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Evaluation of *Trichoderma virens* and *Burkholderia cepacia* for antagonistic activity against root-knot nematode, *Meloidogyne incognita*

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Summary – The bacterium *Burkholderia cepacia* (strain Bc-2) and the fungus *Trichoderma virens* (strain Gl-3) were investigated for activity against the nematode *Meloidogyne incognita*. Culture filtrates from Bc-2 and Gl-3 contained extracellular factors that inhibited egg hatch and second-stage juvenile (J2) mobility. Size fractionation results and lack of detectable chitinase or protease activities from Bc-2 and Gl-3 culture filtrates suggested that the inhibitory factors in the *in vitro* assays were non-enzymic. Tomato root explant cultures of *M. incognita* treated with *T. virens* culture filtrate had 42% fewer eggs and J2 per g of roots than cultures treated with control medium that had not been inoculated with *T. virens*. In glasshouse tests with tomato, Bc-2 and Gl-3 were applied individually as seed coatings and as root drenches in both viable and non-viable formulations. At the 65-day harvest, non-viable *B. cepacia* was the only treatment that suppressed eggs and J2 per g of roots (29% suppression) compared to water controls.

Résumé – ***Evaluation de l'activité antagoniste de* Trichoderma virens *et* Burkholderia cepacia *envers le nématode* Meloidogyne incognita –** La bactérie *Burkholderia cepacia* (souche Bc-2) et le champignon *Trichoderma virens* (souche G1-3) ont été étudiés dans l'optique de leur action envers le nématode *Meloidogyne incognita*. Les filtrats de culture de Bc-2 et de G1-3 contiennent des facteurs extracellulaires inhibant l'éclosion et la motilité des juvéniles de deuxième stade (J2) du nématode. Les résultats de fractionnements relatifs à la taille et la nondétection d'une activité chitinasique ou protéasique dans les filtrats de culture de Bc-2 et G1-3 suggèrent que les facteurs inhibant présents lors des expériences *in vitro* ne sont pas de nature enzymatique. Des élevages de *M. incognita* sur explants de racines de tomate traités avec des filtrats de culture de *T. virens* produisent des œufs et des J2 en nombre inférieur de 42% à celui d'élevages traités par un milieu témoin, non inoculé avec *T. virens*. Lors d'essais en serre sur tomate, Bc-2 et G1-3 ont été appliqués séparément, soit en pralinage des semences, soit sur la tranchée, et en formulation vivante ou non-vivante. A la récolte, après 65 jours, la formulation non-vivante de *B. cepacia* s'est révélée le seul traitement diminuant le nombre d'œufs et de J2 par g de racines: moins 29% par rapport au témoin ne contenant que de l'eau.

Keywords – bacteria, biological control, *Lycopersicon esculentum*, natural products, tomato.

Root-knot nematodes (*Meloidogyne* spp.) are among the most economically important groups of plant-parasitic nematodes, causing damage and yield losses on most cultivated plants (Sasser & Freckman, 1987). Cultural practices, use of resistant cultivars and application of chemical nematicides are primary strategies for disease management, but yield losses persist with numerous crops. Application of microorganisms antagonistic to *Meloidogyne* spp., or of compounds produced by these microbes, could provide additional opportunities for managing disease. Research in this area has resulted in commercial biocontrol preparations reported to act against root-knot nematodes (Stirling, 1991; Fravel, 2000). Continued studies are needed to identify additional organisms that reduce disease caused by these nematodes.

The first goal of the current study was to determine the ability of selected microbes to produce compounds affecting root-knot nematode viability and mobility. De-

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creasing egg hatch and rendering juveniles immobile (or non-viable) could reduce the numbers of nematodes that find roots and feed. The two organisms chosen for this investigation were the fungus *Trichoderma virens* (Miller, Giddens & Foster) v. Arx (=*Gliocladium virens* Miller, Giddens & Foster) and the bacterium *Burkholderia cepacia* (Palleroni & Holmes) Yabuuchi *et al.* (=*Pseudomonas cepacia* (*ex* Burkholder) Palleroni & Holmes). Strains of each microorganism are commercially available for management of fungal plant pathogens, including *Pythium* and *Rhizoctonia* (Fravel, 2000). Additionally, a nematotoxic strain of *B. cepacia* is registered for use against some plant-parasitic nematodes (Stine Microbial Products, marketed by Market VI, L.L.C., Shawnee, KS, USA).

B. cepacia isolate Bc-2 was selected for this research because preliminary tests indicated that this strain was toxic to some species of free-living nematodes (Carta, in press). *Trichoderma virens* isolate Gl-3 was used because of its ability to suppress other soil-borne pathogens (Ristaino *et al.*, 1994; Mao *et al.*, 1997, 1998a, 1998b). Demonstration of activity against nematodes could enhance the usefulness of this strain. After determining activity *in vitro*, a second goal of this study was to determine if either organism is effective against root-knot nematode on tomato (*Lycopersicon esculentum* Mill.) when applied as a viable formulation. A third objective was to test a non-viable formulation of each microbe for ability to suppress nematode populations. These formulations were studied because application of active natural products from microbe cultures can eliminate the need to maintain living organisms throughout the delivery, storage and application processes.

Material and methods

Microorganisms

The organisms used in these studies were *T. virens* isolate Gl-3 (=*G. virens* isolate Gl-3) and *B. cepacia* isolate Bc-2, both from the collection of the Biocontrol of Plant Diseases Laboratory (USDA, ARS, Beltsville, MD, USA).

Preparation of culture filtrates

Strain Bc-2 was incubated until it reached the stationary phase (2 days at 37°C and 250 rpm) in potato dextrose broth (PDB) (Difco Laboratories, Detroit, MI, USA), nutrient broth (NB) (Difco Laboratories), or minimal medium (BL) (Berka & Lessie, 1984) plus 0.2% glycerol, 0.2% chitin, or 0.2% glycerol plus 0.2% chitin. Cultures were centrifuged at 6000 *g* for 10 min and the supernatant passed through a 0.2 μm filter. Gl-3 was incubated until it reached the stationary phase (5 days at 20°C and 250 rpm) in PDB, wheat bran extract (WBE; prepared by boiling 59 g wheat bran in 1 l water for 10 min, followed by straining through coarse muslin), or Weindling's minimal medium (as modified by Jones and Hancock, 1987) plus 0.5% glycerol (WG) or 0.2% chitin, or WG amended with 0.2% chitin. Cultures were filtered through cheesecloth, centrifuged at 10 000 *g* for 10 min, and the supernatant passed through a 0.2 μm filter. All culture filtrates were stored at −20°C until used.

In vitro inhibition assays

Culture filtrates from Bc-2 and Gl-3 were tested in 24-well tissue culture plates for effects on *Meloidogyne incognita* (Kofoid & White) Chitwood egg hatch and on mobility of hatched second-stage juveniles (J2), as described by Nitao *et al.* (1999). *M. incognita* egg masses were collected from roots of glasshouse-grown tomato plants and the eggs subsequently separated and sterilized with 0.525% sodium hypochlorite. In each well, 0.1 ml sterile water containing eggs was combined with 0.9 ml of either a culture filtrate or a control. Treatments were water, PDB, NB, WG, culture filtrates from Bc-2 grown in PDB or NB and culture filtrates from Gl-3 grown in PDB or WG. The experiment was performed twice. Approximately 113 eggs were added per well in the first trial and 201 eggs in the second trial. Each of the eight treatments was placed into five wells per trial. Counts were made of total J2 and of mobile J2 in each well after 3-4 days (hereafter referred to as '3 days') and again after 14 days.

In experiments to determine the effect of size-fractionated culture filtrates on egg hatch and J2 mobility, culture filtrates of Gl-3 or of Bc-2 grown on PDB were fractionated by centrifugation in a Centriprep 3 apparatus (Amicon Corp., Lexington, MA, USA) at 4000 *g* for 5 h (molecular weight cut-off = 3 KDa). The filtrate (<3 KDa fraction) was frozen until used. The retentate (>3 KDa fraction) was washed in a Centriprep 3 apparatus with sterile PDB to remove <3 KDa contaminants and restored to the original volume with sterile PDB. The non-fractionated culture filtrate and the >3 KDa fraction were also frozen until used. Sterile, non-inoculated PDB was size-fractionated as above and frozen until used as

controls. *In vitro* inhibition assays were performed as above with these preparations.

Enzyme assays

Culture supernatant was assayed for chitinase and protease activities. For chitinase activity, 50 mM 2-(*N*-morpholino) ethanesulfonic acid (MES) buffer, pH 5.0, plus 0.2% chitin was mixed with culture filtrate and incubated at 37°C. Liberated *N*-acetylglucosamine equivalents due to chitinase activity were determined by the method of Reissing *et al.* (1955). Protease activity was determined by incubating culture filtrate in 50 mM MES, pH 5.0, plus 0.4% azocoll at 37°C (Chavira *et al.*, 1984). One unit of chitinase activity was defined as the amount of enzyme that released 1 μmole N-acetylglucosamine equivalent/min/ml culture filtrate. One unit of protease activity was the amount of enzyme that increased absorbance at 520 nm/1 unit/h/ml culture filtrate. Lower limits of detection were 2.3 units for chitinase and 0.02 units for protease.

Monoxenic root explant culture assays with Gl-3

Five Petri dishes of Gamborg's B-5 medium (Life Technologies, Grand Island, NY, USA) each received 0.5 ml of culture filtrate from Gl-3 grown in WG, non-inoculated WG (control), or water. Three excised tomato (cv. Rutgers) root tips were placed in each Petri dish. The next day, *ca* 2200 root-knot nematode eggs (obtained from monoxenic root explant cultures) in a 0.2 ml aqueous suspension were added to each Petri dish. The cultures were incubated at 28°C for 35 days following nematode inoculation and fresh root weight per Petri dish, root gall indices, number of eggs per egg mass and eggs and J2 per g of root recorded. Results were taken from five Petri dishes each for the water and the culture filtrate treatments and three Petri dishes for the WG treatment. To collect the data, the medium in each Petri dish was melted for 45 s in a microwave oven and the roots rinsed with warm water. Fresh root weights were determined. To count the number of eggs per egg mass, one or two egg masses were removed from each Petri dish (for a total of ten egg masses for the culture filtrate treatment, seven for the water control, and five for the WG treatment). Root gall indices were recorded following the procedure of Taylor and Sasser (1978): 0 = no galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, and 5 = >100 galls per root system. The roots were subsequently macerated and swirled in 0.525% sodium hypochlorite for 4 min, and the eggs and J2 collected on a 25 μm aperture sieve. The number of eggs and J2 per Petri dish (minus the eggs in the removed egg masses) were counted.

Formulations for glasshouse bioassay

Strain Gl-3 was grown for 14 days in molasses-yeast medium (3.0% molasses and 0.5% brewer's yeast in water; Papavizas *et al.*, 1984). Gl-3 culture (1 L) was added to an autoclaved mixture consisting of 50 g peat and 150 g pyrophyllite (hydrous aluminum silicate). The formulation was vacuum filtered on a linen towel, milled to pass through a 2 mm aperture sieve, and refrigerated at 5°C until used. Strain Bc-2 was grown in 100 ml tryptic soy broth (Sigma, St. Louis, MO, USA) at 37°C and 300 rpm for 14 to 16 h. Cultures were centrifuged, resuspended in 25 ml sterile distilled water, mixed with sterilized peat/pyrophyllite (50 g/150 g) as described for Gl-3, filtered and sieved.

For seed coating, seeds were first rolled in a sticker consisting of 97 ml sterile distilled water, 3 ml Bond (Loveland Industries, Inc., Greeley, CO, USA), 1 g Keltrol (Kelco, San Diego, CA, USA) and 1 ml Celgard Silver Film Coating Polymer (Celpril, Mantaca, CA, USA). Each seed was then rolled in a formulation of either Gl-3 or Bc-2 (sieved prior to use). Colony-forming units (CFU) per seed were determined by dilution plating. In viable preparations, approximately 10^5 CFU of *T. virens* and 10^8 CFU of *B. cepacia* were applied to each seed. Autoclaved preparations did not contain detectable *B. cepacia* or *T. virens*, as determined by dilution plating.

To prepare a root dip, each biocontrol formulation was added at 2% by weight (2 g per 100 ml) to 1% Keltrol. CFU per ml root dip were determined by dilution plating. In viable preparations, Gl-3 and Bc-2 were added at approximately 10^6 and 10^7 CFU per ml, respectively. Autoclaved treatments did not contain detectable *B. cepacia* or *T. virens* CFU.

Glasshouse bioassay in soil

Coated and untreated tomato cv. Orange Pixie seeds were planted in flats of Scotts Terra-Lite Redi-earth Peat-Lite Mix (Scotts-Sierra Horticultural Products Company, Marysville, OH, USA). Seedlings were grown under natural and supplemental lighting (16 continuous h per day) at 18-25°C in the glasshouse. After 2 weeks, seedlings were each fertilized with several grains of Osmocote Plus controlled release fertilizer (Scotts-Sierra Horticultural

Products Company). Three weeks after planting, roots of each seedling were dipped in the same treatment that had been used as a seed coating, and seedlings transplanted into loamy sand (79% sand, 15% silt, 6% clay, 2.84% organic matter, pH 6.5, made from 3 parts compost to 1 part sand). Control seedlings grown from untreated seeds were dipped in sterile water. Immediately following transplanting, nematode inoculum was added near the seedling roots (10 000 eggs in 1 ml water per seedling). Pot diameters were 10.2 cm and volumes were 570 ml; each pot contained *ca* 600 g (air-dried weight) of loamy sand. Pots were arranged in a randomized block design for each harvest date. Four days after transplanting, all plants were fertilized with 2 g of Osmocote Plus. Plants grown for the 65-day harvest were fertilized again 42 days after transplanting. At 35 and 65 days after transplanting, ten pots per treatment were harvested. The six treatments were: Bc-2, Gl-3, non-viable (autoclaved for 20 min at 115°C, 18 p.s.i.) Bc-2, non-viable (autoclaved) Gl-3, Keltrol sticker formulation and a control with no seed treatment. Fresh root weight, fresh and dry shoot weight (the latter determined after drying shoots for 48 h at 60°C), shoot height (from the soil line to the tip of the main stem), root gall indices, and number of eggs and J2 per g of root were determined. Fruit weight and number of fruit per plant were recorded at 65 days only. The experiment was performed twice.

To determine egg and J2 numbers, eggs and J2 in each pot were counted after collection from roots and from a soil subsample (1/40 of the soil obtained after thorough stirring in water of all soil from a pot). The eggs were collected on nested sieves (25 μm aperture sieve nested under a 250 μm aperture sieve), and centrifuged (475 *g* for 3 min) in water and in sucrose (procedure modified from Hussey and Barker, 1973; McClure *et al*., 1973; Taylor and Sasser, 1978). The eggs and J2 produced external to the root tissue (*i.e.*, not surrounded by plant tissues) were combined with the eggs and J2 in the soil subsample, to obtain a count labeled "external eggs and J2". "Internal eggs and J2" (*i.e.*, produced inside the root tissue) were collected by thoroughly macerating roots prior to washing and centrifugation.

After the 65-day harvest, 10% of the root systems of three plants from each treatment were sonicated in 100 ml sterile distilled water and the populations of Gl-3 and Bc-2 determined by dilution plating on TME (Papavizas & Lumsden, 1982) and PCAT (Burbage *et al*., 1982), respectively.

Analysis of data

Data from both trials of the *in vitro* inhibitory assays were combined following multiplication of first-trial data by 1.78 to adjust for lower egg inoculum levels (inoculum levels described in 'In Vitro Inhibition Assays' section), and analysed with SAS Lab (SAS Institute, Cary, NC, USA). Fractionation data and root explant assay data were also analysed with SAS Lab. For the glasshouse 35-day harvest, dry shoot weight, root weight, external eggs and J2 per g of root, internal eggs and J2 per g of root, and combined external plus internal eggs and J2 per g of root were analysed as one factor general linear mixed models using PROC MIXED (SAS). To correct variance heterogeneity for external eggs and J2 per g of root, internal eggs and J2 per g of root, and external plus internal eggs and J2 per g of root, the analysis was done on log transformed values, $\mathrm{Ln}(x + 1)$. Shoot height and fresh shoot weight were also analysed as one factor general linear mixed models. For these two variables, the overall F-tests and mean comparisons were statistically significant ($P \leqslant 0.05$).

For the glasshouse 65-day harvest, shoot height, fresh shoot weight, number of fruit, fresh fruit weight and dry fruit weight were analysed as one factor general linear mixed models. Mean comparisons were done using pair-wise contrasts at the $P \leqslant 0.05$ significance level. Dry shoot weight, root weight, external eggs and J2 per g of root, internal eggs and J2 per g of root, and combined external plus internal eggs and J2 per g of root were analysed as one factor general linear mixed models. Variance heterogeneity occurred in the variables that included eggs and J2 per g of root; to correct this problem the analyses were done on the ranks of the variables. For root weight, the analysis was done on the log-transformed values, Ln(value). Mean comparisons were done using pair-wise contrasts at the $P < 0.05$ significance level.

Results

In vitro inhibition assays

Extracellular factors present in culture filtrates from Gl-3 and Bc-2 inhibited egg hatch and J2 mobility. Culture filtrate from either microbe grown in PDB generally demonstrated activity against the nematode, and filtrate from Gl-3 in WG was effective after the longer incubation period (Fig. 1). Three days after eggs were added to

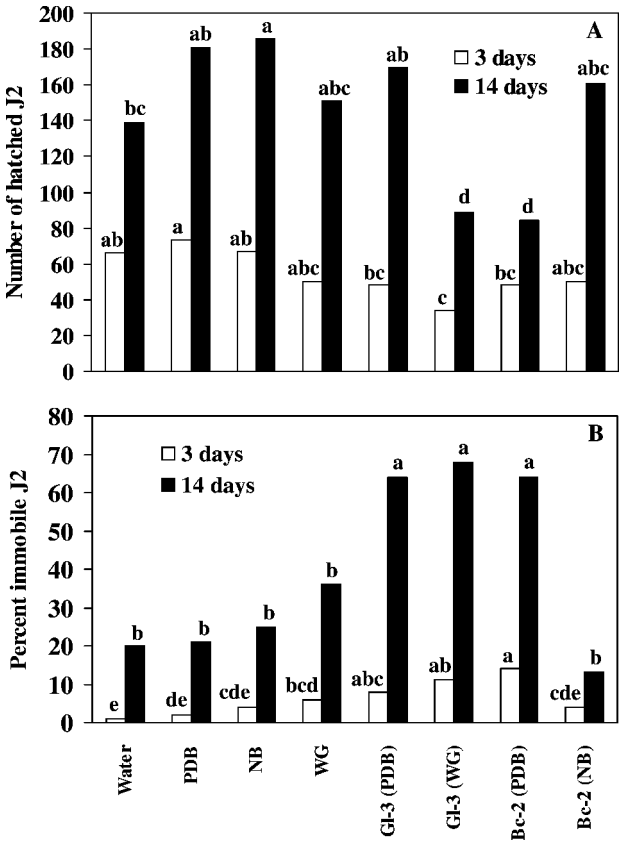

Fig. 1. In vitro *effects of filtrates from cultures of* Trichoderma virens *strain Gl-3 and* Burkholderia cepacia *strain Bc-2 on egg hatch (A) and J2 mobility (B) of* Meloidogyne incognita*. Culture filtrates were applied to* M. incognita *in multiple well tissue culture plates. Media used were PDB: potato dextrose broth; NB: nutrient broth; WG: Weindling's minimal medium with glycerol. Values are means within a well for each treatment. Percentages of immobile J2 were calculated as mean number of immobile J2/(mean number of mobile + immobile J2). For each counting day, bars with the same letter are not significantly different (*$P \leqslant 0.05$*).*

the wells, egg hatch in the culture filtrates from either Bc-2 or Gl-3 grown in PDB was 33% lower than egg hatch in the PDB control (Fig. 1A). Both of these treatments more than tripled the percentage of immobile J2 (Fig. 1B). Other culture filtrates from Bc-2 and Gl-3 did not significantly affect egg hatch or J2 mobility at 3 days compared with appropriate media controls (Fig. 1).

Fourteen days after addition of eggs to the wells, culture filtrate from Bc-2 grown in PDB still significantly decreased egg hatch, resulting in a 53% reduction compared with PDB, and filtrate from Gl-3 in WG effectively reduced egg hatch by 41% compared with the WG control (Fig. 1A). However, filtrate from Gl-3 in PDB did not decrease egg hatch (Fig. 1A). All three culture filtrates, Bc-2 in PDB, Gl-3 in WG, and Gl-3 in PDB, significantly increased the percentage of immobile J2 by 1.75 to 5 times compared with all other treatments (Fig. 1B). As at 3 days, culture filtrate from Bc-2 in NB had no effect on either egg hatch or J2 mobility, indicating that production of extracellular inhibitory factors by Bc-2 was media dependent. At 14 days, NB stimulated egg hatch compared with the water control (Fig. 1A).

Size fractionation experiments with culture filtrates from Gl-3 and Bc-2 grown in PDB were largely inconclusive at 3 days. Only exposure to non-fractionated culture filtrates resulted in significantly lower ($P \leqslant 0.05$) egg hatch than was recorded from controls at that time (data not shown). At 14 days, fewer eggs hatched in the < 3 KDa fraction from Bc-2 and the non-fractionated Bc-2 culture supernatant than in the PDB controls (Fig. 2). This indicates that the hatch inhibitory factor produced by Bc-2 cultures is of low molecular weight and possibly not an enzyme. There was no inhibitory activity in Gl-3 culture supernatants at 14 days. There was an apparent effect of size-fractionation on J2 mobility. Fractionated PDB controls increased the number of immobile J2 at 14 days (data not shown).

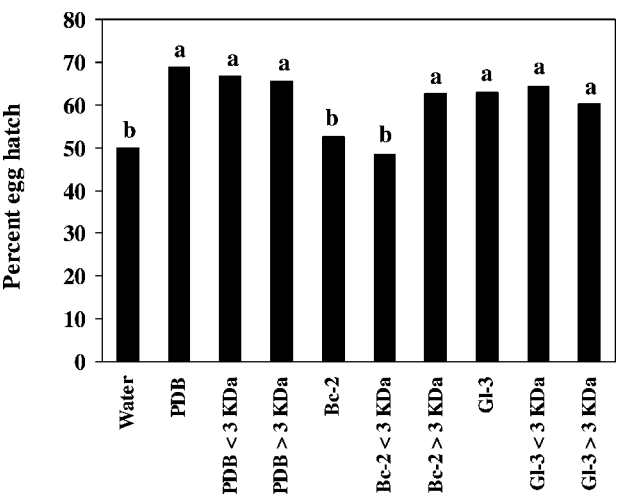

Fig. 2. *Effects on* Meloidogyne incognita *egg hatch of culture filtrates from* Trichoderma virens *Gl-3 and* Burkholderia cepacia *Bc-2 grown in potato dextrose broth (PDB), size fractionated and applied to eggs in multiple well tissue culture plates. Values are means within a well for each treatment. Bars with the same letter are not significantly different (*$P \leqslant 0.05$*).*

Enzyme activities associated with Gl-3 and Bc-2 culture filtrates

Chitinase activity was undetectable in culture filtrates from Gl-3 grown in WG, WG plus 0.2% chitin and in Weindling's medium plus 0.2% chitin. Protease activities were undetectable in culture filtrates from Gl-3 grown in PDB and in WBE. Chitinase activities were also undetectable in culture filtrates from Bc-2 grown on PDB, NB, BL + glycerol, BL + 0.2% chitin and BL + glycerol and 0.2% chitin. Protease activity was also below detectable limits in all culture filtrates from Bc-2.

Monoxenic root explant culture assays with Gl-3

Culture filtrate from Gl-3 grown in WG suppressed numbers of eggs and J2 per g of root by 42% compared with the WG control in root explant culture assays (Table 1). Additionally, this culture filtrate treatment resulted in the largest fresh root weight; the mean for this treatment was 32% greater than the water control and 38% greater than the WG control. No effects were found on root gall indices or on numbers of eggs per egg mass in root explant cultures (Table 1).

Glasshouse bioassays

At 35 days, treatments did not affect nematode populations or plant vigour. Internal numbers of eggs and J2 per g of root (inside root tissue) were highest with viable Gl-3 and non-viable Bc-2 treatments and lowest with the non-viable Gl-3 treatment (Fig. 3A). Application of non-viable Gl-3 resulted in significantly fewer eggs and J2 within root tissue (lower internal counts) than application of viable Gl-3, but none of the treatments significantly affected the internal number of eggs and J2 per g of root compared with the controls. External numbers of eggs and J2 per g root (counted from the rhizosphere and soil) were not affected by treatment and no significant differences were found when internal and external egg and J2 counts were combined (Fig. 3A). Root galling indices averaged 5 (>100 galls per root system) for each treatment. Measure-

Table 1. Meloidogyne incognita *populations and root weights from tomato root explant cultures treated with* Trichoderma virens *strain Gl-3 culture filtrate.*

Treatment	Fresh root weight (g)	Eggs + J2 per g root	Eggs per egg mass	Root gall index
WG	2.1 b	4996 a	614 a	4.3 a
Gl-3 (WG)	2.9 a	2896 b	428 a	4.4 a
Water	2.2 b	3717 ab	556 a	4.4 a

WG = Weindling's medium with glycerol; Gl-3 (WG) = culture filtrate from *T. virens* strain Gl-3 grown in WG. Numbers in columns followed by the same letter are not significantly different at $P \leqslant 0.05$. Eggs+J2 were log transformed for analysis. Means are reported in orginal units.

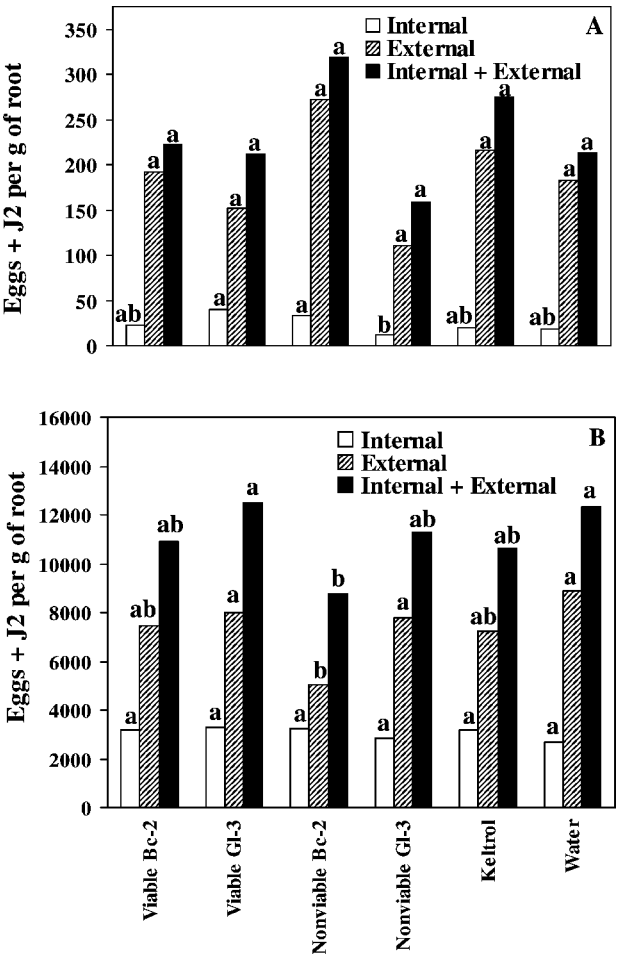

Fig. 3. *Effects of* Trichoderma virens *strain Gl-3,* Burkholderia cepacia *strain Bc-2 and a Keltrol formulation (sticker without Gl-3 or Bc-2) on* Meloidogyne incognita *egg and J2 populations on glasshouse-grown tomato at 35 (A) and 65 (B) days after transplanting. 'Internal' = eggs+J2 per g of root inside root tissue, and 'External' = eggs+J2 collected from the rhizosphere and from a soil sample, also reported per g of root. Within each category (internal, external, and internal+external), bars with the same letters are not significantly different ($P \leqslant 0.05$).*

Table 2. *Effects of* Trichoderma virens *strain Gl-3 and* Burkholderia cepacia *strain Bc-2 on vigour of glasshouse-grown tomato plants at 35- and 65-day harvests.*

Treatment	Fresh shoot weight (g)		Dry shoot weight (g)		Shoot height (cm)		Root weight (g)		Fresh fruit weight (g) (Number of fruit)
	35	65	35	65	35	65	35	65	65
Viable Bc-2	25.6 a	48.8 a	3.1 a	6.8 a	26.1 a	43.3 bc	12.3 a	34.0 ab	50.9 (4) a
Viable Gl-3	25.8 a	47.9 a	3.1 a	6.5 ab	26.8 a	42.6 bc	13.8 a	31.1 b	62.2 (5) a
Non-viable Bc-2	25.5 a	47.1 a	3.1 a	6.7 ab	26.8 a	47.1 ab	14.0 a	36.2 ab	55.8 (4) a
Non-viable Gl-3	23.1 a	52.8 a	2.9 a	7.2 a	26.1 a	50.2 a	12.1 a	37.1 ab	57.7 (4) a
Keltrol	24.5 a	55.4 a	3.0 a	5.8 b	26.6 a	46.4 ab	12.3 a	37.7 a	63.2 (5) a
Water	23.0 a	46.2 a	2.8 a	6.3 ab	26.2 a	39.3 c	13.3 a	31.0 b	70.2 (5) a

Keltrol refers to the sticker formulation containing Keltrol.
Numbers in columns followed by the same letter are not significantly different at $P \leqslant 0.05$.

ments of fresh and dry shoot weights, shoot height and root weight also showed no significant differences among treatments (Table 2).

At 65 days, non-viable Bc-2 was the only treatment that suppressed nematode populations compared with the water control. Populations were not significantly lower than populations in the Keltrol control. With the non-viable Bc-2 treatment, the external numbers of eggs and J2 per g of root, and the combined nematode counts (external plus internal eggs and J2) were suppressed 43% and 29%, respectively, compared with the water control (Fig. 3B). This result was based only on effects on the external nematode population numbers; in contrast with the 35-day results, the internal number of eggs and J2 was not affected by any treatment (Fig. 3). At 65 days, root gall indices averaged 5 per treatment. Fresh shoot weight, numbers of fruit, fresh fruit weight and dry fruit weight (latter not shown) were also not affected by treatment (Table 2). However, dry shoot weight, shoot height and root weight did vary with treatment. Dry shoot weights were largest with the viable Bc-2 and the non-viable Gl-3 treatments and shoot height was greatest with the latter treatment, with a 28% increase compared with the water control. Root weight was greatest with the Keltrol control treatment, showing a 21-22% increase compared with viable Gl-3 and water control treatments.

When sampling was conducted for the presence of Bc-2 and Gl-3 after the 65-day harvest of the second trial, there was no indication that the microbes were present above background levels.

Discussion

B. cepacia strain Bc-2 and *T. virens* strain Gl-3 can produce substances in culture filtrates that significantly inhibit egg hatch and J2 mobility of *M. incognita* in *in vitro* assays. These assays also demonstrated that the medium used for culturing the microbes affected production of inhibitory compounds, as has been recorded with other microorganisms, including a different strain of *T. virens* (Roberts & Lumsden, 1990). The lack of detectable chitinase and protease activity and suppression of egg hatch with low molecular weight fractions (<3 KDa) of Bc-2 culture filtrate, suggest that the suppressive activity in Bc-2 culture filtrate was primarily non-enzymic in nature. Egg hatch and J2 mobility *in vitro* were probably affected by antibiotic production; antibiotics active against other microbes have been identified from both organisms (*e.g.*, Lumsden *et al.*, 1992; Howell *et al.*, 1993; El-Banna & Winkelmann, 1998; Kang *et al.*, 1998). *T. virens* acts against certain plant pathogenic fungi through antibiotic production, although enzyme production may enhance activity of other metabolites (Jones & Hancock, 1988; Roberts & Lumsden, 1990). However, other isolates of *T. virens*, like Gl-3, produced low to non-detectable levels of chitinase and low levels of protease under similar conditions (Roberts & Lumsden, 1990). Yet to be determined is whether enzyme production is different in the soil than it is in the *in vitro* cultures used in the current study. Despite *in vitro* suppressive activity, viable preparations of these microbes were not active against root-knot nematode in the tomato rhizosphere. The current results indicate that they did not colonise the tomato rhizosphere, did not produce antagonistic compounds in sufficient quantity

to affect the nematodes or did not produce the suppressive compounds under the conditions of the glasshouse test. Production of nematode-antagonistic compounds *in vitro* does not necessarily mean that such compounds will be produced in the soil.

Non-viable Bc-2 demonstrated some activity against root-knot nematode on tomato, although the viable treatments did not suppress nematode populations. Culture conditions used for producing the biomass may have stimulated production of inhibitory compounds. It is possible that suppressive compounds were broken down by the viable bacterium on the tomato roots and consequently did not suppress nematode populations.

Eggs and J2 external to the root tissue were affected by the non-viable Bc-2 formulation, while eggs and J2 protected by root tissue were not. This result has been previously observed with interactions between viable biocontrol fungi and *Meloidogyne* spp. on tomato and cantaloupe (Leij & Kerry, 1991; Leij *et al.*, 1992; Meyer, 1999) and with a non-viable biocontrol fungus formulation (Meyer, 1999). In the current study, the suppressive compounds produced by the bacterium presumably did not penetrate root galls to reach the nematodes within.

The results of the glasshouse experiment do not indicate that viable *T. virens* or *B. cepacia* are active in the tomato rhizosphere, at least under the test conditions. Similarly, Noel (1990) reported that *B. cepacia* was not effective against *Heterodera glycines* Ichinohe on soybean. *T. virens* seldom has been studied as a management agent for nematodes, but, in a previous study with cotton, seed treatment with *T. virens* also did not reduce *M. incognita* reproduction (Zhang *et al.*, 1996). Conversely, non-viable Bc-2 did suppress nematode numbers in the current glasshouse test, compared with the water control. The production and use of nematicidal natural products, rather than application of a living bacterium, is of particular interest because of possible health risks associated with use of some strains of *B. cepacia* (Holmes *et al.*, 1998). However, the low suppressive effect of Bc-2 in this study indicates that this strain may not be a likely candidate for intensive future investigations with root-knot nematode on tomato.

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