APPLICATION OF *BURKHOLDERIA CEPACIA* AND *TRICHODERMA VIRENS*, ALONE AND IN COMBINATIONS, AGAINST *MELOIDOGYNE INCOGNITA* ON BELL PEPPER

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ABSTRACT

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Bell pepper (*Capsicum annuum* L.) seeds and seedlings were treated with three potentially beneficial microbes, applied alone and in combinations, to compare effects of these formulations on root-knot nematode (*Meloidogyne incognita*) populations and on plant growth in the greenhouse. Individual treatments (applied as seed coatings and seedling drenches) were formulations of *Burkholderia cepacia* strains Bc-2 and Bc-F, and of *Trichoderma virens* strain Gl-3. Combination treatments were Bc-F+Gl-3, Bc-2+Gl-3, Bc-F+Bc-2, and Bc-F+Bc-2+Gl-3. At transplanting, pepper seedlings were each inoculated with 10 000 *M. incognita* eggs or left uninoculated, and harvested 10 weeks later. Nonviable microbe formulations of each individual strain were also applied; these were tested only on nematode-inoculated plants. No treatment consistently affected plant growth. Numbers of eggs + second-stage juveniles (J2) per g root were significantly lower with the Bc-2, Bc-F, and Gl-3 treatments than in the untreated controls, and highest with the nonviable Gl-3 treatment. This indicates that the viable preparations suppressed *M. incognita* numbers on pepper under the greenhouse test conditions. Importantly, the egg + J2 numbers recorded from combination treatments were not significantly different from untreated controls, suggesting that strain combinations decreased biocontrol effectiveness relative to applications of individual microbes.

Key words: bacteria, biocontrol, *Capsicum annuum*, fungus, management, root-knot nematode.

RESUMEN

Meyer, S. L. F, D. P. Roberts, D. J. Chitwood, L. K. Carta, R. D. Lumsden, and W. Mao. 2001. Aplicación de *Burkholderia cepacia* y *Trichoderma virens*, individualmente y en combinación para controlar *Meloidogyne incognita* en pimiento dulce. Nematrópica 31:75-86.

Semillas y plántulas de pimiento dulce (*Capsicum annuum* L.) se trataron con tres microbios potencialmente beneficiosos, los cuales se aplicaron individualmente o en combinación para comparar sus efectos sobre poblaciones del nematodo nodulador (*Meloidogyne incognita*) y sobre el crecimiento de las plantas en condiciones de invernadero. Tratamientos individuales (aplicados como cubierta a las semillas y remojo de plántulas) estuvieron representados por formulaciones *Burkholderia cepacia* que incluyó las raza Bc-2 y Bc-F, y *Trichoderma virens* raza Gl-3. Los tratmientos combinados fueron: Bc-F+Gl-3, Bc-2+Gl-3, Bc-F+Bc-2, y Bc-F+Bc-2+Gl-3. En el momento del transplante las plántulas de pimiento dulce se inocularon con 10 000 huevos de *M. incognita*. La cosecha se realizó 10 semanas después. También se evaluó formulaciones con microbios no viables de cada raza. Estos tratamientos sólo se aplicaron en plantas inoculadas con nematodos. Los tratamientos evaluados no afectaron consistentemente el crecimiento de las plantas. El número de huevos + segundo estado juvenil (J2) por raíz fue significativamente menor en los tratamienntos Bc-2, Bc-F, y Gl-3 en comparación con los testigos, y los más altos valores en el tratamiento no viable Gl-3. Esto demuestra que las preparaciones

viables suprimen los niveles de *M. incognita* en pimiento dulce cultivado en invernadero. Es importante destacar que el número de huevos + J2 registrado en tratamientos combinados no presentó diferencias significativas con respecto a los testigos no tratados. Esto sugiere que la combinación de cepas disminuyó la efectividad del biocontrol en relación a la aplicación individual de microbios.

Palabras claves: bacteria, biocontrol, *Capsicum annuum*, hongos, manejo, nematodo nodulador de raíz.

INTRODUCTION

Research on microbial pest control agents has identified many organisms capable of affecting plant pests and pathogens, but few biocontrol agents are available for commercial application (Fravel, 1999; Larkin *et al.*, 1998). One factor limiting commercial utilization of biological control organisms is inconsistent performance in the field. Chemical and physical properties of the soil, weather conditions, species of host plant, variations in rhizosphere colonization by biocontrol agents, presence of nontarget plant pathogens, and interactions with soil microflora and fauna all affect the ability of applied beneficial organisms to colonize, disperse, produce necessary compounds, or parasitize plant pathogens (Bourne *et al.*, 1996; Cook and Veseth, 1991; Mazzola and Cook, 1991; Ownley *et al.*, 1991; Shanahan *et al.*, 1992; Siddiqui and Mahmood, 1999; Stirling, 1991; Weller, 1988). One possible means of increasing consistency and efficacy of biocontrol agents is to apply combinations of these microorganisms. This approach has been studied for management of various soilborne pathogens, such as *Fusarium* spp., *Gaeumannomyces graminis*, *Heterodera schachtii*, *Meloidogyne incognita*, *Pythium spp.*, and *Verticillium dahliae* (e.g., Duffy *et al.*, 1996; Duponnois and Mateille, 1999; Hojat Jalali *et al.*, 1998; Leij *et al.*, 1992; Lemanceau and Alabouvette, 1991; Mao *et al.*, 1998a; Mao *et al.*, 1998b; Nagtzaam *et al.*, 1998; Pierson and Weller, 1994; Siddiqui and Mahmood, 1993). It is thought that combinations of biocontrol agents have a greater potential for increased activity against one or more plant pathogens and provide for expression of traits under a wider range of environmental conditions. Additionally, a combination of biocontrol agents may more effectively colonize the rhizosphere (Pierson and Weller, 1994).

While benefits may be considerable, there are also potential drawbacks to application of a combination of organisms. Antagonism between biological control agents through competition and antibiosis may lead to decreased performance in the field (Paulitz, 1990; Pierson and Weller, 1994). The potential benefits and pitfalls must be examined so that efficacious combinations can be identified.

The goal of this research was to compare the efficacy of microbes applied individually and in combinations to act as biocontrol agents for root-knot nematode (*Meloidogyne incognita* [Kofoid and White] Chitwood) on bell pepper (*Capsicum annuum* L.). Pepper was selected for the study because current agricultural practices for that crop rely heavily on methyl bromide application for control of soilborne diseases, and the upcoming ban on methyl bromide use will result in severe crop losses in the absence of replacement management agents. The tested biological control agents, *Trichoderma virens* (Miller, Giddens and Foster) von Arx. (= *Gliocladium virens*) and *Burkholderia cepacia* (Palleroni and Holmes) Yabuuchi *et al.* (= *Pseudomonas cepacia*), were chosen for several reasons. First, culture filtrates from strains of both organisms

suppressed *M. incognita* egg hatch and juvenile mobility during *in vitro* assays, and culture filtrate from *T. virens* suppressed *M. incognita* numbers in tomato root explant cultures (Meyer *et al.*, 2001). Second, *B. cepacia* is sold commercially as a biocontrol agent for some plant-parasitic nematodes (Stine Microbial Products, marketed by Market VI, L.L.C., Shawnee, KS, U.S.A.). A third reason is that a nonviable formulation of *B. cepacia* (isolate Bc-2) demonstrated activity against *M. incognita* populations on greenhouse-grown tomatoes, even though viable formulations of *B. cepacia* strain Bc-2 and *T. virens* were not effective (Meyer *et al.*, 2001). Compounds produced by *B. cepacia* might therefore have potential for use against nematodes on other crops. Additionally, both microbes were selected because *T. virens* and *B. cepacia* were effective in combination for reducing effects of soilborne fungal diseases on pepper (Mao *et al.*, 1998b). Activity against nematodes would enhance usefulness of these agents for managing a broader spectrum of pepper diseases.

The purposes of the current study were to compare: 1) *B. cepacia* and *T. virens* in viable formulations as potential management agents for *M. incognita* on bell pepper; 2) formulations of individual microbes with formulations containing combinations of biocontrol agents, since the latter were more effective than individual agents against other diseases on pepper; 3) activity of individual viable microbes with nonviable agents; and 4) efficacy of two strains of *Burkholderia* for suppressing nematode numbers on bell pepper.

MATERIALS AND METHODS

Microorganisms: The organisms used in these studies were the fungus *T. virens* (Gl-3; USDA, ARS Alternate Crops and Systems Laboratory, Beltsville, MD) and the bacterium *B. cepacia* (isolates Bc-2 and Bc-F; USDA, ARS Sustainable Agricultural Systems Laboratory, Beltsville, MD).

Formulations: Trichoderma virens strain Gl-3 was grown 8 to 14 days in molasses-yeast medium (3.0% molasses and 0.5% brewer's yeast per 1 L water) (Papavizas *et al.*, 1984). Cultures of Gl-3 were formulated in the following proportions: 510 ml Gl-3 culture was added to an autoclaved mixture consisting of 50 g peat and 150 g pyrophyllite (hydrous aluminum silicate). Formulations of Gl-3 were vacuum filtered on a linen towel, the residue was milled to pass through a 10-mesh sieve (2-mm openings), and refrigerated at 5°C until used. Strains Bc-2 and Bc-F were each grown in 100 ml tryptic soy broth (Sigma, St. Louis, MO) at 37°C and 250 rpm on a rotary shaker for 14 to 16 hours, pelleted by centrifugation, resuspended in 25 ml SDW, mixed with sterilized peat:pyrophyllite (50 g:150 g) as described for Gl-3, filtered, and sieved.

For combination formulations, *B. cepacia* strains were resuspended in 25 ml water (as described above). When the two strains were combined, 12.5 ml of each strain were used so that the total number of bacteria in the suspension was consistent among individual preparations. The final suspension was then mixed with the sterilized peat/pyrophyllite mixture. For *Burkholderia* + *Trichoderma* combinations, *B. cepacia* was resuspended in 25 ml water and mixed with the Gl-3 formulation prepared with 50 g peat/150 g pyrophyllite. The moisture in the resulting mixture was reduced by adding 3 g of the sterilized peat/pyrophyllite (1:3) mixture, and air-dried to a moist powder.

The tested agents were applied twice during the experiment to each treated plant: first in seed coatings, and again in seedling drenches. For seed coating, each seed was rolled in a sticker consisting of 97 ml SDW, 3 ml Bond (Loveland Industries,

Inc., Greeley, CO), 1 g Keltrol (Kelco, San Diego, CA) and 1 ml Celgard Silver Film Coating Polymer (Celpril, Mantaca, CA). Each treated seed was then rolled in a formulation of the appropriate treatment. To determine colony-forming units (CFU) per seed, 10 seeds were added to 9 ml SDW, sonicated for 5 minutes, vortexed for 1 minute, and dilution-plated onto TME (Papavizas and Lumsden, 1982) for Gl-3 and onto PCAT (Burbage *et al.*, 1982) for strains Bc-2 and Bc-F.

Drenches were prepared as 50 g dried formulation in 500 ml water. CFU per ml were determined by dilution plating, essentially as described for seed coatings. Nonviable microbe treatments (autoclaved prior to use) were also prepared for seed coatings and drenches of individual strains.

Seed treatments involving *B. cepacia* strains contained ca. 10^6 CFU per seed while seed treatments involving *T. virens* contained ca. 10^4 CFU per seed. Drenches contained ca. 10^8 CFU per ml of either *B. cepacia* strain or ca. 10^4 CFU per ml *T. virens*. Formulations with nonviable microbes did not contain detectable *B. cepacia* or *T. virens* CFU.

Greenhouse assays: Capsicum annuum cultivar 'California Wonder' (a bell pepper susceptible to *M. incognita*) seeds (treated with a seed coat or left untreated) were planted in flats of Scotts Terra-Lite Rediearth Peat-Lite Mix (Scotts-Sierra Horticultural Products Company, Marysville, OH). Seedlings were grown at l8 to 27°C in the greenhouse with 16 h light per 24-h day. Three weeks after sowing, seedlings were drenched with the same potential biocontrol agent(s) that had been used to coat the seeds (500 ml drench formulation per 50 seedlings in a flat). Seedlings from untreated seeds were drenched with water. Five weeks after sowing, randomly selected seedlings were transplanted into pots of sandy soil (93% sand, 3% silt, 4% clay, 0.47% organic matter, pH 6.3, made from 1 part compost to 8.5 parts sand). Pot diameters were 10.2 cm and volumes were 570 ml; each pot contained 1 seedling in ca. 610 g (air-dried weight) of the sandy soil. Immediately following transplanting, nematode inoculum (10 000 *M. incognita* eggs in 1 ml water per seedling) was pipetted into a 2.5-cm-deep hole near the roots of half of the seedlings. Pots were arranged in a randomized block design (two blocks, each containing half the number of pots of each treatment), and maintained in the greenhouse. Twenty-two and 16 plants per treatment were transplanted for the first and second trials, respectively.

Approximately 10 weeks after transplanting, 10 pots per treatment were harvested in each trial of the experiment. Root fresh weight, shoot fresh and dry (48 h at 60°C) weights, shoot height (from the soil line to the tip of the main stem), number of fruit per plant, fruit fresh and dry (3 d at 60°C) weights, and the number of *M. incognita* eggs and J2 per g of root were determined. The experiment was conducted twice. In the first trial, the following eight treatments were tested on pepper inoculated with *M. incognita* and on uninoculated pepper: 1) Bc-F; 2) Bc-2; 3) Gl-3; 4) Bc-F+Gl-3; 5) Bc-2+Gl-3; 6) Bc-F+Bc-2; 7) Bc-F+Bc-2+Gl-3; and 8) untreated. All of the seven tested formulations contained viable microbes. In the second trial, all treatments were repeated, and three additional treatments were tested on pepper inoculated with *M. incognita*: 1) nonviable Bc-F; 2) nonviable Bc-2; and 3) nonviable Gl-3 (formulations were autoclaved prior to use).

Eggs and J2 were counted after collection from roots and soil (procedure modified from Hussey and Barker, 1973; McClure *et al.*, 1973; Taylor and Sasser, 1978). For each pot, all of the soil was washed in 8 L water, and the water suspension was poured through a 20-mesh sieve

(pore size 850 µm) nested on a 500-mesh sieve (pore size 25 µm). Eggs and J2 collected on the 500-mesh sieve were centrifuged in water at 475 × g for 3 min. The supernatant was decanted, a 1 M sucrose solution was added to each tube, the pellet was shaken in the solution, and the suspension centrifuged again at 475 × g for 3 minutes. Eggs and J2 were collected on a 500-mesh sieve. Eggs and J2 attached to, or inside of, root tissues were collected by macerating roots with scissors, swirling the root pieces in 0.525% sodium hypochlorite for 4 minutes, collecting and washing the eggs and J2 on a 500-mesh sieve nested under a 60-mesh sieve (pore size 250 µm), and centrifuging the eggs and J2 as with the soil samples.

Reisolation of biocontrol agents: CFU of *T. virens* and *B. cepacia* strains on roots were determined after harvest of the second trial. Ten percent (by weight) of the root system of each of two plants per treatment (plants not used for nematode counts) was randomly selected and added to 200 ml SDW, sonicated 5 minutes, vortexed 1 minute, and dilution plated onto TME for *T. virens* and onto PCAT for *B. cepacia* isolation. *Trichoderma virens*-like and *B. cepacia*-like colonies were counted.

Analysis of data: Seven variables: shoot height, shoot fresh weight, shoot dry weight, root fresh weight, number of fruit, fruit fresh weight, and fruit dry weight were analyzed as general linear mixed models using PROC MIXED (SAS Institute) with treatment as the fixed factor and harvest date as the random factor. To correct the variance heterogeneity in shoot dry weight and root weight, treatments were grouped into similar variance groups for the analysis. Estimates for synergistic effects (when the combined effect of two microbes is greater than the additive effect of the microbes when applied individually) or antagonistic effects (when the combined effect of two microbes is less than the additive effect) and test of significance were done for the Bc-F+Gl-3, Bc-2+Gl-3, and Bc-F+Bc-2 treatments in nematode-inoculated plants. The additive effect was calculated as "untreated mean + (treatment A mean - untreated mean) + (treatment B mean - untreated mean)." The possibility of a synergistic or antagonistic effect was explored only when a treatment effect was statistically significant.

The variables eggs per g of root, J2 per g of root, and eggs + J2 per g of root were also analyzed as general linear mixed models using PROC MIXED with treatment as the fixed factor and harvest date as the random factor. To meet the assumptions of the models all of the variables were natural log transformed $\ln(x + 1)$. The variables eggs per g of root and eggs + J2 per g of root had heterogeneous variances and their treatments were grouped into similar variance groups for analysis. All means are presented in the original units.

RESULTS

Plant growth: Overall, plant growth was not consistently affected by treatment. Only root fresh weight ($F = 4.35$, $P = 0.0001$) and shoot dry weight ($F = 3.94$, $P = 0.0001$) varied among treatments (Table 1; results are presented for formulations with viable microbes). Treatment did not alter shoot height, shoot fresh weight, number of fruit, fruit fresh weight, or fruit dry weight. Shoot dry weights were similar on control plants with and without nematodes (Table 1). However, application of viable Bc-F or viable Bc-2 to plants inoculated with nematodes resulted in higher shoot dry weights than those recorded from most other treatments, including weights from plants not inoculated with nematodes (Table 1). Shoot dry weight with Bc-F treatment was 58% higher than shoot dry weight from

Table 1. Plant growth responses following treatment with the biocontrol agents *Burkholderia cepacia* (strains Bc-2 and Bc-F) and *Trichoderma virens* (strain Gl-3), applied individually and in combinations for management of *Meloidogyne incognita* on greenhouse-grown bell pepper.[x]

Treatment	Shoot height (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Number of fruit[y]	Fruit fresh weight (g)	Fruit dry weight (g)	Root fresh weight (g)
Meloidogyne incognita							
Bc-F	22.4 a	26.9 a	7.9 a	2 a	53.4 a	3.7 a	31.2 ab
Bc-2	22.3 a	25.5 a	7.5 ab	3 a	65.7 a	4.1 a	22.4 abc
Gl-3	22.1 a	26.0 a	4.4 c	2 a	52.4 a	3.6 a	26.9 ab
Bc-F+Gl-3	21.6 a	26.0 a	4.4 c	2 a	52.6 a	2.8 a	19.4 bc
Bc-2+Gl-3	22.9 a	29.8 a	5.2 abc	2 a	56.1 a	3.0 a	30.8 ab
Bc-F+Bc-2	21.8 a	25.5 a	4.4 c	2 a	56.6 a	3.8 a	20.5 abc
Bc-F+Bc-2+Gl-3	21.7 a	25.3 a	4.4 c	2 a	52.4 a	3.4 a	18.2 bc
Untreated	22.1 a	28.6 a	5.0 bc	2 a	63.7 a	4.1 a	28.8 a[z]
No *Meloidogyne incognita*							
Bc-F	21.7 a	29.2 a	5.1 abc	2 a	53.5 a	3.3 a	18.5 bc
Bc-2	22.7 a	25.7 a	4.6 c	2 a	49.9 a	3.2 a	20.0 bc
Gl-3	22.6 a	27.4 a	4.5 c	2 a	49.9 a	3.3 a	18.7 bc
Bc-F+Gl-3	21.6 a	25.4 a	4.3 c	2 a	50.5 a	3.2 a	16.8 c
Bc-2+Gl-3	21.6 a	23.7 a	3.9 c	2 a	49.9 a	3.2 a	19.7 bc
Bc-F+Bc-2	20.8 a	25.5 a	4.1 c	2 a	57.5 a	3.6 a	19.7 bc
Bc-F+Bc-2+Gl-3	21.2 a	24.4 a	4.4 c	2 a	54.4 a	3.4 a	22.9 abc
Untreated	22.7 a	28.1 a	4.8 c	2 a	57.9 a	3.7 a	22.2 abc

Treatment means with different letters within a column are different at $P \leq 0.0004$ significance level (Experiment-wise error 0.05).

[x]Results are for formulations containing viable microbes.

[y]Rounded to the nearest whole number.

[z]The values 30.8 and 31.2 are listed as 'ab,' and 28.8 as 'a,' because the two higher values were in a group with a larger variance than the variance for the group that included 28.8.

nematode-treated control plants. Application of nonviable Bc-2 or of nonviable Bc-F (trial 2 of the experiment) did not comparably increase shoot dry weights (data not shown). Interactions between biocontrol agents also affected shoot dry weights; two of the three combination treatments tested for synergism or antagonism produced results other than an additive effect of the individual agents (Table 2). Treatment of nematode-inoculated plants with Bc-F+Bc-2 and with Bc-F+Gl-3 resulted in an antagonistic interaction; shoot dry weights were 58% and 40% lower, respectively, than predicted with an additive effect (Table 2). The dry shoots from plants treated with these combinations were similar in weight to shoots with most treatments, including Gl-3 treatment (Table 1).

As with shoot dry weights, root fresh weights were similar for control plants with and without nematodes (Table 1). Although Bc-F and Bc-2 applications enhanced shoot dry weights on nematode-inoculated plants, neither organism significantly increased root weights compared to most treatments (Table 1). Nonviable agents did not affect root weights compared to control plants (data not shown). On nematode-inoculated plants, root fresh weights were reduced 33% and 37%, respectively, by treatment with Bc-F+Gl-3 and with Bc-F+Bc-2+Gl-3, compared with control plants. As with shoot dry weight, the Bc-F+Gl-3 treatment resulted in an antagonistic interaction that reduced root fresh weight (33%) beyond the expected additive effect of the two organisms (Table 2). Conversely, Bc-2+Gl-3 treatment had a synergistic interaction that resulted in a root fresh weight 51% greater than the predicted additive effect on nematode-inoculated plants.

Nematode populations: Numbers of eggs and of J2 per g of root were both affected by treatment (Fig. 1; results are presented for formulations with viable microbes). Results of the analysis of variance were: a) eggs per g of root, $F = 2.04$, $P = 0.0601$; b) J2 per g of root, $F = 4.73$, $P = 0.0001$; c) eggs + J2 per g of root, $F = 2.31$, $P = 0.0346$. Individual biocontrol organisms had the greatest suppressive effect on nematode populations; numbers of eggs per g of root were suppressed 60% to 69% with application of Bc-2, Bc-F, or Gl-3 (Fig. 1), compared to untreated controls and to Bc-F+Bc-2+Gl-3 treatment. Application of biocontrol combinations did not suppress egg numbers. Compared to the untreated control, numbers of J2 per g of root were lower with all microorganism treatments except Bc-F+Bc-2+Gl-3. Application of Bc-F resulted in the lowest number of J2 per g of root, with a 79% decrease compared to the untreated control. This treatment also resulted in the smallest value for eggs per g of root. When egg and J2 counts were combined, the numbers of eggs + J2 per g of root were suppressed by application of the three individual treatments, but not by application of any combination treatment (Fig. 1). Percent reductions compared to the untreated control were 58% (Gl-3), 55% (Bc-2), and 67% (Bc-F). Data from the three nonviable treatments are not shown in Fig. 1, because the three treatments were not included in the first trial of the experiment. Treatment with nonviable Gl-3 or nonviable Bc-F resulted in significantly greater numbers of eggs + J2 per g of root than treatment with the comparable viable agents (data not shown). Additionally, plants treated with the nonviable Gl-3 had significantly more nematodes than untreated control plants (data not shown).

Reisolation of biocontrol agents: After the second trial of the experiment was harvested, *Trichoderma virens*-like and *B. cepacia*-like organisms were not reisolated from treated roots at levels above background levels found on the control roots. There

Table 2. Additive, combined, synergistic and antagonistic effects of biocontrol agent combinations on growth of bell pepper plants inoculated with *Meloidogyne incognita*. Tested agents were *Burkholderia cepacia* strains Bc-F and Bc-2 and *Trichoderma virens* strain Gl-3.

	Shoot dry weight[w]				Root fresh weight			
Treatment	Additive effect[x]	Combined effect[y]	Synergy/ antagonism[z]	*P*-value	Additive effect	Combined effect	Synergy/ antagonism	*P*-value
Bc-F+Gl-3	7.3	4.4	-2.9	0.0009	29.2	19.4	-9.8	0.0340
Bc-2+Gl-3	7.0	5.2	None	0.0898	20.4	30.8	10.4	0.0228
Bc-F+Bc-2	10.5	4.4	-6.1	0.0001	24.7	20.5	None	0.3499

[w]Plant growth variables shown are the only ones in which a synergistic or antagonistic effect was observed.
[x]Values in the "Additive Effect" columns are those expected if two treatments were applied in combination and did not demonstrate synergy or antagonism. Additive effect = untreated mean + (treatment A mean - untreated mean) + (treatment B mean - untreated mean).
[y]Values in the "Combined Effect" columns are the means calculated after treatment with formulations containing two different microbes (as reported in Table 1).
[z]Synergism between the two agents exists when the combined effect of the two microbes is greater than the additive effect of individual applications of the organisms; antagonism exists when the combined effect is a lower value than would be expected with an additive effect (negative values in the table).

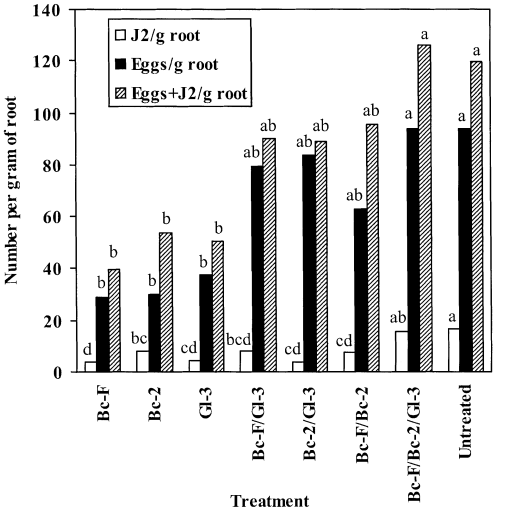

Fig. 1. Number of *Meloidogyne incognita* eggs, second-stage juveniles (J2), and eggs + J2 per g of root following treatment of bell pepper with the biocontrol agents *Trichoderma virens* (strain Gl-3) and *Burkholderia cepacia* (strains Bc-F and Bc-2), applied individually and in combinations. All treatments were applied as seed coatings and as drenches. Results are presented for formulations containing viable microbes. Letters are comparable with each other within a category (categories are indicated by color pattern), but are not comparable among the three categories. Bars within a category and with a common letter are not significantly different ($P = 0.0601$ for eggs per g of root, $P = 0.0001$ for J2 per g of root; $P = 0.0346$ for eggs + J2 per g of root).

was therefore no indication that the applied agents were proliferating on the roots at that time (more than 12 weeks after the last application of viable microbes).

DISCUSSION

Viable formulations of *Burkholderia cepacia* (Bc-2 and Bc-F) and *T. virens* (Gl-3) significantly suppressed numbers of root-knot nematode eggs and J2 produced on roots of pepper plants. Additionally, *B. cepacia* strain Bc-F increased shoot dry weights of nematode-infested plants, compared to controls. Nonviable formulations of the microbes were not active against *M. incognita* populations in this study. Combining biocontrol agents did not result in beneficial synergistic interactions; individual viable agents significantly suppressed nematode populations, while combinations did not. These results differ from those recorded after application of *T. virens* + *B. cepacia* combinations to corn, tomato, and pepper for management of soilborne fungus diseases (Mao *et al.*, 1998a, 1998b). In those tests, combinations of these microorganisms were more effective than individual agents for disease control on corn, for improving pepper plant vigor and suppressing disease, and for increasing yield on tomato (Mao *et al.*, 1998a, 1998b). It is not clear why the combinations did not reduce root-knot nematode populations on pepper in the current study, even though the individual agents were effective. It is possible that the tested strains were antagonistic to each other under the conditions used for this nematode management experiment, or that the combinations tested here suppressed production of nematode-antagonistic compounds.

Trichoderma virens, while studied extensively as a biocontrol agent for fungus diseases of plants, has not been widely tested as an applied agent for nematode management. In an earlier study, seed treatment with *T. virens* did not suppress reproduction of *M. incognita* on cotton (Zhang *et al.*, 1996). *Burkholderia cepacia* is sold commercially as a biological nematicide for management of a number of plant-parasitic nematodes on various crops, including vegetables. However, *B. cepacia* applied as a soybean seed treatment was not effective against soybean cyst nematode (Noel, 1990). While neither viable microbe affected root-knot nematode numbers on tomato in an earlier greenhouse test (Meyer *et al.*, 2001), viable *T. virens* and *B. cepacia* both suppressed nematode populations on bell pepper in the current

study. As with all biocontrol organisms, strain selection, formulation, soil environment, target nematode, nematode population numbers, and host crop affect results. Even among different cultivars of a single host plant, *Zea mays, B. cepacia* biocontrol efficacy varied against damping-off diseases (Hebbar *et al.*, 1998). For both organisms, then, differential performance on different host species was not surprising. The tested microbes were not present on the roots above background levels after harvest in the tomato study (Meyer *et al.*, 2001) or in the current pepper study, so the nematode suppression was not associated with long-term root colonization of the microbes on the pepper roots. Total final numbers of nematodes may have factored into the differential suppression; root-knot nematode populations were higher on tomato treatments at 65 days (Meyer *et al.*, 2001) than on pepper at a comparable harvest time. With root-knot nematode, gall size can also be a factor in determining efficacy of biocontrol agents, because root-knot nematode eggs embedded in plant tissue are generally protected from biocontrol agents (Leij *et al.*, 1992; Leij and Kerry, 1991; Meyer, 1999). The small size of the galls observed at harvest on pepper roots from all treatments in the current study may have enhanced the ability of the applied agents to act against the nematode.

Both organisms used in this study have shown activity against multiple plant diseases (e.g., Bowers and Parke, 1993; Lumsden and Locke, 1989; Mao *et al.*, 1997, 1998a, 1998b). The current results indicate that strains of *B. cepacia* and *T. virens* are also active against *M. incognita* on bell pepper under the conditions of this greenhouse test. Because efficacy of biocontrol agents is strongly influenced by biotic and abiotic factors in the environment, additional studies will indicate whether the activity against root-knot nematode persists with different soil types, nematode numbers, and climatic conditions.

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