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Effects of a Mutant Strain and a Wild Type Strain of *Verticillium lecanii* on *Heterodera glycines* Populations in the Greenhouse

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Abstract: A wild type strain of *Verticillium lecanii* and a mutant strain with increased tolerance to the fungicide benomyl were evaluated in greenhouse experiments for effects on *Heterodera glycines* populations. Nematodes were applied at 300 eggs and juveniles per 4,550-cm^3 pot (two soybean plants in 4,990 g loamy sand per pot) and at both 300 and 10,000 eggs and juveniles per 1,720-cm^3 pot (one soybean plant in 2,060 g sand per pot). With 300 nematodes added per pot, both *V. lecanii* strains significantly reduced nematode populations in loamy sand (fungus applied at 0.02% dry weight per dry weight loamy sand) and sand (0.006% and 0.06% fungus application rates). The mutant strain applied at 0.002% to sand also significantly reduced cyst numbers. When 10,000 nematodes were added per pot, only the mutant strain at 0.06% significantly decreased population. Various media were tested for isolation of the fungus strains from prills, loamy sand, and sand, but the fungi were recovered from few of the greenhouse pots.

Key words: biological control, fungus, *Heterodera glycines*, mutant, nematode, nematode antagonist, soybean cyst nematode, *Verticillium lecanii*.

Numerous fungi have been found associated with cysts and eggs of *Heterodera glycines* Ichinohe (soybean cyst nematode), and studies have been conducted on fungi that have potential to act as antagonists to *H. glycines* (4,9,14,17,21). *Verticillium lecanii* (A. Zimmermann) Viégas is one of the fungi known to be active against species of *Heterodera*, including *H. glycines* (9–11,17). A strain of *V. lecanii* deleterious to soybean cyst nematode (SCN) eggs in a petri dish assay (17) was selected for further study as a potential management agent for SCN. The strain was tested for tolerance to the fungicide benomyl (18), and mutants with increased benomyl tolerance were then induced with ultraviolet radiation (16). This was done because some fungus strains with increased benomyl tolerance may have enhanced biocontrol potential as a result of genetic manipulation (1,3,19,20). Additionally, if the formulation for the biocontrol fungus included a food source to help initiate growth of the beneficial fungus, benomyl could be added to reduce growth of unwanted fungi on the food source.

A mutant that showed enhanced ability to act against SCN in preliminary experiments was selected for more extensive greenhouse tests. Studies conducted on this mutant strain and the wild type strain compared strain abilities to reduce SCN populations on soybean in the greenhouse.

MATERIALS AND METHODS

Fungus inoculum and production of prills: Two strains of *Verticillium lecanii* were tested. The wild type strain was American Type Culture Collection number 58909, and the mutant strain was Beltsville Nematology Laboratory M2S1 (Agricultural Research Service Culture Collection, NRRL 18726). The latter had been induced with ultraviolet radiation from strain 58909 and selected for increased tolerance to the fungicide benomyl (16). The fungi were incorporated into alginate prills (5) for greenhouse tests. For production of inoculum, fungi were grown 1 week on potato dextrose agar (PDA) in petri dishes. Fungus

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colonies were then cut into pieces and homogenized in potato dextrose both (PDB) in a blendor. Fungus (broth) suspension was poured into Erlenmeyer flasks and rotated at 200–240 rpm on orbital shakers at 25 C for 2 days. To harvest the fungi, broth cultures were centrifuged at 9,000 rpm for 10 minutes (13,000*g*) in a Sorvall GSA rotor, and fungi were collected as pellets. The wet mycelium and conidia were stirred in water and homogenized to a slurry in a Virtis "45" mechanical homogenizer (setting 80–90, two 10-second runs). The slurry was then incorporated into alginate prills (5) made with 5 g wheat bran, 100 g wet fungus slurry, and 15 g alginate per liter. Each air-dried prill was approximately 1–2 mm in diameter and weighed about 1.0–1.5 mg. Fungus wet weight to dry weight ratios were determined by weighing a small amount of wet fungus, drying the fungus overnight at 50 C, and weighing again.

Nematode production: Heterodera glycines race 3 was used for greenhouse experiments and was produced on sterile petri dish cultures of excised soybean (*Glycine max* (L.) Merr. cv. Kent) root tips grown on Gamborg's B-5 medium (6,12). The strain, which originated from soybean fields in Tennessee, had been maintained in gnotobiotic cultures at the Beltsville Nematology Laboratory (15). For the current experiments, cysts and egg masses were picked from plates, and eggs were allowed to hatch overnight in 0.5% aqueous chlorhexidine diacetate salt or in sterile water. Eggs and juveniles were then collected on screens (pore size 25 μm), counted, and diluted so that either 300 or 10,000 eggs and juveniles were added to each pot in the greenhouse experiments described below.

Greenhouse experiments: For all experiments, greenhouse temperatures were kept as close as possible to 27 C (16–43 C range). From September through April, supplemental lighting (400-watt, high-pressure sodium bulbs) was used to provide 12–16 hours of daylight per 24-hour period. To determine whether the fungi would be effective under different conditions, two sets of experimental parameters were tested in the greenhouse.

The variables distinguishing Experiment 1 included use of cultivar Kent (susceptible to SCN), germination of seeds in potting mix and growth in the mix for 2 weeks, transplanting of two soybeans into each 4,550-cm^3 pot containing loamy sand, inoculation 2 weeks after transplanting, and termination of the experiment 7 weeks after inoculation.

For Experiment 1, the soybean seeds were planted into Terra-Lite Redi Earth Peat-Lit Mix (Grace Sierra, Horticultural Products Co., Milpitas, CA) in styrofoam flats, and then transplanted 2 weeks after sowing. Experimental units were 20-cm-d pots (4,550 cm^3 volume), each containing two plants in 4,990 g (air-dried weight) loamy sand. The loamy sand was made from compost (16 m^3 top soil, 8.4 m^3 manure, 36 kg 5-10-5 (NPK), and 109 kg high magnesium dolomitic lime) mixed with sand. The sand-compost mixture comprising the loamy sand was 76–81% sand, 13.5–17% silt, 5.5–7% clay, 2.7–3.3% organic matter, pH 6.7–7.2. The loamy sand was tested and found to be free of cyst nematodes before use in greenhouse experiments. The loamy sand was used both steamed and unsteamed (two trials with each treatment).

Two weeks following transplanting, the pots were treated with nematodes and fungi. A 4- to 5-ml water suspension containing a total of 300 juveniles and eggs was pipetted into holes made in the loamy sand near the plant roots in each pot. Prills were also placed into holes near the roots. Holes were refilled following inoculation. Air-dried prills containing viable fungus were added at three rates: 0.15, 0.5, and 5.0 g prills per pot. These amounts were equivalent to 0.003%, 0.01%, and 0.1% dry weight prill per weight of air-dried loamy sand, respectively. This resulted in fungus application rates of approximately 0.0007% (dry weight fungus per dry weight loamy sand), 0.002%, and 0.02%. Control pots were inoculated with nema-

todes only. Four pots were used per treatment in each trial of the experiment; pots were arranged in complete blocks. Each trial was terminated 7 weeks after inoculation.

To determine whether alginate bran prills would affect numbers of cysts produced on plant roots, prills were made with 30 g bran per liter alginate solution and applied at a rate of 5 g prills (dry weight) per 4,550-cm^3 pot (0.1% dry weight prill per weight of air-dried steamed loamy sand). Control pots did not receive prills. Three hundred eggs and juveniles were inoculated into each pot, and four pots were used per treatment. The experiment was conducted three times, resulting in a total of 12 pots per treatment.

The variables distinguishing Experiment 2 included use of cultivar Essex (susceptible to SCN), germination of seeds in sterile sand and growth in the sand for 1 week, transplanting of one soybean into each 1,720-cm^3 pot containing unsteamed sand, inoculation at the time of transplanting, and termination of the experiment 9 weeks after inoculation.

For Experiment 2a, seeds of *Glycine max* cv. Essex were planted into sterile sand in styrofoam flats and transplanted 1 week later. Experimental units were 15-cm-d (1,720 cm^3) pots, each containing one plant in 2,060 g unsteamed sand. This sand contained compost as in Experiment 1, but the final composition of the sand/compost mixture for Experiment 2 was 97% sand, 2.1% silt, 0.9% clay, 0.4% organic matter, pH 6.7–7.2 (referred to herein as "sand"). Inoculation with *H. glycines* and with prills was done the same day as transplanting, through holes made in the sand near the plant roots. Holes were filled after inoculation. Ten pots were used per treatment, and the pots were arranged in two randomized complete blocks. Three hundred nematodes were added to each pot. Air-dried prills containing viable fungus were added at three rates: 0.15 g/pot, 0.5 g/pot, and 5.0 g/pot (0.007%, 0.02%, and 0.2% dry weight prill per weight air-dried sand, respectively). Mutant and wild type treatments were approximately 0.002%, 0.006%, and 0.06% dry weight fungus per weight sand. Control pots received nematodes only. The experiment was repeated once; both trials were terminated 9 weeks after inoculation.

Experiment 2b was conducted with the same parameters as Experiment 2a, except that 10,000 nematodes were added per pot, and the mutant and wild type treatments used were the two highest rates: 0.006% and 0.06% dry weight fungus per weight air-dried sand (0.5 and 5.0 g prills per pot, respectively). Three trials of Experiment 2b were conducted. In trials 1 and 2, both fungus strains were applied at 0.006%. In trials 2 and 3, both strains were applied at 0.06%. Control pots receiving only nematodes were tested in all three trials. In trials 2 and 3, additional controls, consisting of pots receiving autoclaved prills containing nonviable fungus, were tested and applied at 5.0 g prills per pot (0.06% fungus weight per sand weight).

Collection and counting of nematode cysts: To harvest the cysts and females (hereafter referred to as "cysts"), roots and loamy sand or sand were washed over a 20-mesh sieve (pore size 850 μm) nested in a 60-mesh sieve (pore size 250 μm). The cysts were separated from the loamy sand and sand with a centrifugal-flotation technique modified from Jenkins (13). In this technique, cysts were collected on the 60-mesh sieve, and debris was removed by centrifugation in water for 3 minutes at 2,000 rpm (ca. 640*g*). The nematodes were suspended in a 1-M sucrose solution and centrifuged again for the same time and speed. After collection on a 500-mesh sieve, the cysts were washed and suspended in water. For experiments in which 300 nematodes had been added per pot, total numbers of cysts collected from the roots and loamy sand or sand of each pot were counted. For experiments in which 10,000 nematodes were initially added to each pot, counts were made of five 2-ml samples per pot. Total number per pot was estimated from the samples.

Isolation of fungi from loamy sand, sand, and

prills; tests of semi-selective media: At the end of Experiment 1 trials, recovered prills were plated onto agar to check for viable *Verticillium.* Additionally, loamy sand (Experiment 1) and sand (Experiment 2) were diluted in water and plated onto agar media to determine whether *V. lecanii* was proliferating in the pots. Number of pots sampled, media tested, and number of petri dishes used for each experiment are presented for the recovered prills (Table 1) and the dilution experiments (Table 2). Techniques and media are described below.

Prills were recovered from pots that had been treated with 5.0 g dry weight prills. Prills applied at lower rates were difficult to recover and therefore not tested for fungus viability. For these tests, loamy sand was washed from the prills, and the wet prills were spread onto agar media (ca. 0.5–1.0 g wet weight prills/petri dish).

To isolate the fungi from loamy sand or sand, serial dilutions were plated onto agar media. To make the dilutions, loamy sand or sand was removed from pots and stirred in water at a ratio of 10 g to 50 ml water (dilution 1). Serial dilutions were then made with 1 ml of suspension added to 9 ml water for each dilution (dilutions 2–4).

While conducting the fungus isolation experiments, various agar media were tested to determine whether a semi-selective medium for these strains of *V. lecanii* could be developed. The following media were tested: 1. Ausher's medium no. 2 (2). 2. PDA ABE: potato dextrose agar amended with antibiotics, benomyl, and ethanol. PDA ABE was made with 39 g PDA, 970 ml distilled water, and 0.3 g streptomycin sulfate plus 0.3 g tetracycline in 10 ml sterile water. The flask in which the antibiotics were mixed in water was rinsed with 6 ml 95% ETOH, which was added to the medium. Benomyl (Benlate 50 wettable powder or DF, E. I. du Pont de Nemours, Wilmington, DE) was added in two different amounts: a) PDA ABE 100 was made with 0.2 g benlate in 20 ml distilled water per liter medium (100 μg benomyl/ml PDA), and b) PDA ABE 5 contained 0.01 g benlate/L. 3. Selected media were also amended with 0.05 g rose bengal/liter. These media are indicated by the letters RB.

Analysis of data: Analysis of variance (SAS Institute's PROC MIXED, 23) was conducted on the data from the bran-only prill trials.

The data from the separate experiments (1, 2a, and 2b) were subjected to analysis as a one factor design using SAS Institute's PROC GLM (22). The treatment factor was the fungus type combined with the rate of application. The treatments included a negative control. The treatment means were compared using LSD to determine which treatments differed at the 0.05 significance level (see Table 3). The whole-number cyst counts were log transformed by $\log_{10}$ to correct for variance heterogeneity. For presentation, treatment means were transformed back to cyst counts. Back transforming the standard

TABLE 1. Isolation of *Verticillium lecanii* from alginate prills applied to loamy sand.

Loamy sand treatment	Number of pots sampled per fungus strain	Medium	Total number of petri dishes per fungus strain	Number of petri dishes (and pots) from which *V. lecanii* was isolated
Steamed	4 (2 pots per experiment trial)	PDA ABE 5	12	Mutant—11 (4 pots) Wild type—5 (2 pots)
	2 (second trial only)	Aushers	6	Mutant—1 Wild type—2 (2 pots)
Unsteamed	8 (all pots; 4 per experiment trial)	PDA ABE 100	24	Mutant—0 Wild type—0
	8	Aushers	24	Mutant—0 Wild type—1

Samples were taken from pots treated with 5.0 g prills/pot in Experiment 1. Prills were plated onto three petri dishes of each agar per pot.

TABLE 2. Isolation of *Verticillium lecanii* from loamy sand and sand.

Experiment	Treatment[a]	Number of pots sampled per treatment; number per *V. lecanii* strain[b]	Medium	Total number of petri dishes per treatment; total per *V. lecanii* strain	Number of petri dishes (and pots) from which *V. lecanii* was isolated (all application rates combined)
1—steamed loamy sand	Control (no prills), 3 rates mutant, 3 rates wild type	4 (2 pots per trial); 12	PDA ABE 5	20; 60	Mutant—12 (7 pots) Wild type—0
		2; 6[b]	Aushers	8; 24	Mutant—0 Wild type—1
2a—unsteamed sand	Control (no prills)	20	PDA 100 RB	80	0
			PDA ABE 100	80	0
			PDA ABE 100 RB	80	0
			Aushers	80	0
		10[b]	Aushers RB	40	0
	3 rates mutant	20; 60	PDA ABE 100	80; 240	3 (3 pots)
			PDA ABE 100 RB	80; 240	1
	3 rates wild type	20[c]; 59	PDA 100 RB	80[c]; 236	0
			Aushers	80[c]; 236	1
		10; 30[b]	Aushers RB	40; 120	0
2b—unsteamed sand	Control (no prills)	30	PDA ABE 100	120	0
			Aushers	120	0
	2 rates mutant	20; 40	PDA ABE 100	80; 160	1
	2 rates wild type	20; 40	Aushers	80; 160	0

[a] Each application rate is considered a separate treatment. Dilutions 1 and 2 were used for all experiments, and each dilution was plated onto two petri dishes. The only exception was Experiment 1, trial 1, in which dilutions 3 and 4 were tested on PDA ABE 5 for all treatments (two pots per treatment, one petri dish per dilution).
[b] Both trials of the experiment tested unless indicated by b for second trial only.
[c] Exception: 19 pots used for wild type strain applied at 0.006%. Soil was plated onto 76 petri dishes for that treatment.

TABLE 3. Effect of a mutant strain and a wild type strain of the fungus *Verticillium lecanii* on numbers of *Heterodera glycines* cysts produced on soybean in greenhouse pots. Values in each column are mean numbers of cysts per pot, back transformed following analysis of log transformed data.

Dry weight fungus per weight air-dried loamy sand or sand	300 nematodes/pot		10,000 nematodes/pot	
	Experiment 1 Loamy sand[a]	Experiment 2a Sand[b]	Experiment 2b Sand[c]	
Untreated control	97 a	139 a	8,217 a	9,047 a
Mutant 0.0007%	62 ab	—	—	—
Mutant 0.002%	54 ab	77 bc	—	—
Mutant 0.006%	—	85 b	7,797 a	—
Mutant 0.02%	32 b	—	—	—
Mutant 0.06%	—	58 c	—	5,965 b
Wild type 0.0007%	103 a	—	—	—
Wild type 0.002%	68 ab	115 a	—	—
Wild type 0.006%	—	80 b	7,757 a	—
Wild type 0.02%	37 b	—	—	—
Wild type 0.06%	—	74 bc	—	9,833 a
F value	3.02	6.94	0.00	3.10
P value	0.0486	2×10^{-6}	0.997	0.0389

— = not tested. Numbers within a column followed by the same letter are not significantly different at $P = 0.05$. Letters indicating significant differences can be compared within each column but not between columns. Significance is based on analysis of log-transformed ($\log_{10}$) data.

[a] Cultivar Kent. Steamed and unsteamed loamy sand (four trials total); four 20-cm-d pots/treatment per trial. $N = 16$ for each treatment, except wild type at 0.0007% ($N = 15$).

[b] Cultivar Essex. Unsteamed sand, ten 15-cm-d pots/treatment in each of two trials. $N = 20$ for each treatment, except mutant at 0.06% ($N = 19$).

[c] Cultivar Essex. Unsteamed sand, ten 15-cm-d pots/treatment in each of two trials per column. $N = 100$ for each treatment (analysis was conducted on estimates from five aliquants/pot), except untreated controls in first column ($N = 85$) and 0.06% mutant ($N = 99$).

error was not valid, so standard errors were not reported. Steamed and unsteamed loamy sand trials from Experiment 1 were first analyzed separately and were not significantly different: the back transformed cyst count means were 60 for steamed trials ($N = 55$) and 59 for unsteamed trials ($N = 56$), P value = 0.9192, F value = 0.01. Steamed and unsteamed trials were then grouped for analysis of Experiment 1.

RESULTS

When 300 nematodes were added per 4,550-cm^3 pot, the mean number of cysts per pot was 48 for controls not treated with prills and 52 for pots treated with bran-only prills (standard error equal to 9.6 for both treatments, $N = 12$ per treatment). Values were not significantly different ($P = 0.4697$, F value = 0.54), and bran-only prills were not repeated in later experiments.

At the 300-nematode-per-pot initial population level (Experiments 1 and 2a), both the wild type and mutant strains of *V. lecanii* decreased population levels of soybean cyst nematode at one or more fungus application rates (Table 3). Cyst numbers tended to increase with decreasing amounts of fungus. In Experiment 1, significant reductions in cyst numbers occurred when either fungus was applied at the highest rate (0.02%). The mutant strain caused a 67% reduction in cyst numbers compared to untreated controls; application of the wild type strain resulted in a 62% decrease. In Experiment 2a, in which the same number of nematodes was added to each pot as in Experiment 1, both fungus strains were effective when applied at 0.006% and 0.06%. Significant decreases in cyst numbers were 39% and 58%, respectively, with the mutant strain and 42% and 47% at the same application rates with the wild type strain. However, the mutant strain caused a significant re-

duction (45%) at the lowest tested rate (0.002%), while the wild type strain did not. Neither fungus strain significantly decreased nematode numbers at the latter rate in Experiment 1.

In sand with 10,000 nematodes added per pot (Experiment 2b), neither the wild type nor the mutant strain decreased nematode populations when applied at 0.006% (Table 3). The mutant strain caused a significant 34% reduction in cyst numbers at the highest rate tested (0.06% fungus). The wild type strain did not significantly reduce the nematode populations at any tested rate. Consequently, at the 0.06% fungus application rate, application of the mutant strain resulted in a population reduction significantly different from both the wild type strain and from the untreated controls. Autoclaved prills made with fungus were added at the highest application rate and did not significantly reduce cyst numbers ($P = 0.05$): the mean number of cysts was 6,479 for pots with prills containing autoclaved fungus ($N = 96$), compared with 9,047 for pots without prills ($N = 100$).

Isolation of fungus from prills: The study was conducted with prills from Experiment 1. The fungi were more readily isolated from prills in steamed loamy sand than from prills in unsteamed loamy sand (Table 1). The mutant strain was isolated on PDA ABE 5 after both trials in steamed loamy sand, but the wild type strain was isolated only on PDA ABE 5 after trial 1. Both strains were isolated on Ausher's after trial 2, although this medium was not as effective as PDA ABE 5 for isolation of the mutant strain.

Isolation of fungus from sand and loamy sand: In Experiment 1, PDA ABE 5 inoculated with dilutions 1 and 2 was the most effective medium for isolation of the mutant from steamed loamy sand (Table 2). On this medium, the mutant strain applied at 0.002% and 0.02% was isolated after both trials. The wild type strain was isolated only from one petri dish of Ausher's medium (dilution 2) after trial 2. Because the fungi were not isolated from petri dishes inoculated with dilutions 3 or 4, use of these dilutions was discontinued after Experiment 1.

Additional studies (unpubl.) indicated that PDA ABE 5 was less effective than PDA ABE 100 for isolation of the mutant strain from unsteamed potting media; therefore, the higher rate of benomyl was added for Experiment 2. In Experiment 2a, the mutant strain was isolated from the sand (dilution 2) of four pots inoculated with 0.06% fungus (Table 2). Most of the isolations were from PDA ABE 100. The wild type strain was isolated on Ausher's (dilution 1) from a pot inoculated with 0.06% fungus.

In Experiment 2b, the mutant strain applied at 0.006% was isolated from a single petri dish inoculated with dilution 2 (Table 2).

Discussion

The wild type strain of *V. lecanii* that reduced viability of SCN eggs in petri dish assays (17) also reduced nematode cyst populations in greenhouse pot experiments. In the petri dish assays, this strain was not observed parasitizing live eggs, indicating that the fungus may have been decreasing egg viability through production of deleterious compounds, rather than through parasitism. Since unparasitized live and dead eggs are difficult to distinguish from each other, egg counts from greenhouse pots would not accurately represent the number of viable eggs present in the pots. Cyst and female counts were used to indicate whether nematode populations were reduced by the fungi.

Steamed loamy sand was tested so that the fungus-nematode interaction could be examined without other microorganisms initially present. Unsteamed loamy sand and sand were used to determine how the two strains compared when other microorganisms were viable at the beginning of the experiment and as they would be in agricultural soil. Analyses determined that the results in steamed and unsteamed loamy sand were not significantly different, so

the data were combined for analysis. In those trials (Experiment 1), both fungus strains significantly reduced cyst numbers at the highest prill application rate tested. In Experiment 2a (conducted with unsteamed sand), the mutant significantly reduced all fungus application rates tested. The seed germination medium, pot size, sand-to-compost ratio, soybean cultivar, inoculation time, and test period differed between Experiments 1 and 2. The results obtained under the two experimental conditions indicate that the ability of the fungus to reduce nematode populations can vary with test conditions. This was also demonstrated when a higher level of nematode inoculum (as might be encountered in a field in the spring) was applied in Experiment 2b and no significant reductions were observed with application of the wild type strain. Efficacy of the fungus is undoubtedly influenced by a number of factors, including nematode population levels.

Gaspard and Mankau conducted a study of fungus mutants or biotypes as biocontrol agents for plant-parasitic nematodes (7,8). A wild type strain of *Paecilomyces lilacinus* and UV-induced biotypes with resistance or tolerance to benomyl parasitized eggs of *Meloidogyne* spp., but did not significantly reduce numbers of root-knot nematode eggs on tomato. The mutant strain of *V. lecanii* employed in the current study was also induced with ultraviolet light (16). To induce the mutant strain, aqueous conidial suspensions were plated onto PDA or PDA amended with benomyl (100 μg benomyl/ml PDA). This level of benomyl slowed, but did not completely inhibit, growth of the wild type strain. After a 40-sec exposure to UV light (survival rate was 39%), single spore isolates were made from four colonies that grew more quickly on benomyl-amended agar than did the wild type strain. It was later found that these isolates generally grew more slowly than the wild type strain on PDA not amended with benomyl. As indicated by the greenhouse experiments, a tested benomyl resistant strain was more efficacious than the wild type strain for reducing SCN populations, even though benomyl was not applied to the plants. Either the greenhouse conditions prompted a change from the petri dish experiments and caused the mutant strain to grow more rapidly in the pots than the wild type strain, or the increased effectiveness of the mutant strain was a result of some factor not related to faster growth in the greenhouse pots.

Fungus application rates of 0.002%–0.06% are approximately equivalent to 45–1,360 kg/ha. The mutant strain was effective at the 45-kg/ha rate under some of the conditions employed in these experiments. Further tests will be required to determine whether the control effect is reproducible in the field, what application rates are necessary to reduce nematode populations, and whether the fungus reduces populations enough to increase yields. Manipulation of both the fungus culture conditions and the delivery system may increase the efficacy of the mutant strain so that more consistent and increased control could be achieved. For example, because bran provides a food base for the biocontrol fungus and also encourages growth of other fungi and bacteria, other formulations may improve effectiveness of the fungus.

Both the wild type and mutant strains of *V. lecanii* were difficult to isolate from sand, loamy sand, and prills, as they did not compete well on agar plates, even when semi-selective media were used. The problem was even more pronounced when the potting medium had not been steamed. Isolation of the fungi indicates that they can survive in sand and loamy sand, but it is not yet known whether either strain competes well in the soybean rhizosphere.

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