

Field-Based Evaluation of Biopesticide Impacts on Native Biodiversity: Malagasy Coleoptera and Anti-Locust Entomopathogenic Fungi

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ABSTRACT A community of 225 species of Coleoptera was used as a surrogate to evaluate nontarget effects of entomopathogenic fungi under development as biopesticides for use against the Malagasy migratory locust *Locusta migratoria capito* Saussure (Orthoptera: Acrididae). Evaluation of a standard chemical treatment of fenitrothion + esfenvalerate, two indigenous isolates of *Metarhizium flavoviride* Gams & Roszsyol (SP3 and SP9), and an indigenous isolate of *Beauveria bassiana* (Balsamo) Vuillemin (SP16) against an untreated control in a replicated field trial in southern Madagascar showed that one of the isolates of *M. flavoviride* (SP3) and fenitrothion + esfenvalerate had distinct effects on nontarget beetle communities that were similar to each other. The other two isolates had no detectable effects compared with the untreated control. Based on an evaluation of the species affected, the similar effects of SP3 and the chemical pesticide are hypothesized to be the result of a perturbation of predator-prey relationships, with a distinct tendency to be manifested via predators. The data indicate that use of SP9 and SP16 would have minimal detrimental effects on the biodiversity of nontarget beetles, but that SP3 needs further testing.

KEY WORDS beetles, locusts, entomopathogenic fungi, *Locusta migratoria capito*, *Beauveria bassiana*, *Metarhizium flavoviride*

THE MALAGASY MIGRATORY locust *Locusta migratoria capito* Saussure (hereafter referred to as migratory locust) is a severe pest in Madagascar. At low densities, it exists in a solitary phase, mostly on low-value grazing lands, but periodic high-density outbreaks of the migratory phase produce bands that invade critical food crops with devastating effects (Têtefort and Wintrebert 1963, Scherer 1996). Landscape-level solutions are required to alleviate the migratory locust problem, but these solutions are beyond the means of local farmers (Swanson 1997). For decades, international aid agencies have paid for the widespread spraying of broad-spectrum chemical insecticides against migratory locust (Têtefort and Wintrebert 1963, Uvarov 1977, Rakotobe 1996). These programs have applied organochlorine, carbamate, organophosphate, or other insecticides to as much as 800,000 ha per year (Randriamanantsoa 1996). Such extensive use has recently come into question (Rakotobe 1996), at least in part because of the suspected impact on Madagascar's unique biodiversity, but simply stopping control ef-

forts would lead to widespread hardship, and possibly famine in rural Madagascar.

Although one of the world's poorest countries (World Bank 1997), Madagascar is extremely rich in unique native biodiversity, having been declared one of the world's "megadiversity" nations (McNeely et al. 1990). Unfortunately, much of this biodiversity is under siege, due in large part to an expanding population that is sustained mostly by low-input subsistence agriculture (Rauh 1979). When conflicts arise between food production and biodiversity preservation, solutions are not always simple.

Conflicts between food and biodiversity have driven the search for alternatives to chemical control of locusts throughout Africa, and biological control with fungal pathogens is an obvious choice (Greathead 1992). Entomopathogenic fungi are considered excellent candidates for biopesticides due to their safety, relatively limited host range, ease of production, and suitability to large-scale application (Ferron 1978). However, the Malagasy government is quite aware of the unique nature of the biodiversity of their country, and has instituted strict phytosanitary restrictions on importation of exotic fungi to protect against unintended adverse environmental effects (Swanson 1997). Enhancement of native fungi is considered safer, although risks to nontarget species still exist (Goettel et al. 1990, Goettel and Vandenberg 1994). Therefore, in 1993, researchers from Montana State University's Africa Grasshopper/Locust Biocontrol

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Program began a search for naturally occurring entomopathogenic fungi in migratory locust populations (Delgado et al. 1997a, 1997b). Thirty-three Malagasy fungal isolates from migratory locusts were cultured and evaluated for potential use as biopesticides, yielding three candidates for field trials (Delgado et al. 1997a, Delgado et al. 1997b). These included an isolate of *Beauveria bassiana* (Balsamo) Vuillemin, and two of *Metarhizium flavoviride* Gams & Roszsygol.

Nontarget testing is standard practice in biopesticide evaluation (Goettel 1994), but by definition, all such testing before releasing the control agent into the field has been conducted in the laboratory. Goettel (1994) argues that "The value of laboratory results on nontarget susceptibility to fungal agents is dubious as far as establishing safety in the field is concerned, especially if the fungi in question are indigenous." (emphasis added, see also Goettel 1995 and Goettel and Hayek 2001). In laboratory tests, detectable effects on nontargets are limited to direct pathogenicity of the fungi in question (Prior 1997), and are often not corroborated in the field (Goettel and Hayek 2001). Even most of the limited so-called semifield testing (e.g., Peveling and Weyrich 1992) involves capturing field-treated specimens and holding them either in the laboratory or under unnatural situations while caged in the field to evaluate infection rates. Yet biopesticides (and pesticides in general) may have indirect effects on the prey, predators, parasitoids, competitors, and other ecological associates of susceptible species (Goettel et al. 1990, Goettel and Vandenberg 1994, Cohen et al. 1994, Goettel and Hayek 2001, and references therein). Because of the obvious dangers implicit in releasing self-replicating biological entities, Goettel et al. (1990) and Goettel and Johnson (1992) called for expanded field testing of biopesticides to evaluate these risks to nontargets, but actual use of such tests has been limited (Hayek and Goettel 2000, Goettel and Hayek 2001). Given that in our case, multiple candidate fungi were identified, and that the initial search was driven out of concerns for native biodiversity, inclusion of a strict nontarget safety criterion for these fungi was deemed important for final selection of a strain for commercial development.

Obviously, not all organisms in any environment can be included in such a study. Because the fungal genera under study are known to infect a wide variety of arthropods (Goettel et al. 1990), and because we wanted to include the greatest number of species and widest ecological diversity feasible, we chose to use beetles (Coleoptera) for our model system. Beetles represent Earth's most speciose and ecologically diverse Order of organisms (Crowson 1981). Further, they may be collected in a variety of trap types suitable for quantitative studies, and the specimens hold up well in long-term preservatives.

This unparalleled diversity and practicality provides an excellent model group to study nontarget effects of entomogenous fungi. One or more species of beetles are exposed to virtually every conceivable mode of infection, provide a wide variety of gut pH values, and are susceptible to all manner of cascading interactions.

Their total biomass is large, species range in size over several orders of magnitude, and their very wide trophic diversity includes specialist and generalist herbivores, specialist and generalist animal and seed predators, parasites, fungivores, and scavengers. Beetles also provide a wide variety of ecological services involving, for example, the decomposition of carrion, dung, wood, and leaf litter; pollination of a wide variety of plants; population control of various plants and animals; and soil aeration. Their ecological relationships extend to all other terrestrial arthropod Orders, and they live exposed on plants, within plant tissues, on the ground, and below the ground.

Taking advantage of all these traits, we designed a field test that used beetles to evaluate the nontarget effects of these three candidate biopesticides, and tested the hypothesis that they would be less disruptive to the local fauna than the chemical standard now in use. These data were combined with field efficacy tests against migratory locusts to select the final candidate(s) for commercial development.

Materials and Methods

Under constraints imposed by the conditions of a limited release permit from the Government of Madagascar, we designed a field trial to detect effects of environmental perturbations. This permit restricted the total area that could be treated with each candidate strain, the dates when they could be applied, and other technical details that had to be taken into account in our design. Our basic approach had proven highly sensitive in detecting the effects of environmental perturbation in Glacier National Park, USA (M.A.I., L.L.I., and D.L.G., unpublished data), and was applicable under our limitations of time, personnel, geography, and budget.

Our trial was conducted in Toliara (Tuléar) Province of southern Madagascar, 17.88 km NNW (330°) of Betroka, on a 10-ha plot centered at 23° 09' 42"S, 45° 58' 14"E, at an elevation of 825 m. The area was in a grassland/savanna, with many termitaria, subject to grazing, periodic burning, and scattered small-scale planting of food crops. The soil was red clay with sand and scattered quartz, with a surface that was very hard and exposed. Migratory locusts were present in limited numbers.

The site was a 200 by 600-m patch of uniform, uncultivated scrub land. The centerline was oriented north-to-south along the crest of a very slight, almost unnoticeable, rise, with 1–3 m high brush scattered throughout, and an average brush canopy of 2 m. Visible (≈1 mo old) evidence of low-intensity fire was evident in small patches throughout, with burned and unburned elements in all plots. Recent rains had promoted regrowth.

We used a randomized-plot design with two adjacent rows, each with five square 1-ha plots. Plots were randomly chosen to be treated with one of the three candidate strains (SP3, SP9, SP16), a standard chemical treatment (SCT) or a untreated control (Table 1).

Table 1. Plot design for biopesticide impact evaluation

	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5
Row A	SCT	SP9	SP3	Control	SP16
Row B	SP9	Control	SP16	SCT	SP3

Square 1-ha treatment plots were rimmed with color-coded flagging tape, one color per treatment, using a compass, tape, and a Magellan 1500 global positioning unit (San Dimas, CA) to determine corners and center points. The entire site was under 24-h watch by armed guards for the entire period of the trial.

Before treatment, each plot had three flight-intercept traps and three pit-fall traps installed within 5 m of the center to minimize emigration effects from neighboring plots by maximizing distance from the edges. Traps were located by tossing a marker from the center of the plot in at 0°, 120°, and 240° from North. Each trap was labeled with a code (e.g., IAGREEN-fit2) so that the collector and sorter would not know the treatment identity until all data were collected.

Pit-fall traps were constructed of standard, clear, North American 2-liter soda pop bottles, cut off just below the shoulder. The bottom piece was placed in the ground so that the top edge was flush with ground level. A Solo 750-ml (12-oz, Urbana, IL) cup with a white interior was placed in this container, and half-filled with industrial grade (clear) propylene glycol. The top piece of the 2-liter bottle was then placed upside-down into the opening, making a funnel aimed at the center of the cup.

Flight intercept traps consisted of a panel of olive drab camouflaged mosquito netting stretched vertically between iron poles on an east-west axis. The poles were staked with twine, and a rain roof of clear 6-mil plastic sheeting was fixed over the fighter-intercept traps in tent-fly fashion. Three 39 by 31 by 8-cm aluminum baking pans were centered in a line under the panel, and half-filled with industrial grade (clear) propylene glycol. Immediately before treatment, and on each sampling date, the panel was sprayed with 5% permethrin to increase the number of specimens sampled.

Each of plots 1–5 in an area was treated with one of the following.

- (1) No treatment (control).
- (2) The standard chemical treatment of Sumi-combi-Alpha L-25, applied at the rate of 1 liter/ha. This formulation contained 245 g (AI) fenitrothion and 5 g (AI) of esfenvalerate per liter.
- (3–5) Test treatments of 2.5×10^{13} conidial spores in 5 liters of oil per hectare for two isolates of *Metarhizium flavoviride* Gams & Roszsy (designated SP3 [ARSEF #] and SP9 [ARSEF #] hereafter), and an isolate of *Beauveria bassiana* (Balsamo) Vuillemin (designated SP16 [ARSEF #] hereafter).

Fungi were provided as conidia in an oil-based formulation by Mycotech (Butte, MT). For specifics of strain characterization, production, and application methods, see Delgado et al. (1997a, 1997b); in brief, the three fungi were isolated from *L. migratoria capitata*

in Madagascar, and mass-produced in the United States for these trials. The standard chemical treatment chemical was taken from the normal stocks of the Service Antiacridien. All treatments were applied by a five-person team from the Service Antiacridien in Toliara (Tuléar), using mini-ULVA sprayers. Application was on 29 November 1994, between 0530 and 0900 hours, under clear skies and with no wind. The first posttreatment rain fell on 30 November 1994.

The samples were collected in the morning of days 5, 10, and 15 posttreatment—4, 9, and 14 December. An intended fourth sampling period ending 19 December was abandoned because of heavy rains that flooded the traps. All material in each trap was sieved onto a screen cloth, and placed into a Whirl-Pak bag with appropriate data. Each label was annotated with a trap condition code, i.e., perfect condition (code 1), sub-optimal but still functioning (code 2), or not operating or missing (code 3). The collector did not know the treatment identity during collection.

After return of the samples to Montana State University, all beetles were removed from each bag, mounted, labeled, identified, and counted. The association of color with treatment was unknown to the sorter. Data for each trap were entered into an ASCII matrix, available in their complete form at Ivie et al. (1997). Vouchers are deposited in the collection of the senior author, with duplicates in the collections of the various determiners.

Data Analysis. Data were analyzed using a Kruskal-Wallis test to determine treatment effects on total number of specimens and species, and for pair-wise comparisons where those effects were found. The Kruskal-Wallis test is a nonparametric equivalent to a one-way analysis of variance (ANOVA) and may be used to test for intergroup differences where the k groups do not come from normal distributions and/or k population variances are heterogeneous (Zar 1984). Statistical significance can be defined at various levels (Daniel 1990). For our total species- and total specimen-level analyses we define, an $\alpha = 0.01$ as highly significant, an $\alpha = 0.05$ as significant, and an $\alpha = 0.10$ as possibly significant biologically. The Mann-Whitney test was used to compare for effects of Area and Trap Type. The Mann-Whitney is the corresponding test to the Kruskal-Wallis were $k = 2$.

It may be argued that this design meets the definition of pseudoreplication for manipulative field studies specifically disallowed by these tests (Hurlbert 1984), when looking for treatment effects. However, these violations were imposed by externalities, and we feel our solutions appropriately compensate for the resulting problems. Although the limits of pseudoreplication are clear in laboratory experiments, by definition all field experiments on the same planet and in the same atmosphere have some level of pseudoreplication inherent (i.e., all samples are taken from within the same “container”). Hurlbert (1984), see also Heffner et al. 1996) allows for use of pseudoreplicated data, so long as it is acknowledged that treatment effects cannot be separated with certainty from location effect using only the statistical tests. Our

design pushes the limits of just how much of this can be ignored. However, many experimental designs with unique or very expensive elements that lacked appropriate replication have lead to useful insights into the functioning of natural phenomena (Carpenter 1990). In our case, we had specific legal constraints imposed by the fact that our "limited use" permit allowed us only 2 ha per fungal strain for the nontarget tests. Clearly, because we were working with free-ranging populations and real-world limits on the sharpness of the block edges, we needed to maximize the distance between block edges and the sampling points, by making the largest possible radius a requirement. The best solution to this problem is a single block for each treatment, giving a radius of 67 m. However, this would cause impossible problems with lack of interspersation.

Balancing the need to provide interspersation of sampling, we opted for two linear areas randomly divided into treatment blocks of 100 m² (1 ha), with a minimum distance from sampling point to edge of 42.5 m. Even one more block would have reduced the radius to 33 m. At that point the advantages we expected to gain in interspersation would be overcome by the disadvantages of edge effects.

As if this was not enough, our treatments had different rates of expected impact, the standard chemical Treatment being much faster than the fungi. Compounding this, cascade effects could take even longer to appear. These factors dictated that we stretch our sampling over a relatively long period, but doing so increased the possibility that damage to a trap would cause an imbalance in our design via missing data. Therefore, we separated the 20-d sampling period into 5-d intervals, with the traps reset between each interval.

Strict avoidance of pseudoreplication in this instance would have yielded little possibility of discerning real effects (Carpenter 1990). Therefore, we made the decision to treat each trap catch as a sample. Different trap types and periods are perfectly balanced over treatments (see *Results*), so those aspects of using the data in this manner will have no effect on the evaluation of the hypotheses inherent in this study. Add to this that we interspersed to the degree physically possible, and we believe the data can be examined for indications of treatment effects.

Another violation of strict statistical rules is inherent in all trapping methods, i.e., because an individual is removed from the population when captured, it can only be captured in a single trap, therefore the data are not strictly independent. However, given that the traps themselves were located hundreds of body-lengths (to a beetle) apart, we feel our data may have valuable insights into the nontarget effects of biopesticides that outweigh the statistical limitations. A single trap catch in a wild environment has a certain biological reality that cannot be denied.

Given the inherent limitations with our design, an additional test must be added in evaluating our results—the biologically logical criterion—do the results make sense biologically (Carpenter 1990)? To

Table 2. Summary of beetles captured

Variable	Control	SCT	SP3	SP9	SP16	Total
N	36	36	36	36	36	180
Specimens	260	144	191	374	281	1,250
Species	99	62	80	112	82	225
Unique spp.	36	13	26	30	25	—
Global spp.						22

Control, no treatment; SCT, standard chemical treatment; SP3, *Metarhizium flavoviride* strain SP3; SP9, *Metarhizium flavoviride* strain SP9; SP16, *Beauveria bassiana* strain SP16.

look at this, each species is evaluated individually, because a significant variance from the expected can only be seen one species at a time (i.e., there is no reality to the pooling of the species-level results). Suspect but independent data that point qualitatively to a common biological phenomenon can be considered useful in designing and justifying more detailed tests in the future (Carpenter 1990).

To test for biological significance, chi-squared goodness-of-fit statistics were used for effects on individual species, looking for cases where collections were not distributed randomly among the five treatments. This is a multiple contingency table approach, asking if the distribution of specimens among the various treatments varies significantly from random expectations. Only species collected more than twice were included in these analyses (i.e., singletons and doubletons were removed). Again, to avoid missing biologically important information, an $\alpha = 0.10$ was considered significant, an $\alpha = 0.05$ was highly significant, and an $\alpha = 0.01$ was very highly significant for these data.

Results

The three sampling periods yielded 180 samples, with none missing (i.e., all had trap condition <3). Of these, 54 samples were trap condition of 2 (sub-optimal, but functioning).

A total of 1,250 individual beetles of 225 species, representing 38 families, was recovered from the first three sample periods (Table 2). Individual samples contained 0–30 specimens and 0–20 species each. All traps collected beetles over the course of the study. Table 3 summarizes the degree of overlap between treatments.

First, we determined if yields from the different treatments were statistically different. Results (Tables 4 and 5) showed that treatments had highly significant

Table 3. Summary of species shared between treatments

Treatment	Control	SCT	SP3	SP9	SP16
Control	99	38	38	56	38
SCT		62	30	42	31
SP3			80	47	33
SP9				112	50
SP16					82

Control, no treatment; SCT, standard chemical treatment; SP3, *Metarhizium flavoviride* strain SP3; SP9, *Metarhizium flavoviride* strain SP9; SP16, *Beauveria bassiana* strain SP16.

Table 4. Kruskal-Wallis test pairwise comparisons of treatments for number of specimens

Treatment	Control	SCT	SP3	SP9	SP16
Control	1.000	0.003	0.039	0.311	0.922
SCT		1.000	0.343	0.000	0.004
SP3			1.000	0.002	0.049
SP9				1.000	0.267
SP16					1.000
Mean rank	100.31	64.89	75.99	112.15	99.17

Control, no treatment; SCT, standard chemical treatment; SP3, *Metarhizium flavoviride* strain SP3; SP9, *Metarhizium flavoviride* strain SP9; SP16, *Beauveria bassiana* strain SP16. N = 36 for each treatment. Test statistic = 20.10. P < 0.001.

effects for both numbers of individuals (P < 0.001) and number of species (P = 0.005). Pair-wise tests (Tables 4 and 5) indicated that SP9 and SP16 had no significant effects, when compared with the untreated control, for specimens (P = 0.311–0.922), or species (P = 0.430–0.722). However, the standard chemical treatment and SP3 both showed significant (P = 0.003 and P = 0.039, respectively) differences from the untreated control for number of specimens. Comparing the untreated control to the standard chemical treatment for number of species was highly significant (P = 0.010), whereas SP3 was possibly different (P = 0.060) from the control. The significance of this last P value is supported by the fact that the standard chemical treatment and SP3 were not significantly different from each other in their effects on individual numbers (P = 0.343), or species richness (P = 0.476). Thus, over-reliance on the apparent lack of significance at the 5% level may lead to a type II error if used to reject. The untreated control, SP9 and SP16 were not significantly different from each other for specimens (P = 0.267) or species (P = 0.252), but SP3 was highly significantly (P = 0.002) and significantly (P = 0.049) different from SP9 and SP16 for specimens, and highly significantly (P = 0.008) different from SP9 for species. For species, SP3 and SP16 did not differ significantly (P = 0.126).

Other interactions were examined using the same methods. Number of specimens varied with sampling Area (Mann-Whitney statistic 6.24, P = 0.012), but number of species did not (Mann-Whitney statistic 1.40, P = 0.237). We observed nothing that would account for this effect, but in any case, the relationship

Table 5. Kruskal-Wallis test pairwise comparisons of treatments for number of species

Treatment	Control	SCT	SP3	SP9	SP16
Control	1.000	0.010	0.060	0.430	0.722
SCT		1.000	0.476	0.001	0.025
SP3			1.000	0.008	0.126
SP9				1.000	0.252
SP16					1.000
Mean rank	100.14	69.24	77.69	109.51	95.92

Control, no treatment; SCT, standard chemical treatment; SP3, *Metarhizium flavoviride* strain SP3; SP9, *Metarhizium flavoviride* strain SP9; SP16, *Beauveria bassiana* strain SP16. N = 36 for each treatment. Test statistic = 14.73. P = 0.005.

Table 6. Kruskal-Wallis test mean ranks of trap condition to treatment

Variable	Mean rank				
	Control	SCT	SP3	SP9	SP16
Trap condition	93.50	86.00	93.50	86.00	93.50

Control, no treatment; SCT, standard chemical treatment; SP3, *Metarhizium flavoviride* strain SP3; SP9, *Metarhizium flavoviride* strain SP9; SP16, *Beauveria bassiana* strain SP16. N = 36 for each treatment. Test statistic = 1.42. P = 0.841.

of sampling area to treatment was perfectly balanced (n = 36 for each), and should not have any effect on our other treatment relationships. Sample period effects were highly significant for both numbers of specimens (Kruskal-Wallis statistic 24.34, P = 0.000) and number of species (Kruskal-Wallis statistic 13.34, P = 0.001). Again, the relationship of sample period to treatment was perfectly balanced (n = 36 for each), and should not have any effect on other treatment effects. As expected, different trap types were highly significant in the specimens (Mann-Whitney statistic 42.50, P = 0.000) and species (Mann-Whitney statistic 71.39, P = 0.000), but the relationship of treatment and trap type was perfectly balanced (n = 36 for each) and should have no effect on other treatment effects. Lastly, the trap condition is highly significant when compared with the number of specimens (Mann-Whitney statistic 24.89, P = 0.000) and species (Mann-Whitney statistic 16.53, P = 0.000) captured. This information is seldom noted for studies of this type, although our data show its potential importance. Trap condition did not vary across treatment (Kruskal-Wallis statistic 1.42, P = 0.841), due to a relatively even distribution of disturbance across treatments (Table 6).

Tables 7–11 show the results of various combinations of significant treatment effects on the distribution of specimens for 37 individual species (Table 7), the 26 species that differed for the standard chemical Treatment/SP3 versus the control/SP9/SP16 (Table 8), the 11 species effected by the standard chemical treatment versus SP3 (Table 9), 20 species that differed between SP3 versus SP9/SP16 (Table 10), and 17 species that differed between the standard chemical treatment and control versus the three fungal treatments (Table 11).

Discussion

Replicated field trials of fungus-based bioinsecticides on nontarget Coleoptera in Madagascar show that *Beauveria bassiana* SP16 and *Metarhizium flavoviride* SP9 do not significantly impact the number of species or specimens as compared with the untreated control. By contrast, *M. flavoviride* SP3 shows effects that may be as pronounced as the chemical pesticide Sumicombi-Alpha L-25 (fenitrothion + esfenvalerate), which had a significant impact on nontarget beetles, when compared with the untreated control.

Determining the mode of impact of a treatment's effect on a diverse community must be addressed via

Table 7. Number of individuals of species taken in each treatment and the chi-square and *P*-values from a goodness-of-fit test of treatment effect on number of individuals

Species	Control	SCT	SP3	SP9	SP16	Total	χ^2	<i>P</i>
Elaterid sp. 1	19	1	1	1	2	24	52.7	0.000
Eumolpine sp. 1	0	0	16	2	4	22	40.7	0.000
Melolonthinae sp. 13	4	3	5	26	12	50	37.0	0.000
Scydmaenid sp. 4	1	1	2	7	18	29	36.3	0.000
Melolonthinae sp. 6	3	3	6	21	12	45	26.0	0.000
Staphylinid sp. 13	3	4	0	11	0	18	22.6	0.000
Curculionid sp. 2	3	6	3	3	15	30	18.0	0.001
Aphodiinae sp. 7	44	17	22	27	40	150	17.9	0.001
Staphylinid sp. 8	2	1	1	10	3	17	16.8	0.002
Aderid sp. 1	3	0	0	5	9	17	16.8	0.002
Scydmaenid sp. 3	0	0	0	0	4	4	16.0	0.003
Clerid sp. 2	0	0	0	4	0	4	16.0	0.003
Melolonthinae sp. 11	4	0	1	10	5	20	15.5	0.004
Nitidulid sp. 3	10	4	1	10	15	40	15.2	0.004
Elaterid sp. 2	0	0	2	1	6	9	13.8	0.008
Staphylinid sp. 24	5	0	0	1	1	7	12.3	0.015
Pselaphine sp. 3	1	0	5	1	0	7	12.3	0.015
Scaptioid sp. 1	2	5	4	13	5	29	12.2	0.016
Bembidion sp. 5	6	2	3	11	2	24	12.2	0.016
Melyrid sp. 1	5	5	0	7	0	17	12.1	0.016
Cetoniinae sp. 4	8	2	2	6	0	18	12.0	0.017
Staphylinid sp. 6	0	0	0	4	1	5	12.0	0.017
Staphylinid sp. 12	0	0	0	3	0	3	12.0	0.017
Staphylinid sp. 50	0	0	0	3	0	3	12.0	0.017
Alleculine sp. 6	1	2	7	4	0	14	11.0	0.027
Phalacrid sp. 4	2	0	0	5	1	8	10.8	0.030
Lathridiid sp. 1	4	0	0	2	0	6	10.7	0.031
Melolonthinae sp. 14	3	7	1	5	0	16	10.2	0.036
Nitidulid sp. 1	3	4	11	2	5	25	10.0	0.040
Limmichid sp. 1	2	0	4	5	0	11	9.5	0.051
Cetoniinae sp. 1	2	3	9	8	11	33	9.3	0.055
Cicindelinae sp. 2	1	4	0	1	0	6	9.0	0.061
Elaterid sp. 5	1	1	0	4	0	6	9.0	0.061
Stenus sp. 1	6	4	4	10	13	37	8.5	0.074
Coccinellid sp. 2	0	0	0	3	1	4	8.5	0.075
Endomia sp. 1	0	0	0	3	1	4	8.5	0.075
Staphylinid sp. 20	4	0	0	3	1	8	8.3	0.083

Data reported for species collected three or more times where $P \leq 0.10$. Control, no treatment; SCT, standard chemical treatment; SP3, *Metarhizium flavoviride* strain SP3; SP9, *Metarhizium flavoviride* strain SP9; SP16, *Beauveria bassiana* strain SP16.

examination of the individual species affected. The use of grouped taxa (e.g., orders, families, genera) or even functional groups ignore the basic fact that species are the units of evolution, and as such are expected to vary one from another. It is our suspicion that lack of taxonomic expertise and/or the willingness to pay for it is at the heart of the lumping approach. Yet, even using individual species, it would be possible for a treatment to randomly yield similar numbers of species or specimens but not be biologically similar because the two pools do not contain any of the same taxa, i.e., 31 species from A may be completely different from the 31 in B. Thus, our approach requires looking at the individual species involved, and individual species affects were indeed possible to examine. To assist in interpreting the following discussion, a summary of the appearances of individual species in Tables 7–11 is shown in Table 12. Thirty-seven species taken three or more times had *P* values ≤ 0.10 when their distributions across the treatments were tested (Table 7). Of these, 15 were very highly significant, 14 were highly significant, and the remaining eight significant. This is $\approx 1/6$ of the total species and $\approx 1/2$ of the 79 species taken three or more times.

Given that results for the pooled species (Tables 4 and 5) indicate no significant difference between effects of the standard chemical treatment and SP3, examining these same relationships for individual species helps understand what exactly was being effected. If the number of species in each treatment was coincidentally similar (type I error), no (or very few) species would show individually significant differences between these two groups. However, this test (Table 8) showed some level of significant effect on 26 species (nine species were very highly significant, 11 were highly significant, and six more were significant), with more than half (20) of the species significant by some treatment effect in Table 7 reappearing here. Numbers of four of these species actually increased in the standard chemical treatment/SP3 pair relative to the control/SP9/SP16—a predator, a saprophage, an herbivore, and a fungivore. These four species (Eumolpine sp. 1, Nitidulid sp. 1, Alleculine sp. 6, and Pselaphine sp. 3) were the only ones with a distinctly higher number of specimens in the SP3 samples (Table 7). For all four of these species, SP3 differed from the standard chemical treatment (Table 9). Therefore, because the influence of only one of the two treat-

Table 8. Number of individuals of species taken in control + SP9 + SP16 (1) versus control + SP3 treatments (2), and the chi-square and *P*-values from a goodness-of-fit test of these effects on number of individuals

Species	1	2	Total	χ^2	<i>P</i>
Nitidulid sp. 3	35	5	40	12.6	0.000
Aphodiinae sp. 7	111	39	150	12.2	0.000
Melolonthinae sp. 13	42	8	50	12.0	0.001
Aderid sp. 1	17	0	17	11.3	0.001
Scydmaenid sp. 4	26	3	29	10.6	0.001
Melolonthinae sp. 11	19	1	20	10.2	0.001
Elaterid sp. 1	22	2	24	10.0	0.002
Eumolpine sp. 1	6	16	22	9.8	0.002
Melolonthinae sp. 6	36	9	45	7.5	0.006
Staphylinid sp. 8	15	2	17	5.6	0.017
Staphylinid sp. 20	8	0	8	5.3	0.021
Phalacrid sp. 4	8	0	8	5.3	0.021
Stenus sp. 1	29	8	37	5.2	0.022
Staphylinid sp. 24	7	0	7	4.7	0.031
Nitidulid sp. 1	10	15	25	4.2	0.041
Cicindelinae sp. 1	15	3	18	4.1	0.043
Staphylinid sp. 32	6	0	6	4.0	0.046
Lathridiid sp. 1	6	0	6	4.0	0.046
Cicindelinae sp. 3	12	2	14	3.9	0.050
Alticinae sp. 2	12	2	14	3.9	0.050
Nitidulid sp. 2	9	1	10	3.8	0.053
Bembidion sp. 5	19	5	24	3.7	0.055
Alleculine sp. 6	5	9	14	3.4	0.064
Scydmaenid sp. 2	5	0	5	3.3	0.068
Staphylinid sp. 6	5	0	5	3.3	0.068
Pselaphine sp. 3	2	5	7	2.9	0.090

Data reported for species collected three or more times where $P \leq 0.10$. 1 = control (no treatment), *Metarhizium flavoviride* strain SP9, and *Beauveria bassiana* strain SP16. 2 = SCT (standard chemical treatment) and *Metarhizium flavoviride* strain SP3.

ments in Table 8 column 3 accounts for the significance, these four species have nothing to tell us about the similarity of mode of effect between SP3 and the standard chemical treatment.

None of the remaining 22 species from Table 8 appear in Table 9, so their significance of difference from the control/SP9/SP16 group cannot be attributed to effects of either SP3 or the standard chemical treatment alone. Because of these findings, it can be concluded that the similarity of the standard chemical treatment and SP3 is indeed real. Although auteco-

Table 9. Number of individuals of species taken in standard chemical treatment versus SP3 treatments, and the chi-square and *P*-values from a goodness-of-fit test of these effects on number of individuals

Species	SCT	SP3	Total	χ^2	<i>P</i>
Eumolpine sp. 1	0	16	16	16.0	0.000
Pselaphine sp. 3	0	5	5	5.0	0.025
Melyrid sp. 1	5	0	5	5.0	0.025
Melolonthinae sp. 14	7	1	8	4.5	0.034
Cicindelinae sp. 2	4	0	4	4.0	0.046
Staphylinid sp. 13	4	0	4	4.0	0.046
Melolonthinae sp. 10	0	4	4	4.0	0.046
Limnichid sp. 1	0	4	4	4.0	0.046
Nitidulid sp. 1	4	11	15	3.3	0.071
Cetoniinae sp. 1	3	9	12	3.0	0.083
Alleculine sp. 6	2	7	9	2.8	0.096

Data reported for species collected three or more times, where $P \leq 0.10$. SCT, standard chemical treatment; SP3, *Metarhizium flavoviride* strain SP3.

Table 10. Number of individuals of species taken in SP3 versus SP9 + SP16 treatments, and the chi-square and *P*-values from a goodness-of-fit test of these effects on number of individuals

Species	SP3	SP9+SP16	Total	χ^2	<i>P</i>
Eumolpine sp. 1	16	6	22	15.4	0.000
Nitidulid sp. 3	1	25	26	10.2	0.001
Melolonthinae sp. 13	5	38	43	9.1	0.003
Scydmaenid sp. 4	2	25	27	8.2	0.004
Aderid sp. 1	0	14	14	7.0	0.008
Pselaphine sp. 3	5	1	6	6.8	0.009
Nitidulid sp. 1	11	7	18	6.2	0.012
Melolonthinae sp. 6	6	33	39	5.7	0.017
Staphylinid sp. 13	0	11	11	5.5	0.019
Melolonthinae sp. 11	1	15	16	5.3	0.022
Alleculine sp. 6	7	4	11	4.5	0.033
Cicindelinae sp. 3	0	9	9	4.5	0.034
Staphylinid sp. 8	1	13	14	4.3	0.038
Stenus sp. 1	4	23	27	4.2	0.041
Melyrid sp. 1	0	7	7	3.5	0.061
Nitidulid sp. 2	0	7	7	3.5	0.061
Curculionid sp. 2	3	18	21	3.4	0.064
Cicindelinae sp. 1	1	11	12	3.4	0.066
Phalacrid sp. 4	0	6	6	3.0	0.083
Aphodiinae sp. 7	22	67	89	3.0	0.085

Data reported for species collected three or more times, where $P \leq 0.10$. SP3, *Metarhizium flavoviride* strain SP3; SP9+SP16, *Metarhizium flavoviride* strain SP9, and *Beauveria bassiana* strain SP16.

logical data are not available for any of these species, inference to relatives indicates that these species include one detritivore (Aphodiinae), two saprophages (Nitidulidae), three fungivores (Aderidae, Lathridiidae, Phalacridae), four root-feeders/herbivores (Melolonthinae and Alticinae), and 11 predators (Staphylinidae, Carabidae, Scydmaenidae) (Elateridae could not be assigned to a trophic category).

Comparison of SP3 with SP9/SP16 without the control or standard chemical treatment should show how much of the significance can be tied to the fungi

Table 11. Number of individuals of species taken in (1) control+SCT, versus (2) SP3+SP9+SP16 treatments, and the chi-square and *P*-values from a goodness-of-fit test of these effects on number of individuals

Species	1	2	Total	χ^2	<i>P</i>
Scydmaenid sp. 4	2	27	29	13.2	0.000
Melolonthinae sp. 6	6	39	45	13.3	0.000
Melolonthinae sp. 13	7	43	50	14.1	0.000
Elaterid sp. 1	20	4	24	18.8	0.000
Eumolpine sp. 1	0	22	22	14.7	0.000
Cetoniinae sp. 1	5	28	33	8.5	0.004
Elaterid sp. 2	0	9	9	6.0	0.014
Cicindelinae sp. 2	5	1	6	4.7	0.030
Tenebrionid sp. 1	8	4	12	3.6	0.059
Staphylinid sp. 8	3	14	17	3.5	0.060
Aderid sp. 1	3	14	17	3.5	0.060
Melolonthinae sp. 14	10	6	16	3.4	0.066
Staphylinid sp. 6	0	5	5	3.3	0.068
Melolonthinae sp. 11	4	16	20	3.3	0.068
Melolonthinae sp. 10	1	8	9	3.1	0.077
Scraptiid sp. 1	7	22	29	3.0	0.081
Staphylinid sp. 24	5	2	7	2.9	0.090

Data reported for species collected three or more times where $P \leq 0.10$. Control, no treatment; SCT, standard chemical treatment. 2, *Metarhizium flavoviride* strain SP3, *Metarhizium flavoviride* strain SP9, and *Beauveria bassiana* strain SP16.

Table 12. Summary of appearances of individual species in Tables 7–11

Species	Table				
	7	8	9	10	11
Elaterid sp. 1	***	***			***
Eumolpinae sp. 1 ^a	***	***	***	***	***
Melolonthinae sp. 13	***	***		***	***
Scydmaenid sp. 4	***	***		***	***
Melolonthinae sp. 6	***	***		**	***
Staphylinid sp. 13	***		*	**	
Curculionid sp. 2	***			*	
Aphodiinae sp. 7	***	***		*	
Staphylinid sp. 8	***	**		**	*
Aderid sp. 1	***	***		***	*
Scydmaenid sp. 3	***				
Clerid sp. 2	***				
Melolonthinae sp. 11	***	***		**	*
Nitidulid sp. 3	***	***		***	
Elaterid sp. 2	***				**
Staphylinid sp. 24	**		**		
Pselaphinae sp. 3 ^a	**		*	**	***
Scraptiid sp. 1	**				
Bembidion sp. 5	**		*		
Melyrid sp. 1	**			**	*
Cetoniinae sp. 4	**				
Staphylinid sp. 6	**		*		
Staphylinid sp. 12	**				
Staphylinid sp. 50	**				
Alleculinae sp. 6 ^a	**		*	*	**
Phalacrid sp. 4	**		**		*
Lathridiid sp. 1	**		**		
Melolonthinae sp. 14	**			**	
Nitidulid sp. 1 ^a	**		**	*	**
Limmichid sp. 1	*		**		
Cetoniinae sp. 1	*		*		***
Cicindelinae sp. 2	*		**		**
Elaterid sp. 5	*				
Stenus sp. 1	*	**		**	
Coccinellid sp. 2	*				
Endomia sp. 1	*				
Staphylinid sp. 20	*	**			
Cicindelinae sp. 1			**		*
Staphylinid sp. 32			**		
Cicindelinae sp. 3			**		**
Alticinae sp. 2		**			
Nitidulid sp. 2		*		*	
Scydmaenid sp. 2		*			
Melolonthinae sp. 10			**		*
Tenebrionid sp. 1					*

***, Very highly significant; **, highly significant; *, significant; blank, not significant.

^a Species are those considered uninformative (see text).

(Table 10). Under this test, 20 species were significantly affected (six very highly significant, eight highly, six significant). Excluding the four species discarded above, 13 were shared with the 22 from Table 8, and all 16 showed reductions with SP3. These findings lead to the conclusion that SP3 is indeed contributing to the effects detected.

Comparing the three fungal treatments with the standard chemical treatment/control (Table 11) we found significant effects for 17 species (six very highly significant, two highly significant, nine significant). Only one of the uninformative four showed up in this table. Of the others, nine were shared with Table 8, four others with Table 9.

Thus, it would seem that there is indeed an aspect shared by the chemical pesticide and SP3 that is det-

perimental to beetle biodiversity, and that SP9 and SP16 do not share this effect. It seems almost too coincidental that these two formulations, so very different in their mode of action and expected range of susceptible taxa, could cause these effects through direct mortality. If the simple reduction of live migratory locusts or increase in dead/dying migratory locusts were the cause through trophic interaction (hereafter called cascade) effects, then the similarity would have been between the standard chemical treatment and SP9. This pattern is expected because these treatments were more similar in their mortality rates of locusts (Delgado et al. 1997), but that relationship is soundly rejected (Table 5). Perhaps SP3 is more virulent on another species or group that has a key role in the system, and the chemical pesticide causes similar mortality to that species or group. Under this ad hoc scenario, the cascade effects from this keystone species' or group's reduction would be similar. The fact that half (11 of 22) of the species showing this effect are thought to be predators, and that all of them were reduced by SP3/standard chemical treatment, may lend support to this idea. The hypothetical possibility of such indirect effects on predators are discussed by Goettel and Hayek (2001), but without any examples. In any case, these findings emphasize how very little we actually know about the systems we so confidently manipulate. Caution is well advised in the application of any control agents to localities with an endemic and endangered invertebrate fauna.

The potential risk of damage to nontarget biodiversity through use of *M. flavoviride* SP3 as a control agent is difficult to answer. On the one hand, this strain is present in the environment of Madagascar, and using it as a biopesticide is more an example of enhancement than release. However, not all strains are naturally present everywhere in Madagascar, and widespread application at unnaturally high levels could produce novel local ecological perturbations. In fact, a strain may be naturally quite rare and confined to a small geographical and biological niche, and each isolate has a high likelihood of being a unique genotypic clone. It is quite possible that under natural conditions a potentially broad pathogenic isolate could be ecologically restricted to a small, discrete habitat by any number of factors limiting or preventing its spread. Therefore, the possibility of deleterious environmental effects should not be dismissed in evaluation of native entomopathogenic fungi (Goettel et al. 1990, Goettel and Vandenberg 1994). The similarity of effects of one strain of *M. flavoviride* (SP3) to a wide-spectrum chemical pesticide on nontarget communities, whereas another strain of the same fungal species (SP9) produced a dramatically distinct effect shows the importance of strain-based investigation and field-based tests for nontarget effects. Further, the fact that the lower-effect strain showed better control of the target species (Delgado et al. 1997a, 1997b) gives added strength to the biopesticide argument. The assertion that strain virulence against targets is seemingly inversely related to host breadth (Goettel et al. 1990) is supported by our findings in that SP3 had a

greater effect on nontarget beetles, but was less effective against the target migratory locust than the conspecific SP9 (Delgado et al. 1997a, 1997b). Although our results support the further development of SP9 and SP16 for commercial release in Madagascar, we believe that medium-scale tests of nontarget biodiversity effects would be warranted before SP3 is used on a wide scale. The limitations of the experimental design in terms of pretreatment sampling, limited replication and short posttreatment sampling period must argue against dismissing SP3 as detrimental to nontarget biodiversity, but the risk is significant that it should be further examined.

The insight gained from this exercise should be ample evidence of the wisdom and feasibility of field-based evaluation of biopesticides on native biodiversity. Although the limitations imposed on this study were unfortunate, the relatively robust results show the general validity of the method as a way of approaching the problem. We urge that equivalent, properly funded studies, conducted by appropriately trained systematists, be incorporated into all testing of biopesticides.

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