Journal of Nematology 28(1):36–42. 1996.

Application of a Sex Pheromone, Pheromone Analogs, and *Verticillium lecanii* for Management of *Heterodera glycines*

S. L. F. Meyer and R. N. Huettel

Abstract: A mutant strain of the fungus *Verticillium lecanii* and selected bioregulators of *Heterodera glycines* were evaluated for their potential to reduce population densities of the nematode on soybean under greenhouse conditions. The bioregulators tested were the *H. glycines* sex pheromone vanillic acid and the pheromone analogs syringic acid, isovanillic acid, ferulic acid, 4-hydroxy-3-methoxybenzonitrile, and methyl vanillate. A *V. lecanii*-vanillic acid combination and a *V. lecanii*-syringic acid combination were also applied as treatments. Syringic acid, 4-hydroxy-3-methoxybenzonitrile, *V. lecanii*, *V. lecanii*-vanillic acid, and *V. lecanii*-syringic acid significantly reduced nematode population densities in the greenhouse tests. Results with vanillic acid, isovanillic acid, and ferulic acid treatments were variable. Methyl vanillate did not significantly reduce cyst nematode population densities in the greenhouse tests.

Key words: biological control, fungus, *Glycine max*, *Heterodera glycines*, microbial control, nematode management, sex pheromone, soybean, soybean cyst nematode, vanillic acid, *Verticillium lecanii*.

Numbers of chemical nematicides available for control of plant-parasitic nematodes have decreased in recent years. Other management techniques for these crop pests, such as resistance and cultural practices, do not always have the benefits desired. Consequently, efforts have been directed to the development of novel approaches for managing plant-parasitic nematodes. This paper focuses on application of a biological control organism and bioregulatory compounds as potential management agents for *Heterodera glycines* Ichinohe, the soybean cyst nematode.

The microbial pest control agent investigated in this study is the fungus *Verticillium lecanii* (A. Zimmermann) Viégas, which is active against a number of organisms (8,9,13,23). This species colonized cysts, eggs, and second-stage juveniles of *Heterodera schachtii* Schmidt (6,7) and was isolated from cysts of *H. glycines* (4), and reduced viability of *H. glycines* egg populations (18). Mutant strains of *V. lecanii* were induced with ultraviolet light and selected for increased tolerance to the fungicide benomyl (16). In greenhouse studies, a tested mutant strain was more efficacious than the wild-type strain for decreasing *H. glycines* population densities, even though benomyl was not used in the experiments (17,20). That mutant strain was chosen for the research described herein.

Bioregulators, which are compounds that affect an organism's life processes, were also selected for investigation. Pheromones, a type of bioregulator, have been successfully used for managing insect pests (2) but have not been commercially applied for nematode management (15,17). Such compounds, when applied to soil, might disrupt the nematode life cycle. Identification of a sex pheromone isolated from *H. glycines* (15) provided the opportunity to test a bioregulator for its effects on population densities of a plant-parasitic nematode. The sex pheromone, vanillic acid, attracts the males to the females and is involved in the male coiling that occurs before fertilization (12,15). Application of excess pheromone to the soil environment might reduce nematode population densities by inhibiting males from following pheromone gradients to females. The

Received for publication 15 May 1995.

USDA ARS, Nematology Laboratory, Bldg. 011A, Rm. 153, BARC-West, 10300 Baltimore Avenue, Beltsville, MD, 20705-2350.

Mention of a trademark or proprietary product does not constitute a guarantee, warranty, or endorsement by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other similar products.

The authors thank A. DeMilo (Insect Chemical Ecology Laboratory) for original synthesis of pheromone analogs; P. Crowley, S. Blohm, and R. Reise for assistance in the greenhouse and laboratory; and R. Meyer (Nematology Laboratory), M. McIntosh, M. Camp, and S. Douglass (Statistical Consulting and Analysis Services) for assistance with analysis of data.

chances of affecting the life cycle of *H. glycines* are particularly favorable because this nematode is bisexual, requiring fertilization of a female by a male for reproduction to occur. Additionally, limited in vitro studies indicated that some analogs of the sex pheromone decreased numbers of *H. glycines* females produced in root explant cultures (17,22). The analogs appeared to inhibit mating or decrease host-finding by second-stage juveniles. Certain analogs were consequently included in the greenhouse tests described in this paper.

Our objectives were to evaluate a mutant strain of *V. lecanii*, selected bioregulators, and fungus-bioregulator combinations as agents for reducing *H. glycines* population densities in greenhouse studies. The agents were tested individually to determine whether they could significantly decrease numbers of *H. glycines* cysts produced on soybean roots. *Verticillium lecanii* and bioregulators were also applied together to discover whether such combinations, with potential to inhibit more than one stage of the nematode life cycle, were more effective than individually applied agents.

Materials and Methods

Nematode culture: Heterodera glycines race 3 (SCN) was maintained on sterile petri dish cultures of soybean (*Glycine max* (L.) Merr. cv. Kent) root explants grown on Gamborg's B-5 medium (11). Cysts were picked from the petri dishes, and eggs were allowed to hatch overnight in 0.5% aqueous chlorhexidine diacetate or in sterile water. Second-stage juveniles (J2) and unhatched eggs were then collected on a 500-mesh (25-μm pore) sieve, counted, and diluted so that a total of either 300 or 1,000 (eggs and J2) was added per pot in each experiment.

Fungus culture: A wild-type strain of *Verticillium lecanii* (American Type Culture Collection Strain 58909) was irradiated with UV light, and mutant strain M2S1 (Agricultural Research Service Culture Collection, NRRL 18726) was selected for increased tolerance to benomyl (16). Fungal inoculum was produced in 1-liter Erlenmeyer flasks (each containing 250 ml of potato dextrose broth) rotated at 200–240 rpm on orbital shakers at 25 C for 2 days, and harvested by centrifugation at 13,000*g* for 10 minutes in a Sorvall GSA rotor (20). Fungus wet-to-dry-weight ratios were determined by weighing a small amount of fungus before and after drying overnight at 50 C.

Prill production: Verticillium lecanii was incorporated into alginate prills (3) made with wheat bran as a food source for the fungus. For each 1-liter batch of alginate solution, 100 g wet fungus (ca. 6.4 g dry-weight fungus) was homogenized to a slurry in 100 ml water in a mechanical homogenizer. The slurry was then added to a 1-liter alginate solution containing 15 g alginic acid (sodium salt) and 5 g wheat bran (ground to a particle size of less than 1 mm) (20). Viability of fungus in the prills was determined by plating ca. 10 prills onto PDA and counting the number of prills from which fungus colonies were formed. The fungus grew from 100% of the tested prills at the time of each experiment.

Bioregulators used in this study were vanillic acid (*H. glycines* sex pheromone) and analogs of vanillic acid: ferulic acid, 4-hydroxy-3-methoxybenzonitrile, methyl vanillate, isovanillic acid, and syringic acid. Prills were formulated with <0.6% dry weight bioregulator per dry weight of prills, and with bran as a carrier.

Prills without fungus or bioregulators were tested for effects on SCN population densities in greenhouse studies (20). As reported, application of alginate prills did not significantly affect nematode population densities, so controls without prills were used in previously published experiments (20) and in the current study.

Greenhouse experiments: Three separate experiments were conducted in the greenhouse. The two experiments with steamed loamy sand determined whether the bioregulators would affect nematode populations when other microorganisms were not

initially present to immediately produce or breakdown the tested compounds, or to affect the nematodes. The third experiment was conducted with unsteamed loamy sand. Distinctive parameters of each of the three greenhouse experiments are outlined, followed by procedures common to all three experiments.

Experiment 1—Bioregulator Tests: Bioregulators were applied individually. Pots contained steamed loamy sand, and each pot was treated with 1,000 nematodes (J2 and unhatched eggs, combined). Prills were applied at 5 g/pot (0.1% dry prill weight per dry weight loamy sand; ca. 2,270 kg/ha). The experiment was repeated two additional times, with each treatment applied to four pots per repetition. When all three repetitions of the experiment were combined, $N = 12$ for control, syringic acid, and isovanillic acid treatments; $N = 11$ for all other treatments due to plant death.

Experiment 2—Bioregulator Tests: This experiment was similar to No. 1, except that 300 nematode eggs/J2 were added to each pot. The experiment was repeated one additional time; when both repetitions of the experiment were combined, $N = 8$ per treatment.

Experiment 3—Bioregulator, Fungus, and Fungus-Bioregulator Combination Tests: Pots contained unsteamed loamy sand, and each pot was treated with 300 nematode eggs/J2. Syringic acid, vanillic acid, and *V. lecanii* were applied individually, and two combinations were also used as treatments: *V. lecanii*-vanillic acid and *V. lecanii*-syringic acid. The experiment was repeated two additional times, with each treatment applied to four pots per repetition. When all three repetitions of the experiment were combined, $N = 12$ per treatment. Prills were applied at 1 g/pot (0.02% dry prill weight per dry weight loamy sand; ca. 454 kg/ha). The fungus was consequently applied as a 0.004% amendment to dry-weight loamy sand (ca. 90 kg dry-weight fungus/ha).

Procedures common to all three experiments: Soybean (*Glycine max* cv. 'Kent') susceptible to *H. glycines* was used in greenhouse tests. Soybean seeds were germinated in Terra-Lite Redi-Earth Peat-Lite Mix (Grace Sierra, Horticultural Products Co., Milpitas, CA). Two weeks after sowing, seedlings were transplanted into loamy sand (76–81% sand, 13–17% silt, 6–7% clay, 2–3% organic matter, pH 7.0–7.2) made from sand mixed with compost (20). Experimental units were two soybean plants per 20-cm-d pot (pot volume = 4,550 cm^3; weight of air-dried loamy sand per pot ca. 4,990 g). Four pots per treatment were used per repetition of each experiment. Two weeks following transplanting, pots were treated with prills and with aqueous egg/J2 suspension, as previously described (20). Control pots were inoculated with nematodes only. Greenhouse temperatures were maintained near 27 C (range 16–43 C). Supplemental lighting (400-watt, high-pressure sodium bulbs) was used during September–April to provide 16 hours of continuous daylight per 24-hour period. The studies were terminated ca. 7 weeks after inoculation.

Cyst (and female) counts were used as an indicator of effects on SCN population densities, as in prior experiments (20). *Verticillium lecanii* appears to kill eggs through a mechanism other than direct parasitism of live eggs, so that eggs are dead before fungal colonization (18). It is therefore difficult to count viable eggs in a pot because of the difficulty of distinguishing live and dead eggs. The alternative, i.e., counting total numbers of all eggs combined, would not accurately represent the number of viable eggs in each pot. Consequently, cysts and females (referred to as "cysts") were washed from roots and loamy sand and collected in nested sieves (20-mesh, 850-μm pore size nested over 60-mesh, 250-μm pore size). The cysts were then separated from remaining loamy sand by centrifugation (20). Cysts were counted in watchglasses with a stereomicroscope.

Isolation of fungi from loamy sand and prills: As repetitions of Experiment 3 were concluded, loamy sand was plated onto agar to check for the presence of *V. lecanii*. After

collection of cysts, 10 g loamy sand from each pot was stirred in 50 ml water (dilution 1). One ml of this suspension was added to 9 ml water (dilution 2). Approximately 0.05 ml of each dilution was plated onto each of two petri dishes of modified Ausher's medium No. 2 (1) with PCNB replaced by benomyl, and two petri dishes of PDA ABE 100. PDA ABE 100 was composed of PDA (potato dextrose agar; 39 g in 970 ml distilled water) amended with antibiotics, benomyl, and ethanol (20). The tested strains of *V. lecanii* have been difficult to isolate from soil, even though a number of media were tested for this purpose (20). Some success was achieved with PDA ABE 100 for the mutant strain, while modified Ausher's medium was somewhat more effective for the wild-type strain (20). Both media were selected for the current study.

For the first two trials of Experiment 3, prills were recovered from all 24 pots originally treated with *V. lecanii* and plated onto agar to determine whether the fungus was still viable. After most of the loamy sand had been washed from the prills, the wet weight of the retrieved prills varied from 0.2 g to 1.15 g/pot. The prills from each pot were plated onto 1–3 petri dishes of PDA ABE 100 and 1–3 petri dishes of Ausher's medium. In the first trial of Experiment 3, fungus-syringic acid prills from one pot were recovered in such low amounts that they were plated only onto one petri dish of PDA ABE 100 and not onto Ausher's medium.

Analysis of data: All data from experiments were analyzed using SAS (21). Data analyzed were whole-number cyst counts log transformed by $\log_{10}(x + 1)$ to correct for heterogeneity in variance. For each experiment, analysis of variance was conducted for treatments blocked over repetitions. Analysis of variance showed that the treatment effect was statistically significant (P value = 0.0001) for all three experiments (F values were: Experiment 1, 9.96; Experiment 2, 7.46; Experiment 3, 19.97). Treatment means were separated using an LSD procedure (P = 0.05). For presentation, the treatment means were transformed back to cyst counts for readability. However, back-transforming to mean square error would not be valid, so standard errors were not reported.

Results

Cyst counts: In steamed loamy sand (Experiments 1 and 2), application of syringic acid and 4-hydroxy-3-methoxybenzonitrile reduced *H. glycines* population densities when compared to controls at both nematode population levels ($P \leq 0.05$) (Tables 1 and 2). For the 300 and 1,000 egg/J2 treatments, the mean reductions in cyst numbers (based on the back-transformed cyst count means) were 69% and 55%, respectively, with 4-hydroxy-3-methoxybenzonitrile. With syringic acid, the corresponding reductions were 75% and 64%. Isovanillic acid, ferulic acid, and vanillic acid also caused population reductions when the nematodes were added at 1,000 per pot: mean reductions were 39%, 53%, and 80%, respectively ($P \leq 0.05$). Application of methyl vanillate did not result in reductions in nematode numbers.

In unsteamed loamy sand (Experiment 3), both vanillic acid and syringic acid were effective in reducing nematode population levels (Table 3). This occurred even though fewer prills were added than in the

Table 1. Effect of bioregulator treatments on numbers of *Heterodera glycines* cysts produced on soybean roots in steamed loamy sand. Pots were each treated with 1,000 eggs/J2 (Experiment 1).

Treatment	Number of cysts[a]
Untreated control	122 a
Ferulic acid	57 bc
4-Hydroxy-3-methoxy-benzonitrile	55 bc
Isovanillic acid	74 b
Methyl vanillate	127 a
Syringic acid	44 c
Vanillic acid	25 d

[a] Values are mean numbers of cysts per pot, transformed back from log-transformed ($\log_{10}[x + 1]$) data. Numbers followed by the same letter are not significantly different at $P \leq 0.05$, based on analysis of log-transformed data. N = 12 for control, syringic acid, and isovanillic acid treatments; N = 11 for all other treatments.

TABLE 2. Effect of bioregulator treatments on numbers of *Heterodera glycines* cysts produced on soybean roots in steamed loamy sand. Pots were each treated with 300 eggs/J2 (Experiment 2).

Treatment	Number of cysts[a]
Untreated control	64 a
Ferulic acid	74 a
4-Hydroxy-3-methoxy-benzonitrile	20 b
Isovanillic acid	89 a
Methyl vanillate	54 a
Syringic acid	16 b
Vanillic acid	62 a

[a] Values are mean numbers of cysts per pot, transformed back from log-transformed ($\log_{10}[x + 1]$) data. Numbers followed by the same letter are not significantly different at $P \leq 0.05$, based on analysis of log-transformed data. N = eight per treatment.

experiments with steamed loamy sand. Cyst numbers compared to the controls were reduced 49% and 70% after application of vanillic acid and syringic acid, respectively. Application of the mutant strain of *V. lecanii* alone (85% decrease in cyst numbers compared to controls), *V. lecanii*-vanillic acid combination (68% decrease), and *V. lecanii*-syringic acid combination (80% reduction) also resulted in reduced nematode numbers ($P \leq 0.05$) (Table 3).

Isolation of Verticillium lecanii from prills used in Experiment 3: Prills from a total of 24 pots were recovered from loamy sand and plated onto agar. Many of these prills were rapidly overgrown by contaminating fungi and bacteria, but *V. lecanii* was isolated from prills of seven pots. Three of those pots were treated with *V. lecanii* prills, two with prills containing *V. lecanii* plus vanillic acid, and two with prills containing *V. lecanii* plus syringic acid. All colonies were isolated on Ausher's medium.

Isolation of fungus from loamy sand used in Experiment 3: A total of 36 pots were treated with the prills containing fungus in Experiment 3. *Verticillium lecanii* was isolated from loamy sand of two pots. One pot was originally inoculated with fungus alone and the other was treated with *V. lecanii* plus vanillic acid. Both isolations were made on PDA ABE 100, dilution 2.

TABLE 3. Effect of bioregulator treatments, a mutant strain of *Verticillium lecanii*, and *V. lecanii*-bioregulator combinations on numbers of *Heterodera glycines* cysts produced on soybean roots in unsteamed loamy sand. Pots were each treated with 300 eggs/J2 (Experiment 3).

Treatment	Number of cysts[a]
Untreated control	160 a
Vanillic acid	82 b
V. lecanii-vanillic acid	51 c
Syringic acid	48 cd
V. lecanii-syringic acid	32 de
V. lecanii	24 e

[a] Values are mean numbers of cysts per pot, transformed back from log-transformed ($\log_{10}[x + 1]$) data. Numbers followed by the same letter are not significantly different at $P \leq 0.05$, based on analysis of log-transformed data. N = 12 per treatment.

DISCUSSION

These greenhouse experiments demonstrated that *H. glycines* sex pheromone, four of the five tested analogs, the mutant strain of *V. lecanii*, and two fungus-bioregulator combinations reduced nematode population densities. The results with the bioregulators applied individually corroborated previous laboratory experiments conducted on nematodes propagated in vitro (17,22), which had indicated that some bioregulators reduced numbers of females formed on roots when compared to numbers of females on roots of controls. The petri dish studies suggested that at least some analogs were parapheromones—compounds that act in a manner similar to natural pheromones (14)—and were not acting as larvacides or ovicides.

Although some of the bioregulator compounds were effective in both the petri dish and the greenhouse studies, not all of the tested compounds demonstrating activity against SCN consistently reduced nematode population densities. Syringic acid and 4-hydroxy-3-methoxybenzonitrile reduced population densities in all experiments where they were applied, but vanillic acid, isovanillic acid, and ferulic acid did not decrease cyst numbers under all greenhouse test conditions. Action of the latter three compounds appears to

have been influenced by number of nematodes added to the steamed loamy sand. The compounds were ineffective when nematodes were added at 300 eggs/J2 per pot but significantly reduced population densities when more nematodes (1,000 eggs/J2 per pot) were initially added. This is an interesting observation which merits further study to determine the cause. An opposite effect was recorded from an earlier investigation on *V. lecanii* and SCN (20), in which two tested fungus strains were more effective against SCN when 300 nematodes were applied per pot than when 10,000 nematodes were added per pot. Testing of a substantially larger nematode population, as was done in that study, would corroborate whether the pheromone-analogs continued to be more effective as SCN populations increased. The pheromone analog methyl vanillate, while effective in the petri dish experiments, did not decrease *H. glycines* population densities in these greenhouse tests. One of the compounds applied in the greenhouse, 4-hydroxy-3-methoxybenzonitrile, was not tested in the petri dish assays. This analog was effective in the two greenhouse experiments in which it was tested.

Even though vanillic acid was not effective in Experiment 2, it was selected for the combination tests with *V. lecanii* (Experiment 3) because it is a natural sex pheromone of *H. glycines*. Syringic acid was selected because it consistently decreased cyst numbers in the steamed loamy sand tests, and resulted in slightly greater cyst reductions than application of 4-hydroxy-3-methoxybenzonitrile. The mutant strain of *V. lecanii* was selected for these tests because it had demonstrated activity against SCN in previous greenhouse studies (20). In Experiment 3, all of the tested agents were effective for reduction of nematode populations. The fact that vanillic acid was effective when 1 g of prills was added to each pot of unsteamed loamy sand (Experiment 3), but not when 5 g prills was added per pot of steamed loamy sand (Experiment 2), may indicate that there were indirect effects from the disparate microflora populations. Vanillic acid is broken down by microorganisms in the soil, and it is possible that some of those organisms also acted against the nematodes or stimulated other organisms that were antagonistic to nematodes. Alternatively, the vanillic acid could have affected nonfeeding behavior of microorganisms other than nematodes. A third hypothesis is that the prills acted as a food source for organisms not present in the steamed pots. All of these hypotheses assume that the vanillic acid or the prills themselves affected other organisms besides soybean cyst nematode. A different hypothesis is that the vanillic acid was simply less efficacious in higher amounts. This would seem unlikely but could be tested in later experiments.

The fungus was recovered from few pots. Lack of isolation from pots inoculated with *V. lecanii* could be because fungus viability decreased over the course of the experiment, or because the fungus was overgrown by other organisms on the test media. *Verticillium lecanii* was not recovered from control pots.

Studies on nematode pheromones have been conducted for 30 years since Greet (5) demonstrated that such compounds existed. However, vanillic acid is the only sex pheromone that has been structurally identified from a plant-parasitic nematode. The research reported herein is consequently new to the area of plant-parasitic nematode management, and is of interest because application of a sex pheromone to soil could habituate males to the pheromone, thus preventing mating. These greenhouse studies demonstrated that a sex pheromone and pheromone analogs can reduce nematode populations. In addition, the fungus-bioregulator combinations were tested because it was hypothesized (10) that nematode population densities would be greatly reduced when a bioregulator that affected male (or juvenile) behavior was linked with a fungus that decreased egg viability. While this study demonstrated that the mutant fungus, certain bioregulators, and two fungus-bioregulator combinations effectively re-

duced nematode population densities, there was not a synergistic effect with either tested combination. Fungus-bioregulator combinations may or may not improve efficacy under other conditions. Viability studies on one prill batch indicated that the fungus does not survive as long in alginate prills containing vanillic acid as in alginate prills without vanillic acid (19). If this proves to be a consistent result, it would have to be taken into account in commercial uses where storage over many months is an important feature. The greenhouse studies did indicate that some of the tested agents, particularly syringic acid, vanillic acid, 4-hydroxy-3-methoxybenzonitrile, and *Verticillium lecanii*, are candidates for further research.

Literature Cited

1. Ausher, R., J. Katan, and S. Ovadia. 1975. An improved selective medium for the isolation of *Verticillium dahliae*. Phytoparasitica 3:133–137.

2. Bowers, W. S. 1993. Phytochemical contributions to pest management. Pp. 252–257 *in* R. D. Lumsden and J. L. Vaughn, eds. Pest management: Biologically based technologies. Washington, D.C.: American Chemical Society.

3. Fravel, D. R., J. J. Marois, R. D. Lumsden, and W. J. Connick, Jr. 1985. Encapsulation of potential biocontrol agents in an alginate-clay matrix. Phytopathology 75:774–777.

4. Gintis, B. O., G. Morgan-Jones, and R. Rodríguez-Kábana. 1983. Fungi associated with several developmental stages of *Heterodera glycines* from an Alabama soybean field soil. Nematropica 13:181–200.

5. Greet, D. N. 1964. Observations on sexual attraction and copulation in the nematode *Panagrolaimus rigidus* (Schneider). Nature 204:96–97.

6. Hänssler, G. 1990. Parasitism of *Verticillium lecanii* on cysts of *Heterodera schachtii*. Journal of Plant Diseases and Protection 97:194–201.

7. Hänssler, G., and M. Hermanns. 1981. *Verticillium lecanii* as a parasite on cysts of *Heterodera schachtii*. Journal of Plant Diseases and Protection 88:678–681.

8. Harper, A. M., and H. C. Huang. 1986. Evaluation of the entomophagous fungus *Verticillium lecanii* (Moniliales, Moniliaceae) as a control agent for insects. Environmental Entomology 15:281–284.

9. Heintz, C., and R. Blaich. 1990. *Verticillium lecanii* as a hyperparasite of grapevine powdery mildew (*Uncinula necator*). Vitis: 229–232.

10. Huettel, R. N., and S. L. F. Meyer. 1992. Novel management strategies for plant parasitic nematodes. Pp. 273–276 *in* E. C. Tjamos, G. C. Papavizas, and R. J. Cook, eds. Biological control of plant diseases: Progress and challenges for the future. New York: Plenum Press.

11. Huettel, R. N., and R. V. Rebois. 1985. Culturing plant-parasitic nematodes using root explants. Pp. 155–158 *in* B. M. Zuckerman, W. F. Mai, and M. B. Harrison, eds. Plant nematology laboratory manual. Amherst: University of Massachusetts Agricultural Experiment Station.

12. Huettel, R. N., and R. V. Rebois. 1986. Bioassay comparisons for pheromone detection in *Heterodera glycines*, the soybean cyst nematode. Proceedings of the Helminthological Society of Washington 53: 63–68.

13. Hussey, N. W. 1984. Biological control in integrated pest control programs in Europe. Pp. 128–136 *in* G. Allen and A. Rada, eds. The role of biological control in pest management. Ottawa: University of Ottawa press.

14. Inscoe, M. N., B. A. Leonhardt, and R. L. Ridgeway. 1990. Commercial availability of insect pheromones and other attractants. Pp. 631–715 *in* R. L. Ridgeway, R. M. Silverstein, and M. N. Inscoe, eds. Behavior-modifying chemicals for insect management. New York: Marcel Dekker.

15. Jaffe, H., R. N. Huettel, A. B. DeMilo, D. K. Hayes, and R. V. Rebois. 1989. Isolation and identification of a compound from soybean cyst nematode, *Heterodera glycines*, with sex pheromone activity. Journal of Chemical Ecology 15:2031–2043.

16. Meyer, S. L. F. 1992. Induction of increased benomyl tolerance in *Verticillium lecanii*, a fungus antagonistic to plant-parasitic nematodes. Journal of the Helminthological Society of Washington 59:237–239.

17. Meyer, S. L. F., and R. N. Huettel. 1993. Fungi and fungus/bioregulator combinations for control of plant-parasitic nematodes. Pp. 214–221 *in* R. D. Lumsden and J. L. Vaughn, eds. Pest management: Biologically based technologies. Washington, D.C.: American Chemical Society.

18. Meyer, S. L. F., R. N. Huettel, and R. M. Sayre. 1990. Isolation of fungi from *Heterodera glycines* and *in vitro* bioassays for their antagonism to eggs. Journal of Nematology 22:532–537.

19. Meyer, S. L. F., and R. J. Meyer. 1996. Survival of the nematode-antagonistic fungus *Verticillium lecanii* in alginate prills. Nematologica 41:in press.

20. Meyer, S. L. F., and R. J. Meyer. 1995. Effects of a mutant strain and a wild-type strain of *Verticillium lecanii* on *Heterodera glycines* populations in the greenhouse. Journal of Nematology 27:409–417.

21. SAS Institute. 1991. SAS/GRAPH® Software: Usage, Version 6, 1st ed. Cary, NC: SAS Institute Inc.

22. Stern, S., H. Jaffe, A. DeMilo, and R. N. Huettel. 1988. Disruption of mate finding in soybean cyst nematodes with analogs of the nematode sex attractant. Journal of Nematology 20:661.

23. Uma, N. U., and G. S. Taylor. 1987. Parasitism of leek rust urediniospores by four fungi. Transactions of the British Mycological Society 88:335–340.