EFFECTS OF SELECTED FUNGICIDES ON DEVELOPMENT OF SOYBEAN CYST NEMATODE

J. Faghihi,[1]* R. A. Vierling,[2] J. B. Santini,[2] and V. R. Ferris[1]

[1]Departments of Entomology and [2]Agronomy, Purdue University, West Lafayette, IN 47907, U.S.A.
*Corresponding author: jamal@purdue.edu.

ABSTRACT

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Greenhouse screening of soybean seedlings with soybean cyst nematodes takes several months and results can be confounded by fungal infection of the seedlings. *Rhizoctonia solani* was found to be a major problem and resorting to the use of fungicides was necessary. The effect of different fungicides on soybean cyst nematode (SCN) was assessed. An unusually low number of cysts developed on susceptible soybeans treated with Cleary 3336^{F} fungicide in the greenhouse. Laboratory studies revealed a negative correlation between concentration of Cleary 3336^{F} and number of active second stage juveniles (J2). Field studies in 2003, 2004 and 2005 showed no significant effect of Cleary 3336^{F} on yield or SCN population.

Key words: Cleary 3336^{F}, fungicide, *Heterodera glycines*, management, soybean cyst nematode.

RESUMEN

Faghihi, J., R. A. Vierling, J. B. Santini, y V. R. Ferris. 2007. Efectos de algunos fungicidas sobre el desarrollo del nematodo quiste de la soya. Nematropica 37:259-265.

La evaluación de plántulas de soya con nematodos quiste en invernadero toma varios meses y los resultados pueden complicarse con la presencia de infecciones fungosas. Se encontró que *Rhizoctonia solani* es un gran problema y que se requiere el uso de fungicidas. En este estudio se evaluó el efecto de diferentes fungicidas sobre el nematodo quiste de la soya. Se observó una cantidad inusualmente baja de quistes en plantas de soya susceptibles tratadas con el fungicida Cleary 3336^{F} en el invernadero. Los estudios de laboratorio indicaron una correlación negativa entre la concentración de Cleary 3336^{F} y la cantidad de juveniles de segundo estadio (J2) activos. Los estudios de campo de 2003, 2004 y 2005 no mostraron efecto significativo de Cleary 3336^{F} sobre el rendimiento del cultivo o las poblaciones del nematodo quiste de la soya.

Palabras clave: Cleary 3336^{F}, fungicida, *Heterodera glycines*, manejo, nematodo quiste de la soya.

INTRODUCTION

Soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) reduced U.S. soybean yield more during 2003-2005 than did any other disease (Wrather and Koenning, 2006). Resistant cultivars and crop rotation are currently the most effective management tools for SCN. Breeding SCN-resistant cultivars requires continuous bioassay screening in the greenhouse. The key to effective greenhouse screening is decreasing variation among the assays (Faghihi *et al.*, 1995) which can be influenced by abiotic factors including temperature, moisture, and chemical factors such as fertilizer, pesticides, and soil properties. Among the numerous biotic factors that can alter bioassay results are invertebrates and microbial pathogens such as bacteria, fungi and viruses. Inaccurate bioassay screening can have unfortunate consequences for a breeding program by delaying the release of new cultivars.

For a bioassay of SCN infection and growth to be successful, the root system must be healthy and relatively free of other pathogens for nematodes to develop normally. One pathogen encountered in SCN bioassays that severely damages the root system is *Rhizoctonia solani* Kühn. Poor development of the nematodes on the root system of a soybean selection being evaluated can be misinterpreted as resistance when, in fact, the nematode did not have suitable host conditions for penetration and development.

Some fungicides have been shown to have deleterious effects on various nematodes. In laboratory studies, captan induced cellular stress response in *Caenorhabditis elegans*, and adults were more sensitive than juveniles; captafol and folpet also caused stress to the nematode (Jones *et al.*, 1996). Also in laboratory studies, mancozeb was toxic to *C. elegans* (Easton *et al.*, 2001). Root-knot nematode (*Meloidogyne* spp.) population density was reduced 73-100% by butyric acid (Browning, 2006). ZeroTol killed 100% of foliar nematodes in a water suspension (Jagdale and Grewal, 2002) and cinnamaldehyde resulted in 100% death of entomopathogenic nematodes after a four-hour incubation; whereas azoxystrobin had no effect (Krishnayya and Grewal, 2002) on nematodes tested. In field studies, the fungicides benomyl, chlorothalonil, iprodione, thiram and triadimefon showed no significant nematicidal activity in turf (Dernoeden *et al.*, 1990).

Total plant-parasitic nematode population densities in golf courses were greater in fungicide-treated plots than in controls (Walker *et al.*, 2002), and in arctic soils, benomyl decreased the diversity of nematode species (Ruess *et al.*, 2001). In a subsequent study on non-plant parasitic nematode communities, benomyl greatly reduced the abundance of most nematode species, especially *Eudorylaimus* sp. (Ruess *et al.*, 2002).

While Cleary 3336^{F} fungicide (thiophanate-methyl (dimethyl 4,4'-0-phenylene-bis[3-thioallophanate])) is an effective control of *R. solani*, its effect on non-target species such as nematodes is not clear. Our objective was to determine if selected common greenhouse fungicides had an effect on SCN hatching, mobility or development.

MATERIALS AND METHODS

Laboratory Tests

Direct effect of Cleary 3336^{F} on SCN eggs and cysts was studied in the laboratory by exposing them to different concentrations of Cleary 3336^{F} (0, 29, 58 and 116 µg ai/µl) in mini-chambers. The chambers were constructed with Tygon® R-3603 laboratory tubing 1.5 cm (diameter) by 1.5 cm (length). A piece of cloth screen (150 mm opening) was placed inside the chambers to hold eggs or cysts. The chambers were placed in the center of a petri dish (3.5 cm in diameter). Twenty cysts or 3000 eggs were placed on a screen in the mini-chamber. Each dish was filled with one of the four concentrations of Cleary 3336^{F} or deionized water for the control. Treatments were arranged in a completely randomized design with four replications. This experiment was repeated twice producing similar results each time.

At 24 and 48 hours after the experimental units were established, the mini-chambers were removed from the dishes. Active and inactive juveniles that had hatched and migrated through the screen were counted. The cysts and eggs that remained in the chambers after the 48 hour harvest were rinsed with tap water and the concentrated inoculum was poured on the roots of seven day old susceptible soybean plants, cultivar Williams 82. The plants were grown in the greenhouse in seedling trays for about 6 weeks.

Cysts on the roots were collected by spraying the roots with water. Cysts in the soil were recovered by decanting and sieving (Faghihi *et al.*, 1986).

Greenhouse Test

Soybeans were grown in seedling trays with 2.5 cm diameter cells filled 2/3 full (4 cm) with a sand/soil (3/1) mixture. Two Williams 82 seeds were placed on the surface of the soil in each cell and the cell was drenched with 5 cm^3 water solution of a single concentration of Cleary 3336^F fungicide. In the first experiment the fungicide was applied at rates of 0, 2, 4, 5, 10, 14, 19, and 24 µg ai/µl. In the second experiment rates were changed to 0, 15, 30, 60, 120 and 240 µg ai/µl.

In order to determine the effect of other selected fungicides, a third experiment was conducted using two concentrations of Cleary 3336^F (24 and 48 µg ai/µl) and the label recommended concentrations of azoxystrobin, metalaxyl and cholorothalonil (Table 1). The SCN inoculum, either 20 cysts mixed with soil, or 3000 eggs in water, was poured on top of the seeds at planting. Seeds were then covered with the soil/sand mixture. Seedlings were reduced to one plant/cell after soybean germination. Plants were maintained in the greenhouse at 24°C for 6 weeks, and new cysts produced on each plant were counted, as described above. The experiment was completely randomized with 3 or 5 replicates.

Field Tests

These experiments were conducted in infested fields at the Purdue Agronomy Center for Research and Education from 2003-2005. Soybean seeds were hand-placed 2.5 cm apart in furrows 4 cm deep. Each plot was 4.5 m long and 0.76 m wide. In 2003, five concentrations of Cleary 3336^F (0, 10, 24, 34 and 48 µg ai/µl) were sprayed in-furrow and on top of the seeds. In 2004 and 2005, four concentrations of Cleary 3336^F (0, 29, 58 and 116 µg ai/µl) were used. The seeds were covered immediately with soil. Soil samples were taken next to the rows (6 cores/treatment/plot) at planting and at harvest to estimate the density of SCN in the field. All plants in each row were hand-harvested after maturity for yield determination. The experi-

Table 1. Effect of fungicides on number of soybean cyst nematode using two different inoculum forms in the greenhouse.

	Inoculum	
Fungicides[y]	Eggs	Cysts
Cleary 3336^F (48 µg ai/µl)	0.3 ae[z]	0.1 ad
Cleary 3336^F (24 µg ai/µl)	0.9 be	0.8 bde
azoxystrobin	2.0 c	1.5 ce
cholorothalonil	1.8 cd	0.6 abd
metalaxyl	0.7 abe	1.3 bce
No fungicide	1.5 bcd	1.4 ce

[y]Application rates: Cleary 3336^F 24 and 48 µg ai/µl water, azoxystrobin 0.3 kg ai/ha, metalaxyl 0.03 cm^3 ai/100 g seed, cholorothalonil 1.7 kg ai/ha.

[z]Means log10 (X+1) in the column followed by the same letter are not different ($P = 0.05$).

mental design was completely randomized with five replications.

Data Analysis

Laboratory and greenhouse SCN counts were transformed to $\log_{10}$ (X+1) before analyses to achieve homogeneity of variance among the treatments. Data were analyzed with ANOVA and means were separated by the LSD test ($P = 0.05$). SCN counts over different concentrations of Cleary 3336^F were also described in linear models. Non-transformed means are shown in tables for clarity.

RESULTS AND DISCUSSION

Laboratory Tests

Following 24 hours of exposure to Cleary 3336^F, the number of active J2's that hatched from eggs was ($P = 0.05$) lower compared with the check (Fig. 1). There was no difference in the number of inactive juveniles among treatments. However, the number of new cysts recovered from cells that were inoculated with eggs and juveniles, originally treated with the higher concentration of Cleary 3336^F, was negatively correlated with the concentrations of Cleary 3336^F (Fig. 2). No differences ($P = 0.05$) in number of new cysts were found in the lower concentrations of Cleary 3336^F, or in the number of J2's emerged from cysts that were treated with the different concentrations of Cleary 3336^F.

Greenhouse Tests

Greenhouse studies showed a dramatic effect of Cleary 3336^F on the number of soybean cyst nematodes produced on Williams 82. The numbers of new cysts declined ($P = 0.001$) as the concentration of Cleary 3336^F increased. This reduction occurred when either eggs or cysts were used as inoculum (Figs. 3 and 4). When other fungicides and Cleary 3336^F were compared with the check, only the higher concentration of Cleary 3336^F consistently reduced the development of SCN ($P = 0.05$) (Table 1).

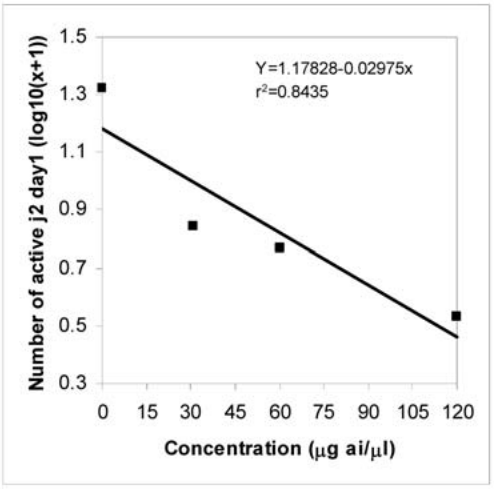

Fig. 1. Number of J2's that remained active after 24 hrs of exposure to different rates of Cleary 3336^F in the laboratory.

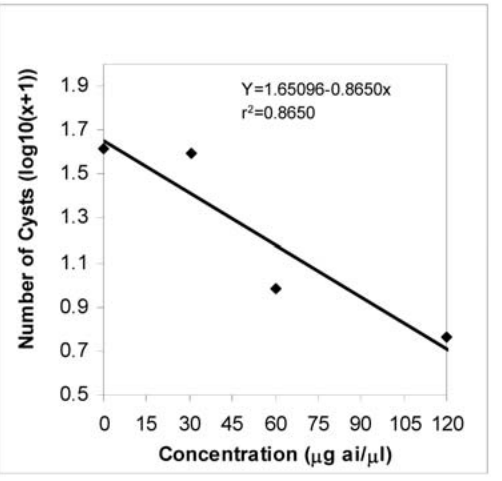

Fig 2. Number of new cysts developed from SCN J2's that were exposed to different rates of Cleary 3336^F in the laboratory.

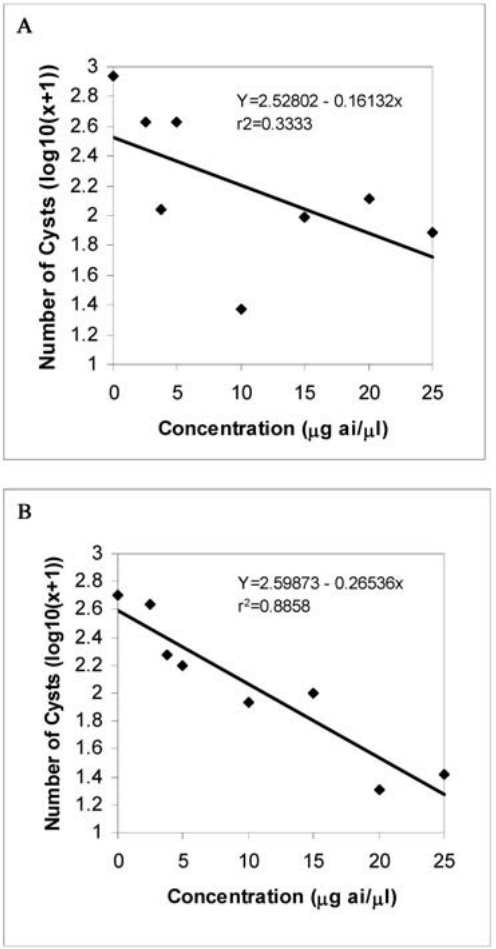

Fig. 3. Effect of Cleary 3336^{F} on formation of new cysts from roots inoculated with two types of SCN inoculum, A) eggs and juveniles, B) cysts, in the greenhouse.

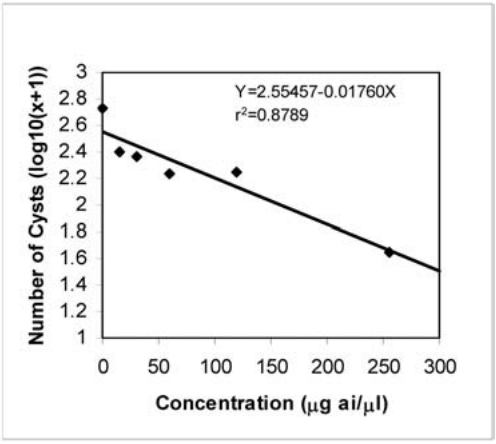

Fig. 4. Effect of higher concentration of Cleary 3336^{F} on soybean cyst nematode cyst formation in the greenhouse tests.

Field Tests

Although the 2003 and 2004 yields appeared to be higher in plots treated with Cleary 3336^{F}, these differences were not statistically significant. In 2005, no differences were observed in yield among plots treated with Cleary 3336^{F} and untreated checks (Table 2). In 2003, the ratio of the final SCN population density over the initial population density was higher in untreated checks, but was not statistically significant. No clear trend in SCN population density ratio could be established among the treatments in 2004 and 2005 field experiments (Table 2).

The fungal destruction of the root system can directly and obviously influence the outcome of SCN assay experiments. The use of fungicides to control root-destroying fungi might also influence assay results indirectly by affecting SCN development, as is shown in these experiments. In the greenhouse, our experiments showed that Cleary 3336^{F}, unlike the other fungicides we tested, effectively interfered with the development of SCN. In the laboratory, treatment of SCN eggs with Cleary 3336^{F} affected the mobility of second stage juveniles. Fewer juveniles hatched from cysts that were exposed to Cleary 3336^{F}, which suggests that Cleary 3336^{F} may inhibit hatching. Low numbers of juveniles in all treatments of these experiments prevented further study of affected nematodes. Our field studies showed no apparent effect by Cleary 3336^{F} on the populations of soybean cyst nematodes sampled at harvest but

Table 2. Effect of Cleary 3336[F] on soybean yield and population density of soybean cyst nematode in 2003-2005.

	2003		2004		2005	
Treatments[w]	Yield kg/ha	Eggs/100 cm^3 soil final/initial[x]	Yield kg/ha	Eggs/100 cm^3 soil final/initial[x]	Yield kg/ha	Eggs/100 cm^3 soil final/initial[x]
0	2295 a[y]	20.4 a	3268 a	42.7 a	3078 a	48.3 a
10	2555 a	9.5 a	NT[z]	NT	NT	NT
24	2362 a	8.7 a	NT	NT	NT	NT
29	NT	NT	3320 a	19.3 a	2923 a	19.1 a
34	2376 a	9.1 a	NT	NT	NT	NT
48	2479 a	13.6 a	NT	NT	NT	NT
58	NT	NT	3389 a	32.0 a	2803 a	11.5 a
116	NT	NT	3835 a	72.3 a	2939 a	22.2 a

[w]Application rates: µg ai/µl of Cleary 3336[F] dissolved in deionized water.
[x]Ratio of population density of SCN at harvest over the population density of SCN at planting.
[y]Means in the column followed by the same letter are not different ($P = 0.05$).
[z]NT = this treatment was not applied.

higher yield was shown in treated plots in 2003 and 2004. This suggests that Cleary 3336[F] fungicide might protect soybean seedlings, from SCN or soil-born fungi early in the season and a better soybean root system may have developed as the result of fungicide treatment. However, if suitable conditions for soybean growth are present when the effectiveness of the fungicide diminishes, soybean cyst nematode will quickly recover. This was apparent in 2003 and 2004 field data. In 2003, the conditions for soybean growth were not as suitable as in 2004. The 2003 growing season was dry, while abundant moisture was present in the 2004 growing season. This difference is reflected in the number of soybean cyst nematodes present at the end of each season. The effect of Cleary 3336[F] on SCN and yield was absent in 2005. We might speculate that cooler temperatures in early spring 2005 prevented full nematode activity, which mitigated any effect from Cleary 3336[F].

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