Evaluation of *Beauveria bassiana* for Control of *Oebalus pugnax* (Hemiptera: Pentatomidae) in Rice[1]

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Abstract Isolates of *Beauveria bassiana* (Balsamo) Vuillemin were tested for biological control of rice stink bug, *Oebalus pugnax* (F.), in the laboratory, in small-plot field experiments compared with conventional insecticides, and in a large-plot experiment to determine the spread and persistence of the fungus. Isolate RSB was found in a naturally- infected *O. pugnax* in a rice field near Crowley, LA, and isolates LRC28 and LRC21 were obtained for their relatively good growth at high temperatures. The soil-derived isolate LRC28 was more virulent to *O. pugnax* adults than isolate RSB in a laboratory experiment. The fungal isolates, applied at $5.0\text{-}5.7 \times 10^{12}$ conidia/ha, did not differ from one another in reducing insect numbers or in infecting rice stink bugs in the small-plot experiments, although isolates LRC28 and RSB, but not LRC21, occasionally differed from the control. The overall impact of *B. bassiana* was moderate on *O. pugnax* nymphs and minimal on adults in the small-plot experiments. A single application of *B. bassiana* reduced rice stink bug nymphs on six of nine sampling dates and adults on two of nine sampling dates from 2-10 d after application in the three small-plot experiments, and prevalence of the fungus was higher in the *B. bassiana* treatment than in controls for nymphs on four dates versus none for adults. A single application of chemical insecticide reduced total rice stink bug numbers more than *B. bassiana* for at least 7 d in small-plot experiments, whereas a double application was more effective than *B. bassiana* for 10 d against nymphs. *Beauveria bassiana* was nearly as effective as a single application of chemical insecticide in suppressing rice stink bug numbers 7-8 d after application. Mixtures of *B. bassiana* and chemical insecticide provided better control of rice stink bug than a single application of either material alone. Fungal epizootics lasted 17-22 d after application, and a low level of fungus recycling occurred in all of the field experiments. In an experiment to monitor spread, *B. bassiana* moved rapidly after its application, probably because of host transport. However, disease prevalence did not differ with distance from the treated plot. Disease prevalence was significantly greater in *O. pugnax* and *Lygus* spp. than in orthopterans. High temperatures probably were the major factor limiting *B. bassiana* epizootics in the current research. Thus, *B. bassiana* has potential for integrated management programs of *O. pugnax* in rice, because it was moderately effective against nymphs and had an additive effect with insecticides.

Key Words Rice stink bug, *Oebalus pugnax*, *Beauveria bassiana*, fungi, microbial control, biological control

The rice stink bug, *Oebalus pugnax* (F.), is a major pest of rice in the southern United States (Swanson and Newsom 1962, McPherson and McPherson 2000). This pest feeds on plant reproductive structures such as flowers and developing seeds

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(McPherson and McPherson 2000). Management to reduce *O. pugnax* numbers is essential because even moderate populations can inflict severe damage in yield as well as quality of rice (Bowling 1967, Patel et al., unpubl. data, Swanson and Newsom 1962). Current *O. pugnax* management programs rely on broad-spectrum chemical insecticides, and management is becoming increasingly difficult due to restrictions on use of some materials and environmental or human safety concerns (Todd et al. 1994). Resistance of *O. pugnax* to insecticides has been reported in Texas (Drees and Plapp 1986).

The use of fungi as biological agents against *O. pugnax* is a promising alternative to chemical control. Among many entomogenous fungi, *Beauveria bassiana* (Balsamo) Vuillemin is potentially the most useful in stink bug control. The primary reasons for interest in this fungus (Fuxa 1987) include its portal of entry by contact instead of ingestion, wide host range, replication in target insects (Ferron 1978, Roberts and Humber 1981), safety to nontarget organisms (Hokkanen and Lynch 1995), in vitro mass-culture (Jackson et al. 2000), numerous strains (St. Leger et al. 1992), and commercial availability (Jaronski 1997). It is a common, soil-borne entomopathogenic fungus that occurs worldwide (Fuxa and Kunimi 1997, McCoy et al. 1988). It naturally infects *O. pugnax* (Patel et al., unpubl. data) and other stink bugs (Moscardi et al. 1988), but is not known to cause natural epizootics in pentatomids. One potential problem with *B. bassiana* is that this fungus generally does not grow well at temperatures up to 30-35°C (Fargues et al. 1997), which are common in North American rice fields. Infections of certain species of stink bugs by *B. bassiana* have been investigated under laboratory (Moscardi et al. 1985, Sosa-Gomez et al. 1997) or field conditions (Sosa-Gomez and Moscardi 1998), but the potential of this fungus for microbial control of *O. pugnax* has not been studied. Also, little is known about the spread and persistence of this fungus after its application in the field.

The purposes of the current study were: (1) to compare the virulence of *B. bassiana* isolates to *O. pugnax*; (2) to determine its efficacy against rice stink bug nymphs and adults in field tests; (3) to determine whether combinations of chemical insecticides and *B. bassiana* isolates were more effective against *O. pugnax* than the separate materials; and (4) to determine the spread and persistence of *B. bassiana* after its release in the field.

Materials and Methods

Fungal isolates. Isolates of *B. bassiana* were selected for the experiments based on their tendency to sporulate on Sabouraud's dextrose agar + yeast (SDAY) (Becton, Dickinson & Co., Sparks, MD) at high temperatures (30-35°C). Isolates LRC21 and LRC28 were provided by the Lethbridge Research Center, Alberta, Canada. LRC21 and LRC28 were used in our experiments because they exhibited the greatest growth at high temperatures among *B. bassiana* isolates in a previous study (Fargues et al. 1997). Isolate RSB originated from a rice stink bug collected from rice field of Crowley, LA, in 2001.

Virulence bioassay. The bioassay techniques were adapted from those of Sun et al. (2002). The fungi were passaged in live hosts, stored on silica gel crystals at –20°C (Humber 1997), and grown on SDAY at 27°C. Conidia were harvested under sterile conditions by flooding the plate with 10 ml sterile distilled H_2O and then scraping the colony with sterile forceps. Conidia were stirred into suspension for 25 min in 300 ml 0.05% Triton X-100 and distilled H_2O, and the suspension was filtered through sterile

cheesecloth to remove debris. Conidial concentrations were ascertained with a hemocytometer under a compound microscope. All suspensions were stored at 4°C for up to 1 wk until used in assays. Viability of conidia at the time of treatment was confirmed by mixing a drop of suspension into a drop of Sabouraud's broth on a microscope slice and incubating under high humidity for 24 h at 27°C (Goettel and Inglis 1997).

Rice stink bugs were collected from rice fields near Crowley, LA. Collected bugs were maintained on cut panicles of barnyard grass, *Echinochloa* spp., in a glass aquarium in the laboratory for at least 2 d before being used in assays. For the bioassay, rice stink bugs in batches of 15 or 20 in a Petri dish were anesthetized by refrigerating them at 4°C for 5 min. Petri dishes with rice stink bugs were then shifted to a cold plate (Tissue Tek® II, Miles Inc. Diagnostics Division, Elkhart, IN), and 2 µL of conidial suspension was applied to the intersegmental region on the ventral surface of the abdomen of each bug with a micropipetter (P100, Eppendorf Inc., Hamburg, Germany). The bioassay of each fungal isolate included six fungal doses plus a control. The range of doses (4×10^2, 4×10^3, 4×10^4, 4×10^5, 4×10^6, and 4×10^7 conidia/bug) was determined in a preliminary test. The experiment had three replications over time; two replications of 15 insects and one replication of 20 insects were treated with each dose, and 0.05% Triton X-100 in distilled water served as the control. Inoculated bugs were transferred to a cut panicle of barnyard grass, *Echinochloa* spp., in an assay cell, one bug per cell. Each assay cell consisted of a 30-ml cup (UR1®, Sweetheart Cup Co. Inc., Owing Mills, MI) with two pieces of wet filter paper (Whatman #1, diam 30 mm), on which three pieces of 2-3 cm sections of panicles of barnyard grass were provided as food. The assay cells were closed with transparent lids (LUR1®, Sweetheart Cup Co. Inc., Owing Mills, MI) and maintained at room temperature and 16 h of daily illumination. The wet filter paper maintained the humidity within each cell at or near saturation. Food was changed every other day. The insects were examined daily for 12 d. Percentage mortality was calculated as the number of stink bugs that grew *B. bassiana* mycelium and conidia divided by the number of individuals treated.

Small-plot field experiments. Experiments were conducted at the Louisiana State University AgCenter Rice Research Station at Crowley, LA, during the summers of 2001, 2002, and 2003. The soil type was a silt loam (fine, montmorillonitic). The experimental design was a randomized complete block with four replications in 2001 and 2003 and five replications in 2002. Table 1 provides dates of agronomic practices and data collection. All seeds were treated with Icon® (fipronil, Bayer Cropscience, Monheim, Germany) to control rice water weevils. The total nitrogen fertilization rate was 120 kg/ha, with the majority of fertilizer applied before flooding. Other agronomic practices used were typical of those used in southwest Louisiana. Each plot measured 1.2 × 6.1 m in all years (7 rows at 0.17 m spacing). A buffer of at least 3.1 m was established between adjacent plots within each replication and 3.7 m between replications. Table 2 provides a list of treatments and application rates.

The plots were treated when rice headed (approximately 75% panicle emergence) and rice stink bugs were found in the plots. Treatments were applied in the evening to reduce exposure of *B. bassiana* conidia to the sun and to provide the spores with the high nighttime humidity. Applications were made with a CO_2 backpack sprayer at 2.3 kg/cm^2 and a flat fan Teejet 8002VS nozzle. Conidia were suspended in 1% (v/v) water/peanut oil; no adjuvant was used with chemical insecticides. The chemical insecticides used were pyrethroids: Karate Z® (lambda-cyhalothrin, Syngenta Crop

Table 1. Dates of selected agronomic practices and sampling during the 2001, 2002, and 2003 field tests

Practice	2001	2002	2003
Planting (drill-seeding)	10-Apr.	8-May	22-Apr.
Permanent flood	22-May	31-May	21-May
Application of treatments	23-Jul.	5-Aug.	21-Jul.
Sampling*	2, 4, 8, 16, and 22	2, 7, 11, and 17	2, 4, 8, 10, 14 and 18

* Days post-application.

Protection, Greensboro, NC), Mustang Max® (zeta-cypermethrin, FMC Corporation, Philadelphia, PA), and Prolex (gamma-cyhalothrin, Dow AgroSciences, Indianapolis, IN).

Rice stink bugs were sampled with a sweep net (38 cm diam), 10 sweeps per plot per sampling date. Collected insects were placed individually in 30-ml cups and returned to the laboratory, where they were reared. A wet filter paper (Whatman #1, diam 30 mm) was placed in the diet cups to maintain high humidity. Cut panicles of barnyard grass were provided as food every second day. The cups were maintained at 27°C, 14:10 L:D. Mortality was observed every alternate day. Dead individuals were moved to multiwell cell culture plates (BD Falcon, BD Biosciences, Franklin Lakes, NJ), which were wrapped in wet paper towels and placed in a closed plastic container to facilitate fungal growth by providing high humidity. The plates were maintained at 27°C. The insects were recorded as killed by *B. bassiana* if the cadavers exhibited external growth of the fungus within 12 d.

Large-plot spread experiment. The spread of *B. bassiana* released in rice fields at the Louisiana State University AgCenter Rice Research Station (Crowley, LA) was evaluated during the summer of 2003. The isolate LRC28 was chosen for this study because it performed well in the small-plot field experiments. The experiment was repeated after an interval of 27 d, because rice was planted on different dates in the fields being used for the two runs of the experiment, which in turn affected the time of panicle emergence and rice stink bug infestation. Each experimental run included a 4.6 × 4.6 m treatment plot, which was treated with *B. bassiana* at a rate of 5.6×10^{12} conidia/ha immediately after the appearance of rice stink bugs. Extensive pretreatment sampling detected only one infected insect, 2 yrs before this experiment. The fungus-treated plot was in the center of an untreated, 28 × 28 m plot for monitoring fungal spread. The 20 × 20 m control plot was 110 m from the fungus-treated plot and was not treated with *B. bassiana*. Sites for sampling fungus spread were established in the four cardinal directions at 4.6 m and 9.1 m from the treated plot. Sampling dates were 1, 5, 9, 13, 18, and 23 d after treatment.

Rice stink bugs, grasshoppers, and lygus bugs were sampled on every date from all of the spread-sampling sites, the treated plot, and the control plot. Each sample consisted of 10 sweeps per site with a sweep net (38 cm diam). One random sample was collected within the treated plot; four random samples were collected from the control plot; and one sample was collected at each spread sample-site.

Throughout the experiment, precautions were taken to minimize the chances of

Table 2. Treatments and rates in the 2001, 2002, and 2003 field tests

2001			2002			2003		
Treatment*	Group**	Rate†	Treatment*	Group**	Rate†	Treatment*	Group**	Rate†
Fury®	I	9.52	Fury®	I	8.16	Mustang Max®	I	11.34
Karate®	I	11.34	Karate®	I	13.61	Prolex®	I	9.07
LRC21	F	5.3×10^{12}	LRC28	F	5.0×10^{12}	Karate®	I	18.14
LRC28	F	5.3×10^{12}	RSB	F	5.0×10^{12}	Karate® (twice)	T	18.14
RSB	F	5.3×10^{12}	Karate® + LRC28	M	13.61+	LRC28	F	5.7×10^{12}
Control	C	—			5.0×10^{12}	LKLRC‡	M	9.07+
			LKLRC‡	M	9.07+			5.7×10^{12}
					5.0×10^{12}	Control	C	—
			Control	C	—			

* Fury® (Zeta-Cypermethrin, FMC Corp.), Karate® (lambda cyhalothrin, Syngenta), Mustang Max® (Zeta-Cypermethrin, FMC Corp.), Prolex® (Gamma cyhalothrin, Dow AgroSic.), LRC21, LRC28, RSB: isolates of *B. bassiana* (see text). The chemical-fungus combined treatments were applied in sequence, not mixed in the sprayer tank.

** Groups of treatments used for statistical analysis. I = chemical insecticide; F = fungal isolate; M = insecticide plus fungal isolate; T = chemical insecticides applied twice, the second application made a week after the application dates mentioned in Table 1; and C = control.

† Rates of treatments; AI/ha for chemical insecticides and conidia/ha for fungal isolates.

‡ Reduced rate of Karate® + LRC28.

samplers contributing to fungal spread. Foot traffic in the fields was limited to that of the samplers. The samplers always walked from sites least likely to have fungus (e.g., open spaces in the field and spread-sampling sites most distant from treated plots) to sample sites with increasing chance of having infected insects. Samplers always exited the field along the same path, in one direction away from fungal-treated plots. Sweep nets were changed frequently during each sampling date to prevent contamination to uninfected insects, and they were autoclaved before the next sample date.

The collected insects were maintained and mycoses determined in the laboratory with the same procedures described for the small-plot field experiments.

Data analysis. The bioassay data were subjected to probit analysis (PROC PROBIT, SAS Institute 1996) after correction for control mortality with Abbott's formula (Abbott 1925). The mortality data from the three replicates for each dosage were combined into one data point for this analysis.

Within each year of the small-plot experiments, a mixed-model, split-plot repeated measures analysis was used to test the effect of treatments on numbers of adults, nymphs, or total rice stink bugs. The data were analyzed by PROC MIXED of SAS (Littell et al. 1996), with block (replicate) as a random effect, treatment as fixed effect, and sampling date (days post application) as a repeated measure. Treatments were grouped (Table 2): I, insecticides applied once; F, fungal isolates applied once; M, combined insecticides and fungal isolates applied once; T, insecticides applied twice; C, untreated. Treatments nested within these groups (Table 2) were analyzed for differences. If they were not significantly different, then inferences were made about the groups instead of individual treatments. The slice statement of SAS was used to detect significant differences by days post application for interactions of days and groups of treatments. Means within groups of treatments were separated by the Fisher's protected LSD test (Milliken and Johnson 1984).

Within each year, data on mortality of rice stink bug by *B. bassiana* were subjected to logistic regression analysis by PROC LOGISTIC (SAS Institute 1996) to determine the effects of treatments (isolates of *B. bassiana*), days post application, and treatment-by-days postapplication interaction. A backward elimination method was used for model building. When no adults or nymphs were present, a value of 0.0001 was used for purposes of analysis. Untransformed values for the means and standard errors of the means (SE) are presented in the tables. Mean mortality rates were separated by Fisher's protected LSD test (Milliken and Johnson 1984).

Mortality data from the large-plot spread experiments were subjected to logistic regression analysis by PROC LOGISTIC (SAS Institute 1996) to determine the effects of species, day post application, distance, and direction from the fungus-treated plot on fungus prevalence. In a preliminary analysis, data inside the fungus-treated plot (distance = 0) were eliminated to determine whether compass direction influenced fungal spread. A backward elimination method was used for model building. Direction was not significant and was, therefore, removed from the final model. Further analysis was performed on the entire data set, including the treated plot. Wald confidence limits (SAS Institute 1996) were used to compare prevalence rates among different host species.

Results

Virulence bioassay. Table 3 summarizes the LD_{50} parameters from the virulence bioassays, which indicated that both the isolate LRC28 and the isolate RSB were

Table 3. Log-dose-probit parameters for isolates of *B. bassiana* against rice stink bug

Fungal isolate*	Slope ± SE	LD_{50} (95% FL)† (conidia × 10^5 per insect)	χ^2‡
LRC28	0.62 ± 0.06	0.42 (0.21-0.80)	5.95
RSB	0.59 ± 0.06	1.93 (1.01-3.80)	9.77

* Observed mortalities for each isolate were corrected with Abbot's (1925) formula.
† Fiducial limits.
‡ Heterogenity about regression (df = 16); table entries were not significant at $a = 0.05$.

virulent to rice stink bug. Fiducial limits (95%) did not overlap, indicating that the LD_{50}s (median lethal doses) for these two isolates were different. Isolate LRC28 was more virulent than isolate RSB. No mortality attributable to *B. bassiana* infection occurred in the control, and total mean control mortality was 9%.

Small-plot field experiments. Treatments within each group did not differ in their effects on numbers of nymphs or adults or total rice stink bugs, whereas groups of treatments and time (days post application) significantly affected the numbers (Table 4). There were significant interactions between treatment groups and days post application.

In 2001, applications of *B. bassiana* significantly reduced densities of rice stink bugs, but the effect was not as strong as chemical insecticides (Table 5). The chemical insecticide- and fungus-treated plots were infested with significantly lower numbers of nymphs than in control plots through day 8 post application. There were more bugs of all stages in the *B. bassiana*-treated plots than in the chemical insecticide-treated plots through day 4, but these numbers were not significantly different afterward. Adults, nymphs, and total rice stink bugs were reduced by at least 50% in *B. bassiana* plots compared with control plots on day 8. The proportion of nymphs to adults in the fungus-treated plots was 0.41 on day 2, 0.36 on day 4, 0.38 on day 8, 0.51 on day 16, and 0.38 on day 22.

In 2002, a combined chemical insecticide/*B. bassiana* treatment at times was more effective than individual applications of chemical insecticides or *B. bassiana* in reducing numbers of rice stink bugs (Table 6). Plots treated with chemical insecticide plus *B. bassiana* had significantly fewer bugs of all stages than in the control and *B. bassiana*-treated plots through day 7. Chemical insecticide-treated plots were infested with fewer bugs of all stages than in *B. bassiana* treated plots on day 2. These numbers were not significantly different afterward except that the *B. bassiana* plots and chemical insecticide plus *B. bassiana* treated plots were infested with fewer adults or total rice stink bugs than the chemical insecticide-only plots on day 17. Fungal prevalence was lower in nymphs in the chemical insecticide/*B. bassiana* treatment than in *B. bassiana* plots on day 7 in 2002 and days 2-4 in 2003. *Beauveria bassiana* plots had fewer nymphs (day 2) and adults or total rice stink bugs (day 7) compared with the control plots. The proportion of nymphs to adults in the fungus-treated plots steadily decreased from 0.74 on day 2-0.46 on day 17. A new treatment (chemical insecticide applied twice per plot) was evaluated in 2003 in addition to the treatments of previous years (Table 7). Two applications of chemical insecticides

Table 4. Analysis of variance for numbers of rice stink bugs as dependent variable in small-plot field experiments, 2001-2003

Year	Tested effects*	df	Nymphs		Adults		Total RSB	
			F	$P > F$	F	$P > F$	F	$P > F$
2001	Group**	2, 6	22.64	0.0016	28.30	0.0009	74.65	<0.0001
	Treatment (Group)†	3, 9	1.83	0.2119	1.65	0.2470	1.85	0.2084
	Time‡	4, 72	11.21	<0.0001	7.16	<0.0001	19.56	<0.0001
	Group × Time	8, 72	6.06	<0.0001	7.61	<0.0001	17.35	<0.0001
	Treatment (Group) × Time	12, 72	1.08	0.3897	0.76	0.6933	1.60	0.1116
2002	Group**	3, 12	19.45	<0.0001	6.05	0.0095	22.18	<0.0001
	Treatment (Group)†	3, 12	1.97	0.1717	3.24	0.0602	0.86	0.4876
	Time‡	3, 84	15.14	<0.0001	14.20	<0.0001	14.09	<0.0001
	Group × Time	9, 84	11.78	<0.0001	5.44	<0.0001	8.49	<0.0001
	Treatment (Group) × Time	9, 84	0.70	0.7090	1.99	0.1660	0.85	0.5739
2003	Group**	4, 6	23.50	0.0008	3.07	0.1068	35.25	0.0003
	Treatment (Group)†	2, 6	1.95	0.2225	1.24	0.3550	0.66	0.5515
	Time‡	5, 105	0.56	0.7299	1.05	0.3919	2.20	0.0601
	Group × Time	20, 105	1.95	0.0156	0.92	0.5633	3.95	<0.0001
	Treatment (Group) × Time	10, 105	1.11	0.3598	2.00	0.0405	0.50	0.8840

* Effects were tested by repeated measures analysis in PROC MIXED of SAS (Littell et al. 1996) of data in Tables 5, 6, and 7. Analysis was performed separately for adults, nymphs, or total rice stink bugs for each year.

** Test for differences between groups; groups of treatments are shown in Table 2.

† Test for differences among treatments within each group.

‡ Sampling dates (days post application).

Table 5. Mean number (±SE) of rice stink bugs in the 2001 small-plot field experiment

Days post application	Treatment*†	Nymphs	Adults	Total RSB
2	Insecticide	1.1 ± 0.2 c	1.0 ± 0.3 b	2.1 ± 0.6 b
	B. bassiana	4.3 ± 0.3 b	6.3 ± 0.7 a	10.6 ± 0.7 a
	Control	5.8 ± 0.9 a	6.3 ± 1.3 a	12.0 ± 0.7 a
	F	34.33	29.93	86.09
	P	<0.0001	<0.0001	<0.0001
4	Insecticide	1.3 ± 0.2 c	0.9 ± 0.4 b	2.1 ± 0.4 b
	B. bassiana	2.8 ± 0.4 b	5.0 ± 0.7 a	7.8 ± 0.5 a
	Control	4.0 ± 0.4 a	5.0 ± 0.6 a	9.0 ± 0.9 a
	F	10.53	18.48	39.50
	P	<0.0001	<0.0001	<0.0001
8	Insecticide	1.4 ± 0.4 b	1.4 ± 0.3 b	2.8 ± 0.5 b
	B. bassiana	1.5 ± 0.2 b	2.4 ± 0.4 b	3.9 ± 0.3 b
	Control	3.0 ± 0.0 a	5.5 ± 0.9 a	8.5 ± 0.9 a
	F	3.95	9.38	18.91
	P	0.0236	0.0002	<0.0001
16	Insecticide	3.0 ± 0.2 a	2.8 ± 0.5 a	5.8 ± 0.5 a
	B. bassiana	2.8 ± 0.4 a	2.7 ± 0.3 a	5.4 ± 0.5 a
	Control	3.8 ± 0.9 a	3.8 ± 0.8 a	7.5 ± 1.55 a
	F	1.44	0.76	2.72
	P	0.2447	0.4737	0.0723
22	Insecticide	2.8 ± 0.3 a	5.1 ± 0.4 a	7.9 ± 0.2 a
	B. bassiana	3.0 ± 0.2 a	4.9 ± 0.4 a	7.9 ± 0.3 a
	Control	4.3 ± 0.9 a	4.5 ± 0.5 a	8.8 ± 0.5 a
	F	3.11	0.21	0.50
	P	0.0505	0.8094	0.6091

* Analysis of variance, repeated measures, in PROC MIXED with the slice statement of SAS (df = 2, 72 in every ANOVA). Means in each column within each day post application followed by the same letter did not differ at $\alpha = 0.05$ (Fisher's protected LSD test).

† Groups of treatments (Table 2) were used for inferences because treatments within each group were not significantly different ($P > 0.05$, Table 4). "Insecticide" treatment refers to the chemical insecticide treatment.

significantly reduced numbers of nymphs or total rice stink bugs for the first 14 days compared with the control and *B. bassiana* treatment. *Beauveria bassiana*-treated plots had significantly fewer nymphs (day 8, 10) and total rice stink bugs (day 8) than control plots. Plots treated with chemical insecticides once had significantly lower

Table 6. Mean number (±SE) of rice stink bugs in the 2002 small-plot field experiment

Days post application	Treatment*†	Nymphs	Adults	Total RSB
2	Insecticide (INS)	0.2 ± 0.2 c	0.7 ± 0.3 b	0.9 ± 0.4 b
	INS + *Bb*	1.3 ± 0.4 c	1.2 ± 0.3 b	2.5 ± 0.6 b
	B. bassiana (*Bb*)	7.3 ± 1.6 b	2.6 ± 0.3 a	9.9 ± 1.6 a
	Control	9.4 ± 1.2 a	2.8 ± 0.9 a	12.2 ± 1.4 a
	F	42.33	5.99	42.45
	P	<0.0001	0.0010	<0.0001
7	Insecticide (INS)	1.4 ± 0.8 b	0.8 ± 0.3 bc	2.2 ± 0.7 bc
	INS + *Bb*	0.4 ± 0.2 b	0.6 ± 0.2 c	1.0 ± 0.2 c
	B. bassiana (*Bb*)	2.5 ± 0.4 ab	1.8 ± 0.2 b	4.3 ± 0.4 b
	Control	4.4 ± 1.2 a	3.4 ± 0.5 a	7.8 ± 1.2 a
	F	5.40	7.27	10.72
	P	0.0019	0.0002	<0.0001
11	Insecticide (INS)	1.1 ± 0.2 a	1.5 ± 0.2 a	2.6 ± 0.2 a
	INS + *Bb*	0.9 ± 0.2 a	1.6 ± 0.3 a	2.5 ± 0.4 a
	B. bassiana (*Bb*)	1.4 ± 0.2 a	1.4 ± 0.2 a	2.8 ± 0.1 a
	Control	2.6 ± 0.7 a	2 ± 0.3 a	4.6 ± 0.9 a
	F	0.95	0.29	1.07
	P	0.4189	0.8295	0.3681
17	Insecticide (INS)	2.2 ± 0.6 a	4.6 ± 0.8 a	6.8 ± 1.1 a
	INS + *Bb*	2.0 ± 0.3 a	2.0 ± 0.5 c	4.0 ± 0.5 c
	B. bassiana (*Bb*)	2.1 ± 0.2 a	2.5 ± 0.4 bc	4.6 ± 0.6 bc
	Control	3.2 ± 0.6 a	3.4 ± 0.7 ab	6.6 ± 1.1 ab
	F	0.49	8.57	3.05
	P	0.6899	<0.0001	0.0329

* Analysis of variance, repeated measures, in PROC MIXED with the slice statement of SAS (df = 3, 84 in every ANOVA). Means in each column within each day post application followed by the same letter did not differ at $\alpha = 0.05$ (Fisher's protected LSD test).

† Groups of treatments (Table 2) were used for inferences because treatments within each group were not significantly different ($P > 0.05$, Table 4). "Insecticide" treatment refers to the chemical insecticide treatment.

numbers of nymphs than *B. bassiana* plots through 10 d and lower numbers of total rice stink bugs through 8 d. On day 14, chemical insecticide plus *B. bassiana*-treated plots had significantly fewer nymphs than chemical insecticide-treated (once) plots, *B. bassiana* plots, and control plots. The proportion of nymphs to adults in the fungus-treated plots was 0.55 on day 2, 0.44-0.47 from day 4 to day 14, and 0.39 on day 18.

Significant numbers of rice stink bugs sampled from plots and reared in the laboratory exhibited signs of mycosis by *B. bassiana* (Tables 8-11). There was a significant treatment effect on disease prevalence in nymph and total bugs, but not adults, and mortality differed over time (Table 8). Mortality in nymphs or total rice stink bugs in the plots treated with LRC28 was significantly higher than in controls on at least one sampling date in each of the 3 yrs (Tables 9-11). Isolate LRC21 did not differ from other treatments. Isolate RSB differed from the control only in day-2 total rice stink bugs in 2001, and the three isolates did not differ from one another (Table 9). Control mortality was never higher than 6.2% and was always zero after day 7 (Tables 9-11). Isolate LRC28 caused mortality up to 10-22 d (Tables 9-11), whereas LRC21 caused mortality for only 8 d (Table 9). Isolate RSB killed rice stink bugs throughout both experiments in which it was applied except for the last sampling date in 2001 (Tables 9 and 10). Mean time to death by *B. bassiana* infection in field-collected *O. pugnax* returned to the laboratory was 4.2 d (range 3-6 d) for nymphs and 5.1 d (range 3-8 d) for adults. There was little variation in the mean and range of time to death among the three experiments.

The percentage nymphal mortality by isolates LRC28, LRC21, and RSB averaged 44, 26, and 26, respectively, during the first 8 d in 2001 (Table 9). The average percentage mortality of total rice stink bugs through 8 d in 2001 was 23% by LRC28, 16% by RSB, and 15% by LRC21. Through 11 d in 2002, nymphal mortality by isolate LRC28 averaged 39%, followed by the isolate LRC28 applied with Karate Z (37%), the RSB isolate (22%), and LRC28 applied with Karate at a reduced rate (13%) (Table 10). Mortality of total rice stink bugs through 11 d in 2002 by both LRC28 and isolate RSB averaged 17-18%, followed by LRC28 applied with Karate (12%), and Karate at ½X (9%). Through 10 d in 2003, nymphal mortality by LRC28 averaged 22% (Table 11), and mortality of total rice stink bugs by LRC28 averaged 13%, followed by the isolate LRC28 applied with Karate at ½X (6%).

Large-plot spread experiment. *Beauveria bassiana* spread rapidly after its application, but the epizootic ended completely by day 23 (Table 12). *Lygus* spp., *Conocephalus* spp., and *Melanopsis* spp., as well as *O. pugnax*, all became infected. Disease prevalence did not differ with direction and distance when the treated plot was not included in the analysis ($P > 0.05$). However, when the treated plot (distance = 0 m) was included and direction excluded in the final analysis, disease prevalence differed with species, time, and distance ($P < 0.05$). Disease prevalence decreased over time and distance from the treated plot. Disease prevalence in *O. pugnax* and *Lygus* spp. did not differ ($P < 0.05$), but prevalence in *O. pugnax* or *Lygus* spp. was greater than in *Conocephalus* spp. and *Melanopsis* spp. ($P < 0.05$). The fungus did not spread into the control plots, a distance of 110 m, by the end of the experiment (23 d after application). Time to death for field-collected *O. pugnax* returned to the laboratory averaged 4.9 d (range 4-7 d), for *Lygus* spp. 4.1 d (range 3-6 d), and for *Conocephalus* spp. and *Melanopsis* spp. 6 d (range 4-8 d). These means and ranges of time to death were similar among all sampling dates.

Discussion

Isolates of *B. bassiana* that we tested in our bioassays, LRC28 and RSB, both infected *O. pugnax*, but their virulence differed by almost 5X (Table 3). The rice stink bug-derived isolate RSB was less virulent to *O. pugnax* adults than the soil-derived isolate LRC28. This suggests that the host of origin may not be a reliable indicator of

Table 7. Mean number (±SE) of rice stink bugs in the 2003 small-plot field experiment

Days post application	Treatment*†	Nymphs	Adults	Total RSB
2	Insecticide (INS)	0.6 ± 0.2 b	1.2 ± 0.3 b	1.7 ± 0.4 b
	Insecticide (twice)	0.2 ± 0.2 b	1.7 ± 1.1 ab	2.0 ± 1.4 b
	INS + *Bb*	0.0 ± 0.0 b	2.0 ± 0.4 ab	2.0 ± 0.4 b
	B. bassiana (*Bb*)	3.0 ± 0.6 a	2.5 ± 0.5 ab	5.5 ± 0.5 a
	Control	3.2 ± 0.6 a	3.5 ± 0.9 a	6.7 ± 1.1 a
	F	14.50	2.43	9.86
	P	<0.0001	0.0524	0.0001
4	Insecticide (INS)	0.1 ± 0.1 b	2.3 ± 0.5 a	2.4 ± 0.5 c
	Insecticide (twice)	0.2 ± 0.2 b	2.5 ± 0.5 a	2.7 ± 0.6 bc
	INS + *Bb*	0.0 ± 0.0 b	2.2 ± 0.5 a	2.2 ± 0.5 c
	B. bassiana (*Bb*)	2.2 ± 0.2 a	2.7 ± 1.1 a	5.0 ± 1.0 a
	Control	2.7 ± 0.6 a	2.7 ± 0.2 a	5.5 ± 0.5 a
	F	11.14	0.14	4.19
	P	<0.0001	0.9673	0.0035
8	Insecticide (INS)	0.2 ± 0.1 c	1.7 ± 0.6 a	1.9 ± 0.6 c
	Insecticide (twice)	0.0 ± 0.0 c	1.2 ± 0.5 a	1.2 ± 0.5 c
	INS + *Bb*	0.0 ± 0.0 c	1.7 ± 0.5 a	1.7 ± 0.5 c
	B. bassiana (*Bb*)	1.7 ± 0.2 b	2.2 ± 0.9 a	4.0 ± 0.8 b
	Control	3.5 ± 0.6 a	3 ± 0.7 a	6.5 ± 1.3 a
	F	14.17	0.98	7.66
	P	<0.0001	0.4230	<0.0001
10	Insecticide (INS)	0.0 ± 0.0 c	2.7 ± 0.7 a	2.7 ± 0.7 b
	Insecticide (twice)	0.0 ± 0.0 c	0.2 ± 0.2 b	0.2 ± 0.2 c
	INS + *Bb*	2.2 ± 0.2 ab	2.2 ± 0.3 a	4.5 ± 0.3 ab
	B. bassiana (*Bb*)	1.7 ± 0.2 b	2.0 ± 0.6 ab	3.76 ± 0.8 ab
	Control	3.0 ± 0.4 a	2.7 ± 0.6 a	5.7 ± 0.9 a
	F	13.47	2.69	6.51
	P	<0.0001	0.0353	0.0001

(Table continues)

the probable virulence of a specific fungal isolate to a specific host. On the other hand, *B. bassiana* isolated from an isopteran was more virulent than isolates from hosts in other phylogenetic groups to the termite *Coptotermes formosanus* Shiraki (Wells et al. 1995).

Table 7. Continued.

Days post application	Treatment*†	Nymphs	Adults	Total RSB
14	Insecticide (INS)	2.2 ± 0.6 a	1.6 ± 0.3 a	3.7 ± 0.7 a
	Insecticide (twice)	0.0 ± 0.0 b	0.5 ± 0.3 a	0.5 ± 0.3 b
	INS + *Bb*	1.0 ± 0.4 b	1.7 ± 0.2 a	2.7 ± 0.5 ab
	B. bassiana (*Bb*)	2.2 ± 0.5 a	2.5 ± 0.6 a	4.7 ± 1.0 a
	Control	2.5 ± 0.6 a	2.5 ± 0.5 a	5.0 ± 0.4 a
	F	6.58	1.47	4.95
	P	<0.0001	0.2171	0.0011
18	Insecticide (INS)	1.4 ± 0.3 a	2.7 ± 0.2 a	4.2 ± 0.3 a
	Insecticide (twice)	0.8 ± 0.5 a	1.5 ± 0.6 a	2.2 ± 0.5 a
	INS + *Bb*	1.3 ± 0.2 a	2.2 ± 0.6 a	3.5 ± 0.6 a
	B. bassiana (*Bb*)	1.3 ± 0.2 a	2 ± 0.7 a	3.2 ± 0.9 a
	Control	2.3 ± 0.5 a	3 ± 0.4 a	5.2 ± 0.5 a
	F	1.58	0.91	1.94
	P	0.1848	0.4602	0.1098

* Analysis of variance, repeated measures, in PROC MIXED with the slice statement of SAS (df = 4, 105 in every ANOVA). Means in each column within each day post application followed by the same letter did not differ at $\alpha = 0.05$ (Fisher's protected LSD test).

† Groups of treatments (Table 2) were used for inferences because treatments within each group were not significantly different ($P > 0.05$, Table 4). "Insecticide" treatment refers to the chemical insecticide treatment.

Table 8. Analysis of variance for mortality of rice stink bugs by *B. bassiana* as the dependent variable in the small-plot field experiments, 2001-2003

			Nymphs		Adults		Total RSB	
Year	Tested effects*	df	χ^2	$P < \chi^2$	χ^2	$P < \chi^2$	χ^2	$P < \chi^2$
2001	Treatment	3	13.60	0.0035	3.79	0.2855	16.06	0.0011
	Time‡	1	14.65	0.0001	6.89	0.0087	19.96	<0.0001
2002	Treatment	4	13.30	0.0099	3.75	0.4400	20.93	0.0003
	Time‡	1	6.86	0.0088	9.72	0.0018	19.49	<0.0001
2003	Treatment	2	7.10	0.0287	3.85	0.1452	11.08	0.0039
	Time‡	1	4.63	0.0314	4.93	0.0264	10.10	0.0015

* Logistic regression analysis in PROC LOGISTIC (SAS Institute 1996) of data in Tables 9, 10, and 11. Analysis was performed separately for adults, nymphs, or total rice stink bugs for each year. Interactions of treatment by time were not significant in any year by the χ^2-test ($P > 0.05$).

‡ Sampling dates (days post application).

Table 9. Percentage infection (±SE) of rice stink bugs by *B. bassiana* in the 2001 small-plot field experiment

Days post application	Treatment*†	Nymphs	Adults	Total
2	LRC21	21.2 ± 14.2 a	8.3 ± 8.3 a	16.2 ± 6.2 ab
	LRC28	39.6 ± 6.2 a	21.3 ± 4.9 a	28.2 ± 5.0 a
	RSB isolate	35.8 ± 6.3 a	12.3 ± 4.4 a	20.8 ± 3.7 a
	Control	0 ± 0 a	6.2 ± 6.2 a	3.8 ± 3.8 b
4	LRC21	31.2 ± 23.7 ab	8.3 ± 8.3 a	15.3 ± 6.1 a
	LRC28	54.2 ± 20.8 a	10.0 ± 10.0 a	20.5 ± 9.0 a
	RSB isolate	24.4 ± 10.9 ab	8.3 ± 8.3 a	13.5 ± 5.9 a
	Control	5.0 ± 5.0 b	6.2 ± 6.2 a	5.8 ± 3.5 a
8	LRC21	25.0 ± 25.0 a	0 ± 0 a	12.5 ± 12.5 a
	LRC28	37.5 ± 23.9 a	8.3 ± 8.3 a	19.6 ± 7.1 a
	RSB isolate	16.7 ± 16.7 a	6.2 ± 6.2 a	12.5 ± 8.0 a
	Control	0 ± 0 a	0 ± 0 a	0 ± 0 a
16	LRC21	0 ± 0 a	0 ± 0 a	0 ± 0 a
	LRC28	12.5 ± 12.5 a	12.5 ± 12.5 a	10.0 ± 10.0 a
	RSB isolate	8.3 ± 8.3 a	0 ± 0 a	5.0 ± 5.0 a
	Control	0 ± 0 a	0 ± 0 a	0 ± 0 a
22	LRC21	0 ± 0 a	0 ± 0 a	0 ± 0 a
	LRC28	12.5 ± 12.5 a	0 ± 0 a	3.6 ± 3.6 a
	RSB isolate	0 ± 0 a	0 ± 0 a	0 ± 0 a
	Control	0 ± 0 a	0 ± 0 a	0 ± 0 a

* Mortality of rice stink bugs sampled on the given days post application and reared in the laboratory. Logistic regression analysis in PROC LOGISTIC was used to analyze the effect of treatments and days post application on mortality. The slice statement of SAS was used to detect significant differences by days post application for interactions of days and treatment. Means in each column within each day post application followed by the same letter did not differ at $\alpha = 0.05$ (Fisher's protected LSD test).

† Mortality data from the insecticide plots were not used because mortality in these plots was negligible; PROC LOGISTIC of SAS fails when this mortality is included.

Fungal isolates did not differ from one another in reducing insect numbers (Tables 5 and 6) or percentage infection (Tables 9 and 10) of rice stink bugs in the small-plot field experiments, although isolates LRC28 and RSB, but not LRC21, occasionally differed from the control (Tables 9-11). In view of the laboratory differences between LRC28 and RSB in LD_{50}'s (Table 3), this suggests that virulence might not be the most important criterion for selecting fungal pathogens to control this pest. Similarly, fungal virulence did not play a defining role in epizootics by *B. bassiana* in a laboratory population of *C. formosanus* (Sun et al. 2003). It has been hypothesized that virulence

Table 10. Percentage infection (±SE) of rice stink bugs by *B. bassiana* in the 2002 small-plot field experiment

Days post application	Treatment*†	Nymphs	Adults	Total
2	LRC28	34.6 ± 4.6 a	30.0 ± 13.33 a	31.8 ± 4.7 a
	LRC28 + Karate 1X	50.0 ± 50.0 a	33.3 ± 33.3 a	40.0 ± 24.5 a
	LRC28 + Karate 1/2X	16.7 ± 10.5 a	10.0 ± 10.0 a	15.0 ± 10.0 ab
	RSB isolate	27.4 ± 8.1 a	24.0 ± 11.2 a	27.2 ± 3.1 ab
	Control	2.0 ± 2.0 a	3.3 ± 3.3 a	3.3 ± 2.1 b
7	LRC28	50.0 ± 22.3 a	6.7 ± 6.7 a	22.7 ± 11.3 a
	LRC28 + Karate 1X	0 ± 0 b	0 ± 0 a	0 ± 0 a
	LRC28 + Karate 1/2X	25.0 ± 25.0 ab	0 ± 0 a	20.0 ± 20.0 a
	RSB isolate	30.7 ± 9.5 ab	10.0 ± 10.0 a	25.0 ± 8.3 a
	Control	0 ± 0 b	4.0 ± 4.0 a	3.3 ± 3.3 a
11	LRC28	30.0 ± 20.0 a	0 ± 0 a	13.3 ± 81.6 a
	LRC28 + Karate 1X	25.0 ± 25.0 a	0 ± 0 a	10.0 ± 10.0 a
	LRC28 + Karate 1/2X	0 ± 0 a	0 ± 0 a	0 ± 0 a
	RSB isolate	10.0 ± 10.0 a	10.0 ± 10.0 a	13.3 ± 8.2 a
	Control	0 ± 0 a	0 ± 0 a	0 ± 0 a
17	LRC28	6.7 ± 6.7 a	0 ± 0 a	3.3 ± 3.3 a
	LRC28 + Karate 1X	0 ± 0 a	0 ± 0 a	0 ± 0 a
	LRC28 + Karate 1/2X	20.0 ± 20.0 a	0 ± 0 a	3.3 ± 3.3 a
	RSB isolate	10.0 ± 10.0 a	0 ± 0 a	6.7 ± 6.7 a
	Control	0 ± 0 a	0 ± 0 a	0 ± 0 a

* Mortality of rice stink bugs sampled on the given days post application and reared in the laboratory. Logistic regression analysis in PROC LOGISTIC was used to analyze the effect of treatments and days post application on mortality. The slice statement of SAS was used to detect significant differences by days post application for interactions of days and treatment. Means in each column within each day post application followed by the same letter did not differ at $\alpha = 0.05$ (Fisher's protected LSD test).

† Mortality data from the insecticide plots were not used because mortality in these plots was negligible; PROC LOGISTIC of SAS fails when this mortality is included.

Table 11. Percentage infection (±SE) of rice stink bugs by *B. bassiana* in the 2003 small-plot field experiment

Days post application	Treatment*†	Nymphs	Adults	Total
2	LRC28	31.2 ± 12.0 a	16.7 ± 16.7 a	24.3 ± 12.0 a
	LRC28 + Karate 1/2X	0 ± 0 b	20.8 ± 12.5 a	20.8 ± 12.5 ab
	Control	0 ± 0 b	6.2 ± 6.2 a	2.8 ± 2.8 b
4	LRC28	33.3 ± 11.8 a	16.7 ± 16.7 a	25.0 ± 10.2 a
	LRC28 + Karate 1/2X	0 ± 0 b	8.3 ± 8.3 a	8.3 ± 8.3 a
	Control	6.2 ± 6.2 b	0 ± 0 a	4.2 ± 4.2 a
8	LRC28	12.5 ± 12.5 a	8.3 ± 8.3 a	16.7 ± 11.8 a
	LRC28 + Karate 1/2X	0 ± 0 a	0 ± 0 a	0 ± 0 a
	Control	0 ± 0 a	0 ± 0 a	0 ± 0 a
10	LRC28	12.5 ± 12.5 a	8.3 ± 8.3 a	10.0 ± 5.8 a
	LRC28 + Karate 1/2X	8.3 ± 8.3 a	0 ± 0 a	5.0 ± 5.0 a
	Control	0 ± 0 a	0 ± 0 a	0 ± 0 a
14	LRC28	8.3 ± 8.3 a	0 ± 0 a	3.6 ± 3.6 a
	LRC28 + Karate 1/2X	0 ± 0 a	0 ± 0 a	0 ± 0 a
	Control	0 ± 0 a	0 ± 0 a	0 ± 0 a
18	LRC28	0 ± 0 a	0 ± 0 a	0 ± 0 a
	LRC28 + Karate 1/2X	0 ± 0 a	12.5 ± 12.5 a	6.2 ± 6.2 a
	Control	0 ± 0 a	0 ± 0 a	0 ± 0 a

* Mortality of rice stink bugs sampled on the given days post application and reared in the laboratory. Logistic regression analysis in PROC LOGISTIC was used to analyze the effect of treatments and days post application on mortality. The slice statement of SAS was used to detect significant differences by days post application for interactions of days and treatment. Means in each column within each day post application followed by the same letter did not differ at $\alpha = 0.05$ (Fisher's protected LSD test).

† Mortality data from the insecticide plots were not used because mortality in these plots was negligible; PROC LOGISTIC of SAS fails when this mortality is included.

may not be the most important factor for the slow-acting microbial agents to succeed in insect control (Fuxa 1987, Fuxa et al. 1998).

The overall impact of *B. bassiana* was moderate on *O. pugnax* nymphs and minimal on adults in the small-plot field experiments. A single application of *B. bassiana*

Table 12. Mean percentage infection (n)* of hemipterans and grasshoppers by *B. bassiana* in the large-plot spread experiment in 2003

Distance (m)**	Days after application					
	1	5	9	13	18	23
			Oebalus pugnax†			
0	50.0 (10)	36.4 (11)	23.1 (13)	6.7 (15)	7.1 (14)	0 (9)
4.6	7.1 (28)	7.5 (53)	1.7 (58)	1.4 (72)	0 (61)	0 (29)
9.1	3.6 (28)	6.4 (47)	1.9 (54)	1.7 (60)	0 (61)	0 (26)
			Lygus spp.†			
0	66.7 (3)	33.3 (3)	20.0 (5)	0 (2)	0 (3)	0 (2)
4.6	11.1 (9)	6.7 (15)	0 (21)	0 (18)	0 (19)	0 (10)
9.1	14.3 (7)	0 (18)	4.5 (22)	0 (20)	0 (16)	0 (10)
			Conocephalus spp. and *Melanopsis* spp.†			
0	16.7 (36)	6.7 (45)	10.0 (10)	0 (10)	0 (6)	0 (6)
4.6	3.3 (184)	1.2 (164)	1.6 (126)	1.3 (75)	0 (46)	0 (28)
9.1	2.9 (174)	1.2 (166)	0.8 (126)	0 (78)	0 (36)	0 (21)

* Data are averages of two replicates. Direction (df = 3, $\chi 2 = 1.1723$, $P = 0.7597$) and distance (df = 1, $\chi 2 = 0.7867$, $P = 0.3751$) were nonsignificant independent variables in logistic regression analysis (PROC LOGISTIC, $P > 0.05$) when the treated plot (distance = 0) was excluded from the preliminary analysis. When direction was excluded and the treated plot was included in the final analysis (model 2), species, day after application, and distance were significant independent variables in logistic regression analysis (PROC LOGISTIC, $P < 0.05$). Logistic regression analysis (PROC LOGISTIC, $P < 0.05$) included percentage infection (y, Model 2), species ($df = 2$, $\chi 2 = 23.2885$, $P < 0.0001$), day (slope = −0.173, df = 1, $\chi 2 = 44.8571$, $P < 0.0001$), distance (slope = −0.0876, df = 1, $\chi 2 = 34.4573$, $P < 0.0001$). No infection by *B. bassiana* was observed in the control plots.

** Distance from the fungus-treated plot; 0 = within the plot.

† Wald confidence limit (95%) for species comparisons: *Conocephalus* spp. and *Melanopsis* spp. vs. *O. pugnax* (0.151, 0.479); *Lygus* spp. vs. *O. pugnax* (0.477, 2.434); *Conocephalus* spp. and *Melanopsis* spp. vs. *Lygus* spp. (0.110, 0.568).

reduced rice stink bug nymphs on six of nine sampling dates and adults on two of nine sampling dates from 2-10 d after application (Tables 5-7), and prevalence of the fungus was higher in the *B. bassiana* treatment than in controls for nymphs on four dates versus none for adults (Tables 9-11). Similarly, *B. bassiana* was more effective against nymphs than adults of *Lygus hesperus* Knight (Noma and Strickler 1999). Thus, adults may be less susceptible than nymphs to this fungus. Another possible explanation for the current results is that mobile, uninfected adults from other plots flew into, or infected adults moved out of, *B. bassiana*-treated plots. Adult movement or drift from spray treatments may explain the low prevalence of infection of adults in the control plots in all 3 yrs of our study (Tables 9-11).

A low level of fungus recycling, or replication in treated insects followed by infection of new hosts, may have occurred in the small-plot and spread field experiments. Insects infected by *B. bassiana* on day 1 or day 2 in these experiments died and produced conidia within 3-8 d in laboratory conditions, whereas epizootics in the field lasted 17-22 d after fungal application (Tables 5-7). The prevalence rates in the current experiments can be considered as epizootic (Fuxa and Tanada 1987) because only one stink bug infected with *B. bassiana* was detected in extensive pre-

liminary sampling. Thus, whereas temperatures conducive to fungal growth may have been limited during these 17-22 d, these data still suggest that the insects were infected by recycled conidia during at least the latter half of each of the current field experiments. In spite of the recycling, fungal prevalence decreased even though the proportion of nymphs, the susceptible stage, was always 0.36 or greater throughout all three small-plot experiments (Tables 5-7).

Chemical insecticides gave better control of *O. pugnax* than *B. bassiana* for 2-10 d in the three experiments (Tables 5-7). A single application of chemical insecticide reduced rice stink bug populations to lower numbers than *B. bassiana* by 7 d in two experiments (Tables 5 and 6) and by 10 d in the third experiment (Table 7), whereas a double application was more effective than *B. bassiana* for 10 d against nymphs (Table 7). These results are similar to those in another study of *B. bassiana* and conventional insecticides (Bifenthrin or Oxydemetonmethyl) in *L. hesperus* (Noma and Strickler 1999).

Beauveria bassiana was nearly as effective as a single application of chemical insecticide in suppressing rice stink bug populations 7-8 d after application in the small-plot field experiments (Tables 5-7); in one case (Table 6, day 17), the fungus was superior to the chemical in suppressing the bugs by the end of the experiment. This supports the concept that *B. bassiana* is a slow-acting agent that must be used to advantage where immediate control is not required (Fuxa 1987).

If the economics are favorable, mixtures of *B. bassiana* and chemical insecticide may provide better control of rice stink bug than a single application of either material alone. This was most evident in nymphs on day 14 (Table 7). This may be an additive effect with the chemical insecticide suppressing the population for 2-10 d and *B. bassiana* taking over 7-8 d after application. Chemicals also may act as stressors to enhance the efficacy of mycopathogens (Anderson et al. 1989, Hassan et al. 1989, Quintela and McCoy 1998a). Another possibility is synergism, such as that between imidacloprid and *B. bassiana* in termites (Boucias et al. 1996) and in larvae of the root weevil, *Diaprepes abbreviatus* L. (Quintela and McCoy 1998b).

The large-plot spread experiment generally had similar patterns of epizootics (Table 12) as the small-plot experiments (Tables 9-11), with prevalence of *B. bassiana* infections decreasing steadily to 0 by day 23 in spite of the recycling. Spread of *B. bassiana* up to 9.6 m within 24 h after application may have been caused by high mobility of the treated insects and perhaps, to a lesser degree, by spray drift. Further spread may have been impeded by a limited source of inoculum in the relatively small treated area as well as the low level of pathogen recycling.

Prevalence of *B. bassiana* was significantly greater in the hemipterans than in the orthopterans in the spread study (Table 12). This is probably due to differential physiological susceptibility, but differences in host mobility, behavior, life cycles, and population density may also have affected prevalence. Behavioral thermoregulation can inhibit *B. bassiana* mycosis in grasshoppers (Inglis et al. 1996b), but it is unknown whether this occurs in rice stink bug and *Lygus* spp. Infection and production of conidia by *B. bassiana* in several species of insects in rice in the current research seemingly is promising for enhanced control of *O. pugnax*.

High temperatures probably were a major factor limiting *B. bassiana* epizootics in the current research. Temperatures above 35°C are known to inhibit growth and development of *B. bassiana* (McCoy et al. 1988), delay germination of its conidia, and decrease mycosis (Inglis et al. 1996b). Isolates LRC21 and LRC28, which were selected for our experiments based on their relatively good growth at high tempera-

tures, grew best at 28-30°C on a semisynthetic medium in the laboratory conditions, but their growth rates were reduced by 27-48% at 32°C, by 61-92% at 35°C, and by 100% above 35°C (Fargues et al. 1997). During 3 yrs of the current study, daytime high temperatures were greater than 32°C on at least 20 of the 30 days after application of *B. bassiana* in each of the four experiments, with temperatures as high as 36-37°C on some dates (Anonymous 2005).

There are several other explanations for the limited efficacy of *B. bassiana* against rice stink bug in the current field experiments. UV-B radiation in the field environment rapidly deactivates conidia and slows their germination on insect cuticle (Inglis et al. 1996a, Rangel et al. 2004). UV-B radiation should not have affected sprayed conidia, because the treatments were applied in the evening in our experiments. However, radiation might have affected recycled conidia later during the experiments. Additionally, the small plots in the current research may have been disadvantageous if infected bugs emigrated, thereby depriving that plot of further inoculum through fungal recycling.

Our results indicate that *B. bassiana* has potential for integrated management programs of rice stink bug in rice, considering its high infection rates and moderate efficacy against nymphs, its possible additive effect with chemical insecticides, and its wide host range in rice insects. In future trials, the fungus should be sprayed in very large plots or even entire fields to eliminate negative effects of bug movement on evaluation and recycling. Similarly, inoculation earlier in the season may provide better control of rice stink bug than in the current research. An interesting continuation of current research would be to study sublethal effects of *B. bassiana* on rice stink bug. *Beauveria bassiana* is known to affect feeding and oviposition of *L. hesperus* in alfalfa (Noma and Strickler 2000). If such research demonstrated that *B. bassiana* significantly reduces feeding and/or oviposition of infected bugs, it would add to the potential of *B. bassiana* as a microbial agent for control of rice stink bug.

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