma loftini* (Dyar) (Lepidoptera: Pyralidae), which causes damage in south Texas estimated at between \$10 and \$20 million annually. We also performed bioassays against the sugarcane borer, *Diatraea saccharalis* (F.) (Pyralidae), the key pest in other sugarcane growing areas. In the bioassays, *E. loftini* was substantially more susceptible to *B. bassiana* than *D. saccharalis*, based on both 5-d LD₅₀ values and survival times. A commercial oil-based formulation of *B. bassiana* was evaluated in the field using the following treatments: oil alone (control), *B. bassiana* + oil, and *B. bassiana* + Silwet L-77 carrier at an application rate of 5×10^{13} spores per hectare. Neither numbers of *E. loftini* per stalk, nor stalk damage ($\approx 20\%$ bored internodes) were significantly affected by treatment. The application of *B. bassiana* + Silwet significantly affected the numbers of internodes showing high damage, but not those with low or medium damage. Analysis of yield data and juice quality showed no significant treatment effects. We conclude that the application of *Beauveria* + Silwet offers the best chances for reducing damage caused by *E. loftini* of those treatments tested. However, reductions in insect incidence or damage did not result in measurable increases in yield or sugar quality, probably because of insufficient coverage. Effective control of stalkboring pyralids in sugarcane using *B. bassiana* will likely require improvements in delivery technology.

KEY WORDS *Beauveria bassiana*, *Eoreuma loftini*, *Diatraea saccharalis*, sugarcane, biological control

THE MEXICAN RICE BORER, *Eoreuma loftini* (Dyar) (Lepidoptera: Pyralidae) is the primary insect pest of sugarcane (*Saccharum* spp.) in the Lower Rio Grande Valley of Texas. Since its entry from Mexico in the early 1980s (Johnson 1984), *E. loftini* displaced another stalkboring pyralid, the sugarcane borer, *Diatraea saccharalis* (F.), as the dominant pest of sugarcane (Meagher et al. 1994). Currently, *E. loftini* accounts for $>95\%$ of the endemic stalkborer population, and damages $\approx 20\%$ of cane internodes (Legaspi et al. 1997). Sugarcane is an important commodity crop in the Lower Rio Grande Valley, accounting for $\approx 10\%$ of the agricultural economy, with an annual economic value of $\approx \$64$ million. All sugarcane planted in Texas is grown in this region. Of $\approx 18,200$ ha planted to sugarcane in the 1996-1997 growing season, only ≈ 80 ha ($<0.5\%$) were treated with insecticides be-

cause these are widely regarded as ineffective against the stalkborers. Chemical treatment of sugarcane is hampered by several factors, including high plant biomass, prolonged insect activity in a benign climate, and the cryptic lifestyle of the pest, where larvae tunnel within stalks and pack the entrances with frass. The larval habit of tightly packing its tunnels with frass prevents physical access by control agents (Smith et al. 1993, Meagher et al. 1998). Sugarcane growers in Texas largely accept the damage by stalkborers, estimated at between \$10 and \$20 million annually (Legaspi et al. 1997, 1999). Nonchemical measures, such as resistant varieties and biological controls, must be evaluated to mitigate the losses caused by stalkborers.

One promising avenue for research is the use of entomopathogenic fungi as biological control agents of insect pests in sugarcane. Field evaluations have been performed using *Metarhizium anisopliae* (Metschnikoff) Sorokin (Deuteromycotina: Hyphomycetes) against several insect pests of sugarcane (e.g., Allard et al. 1990, Jackson and Glare 1992, Samson et al. 1994). In northeastern Brazil, *M. anisopliae* commonly cycles in *D. saccharalis* populations, reach-

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ing an incidence of $\approx 10\%$ during some months of the year (Alves 1986). *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) has been tested in the laboratory and field against numerous pests in various cropping systems [e.g., European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) (Feng et al. 1988); insect pests of cruciferous pests (Butt et al. 1994); house flies, *Musca domestica* L. (Diptera: Muscidae) (Geden et al. 1995); migratory grasshoppers (Orthoptera: Acrididae) (Brinkmann et al. 1997); Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Homoptera: Aphididae) (Vandenberg 1996); and Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) (Poprawski et al. 1997)] as well as for effects on nontarget organisms (Goettel et al. 1990). In this article, we report the results of laboratory and field evaluation of a commercial formulation of *B. bassiana* against the sugarcane stalkborers *E. loftini* and *D. saccharalis*.

Materials and Methods

Laboratory Bioassay. The colonies of both sugarcane stalkborers, *E. loftini* and *D. saccharalis*, were reared on an artificial diet (Martinez et al. 1988) at the Texas Agricultural Experiment Station, Weslaco, TX. *B. bassiana* (strain GHA) was tested as a wettable powder formulation registered in the United States (Mycotrol, Mycotech, Butte MT) for control of whiteflies (Homoptera: Aleyrodidae) and other homopteran pests. Test insects were sprayed with spores suspended in the organosilicone surfactant Silwet L-77 (Loveland Industries, Greeley, CO). Suspensions were prepared by placing 5–25 mg of technical powder into 10 ml of 0.03% aqueous Silwet solution, adding 1 g of glass beads (2 mm diameter), and shaking vigorously for 2 min. All suspensions were used within a few hours of preparation.

Fungal applications were made using a Potter Precision Laboratory Spray Tower (Burkard, Hertfordshire, England). One-milliliter aliquots of spore suspensions were sprayed at 0.7 kg/cm² (10 psi) using a fine nozzle (0.25 mm diameter). Each assay measured the effects of 5 dosages of spores ranging from ≈ 3 to 872 spores per square millimeter using 6 replicates per dosage. The control consisted of Silwet alone, and was used to correct for treatment effects (Abbott 1925). Each replicate consisted of 10 larvae (1st, 2nd, and 3rd instars of *E. loftini* and *D. saccharalis*) in a petri dish (5 cm diameter) with a slice of sugarcane stalk (2 mm thick). From the initial high dosage, a series of 2 serial dilutions was prepared, representing intermediate and low dosages. The dishes were maintained in the laboratory under ambient temperatures ($\approx 26^\circ\text{C}$) and were checked daily for mortality over 9–10 d. All dead stalk borers were removed and retained in a petri dish containing water agar to allow development of overt mycosis. Probit analysis was done on cumulative mortality (5 d after treatment). After spraying with the medium dosage, a plate of Sabouraud dextrose agar supplemented with 1% yeast extract (SDAY) was sprayed with an additional aliquot for viability assess-

ment. The SDAY plates were incubated at 25°C for ≈ 24 h. Afterward, 100 spores from each of 3 areas of the plate were observed (magnification = 500 \times) and scored for germination. The dosage applied to each sprayed section of stalk was estimated by direct counting (magnification = 500 \times) of spores deposited on the surfaces of agar sample blocks placed in the spray arena of the tower. All spore counts were adjusted for percentage of viability and expressed in counts per square millimeter.

Field Trials. The sugarcane variety 'NCo 310' was planted on 15 September 1995 in fields located at the Texas Agricultural Experiment Station in Weslaco, TX. Standard agronomic and management practices for the region were followed (Rozeff et al. 1998). The experimental field consisted of 16 rows measuring 90.8 m, with inter-row spacing of 1.5 m. The field was divided into 12 plots ([2 treatments + 1 control] \times 4 replicates in a randomized complete block design), each consisting of 3 rows of sugarcane measuring 21.3 m. Buffers consisting of 2 rows of untreated sugarcane separated the plots. A tractor-mounted sprayer was used to apply the treatments or carrier control. The sprayer consisted of 2 booms, each measuring 4.6 m and capable of being raised to a height of 4.5 m. Each boom had 9 nozzles oriented downward and arranged in sets of 3, each set spraying 1 row of cane at an inter-row distance of 1.5 m. The sprayer delivered 14.4 liters of spray volume to each plot at a pressure of 5.25 kg/cm² (75 psi) on each of 4 dates. The control and treatments were as follows: (1) oil alone (no *B. bassiana*), (2) *B. bassiana* + oil (Mycotrol ES [oil-based emulsifiable suspension] formulation), and (3) *B. bassiana* + Silwet (Mycotrol WP [wetable powder] *B. bassiana* + 0.03% aqueous Silwet surfactant). The application rate for fungal treatments was 5×10^{13} spores per hectare. Spray application dates (1996) were 31 May, 3 June, 10 July, and 30 September. Estimates were made of plant height at the time of sampling.

Sampling of sugarcane for insect damage was done on 10 and 17 June, 8 and 18 July, 1 and 14 August, 10 and 25 September, and 7 October 1996. Insect damage consisted of feeding and associated deleterious effects, most notably secondary infection caused by the sugarcane red rot fungus *Glomerella tucumanensis* Arx. & Mullar (= *Colletotrichum falcatum* Went.). At each sampling time, 10 stalks from each plot were sampled destructively. Cane was examined for borer damage by splitting each stalk and counting numbers of damaged and undamaged nodes (reported as percentage of bored internodes). Damage to internodes was graded as low (superficial feeding, mostly to external plant tissue), medium (borer tunneling into stalk and damaging at most 50% of the internal plant tissue), or high (almost the entire internode consumed by insect feeding or associated damage). The numbers and species identities of all borers collected were recorded. Numbers of borers found inside and outside the stalks were also recorded because significant numbers outside the stalks are more exposed to control agents and may indicate a suitable time for spraying. Dead stalkborers were removed and retained in a petri dish containing water-agar mixture to check for mycosis.

Table 1. Probit analysis results for 1 isolate of *B. bassiana* against the larval instars of *E. loftini* and *D. saccharalis*

Instar	<i>n</i> ^a	LD ₅₀ ^b	95% CL	Slope ± SE	χ ² (df)	h ^c
<i>E. loftini</i> ^d						
1st	290	4.7	2.8–7.5	1.2 ± 0.2	15.0 (28)	0.54
2nd	290	55.2	17.8–92.4	2.2 ± 0.4	75.4 (28)	2.69
3rd	293	31.4	13.2–50.6	1.7 ± 0.3	44.8 (28)	1.67
<i>D. saccharalis</i> ^e						
1st	296	72.1	41.8–123.9	1.1 ± 0.2	33.3 (28)	1.19
2nd	299	384.3	271.3–647.4	1.7 ± 0.5	25.7 (28)	0.92
3rd	290	777.0	523.5–1,596.8	1.8 ± 0.3	41.4 (28)	1.48

^a One assay for each instar; 10 insects per replicate, 6 replicates per dosage, 5 dosages per assay (starting *n* = 300; differences in reported *n* due to loss of larvae incurred during experiment).

^b Analysis done on log₁₀ spores per mm².

^c Heterogeneity factor.

^d Control mortality = 3.3%.

^e Control mortality = 8.5%.

Harvesting of sugarcane was on 28 October, the stalks were collected for juice analysis on 29 October and the field was burned on 30 October. Cane stalks for yield data were collected on 31 October and 2 November. Total plot weight of cane was obtained by harvesting and weighing 21.4 m of cane from each plot. Juice quality (sucrose content; measured using a polarimeter and recorded as percentage 'pol;'; Chen 1985) and sugar yield data were obtained following the methods described in Legaspi et al. (1999).

Statistical Analysis. Median lethal dosages (LD₅₀ values) from laboratory bioassays were estimated 5 d after treatment using the POLO PC computer program developed by Russell et al. (1977) (LeOra Software 1987). LD₅₀ values were calculated separately for each instar of both species. Replicates were conducted on different dates using different generations of insects. Mean survival time of the stalkborers sprayed at the high dosage was calculated by averaging the posttreatment survival times. (The high dosage treatments were selected because they induced highest mortalities.) For each instar, survival times were compared between species using *t*-tests. The numbers of stalkborers and percentage of damaged internodes were analyzed as a two-way analysis of variance (ANOVA), where time and treatment were independent variables using Systat statistical software (SPSS 1997). Percentage data were transformed (arc sine square root) before analysis, but are presented as untransformed means ± SE. Yield and juice quality data were analyzed for treatment effects by ANOVA. When treatment effects were significant, means were separated using Tukey honestly significant difference (HSD) test (*P* < 0.05).

Results and Discussion

Laboratory Bioassay. Spore viability was 90–95% during laboratory bioassays. Results of the bioassays, including probit-log dosage regression coefficients and LD₅₀ values are shown in Table 1. In both stalkborers, an inverse relationship was observed between larval instar and susceptibility to *B. bassiana*, except

Table 2. Mean survival time for stalkborers sprayed at high spore dosages (402–872 spores per mm²) of 1 isolate of *B. bassiana*

Instar	Mean days survival (±SE)				<i>t</i>	df	<i>P</i>
	<i>n</i>	<i>E. loftini</i>	<i>n</i>	<i>D. saccharalis</i>			
1st	53	3.8 ± 0.19	52	4.6 ± 0.28	2.5	90.4	0.014
2nd	57	3.3 ± 0.16	43	4.8 ± 0.31	4.3	63.5	<0.01
3rd	60	4.3 ± 0.11	58	6.4 ± 0.16	10.9	103.6	<0.01

for 2nd-instar *E. loftini*. The 1st-instar stage was the most susceptible in both species. This result is not uncommon, because younger instars often are more susceptible to *B. bassiana* (e.g., Feng et al. 1985, Vandenberg et al. 1998). Survival time data for stalkborers receiving the high dosage are shown in Table 2. Statistical analysis shows that for every instar, *E. loftini* survived for shorter periods after exposure to *B. bassiana* than *D. saccharalis*. Based on these data, *E. loftini* was substantially more susceptible to *B. bassiana* strain GHA than was *D. saccharalis*, and died more rapidly as a result of infection. These results identify this strain as a possible biological control agent of pyralid stalkborers of sugarcane. The extremely high susceptibility of *E. loftini* is a positive result with respect to Texas, because this species is the more important of the 2 pests in the Lower Rio Grande Valley (Legaspi et al. 1997, Spurgeon et al. 1997, Meagher et al. 1998). It is also possible that even a limited screening of available *B. bassiana* isolates might identify strains with high virulence against *D. saccharalis* as well as *E. loftini*.

Field Trials. Sugarcane plant heights in the months of May, June, July, and September were estimated to be 0.8, 1.1, 1.4, and 2.4 m, respectively. The total number of borers recorded from field sampling was 1,653, of which only 9 were identified as *D. saccharalis* (0.5%), which agrees with the results of previous surveys (e.g., Meagher et al. 1998). Mean numbers of borers per stalk are shown in Fig. 1. Mean numbers of *E. loftini* per stalk were significantly affected by time (*F* = 15.9; df = 8, 1,053; *P* < 0.01), but not by treatment (*F* = 0.7; df = 2, 1,053; *P* = 0.48) (interaction: *F* = 3.8; df = 16, 1,053; *P* < 0.01) (Fig. 1A). The proportion of borers found outside the stalks was close to 0.7 early in the season (June), but declined to ≈0.3 for the remainder of the sampling period (Fig. 1B). The high proportion of borers found outside the stalks suggests that chances for successful control may be increased by applying the control agents early in the growing season when borers are most likely to come in contact with spores. Despite 2 early season applications of *Beauveria*, only 32 (<2%) insects were found to have symptoms of mycosis (one in the control). However, mycosis is probably underestimated because many infected larvae (especially smaller ones) most likely escaped collection.

Similarly, percentage of bored internodes was affected by time (*F* = 59.3; df = 8, 1,053; *P* < 0.01), but not treatment (*F* = 2.6; df = 2, 1,053; *P* = 0.07) (interaction *F* = 1.7; df = 16, 1,053; *P* < 0.05) (Fig. 1C). Overall percentage of damage in the control was 22.5% (989 damaged internodes per 4,387 total internodes). Overall damage in the *Beauveria* + oil treatment was

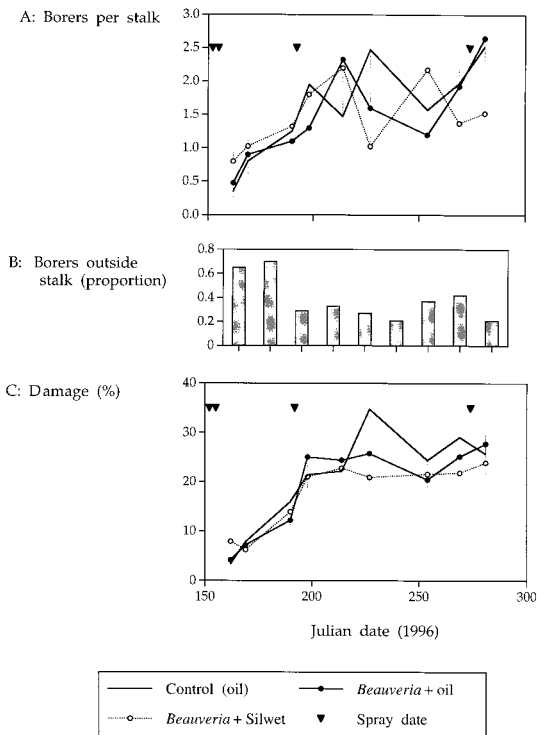


Fig. 1. (A) Mean numbers of *E. loftini* per stalk ($n = 40$, mean \pm SE) by treatment. (B) Overall proportion of borers found outside stalks. (C) Percentage of bored internodes ($n = 40$, mean \pm SE) by treatment.

20.8% (934/4,487), and in the *Beauveria* + Silwet treatment was 19.1% (863/4,508). Total numbers of internodes (summed across replicates) displaying low, medium, and high damage are shown in Fig. 2. Internodes showing low damage were significantly affected by time (two-way ANOVA; $F = 27.7$; $df = 8, 1,053$; $P < 0.01$), but not treatment ($F = 1.2$; $df = 8, 1,053$; $P = 0.26$) (interaction: $F = 2.5$; $df = 16, 1,053$; $P < 0.01$). Likewise, numbers of internodes showing medium damage were affected by time ($F = 17.3$; $df = 8, 1,053$; $P < 0.01$), but not treatment ($F = 1.9$; $df = 2, 1,053$; $P = 0.14$) (interaction: $F = 1.8$; $df = 16, 1,053$; $P < 0.05$). However, number of internodes showing high damage were significantly affected by both time ($F = 65.4$; $df = 8, 1,053$; $P < 0.01$) and treatment ($F = 4.0$; $df = 2, 1,053$; $P < 0.05$) (interaction: $F = 6.6$; $df = 16, 1,053$; $P < 0.01$). The damage in the treatment consisting of *Beauveria* + Silwet was significantly lower than the control (oil only) (Tukey HSD, $P < 0.05$). All other means comparisons were not statistically significant.

In field tests conducted in Brazil, applications of *M. anisopliae* at the rate of 1×10^{13} spores per hectare caused 58% mortality of *D. saccharalis* (Alves 1986) and preparations of *B. bassiana* containing 3.7×10^8 spores per milliliter reduced *D. saccharalis* damage by 45% (Alves et al. 1985). We have shown that *D. saccharalis* is less susceptible to *B. bassiana* than *E. loftini* in the laboratory. The effectiveness of fungal applications in Brazil

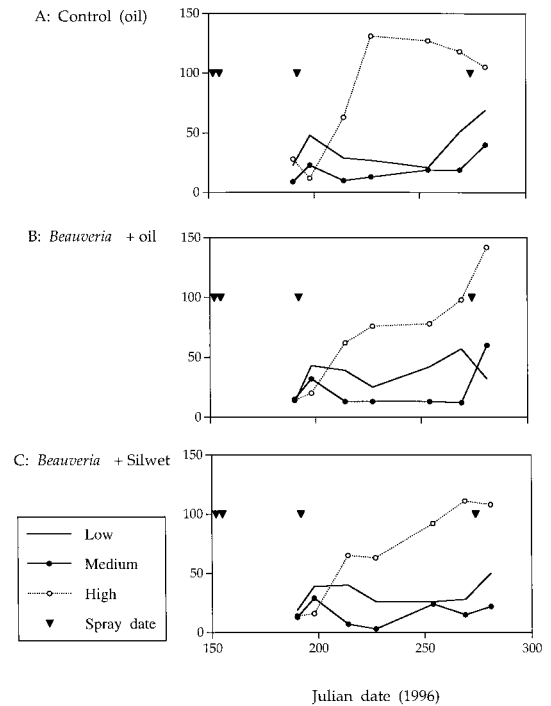


Fig. 2. (A) Total number of internodes showing low, medium and high damage in control. (B) Total number of internodes showing low, medium and high damage in *Beauveria* + oil treatment. (C) Total number of internodes showing low, medium and high damage in *Beauveria* + Silwet treatment.

may be attributed to greater contact between the host and pathogen. *D. saccharalis* is a larger host than *E. loftini*; its tunnels also are larger and, unlike *E. loftini*, usually free of the frass that may obstruct access by the control agents (Smith et al. 1993, Meagher et al. 1998). However, because their application method, target insect, fungal isolate, and dosage used were different, direct comparisons are impossible.

Analysis of yield data and juice quality ($n = 4$) showed no significant treatment effects. Mean weight per stalk was ≈ 1.6 kg (± 0.06 SE), regardless of treatment ($F = 0.006$; $df = 2, 9$; $P = 0.99$). Mean percentage of sucrose was 12.2 (± 0.8), 13.1 (± 0.7), and 12.7% (± 0.7) for the control, and *Beauveria* with oil, and Silwet, respectively ($F = 0.4$; $df = 2, 9$; $P = 0.67$). Mean total plot weight of 21.4 m of cane was 274.1 (± 37.6), 270.7 (± 15.8), and 296.7 kg (± 6.2), for the control, *Beauveria* with oil, and Silwet, respectively ($F = 0.35$; $df = 2, 9$; $P = 0.71$).

We have demonstrated in laboratory tests that sugarcane stalkborers are susceptible to *B. bassiana*. However, laboratory experiments provide information only on selected populations of insects submitted to maximum challenge tests. Evaluation and prediction of effects from these laboratory studies may not be realistically applicable to agroecosystems. The complexity and cost of field studies nevertheless underscore the need for initial pathogenicity tests in the

laboratory, although it is clear from our field results that extrapolation of bioassay data to practices in the field is difficult and conjectural. Moreover, the effectiveness of microbial pesticides in the field depends on several factors, including the innate susceptibility of the target pest, degree of exposure to the pesticide, prevailing environmental conditions, and physiological interactions between the host and pathogen (Fuxa and Tanada 1987, Tanada and Kaya 1993). The degree of exposure of the target depends largely on the probability that the pest will come in contact with the microbial pesticide. This probability is in turn dependent on other factors, such as the persistence of the microbial agent in the field, distribution of the microbial agent within the crop canopy, and the behavior and morphology of the target pest. Achieving effective spray coverage in the dense sugarcane canopy, one of the previously mentioned constraints to successful use of chemical controls, is certainly no less a problem with mycoinsecticides. At the same time, however, the dense sugarcane canopy and 12- to 18-mo crop cycle should provide highly favorable conditions for establishment, augmentation, and spread of fungal pathogens in stalkborer populations.

Previous comparisons between laboratory and field efficacies of commercial formulations of *B. bassiana* have yielded similar results. Ignoffo et al. (1979) tested Boverin (a *B. bassiana*-based mycoinsecticide produced in the former Soviet Union), on 3 lepidopteran pests of cole crops: *Pieris rapae* (L.) (Pieridae), *Plutella xylostella* (L.) (Plutellidae), and *Trichoplusia ni* (Hübner) (Noctuidae). LC₅₀ values in the laboratory bioassays were 0.25, 0.023, and 0.27% of Boverin, respectively, for the 3 pests. However, little or no control was obtained in field tests using 1.0% Boverin on collard and soybean crops. To be competitive with alternative controls, the specific activity against the pests would need to be increased ≈50-fold (Ignoffo et al. 1979). Despite significant mortality in laboratory bioassays, the failure of fungal mycoinsecticides to induce field epizootics is commonly reported (Dorschner et al. 1991). Many failures are likely the result of unfavorable relative humidity and temperature conditions in the field, relative to those in the laboratory. In bioassays using *B. bassiana* against the hop aphid, *Phorodon humili* (Schrank) (Homoptera: Aphididae), relative humidity in petri dishes approached 100%, was variable (50–95%) in caged whole-plant experiments, but averaged only 30% in the field (Dorschner et al. 1991). Temperatures in the laboratory and cage experiments also were relatively constant at 21–22°C, but often exceeded 30°C in the field.

The pathogenicity of *B. bassiana* to nontarget or beneficial insects is reviewed by Goettel et al. (1990). The extensive host ranges of fungal pathogens in general, and *B. bassiana* in particular, necessitate host specificity studies relevant to a given field situation. However, in comparison with chemical insecticides, the risks of entomopathogenic fungi to nontarget organisms is generally minimal (Goettel et al. 1990). In the case of beneficial insects in sugarcane, laboratory studies using *Beauveria* + oil, *Beauveria* + Silwet, and

an untreated control showed no deleterious effects on the longevity of *Allorhogas pyralophagus* Marsh (Hymenoptera: Braconidae), a parasitoid imported from Mexico against *E. loftini* (J.C.L., unpublished data).

In summary, laboratory bioassays showed that *E. loftini* was more susceptible to *B. bassiana* than was *D. saccharalis*, and died more rapidly following infection. The field results did not show treatment likely to result in economic benefit. The significant time effects are to be expected because the insect populations generally increase with time. However, significant treatment effects were limited to declining incidence of severe internode damage between the control and *Beauveria* + Silwet treatment. The same trend was found in the percentage of bored internodes, which was also found to be lower in the *Beauveria* + Silwet treatment, compared with the control, although the effect was not strictly significant ($P = 0.07$). Reductions in insect incidence or damage did not result in measurable increases in yield or sugar quality, probably because of insufficient coverage. Damage levels of ≈20% bored internodes typically reported are estimated to cause economic loss of over \$1,100/ha (Legaspi et al. 1999). A reduction in damage levels to ≈5% bored internodes could bring economic losses to less than \$300/ha. Effective use of *B. bassiana* formulations against sugarcane pests will likely require improvements in technology for delivering the fungus.

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