BIOLOGICAL CONTROL

The Impact of Fungicides on *Nomuraea rileyi* (Farlow) Samson Epizootics and on Populations of *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae), on Soybean

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Impacto de Fungicidas Sobre Epizootias de *Nomuraea rileyi* (Farlow) Samson e Sobre Populações de *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae), em Soja

RESUMO - O fungo *Nomuraea rileyi* (Farlow) Samson é um dos inimigos naturais mais importantes de lagartas da soja, principalmente em condições de tempo úmido. Surtos de oídio têm demandado a aplicação de fungicidas na soja, especialmente nas etapas iniciais do ciclo agrícola, época na qual a interferência sobre *N. rileyi* pode favorecer a ocorrência de lagarta por redução do inóculo desse fungo. Ensaios de laboratório mostraram que benomil, difenoconazole, enxofre e carbendazim reduziram a germinação de *N. rileyi* sendo o último menos deletério. Para avaliar o impacto dos fungicidas utilizados no controle do oídio sobre *N. rileyi*, foram realizadas aplicações com difenoconazole (75 g i.a./ha) ou benomil (262,5 g i.a./ha) em parcelas de soja. No ensaio realizado em 1997/1998 foi realizada apenas uma aplicação de fungicida, estando as plantas no estágio R1-R2. No ensaio realizado em 1998/99 realizaram-se duas aplicações, espaçadas de 19 dias, quando as plantas estavam nos estágios V5 e V7, respectivamente. O número de lagartas foi significativamente maior nas parcelas tratadas com os fungicidas que nas parcelas testemunhas. Em 1997/98, a parcela tratada com benomil apresentou população mais elevada da lagarta-da-soja que nas parcelas testemunhas ou na tratada com difenoconazole. Em 1998/99, a população da lagarta-da-soja foi maior entre o sétimo e décimo segundo dia após a primeira aplicação, e continuou alta até o décimo nono dia após a segunda aplicação. Os fungicidas causaram um atraso de 2 a 14 dias na iniciação da epizootia de *N. rileyi*.

PALAVRAS-CHAVE: Fungo entomopatogênico, difenoconazole, benomil, carbendazim, *Microsphaera diffusa*, controle biológico

ABSTRACT - The fungus *Nomuraea rileyi* (Farlow) Samson is one of the most important natural enemies of soybean caterpillars, mainly under humid weather conditions. Outbreaks of the fungus *Microsphaera diffusa* Cooke & Peck have demanded fungicide applications on soybeans, which could result in outbreaks of noctuid populations by reduction of the natural inocula of *N. rileyi*. The recommended fungicides have shown to be detrimental to beneficial fungi, reducing infection, delaying epizootics, and resulting in increased host population densities. In laboratory assays, benomyl, difenoconazole, sulphur and carbendazim affected conidial germination of *N. rileyi*, being the latter less deleterious. To assess the impact of fungicides used to control *M. diffusa*, on *N. rileyi*, two tests were carried on, spraying difenoconazole (75 g a.i./ha) and benomyl (262.5 g a.i./ha) on soybean plots. In the 1997/98 trial, fungicide was sprayed once on soybean plants at R1-R2 developmental stages. In the 1998/99 test, two applications were made, when plants were at V5 and V7 developmental stages, respectively. The number of VBC larvae was significantly higher in the fungicide treated plots than in the control plots. In the 1997/98 test, benomyl treated plots resulted in higher populations of VBC than in the control or in the difenoconazole plots. In the 1998/99 test, VBC population was higher from 7 to 12 days after the first application, and remained high until 19 days after the second application. In general, fungicide treatments delayed the begining of *N. rileyi* epizootics from 2 to 14 days.

KEY WORDS: Entomopathogenic fungi, difenoconazole, benomyl, carbendazim, *Microsphaera diffusa*, biocontrol

In Brazil, soybean is cultivated over 14 million ha and the major key pest is the velvetbean caterpillar (VBC), *Anticarsia gemmatalis* Hübner. The majority of the insecticide applications in soybeans are directed against this insect. Usually VBC occur at higher intensities from the mid to late vegetative stages (mid December) to the end of flowering stage of the crop (mid January), but populations of the insect can be detected within few days after plant emergence. When relative humidity is high, the entomopathogenic fungus, *Nomuraea rileyi* (Farlow) Samson maintains the pest population below economic injury levels (Moscardi & Sosa-Gómez 1993).

Fungicide applications against soybean pathogens were usually not recommended in Brazil until 1997, because fungal diseases could mostly be controlled through resistant varieties. In the 1995/96 growing season an outbreak of the powdery mildew, caused by *Microsphaera diffusa* Cooke & Peck, led to the recommendation of fungicides. The chemical control of phytopathogenic fungi is critical, because diseases as the powdery mildew sometimes can cause yield losses of 30 to 40% (Embrapa 2001).

Among these fungicides, benomyl has shown to be detrimental to beneficial fungi, for example reducing infection rates of *Erynia neoaphidis* (Remaudière & Hennebert) (Wilding 1982) and *N. rileyi* (Johnson *et al*. 1976), delaying the epizootics, and resulting in increased population densities (Stansly & Orellana 1990).

In Brazil, as in other regions of the world, the entomopathogenic fungus *N. rileyi* is one of the most important natural control agent of the soybean caterpillars *A. gemmatalis*, *Pseudoplusia includens* (Walker) and *Rachiplusia nu* (Guenée). Therefore, our interest was to determine whether fungicide applications for *M. diffusa* control could reduce the inoculum of *N. rileyi* and consequently favor the increase of VBC populations. We studied the impact of recommended rates of difenoconazole and benomyl on *N. rileyi*, assessing the effect of the fungus on the VBC populations. Our purpose was to know whether single or repeated fungicide applications could suppress *N. rileyi* inocula increasing caterpillar populations. The knowledge of the effect of different fungicides on *N. rileyi* would allow the selection of the less deleterious to be used in integrated management programs.

Material and Methods

Laboratory Studies. The effects of the fungicides were assessed in the laboratory using the following *N. rileyi* isolates: Nr250 (Londrina, PR), Nr304 (Planaltina, DF) and Nr424 (Assis, SP) (Sosa-Gomez 2002). The fungicides were diluted in water, taking into account the recommended dose per ha, considering 100 L as the volume of application per ha. The fungicide concentrations (mg a.i./ml) in the fungicide suspension were: Kumulus (sulphur): 20 mg/ml; Derosal (carbendazim): 0.0025 mg/ml; Benlate 500 (benomyl): 2.25 mg/ml; and Score (difenoconazole): 0.00037 mg/ml. Conidia obtained from two week fungal colonies were exposed to the fungicide suspensions during 4h and sprayed onto microscope slides precoated with a thin layer of SMAY medium (sabouraud, maltose, agar and yeast extract). The slides were incubated at 26 ± 1,5°C for 18-24h in a humid chamber. After that, germinated and ungerminated conidia were quantified and data analyzed using Tukey test (Jandel Scientific 1994).

Field Studies. These studies were carried out for two consecutive seasons in the experimental station of Embrapa, in Londrina, Paraná State. Soybeans (variety BR-37) were sown under no till system on November 11 (1997) and on November 12 (1998). The experimental area was divided in three plots of 2666 m^2 each. The fungicides were applied at the following doses: difenoconazole- 75 g a.i./ha and benomyl-262.5 g a.i./ha, both in 300 L of water in the 1997/98 test and in 125 L in the 1998/99 test. The control plot was treated with water. Insects were sampled twice a week by the shake-cloth method (Kogan & Pitre 1980), in 15 sites/plot, before and after the application of fungicides. In the 1997/98 season, the fungicides were applied on January 9 (1998), one day after the first death by *N. rileyi* was detected. This date was selected because most of the plants were at the R1-R2 developmental stage. In the 1998/99 season, the first spraying was made on December 23 (1998) (plant developmental stage V5), five days before the first death caused by *N. rileyi*. The second spraying was made on January 1st (1999) (plant developmental stage V7). The number of alive and *N. rileyi* killed larvae per shake-cloth was recorded. In each sampling, live VBC larvae were collected and taken to the laboratory to access and confirm fungal infection. Larvae from each treatment were fed on leaves in the laboratory for one week. Visual estimates of defoliation and soybean phenological stages (Ritchie *et al*. 1982) were recorded at each sampling date. Precipitation was recorded using a tipping bucket rain gage (Model TE525). Relative humidity (RH) was determined using a RH probe (Model HMP35C). Data were recorded in a 21X Micrologger (Campbell Scientific, Inc., Logan, Utah, USA).

Differences among treatments were detected by Tukey test for the conidial germination data. Field data were analyzed using the Kruskal-Wallis one-way analysis of variance on ranks and the means were compared by the Student-Newman-Keuls test or Dunn´s test (Jandel Scientific 1994).

Results

Laboratory Studies. In general benomyl, difenoconazole, and sulphur reduced conidial germination. Carbendazim was less deleterious to the *N. rileyi* isolates (Table 1). The germination of the Nr250 isolate suggests that this isolate was not affected by carbendazim, and that interactions between fungicides and isolates occur.

Table 1. Percentages (±SEM) of conidial germination comparisons among isolates of *N. rileyi* conidia exposed during 4h to a fungicide suspension in water. Germination on SMAY medium after 18-24h at 26 ± 1.5°C.

Fungicides	*N. rileyi* isolates (Nr250, Nr304, Nr424)[1]	Nr 250 isolate
Control	36.6 ± 8.43 a	13.3 ± 1.06 a
Carbendazim	21.6 ± 3.70 b	11.9 ± 0.07 a
Benomyl	18.7 ± 8.10 bc	5.3 ± 1.71 b
Sulphur	18.3 ± 7.98 c	1.8 ± 1.06 b
Difenoconazole	11.3 ± 2.76 c	1.0 ± 0.55 b

[1]Means of three isolates

Means in a column followed by the same letter are not significantly different by Tukey test ($P < 0.05$).

Field Studies. In the field test of 1997/98, the first death by *N. rileyi* was detected on January 8, when no fungicide treatments had been applied in the field. Six days after treatment (Jan/15/98), the mean number of live larvae was 38% higher in the area treated with benomyl (Student-Newman-Keuls test, P = 0.003) than in the control and in the difenoconazole treated area (Fig. 1). The number of larvae remained high until 13 days after treatment (Jan/22/99). Afterwards, differences among treatments were not significant, due to an overall decrease in *A. gemmatalis* population in the experimental area. The highest mean number of dead larvae attacked by *N. rileyi* was collected in Jan/29/98 in the control plot. As a whole, in all sampling dates, there were no statistical differences related to the mean number of larvae dead by *N. rileyi* between treated and untreated plots. Only in one date (Jan/22/99) the mean number of dead larvae by *N. rileyi* was significantly lower in the difenoconazole treated plot than in the benomyl and control plots (Student-Newman-Keuls test, P = 0.04). A two-day delay on *N. rileyi* occurrence was observed in larvae taken to the laboratory from difenoconazole treated plots (Table 2). The mean percentages of infection were 18.6; 19.4 e 15.6 for the control plot, difenoconazole and benomyl treated plots respectively, with no significant differences between them (Kruskal-Wallis ANOVA on Ranks, P = 0.84).

In the 1998/99 field test, the first case of *A. gemmatalis* mortality by *N. rileyi* was observed on Jan/07/99, in the samples collected in Dez/30/98 and kept in the laboratory. Under field conditions, the first case of *N. rileyi* disease was observed on Jan/11/99. In this test the effect of the fungicides was evident. Seven days after fungicide applications (Dez/30/98) the mean number of VBC larvae was two times higher (Student-Newman-Keuls test, P < 0.001) in the benomyl treated plots than in the control plots (Fig. 2). Difenoconazole treated plots also showed significantly higher mean number of VBC from January 11 to January 25. The number of larvae remained higher in the fungicide treated plots during 13 days after the first spraying. Differences among number of live larvae were evident until 19 days (Jan/25/99) after the second application: the number of larvae was superior to the control plot in both

Table 2. Percentages of *N. rileyi* infection on *A. gemmatalis*, at each sampling date, observed in larvae taken to the laboratory, and maintained on soybean leaves from respective plots.

Date	Control	Difenoconazole	Benomyl
Growing season 1997/98			
Jan/13/98	21	0	12
Jan/15/98	16	35	12
Jan/20/98	30	12	17
Jan/27/98	12	25	32
Jan/29/98	14	25	5
Growing season 1998/99			
Dez/30/98	7	0	0
Jan/05/99	5	0	0
Jan/07/99	0	0	0
Jan/11/99	0	0	0
Jan/14/99	3	7	3
Jan/18/99	30	14	28
Jan/21/99	15	20	20
Jan/25/99	50	25	25
Jan/28/99	20	26	43
Fev/01/99	60	50	29

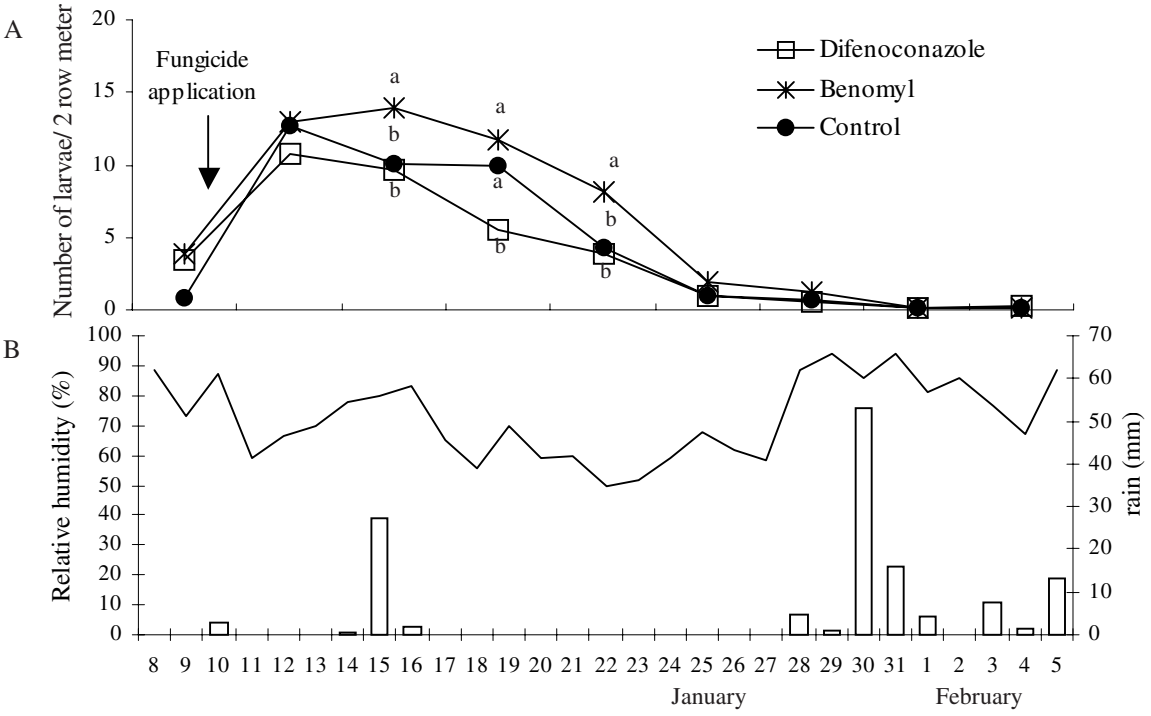

Figure 1. (A) Mean number of *A. gemmatalis* larvae sampled in 2 m row of soybean. Growing season 1997/98. (B) Rainfall (bars) and relative humidity (line) during the 1997/98 growing season. Means followed by the same letter at each sampling date do not differ statistically by the Student-Newman-Keuls test (P = 0.05).

areas treated with fungicides.

In the growing season of 1997/98 the maximum density of the VBC population was similar to that observed in 1998/99, however decreased to nearly zero at the end of January 1998. In the 1998/99 field test the VBC populations reached a similar density in the beginning of January 1999 and remained with some fluctuations in a plateau until February 25, declining rapidly close to zero after February 15 (data not shown).

The mortality of larvae collected from treated and non-treated areas and kept in the laboratory did not differ statistically by the Kruskal-Wallis ANOVA on ranks (P = 0.77). However, the first case of the mycosis was detected in the untreated subplots on Dez/30/98, while in the plots treated with fungicide, larvae killed by *N. rileyi* were found two weeks later (Jan/14/99) (Table 2). After the end of January mortality by *N. rileyi* was not considered due to VBC low numbers. The rain was more evenly distributed and the relative humidity was most favorable to *N. rileyi* in the 1998/99 season than in the 1997/98 season.

Discussion

In vitro, difenoconazole, sulphur and benomyl seem to be more detrimental to *N. rileyi* than carbendazim. Ignoffo *et al.* (1975) found that benomyl was not among the most active fungicides against *N. rileyi*, but its effect was sufficient to inhibit infection on *Trichoplusia ni* larvae. In the present work, nevertheless, in the benzimidazole group there were differences; carbendazim, a metabolite of benomyl, did not suppress *N. rileyi* mycelial growth as benomyl did. Carbendazim was less harmful *in vitro* to the three isolates tested, showing selectivity at least for this isolate, obtained from the same region where the field study was performed.

In the 1997/98 field test, the density of VBC was significantly higher during three sampling dates (eight days) in the benomyl treated subplots, suggesting its deleterious effect on *N. rileyi*. Difenoconazole did not cause perceivable effects on the fungus through this season. During the 1998/99 field test, when the density of VBC reached 15 larvae per two row meter and remained high through the season, the deleterious effects of both fungicides on *N. rileyi* were more evident. Plots treated with fungicides showed higher VBC larval density during 25 days: 38% higher in the benomyl plot than in the control (Jan/05/99) and 63% higher in the difenoconazole plot than in the control (Jan/21/99). The longest residual effect of the fungicides on *N. rileyi* observed in 1998/99 could be due to the double application in this season. In the 1997/98 season the rapid decline of VBC populations hampered detection of statistical differences among treatments.

The effect of the fungicides was more evident in the field test of 1998/99 as the fungicides were applied twice in this season, and as well as because they were applied earlier than in 1997/98. Thus, it is possible that due to the earlier application of fungicides in the 1998/99 season the initial inoculum of *N. rileyi* was depleted, delaying the epizootic. Fungicide treatments are usually recommended to control fungal diseases as *Septoria glycines* (Hemmi) and *Cercospora kikuchii* (Mastsumoto & Tomoyasu) Gardner during the developmental stages R5.1 or R5.5, when *N. rileyi* already performed its role as caterpillar control agent. Therefore, during this phase of the soybean crop, the deleterious effect of these fungicides is not important. However, early fungicidal applications directed against *M. diffusa* in the season

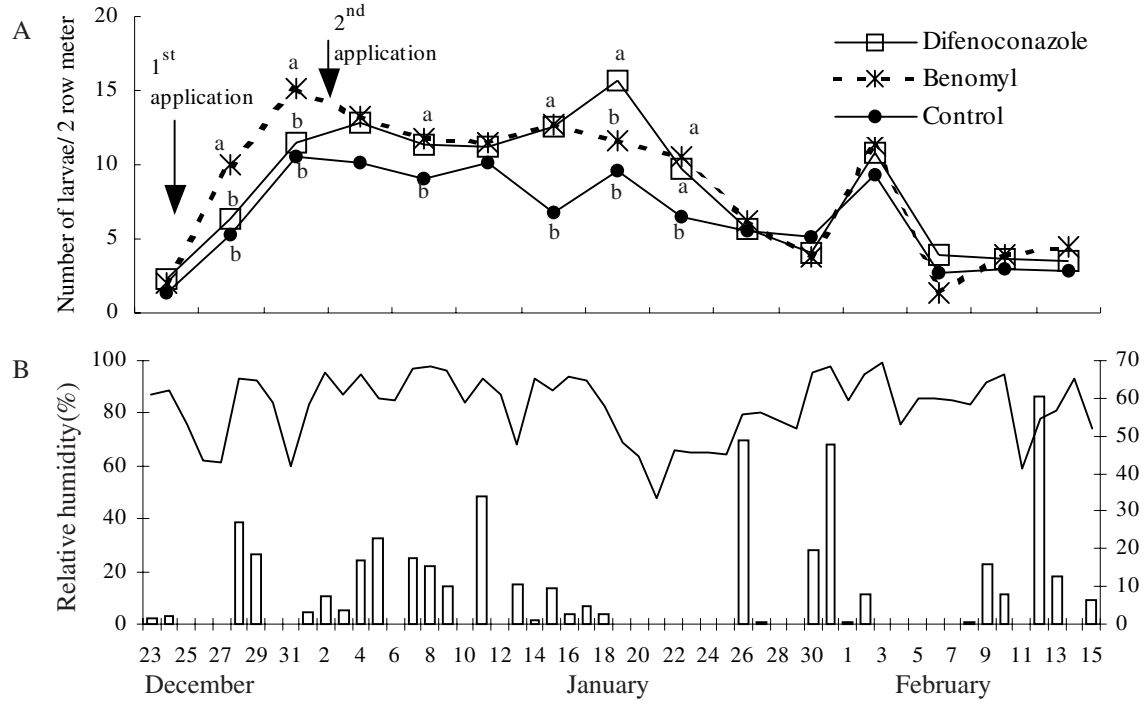

Figure 2. (A) Mean number of *A. gemmatalis* larvae sampled in 2 m row of soybean. Growing season 1998/99. (B) Rainfall (bars) and relative humidity (line) during the 1998/99 growing season. Means followed by the same letter at each sampling date do not differ statistically by the Student-Newman-Keuls test (P = 0.05).

(vegetative and early reproductive stages) could result in adverse effects, such as delaying *N. rileyi* epizootics and promoting VBC population increase.

The percentages of infection observed in the laboratory and the number of dead larvae collected in 2 m of row (larvae that remained attached to the plants or that fell down on the cloth) were not significantly different among treatments, in both field tests. However, considering the larval samples taken to the laboratory in both seasons, the initiation of the epizootic was delayed. The percentages of dead larvae by *N. rileyi* seemed to be higher than that observed in the 1997/98 trial, as well as, the mortality by the fungus was observed earlier in the laboratory in 1998/99, possibly due to more humid conditions in this season (Figs. 1 and 2), because *N. rileyi* needs relative humidity above 70% for conidiogenesis (Kish & Allen 1978).

In both seasons, the density of VBC did not surpass the economic threshold level and the *N. rileyi* inhibition was not enough to favor VBC populations to the point that VBC could cause economic damage to soybean. In both seasons the defoliation level did not surpass 10%. However, the use of non-selective fungicides to *N. rileyi* could be detrimental to yield and to the IPM program in Brazil, especially in seasons of high VBC incidence.

The deleterious effect of benomyl on *N. rileyi* has already been shown by Johnson *et al.* (1976) and Horton *et al.* (1980) for the conditions of southern USA. Stansly & Orellana (1990) observed that chlorothalonil and benomyl inhibited *N. rileyi*, causing significantly higher populations of VBC and soybean looper. We also observed that difenoconazole, inhibitor of demethylation, showed deleterious effect on *N. rileyi* in the laboratory and in the field. Both fungicides, which strongly inhibit growth of *N. rileyi in vitro*, delayed the appearance of early cases of the disease and favored the population increase of the VBC in the field.

Although benomyl or difenoconazole may delay *N. rileyi* infections on caterpillar populations in experimental plots, these fungicides may not prevent outbreaks of this entomopathogenic fungus on lepidopterous populations in soybean. Since our laboratory data suggest that benomyl and difenoconazole are more deleterious to *N. rileyi*, early season fungicide applications against powdery mildew outbreaks, during early vegetative stages of soybean, could preferably be made with less detrimental fungicides as carbendazim.

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