Field Manipulation of *Nomuraea rileyi* (Moniliales: Moniliaceae): Effects on Soybean Defoliators in Coastal Ecuador

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J. Econ. Entomol. 83(6): 2193–2195 (1990)

ABSTRACT Attempts to influence the prevalence of the entomophathogenic fungus *Nomuraea rileyi* (Farlow) Samson in populations of the velvetbean caterpillar, *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae), and the soybean looper, *Pseudoplusia includens* (Walker) (Lepidoptera: Noctuidae), were made in two field experiments with soybean (*Glycine max* L.) on Ecuador's humid coastal plain. Larval numbers and mortality were compared in large replicated plots sprayed with either conidia or the fungicides benomyl and chlorothalonil, and in untreated controls. *N. rileyi* conidia treatment caused a short-lived increase in larval mortality and no change in population levels of velvetbean caterpillar or soybean looper. The fungicide treatment persistently inhibited *N. rileyi*, causing significantly higher populations of the two defoliators.

KEY WORDS Insecta, fungicide, soybean defoliators, *Nomuraea rileyi*

SOYBEAN HAS become an increasingly important crop in recent years on Ecuador's central coastal plain, where more than 66,000 ha were planted in 1987 (del Salto & Tschirley 1988). The major production area is the environs of Quevedo (Los Rios Province), where two or three crops per year can be grown. Insect pests include a complex of defoliators, principally the velvetbean caterpillar, *Anticarsia gemmatalis* Hübner; the soybean looper, *Pseudoplusia includens* (Walker); *Omiodes (Hedylepta) indicata* F. (Lepidoptera: Tortricidae); *Ceratoma facialis* Erickson (Coleoptera: Chrysomelidae); the tortricid pod and stem borers *Epinotia aporema* Walsingham and *Cydia fabivora* Meyrick; and several species of pod-feeding stinkbugs, including *Euschistus crenator* F., *E. bifistolus* Palisot de Beauvois, and *Piezodorus guildinii* Westwood (Hemiptera: Pentatomidae) (Stansly & Mendoza 1988). Many growers spray two or three times per crop, mostly to control defoliators.

The use of economic thresholds, ranging from 45% defoliation plus two defoliators per meter of row in the two- to four-leaf stage to 17% plus 20 defoliators per meter of row in the pod-fill stage (Stansly & Mendoza 1988), has reduced the number of necessary sprays to one or less per crop. Even when the threshold is reached, the decision to spray may be postponed when velvetbean caterpillar and soybean looper are the predominant defoliators and 10% of their larvae are mummified by the entomopathogenic fungus *Nomuraea rileyi* (Farlow) Samson. This guideline was adopted because, in the past, appearance of numerous mummified larvae usually indicated an epizootic of *N. rileyi* would develop before economic damage occurred (Stansly & Mendoza 1988). Given the importance of *N. rileyi* to integrated pest management in this humid region (mean annual RH 85%), more information was needed on the relationship between control practices, prevalence of the pathogen, and population dynamics of the two susceptible noctuid defoliators. The following study was undertaken to determine if the epizootic process could be accelerated by additional inocula as a means of limiting the severity of occasional outbreaks of defoliating noctuids, and interrupted by fungicide applications sometimes used for control of frog eye leaf spot caused by *Cercospora sojina* Hara.

Materials and Methods

The experiment was conducted at two study sites located ≈25 km from each other on a NE–SW transect nearly bisected by the city of Quevedo (1°01′ S, 79°35′ W; 75 m above mean sea level; mean temperature, 25°C; mean rainfall, 2,100 mm/yr) during the early dry season (May, June, and July). Rainfall for these months at the Instituto Nacional de Investigaciones Agropecuarias Experimental Station near Finca San Luis (the SW site) was 262.4, 0.1, and 2.0 mm, respectively, and evaporation was 95, 83, and 77 mm. Relative humidity reached 100% on 50 occasions and did not go below 53%. Temperature varied between the extremes of 18 and 34°C. Conditions at the NE site (Hacienda Nueva Esperanza) were similar, although somewhat wetter and more humid.

The respective owners prepared their soil for commercial production by disking in crop residues (soybean) from the last harvest (less than a month

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previous) and planting immediately to soybean (INIAP variety 303 selected from crosses of Davis and Jupiter) on 6 May 1987 at Nuevo Esperanza in 60-cm rows, and 27 May 1987 at San Luis in 50-cm rows. Final plant densities obtained were 125,000 per hectare at Nueva Esperanza and 200,000 per hectare at San Luis. The pre-emergence herbicides alachlor (2 liters/ha) and linuron (1 kg/ha) were applied, and the plots were weeded manually twice.

A randomized complete-block field plot design was used at each site with three treatments and five replications. Each plot measured 40 by 50 m with 10 m between plots. The three treatments were (1) an application of *N. rileyi* conidia, (2) applications of fungicide, (3) no applications (untreated control). The conidia applications were made when mean numbers of larvae (velvetbean caterpillar + soybean looper) exceeded two per meter of row. This occurred 1 wk after the first flowering at both sites. Plots assigned to this treatment were sprayed with a suspension prepared by blending cadavers on which the fungus had recently sporulated with refrigerated *N. rileyi* conidia in a 1% solution of Triton ACT to give a final concentration of 2.5×10^7 conidia per milliliter, as determined with a hemocytometer. Conidial germination was estimated at 64% (SEM = 0.9%, 48 hr) by streaking conidia from five cadavers on slides coated with Sabouraud maltose agar fortified with 2% yeast extract. The conidia suspension was applied near dusk within 3 h of preparation using a motorized backpack sprayer calibrated to deliver 20 liters/ha (5×10^{11} conidia per hectare; 3.2×10^{11} viable conidia per hectare). Each plot assigned to fungicides was treated with the backpack sprayer every 14 d starting just before flowering with a mixture of benomyl 50% wettable powder (Benlate, E. I. du Pont de Nemours & Co., Agricultural Products Department, Wilmington, Del.) and chlorothalonil 50% emulsifiable concentrate (Bravo 500, Fermenta Plant Protection Company, Painesville, Ohio) at a rate of 250 g(AI)/ha. Benomyl was chosen because it is commonly used to control *C. sojina* in soybean and because it inhibits *N. rileyi* both in vivo and in vitro (Ignoffo et al. 1975). Chlorothalonil was included because it inhibited *N. rileyi* in vitro more than any other fungicide tested by Ignoffo et al. (1975).

Larvae were sampled weekly from the seven-node stage through the onset of senescence (nine samples at Nueva Esperanza and eight at San Luis) by shaking plants from 1 m of row over a drop cloth at three randomly chosen locations in each plot. Mortality of velvetbean caterpillar and soybean looper caused by *N. rileyi* was determined by rearing an average of 24 larvae per plot (a range of 10–37 larvae) on four sample dates, 1 d before, and 1, 5, and 14 d after conidia application. The larvae were placed individually in 2-oz. (57-ml) clear-plastic medicine cups partly filled with pinto bean diet (Leppla 1985) and maintained in the laboratory at 23±5°C until adult emergence, parasite emergence, or death. Larval mortality was attributed to the presence of either *N. rileyi* (conidia, or mycelia, or both), insect parasites (parasite emergence), or unknown causes. Yield data were obtained by hand harvesting 200 m^2 per plot in two randomly chosen subplots of 100 m^2 and later separating the seed with a motorized thresher.

Number of larvae sampled from the field were analyzed by species and location using a split-plot design with sample dates as subplots and treatments as whole plots (Freund et al. 1986). Orthogonal contrasts were used for planned comparisons of means (SAS Institute 1985). Correlation between mortality caused by *N. rileyi* and mortality caused by parasitism was determined using a correlation procedure (SAS Institute 1985), with variables being the number of field-collected, laboratory-reared larvae dying from these causes. Percent mortality data were transformed by the arcsine square root function (Steel & Torrie 1980) and analyzed with analysis of variance by sampling date using Duncan's multiple range test for mean differences (SAS Institute 1985). Data were combined over locations and species for the analysis of variance because the effects of these factors were not significant ($F = 1.69$, $P > 0.26$, and $F = 0.13$, $P > 0.72$, respectively; df = 1, 237).

Results and Discussion

Larval Populations. Populations of velvetbean caterpillar and soybean looper were typically low throughout most of the crop cycle. The two species combined reached a peak of 3.3 larvae per meter (SEM = 1.0, $n = 5$) in control plots 2 wk after flowering at Nueva Esperanza and 5.5 per meter (SEM = 0.29, $n = 5$) 2 wk later at San Luis. Of the larvae sampled throughout the season at Nueva Esperanza, velvetbean caterpillar accounted for 48% and soybean looper accounted for 52% ($n = 135$, SEM = 2.2 for both). At San Luis, we found 66% velvetbean caterpillar and 34% soybean looper ($n = 120$, SEM = 2.4 for both).

From 2 wk after an application of conidia until crop maturity, mean number of live larvae varied significantly with treatment for both species at Nueva Esperanza and for velvetbean caterpillar at San Luis. Orthogonal comparisons showed that this difference occurred primarily between the fungicide treatment against the conidia and control treatments combined, whereas conidia and control treatments did not differ significantly (Table 1).

Larval Mortality. Parasites reared from larvae or pupae of velvetbean caterpillar were *Glyptapanteles caffreyi* Muesebeck, *Zelomorpha* sp., and *Rogas vaughani* Muesebeck (Hymenoptera: Braconidae) (determined by P. M. Marsh, US. National Museum, Washington, D.C.); *Euplectrus* sp. (Hymenoptera: Eulophidae) (determined by M. E. Schauf, U.S. Natural History Museum, Washington, D.C.); and *Eucelatoria* sp. (Diptera: Tachin-

Table 1. Mean number of velvetbean caterpillar and soybean looper larvae per meter of row sampled weekly beginning 2 wk after spraying soybean plants with conidia of *N. rileyi* until crop maturity at two sites in Ecuador

Site[a]	Treatment			F^b	P
	Conidia, mean ± SEM	Control, mean ± SEM	Fungicide, mean ± SEM		
	Velvetbean caterpillar				
NE	1.0 ± 0.15	0.9 ± 0.14	1.4 ± 0.16	7.6	0.008
SL	2.8 ± 0.36	2.9 ± 0.31	3.8 ± 0.27	9.3	0.004
	Soybean looper				
NE	1.6 ± 0.22	1.4 ± 0.13	2.0 ± 0.17	6.0	0.018
SL	1.5 ± 0.19	1.5 ± 0.20	1.4 ± 0.11	0.4	NS

NS, not significant.
[a] NE, Nueva Esperanza; SL, San Luis.
[b] *F* values and probabilities from orthogonal comparisons between fungicide treatment and pooled control and conidia treatments (df = 1, 83 for NE site; df = 1, 68 for SL site [SAS Institute 1985]).

idae) (determined by N. Woodley, U.S. National Museum, Washington, D.C.). *Litomastix truncatella* Dalman (Hymenoptera: Encyrtidae), *Euplectrus* sp. (determined by M. E. Schauf), *Aleiodes* n. sp., and *Cotesia* sp. (Hymenoptera: Braconidae) (determined by P. M. Marsh) were reared from soybean looper. Mortality caused by parasites was inversely correlated with mortality caused by *N. rileyi* ($r = -0.42$, $n = 120$, $P < 0.0001$).

There were highly significant treatment effects on mortality caused by the fungus for all three postapplication sample dates. All three treatments differed significantly 1 and 5 d after application. However, control and conidial application treatments were not significantly different 14 d after application (Table 2).

We interpret these results to mean that an initial increase in larval mortality induced by conidial treatment disappeared after 2 wk, but suppression of *N. rileyi* by repeated fungicide treatment persisted. Fungal suppression probably resulted in the higher larval populations observed in chemically treated plots. However, economic injury levels were not reached during the experiment, and no significant yield differences occurred among treatments ($F = 1.5$, df = 2, $P = 0.24$).

We conclude that spraying soybean with *N. rileyi* to control velvetbean caterpillar and soybean looper is not warranted under such conditions as those of our study because, although fungus-induced mortality was increased temporarily, pest populations were not measurably reduced. On the other hand, suppression of *N. rileyi* in fungicide-treated plots did allow pest populations to increase, and this process could lead to economic injury. Coastal Ecuador's climate favors efficient development and propagation of *N. rileyi*. Agricultural practices should also be geared to favor, or at least not to interfere with, this important aid to pest management.

Acknowledgment

We thank the owners of both study sites, G. Coello and O. Ojas, for their cooperation. Both D. G. Boucias and J. R. Harper gave technical advice, and J. R. Harper provided a helpful review. Funding was provided by the United States Agency for International Development through the project "Rural Technology Transfer System," Contract 518-0032-C-00-1040, administered by the Institute of Tropical Agriculture of the University of Florida. This research is Florida Agricultural Experiment Station Journal Series R-00431.

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Received for publication 30 January 1989; accepted 5 June 1990.

Table 2. Percent mortality (mean ± SEM) of velvetbean caterpillar + soybean looper caused by *N. rileyi* in two soybean fields in Ecuador

Treatment	1 d before application of conidia	Time from application of conidia		
		1 d	5 d	14 d
Conidia	10.2 ± 2.6a	39.1 ± 4.7a	50.7 ± 2.4a	42.3 ± 3.5a
Fungicide	9.3 ± 5.0a	4.2 ± 0.9c	4.1 ± 2.3c	10.6 ± 1.2b
Control	3.6 ± 1.3a	16.8 ± 2.0b	34.5 ± 4.6b	39.6 ± 4.1a

Data were analyzed using arcsine square root transformation. Within a column, means ± SEM followed by the same letter are not significantly different ($P > 0.05$; Duncan's multiple range test [SAS Institute 1985]).