Trichoderma spp. as Biocontrol Agents of Rhizoctonia Damping-off of Radish in Organic Soil and Comparison of Four Delivery Systems

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ABSTRACT

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Two hundred twenty-five isolates of *Trichoderma* were tested in the greenhouse as antagonists for biocontrol of *Rhizoctonia solani* on radish. Isolates were obtained from organic and mineral rhizosphere soils of radish, from organic and mineral surface soils, by baiting techniques, or from other laboratories. Seven isolates were chosen for use in two field studies in 1983, and three isolates were used in four 1984 field studies. At one location in 1983, two isolates of *T. hamatum* resulted in disease indices of 34 and 43, compared with 82 in the control. In 1984, the same two isolates resulted in disease indices of 23 and 25, compared with 52, 57, and 58 in controls. Four delivery systems were evaluated at two locations for applying antagonists in the field: fluid drilling a gel matrix containing seed and a 5-day-old liquid culture of antagonist produced on a shake table; coating ungerminated conidia onto seed with 2% methylcellulose before planting; applying germinated conidia in a wheat bran/sand preparation to the seed furrow before planting; and drenching the furrow before planting with a 5-day-old liquid culture of the antagonist produced on a shake table. The fluid drilling technique was superior to the others in both 1983 and 1984 Hartville field studies. Isolates of *Trichoderma* obtained from organic soils were better antagonists in organic soil field trials than those from mineral soils. Many biocontrol treatments provided better control of Rhizoctonia damping-off than treatment with pentachloronitrobenzene at 5.3 kg a.i./ha in 1984 tests.

Additional key words: gel seeding, *Raphanus sativus*.

Damping-off caused by *Rhizoctonia solani* Kühn is economically important in some seasons on radish (*Raphanus sativus* L.) grown in organic soils. Although fungicides are available for control of *R. solani*, these have not been successful in organic soils because of adsorption of these compounds to the abundant organic matter (26).

Use of microorganisms as antagonists of *R. solani* has been investigated as an alternative control method, and several examples of bacterial and fungal biological control agents have been reported (3,6,19,25,31). *Trichoderma* spp. have been shown to control *R. solani* on a variety of crops in greenhouse (3,4,16,18,19,31) and field studies (4,11,12), and on radish in several greenhouse studies (2,18,19,20,25,28,31,35). Baker (2) and others (20,35), under controlled conditions, have developed soils suppressive to *R. solani* through monoculture of radish, and have correlated suppressiveness with high population densities of *Trichoderma* spp. Also, Kuter et al (25) and Nelson and Hoitink (28) have correlated high population densities of *Trichoderma* with suppression of *R. solani* in container media amended with composted hardwood bark. Effective control of seed decay and damping-off caused in radish by *R. solani* and Pythium spp. has been obtained with *Trichoderma hamatum* (Bon.) Bain applied as a seed treatment in greenhouse studies (18,19). Henis et al (20) have suggested successive plantings, pentachloronitrobenzene (PCNB), and *T. harzianum* Rifai as an integrated approach to control of *R. solani* damping-off of radish. Others also have suggested *Trichoderma* spp. as a component in integrated control systems for *R. solani* (12,24,31).

Once successful antagonists are identified, practical delivery systems must be developed. Delivery systems commonly used in previous research involved coating seed with conidia of *Trichoderma* (5,11,18,19,31,34) or applying conidia in wheat (*Triticum aestivum* L.) bran preparations (4,12,16,34). Gel seeding or fluid drilling, a technique by which pregerminated seed are extruded into soil in a fluid matrix, also has been used as a delivery system for biological control agents (6,7,13,15,17). Various other antagonist delivery systems also have been suggested (1,14,22,30).

This study was initiated to investigate the use of *Trichoderma* spp. as biocontrol agents of *R. solani* on radish grown in organic soils. *Trichoderma* spp. were chosen as potential antagonists because of their previous successful use as biological control agents against *R. solani* and because of their optimal growth at pH 5.0–5.5 (3,8,17,19) which is the characteristic pH of Ohio organic soils. Goals of this study were to collect isolates of *Trichoderma* spp. from radish-production soils, to test them as potential antagonists of *R. solani* in greenhouse and field trials, and to evaluate four delivery systems for introduction of the antagonists into field soils.

MATERIALS AND METHODS

Isolate collection. Isolates of potentially antagonistic *Trichoderma* spp. were obtained by serial dilution from organic soil (Rifle peat: 15% silt, 1% fine sand, 9% clay, 75% organic matter, pH 5.4) collected from the upper 8 cm of commercial radish fields, from rhizosphere soil of radishes grown in these fields, or from various mineral soils. Media used for dilutions were acidified (pH 4–5) potato-dextrose agar; peptone-dextrose agar containing rose bengal, streptomycin sulfate, and formalin (27); or a medium selective for *Trichoderma* (10).

Other potential antagonists were acquired by using a baiting technique. *Rhizoctonia solani* AG-4 (isolate 1), isolated from a radish hypocotyl, was maintained on malt-extract agar (MA) at 20–25 C and used throughout this research. Inoculum of *R. solani* was prepared from an air-dried, chopped, potato-soil mix described by Ko and Hora (23). Their procedure was modified by

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grinding the mixture with a mortar and pestle and passing it through 1-mm and 2-mm sieves to obtain inoculum pieces 1–2 mm in diameter (28). Sacks of white linen material (12 × 9 cm), were filled with 2–3 g of this inoculum as a bait and stitched closed with monofilament nylon fishing line. Twenty sacks were buried in 30-cm-diameter pots (five sacks per pot) containing Rifle peat soil collected from commercial radish fields at Celeryville, OH. Pots were kept in a greenhouse at 18–24 C under a 12-hr photoperiod and watered daily. After 1 wk, one sack was removed from each pot; the remaining sacks were individually removed at 5-day intervals thereafter. Contents of each sack were placed into 100 ml of sterile 0.1% water agar (WA), and serial dilutions were made and plated on the medium selective for *Trichoderma*. Colonies of *Trichoderma* spp. were subcultured after 1–2 wk on MA. All cultures of *Trichoderma* spp. were identified by using the system of Rifai (33). Several cultures of *Trichoderma* spp. used in these tests were obtained from other laboratories.

Screening potential antagonists. Two hundred twenty-four isolates of *Trichoderma* spp. were tested in the greenhouse for antagonism to *R. solani*. They were grown on either MA or molasses yeast agar (20 g of molasses, 5 g of dried brewer's yeast, 20 g of agar; and 1 L of water) (MYA) at 20–25 C under continuous fluorescent light. After 7–10 days, conidia were removed in 5–7 ml of sterile distilled water by agitation with a sterile bent glass rod and then poured into a sterile test tube and agitated for approximately 15 sec with a vibrating agitator. Conidial concentrations were determined with a hemacytometer and sterile distilled water was added to bring concentrations to 3×10^6 conidia per milliliter. Four milliliters of each conidial suspension was added to individual 40-g quantities of a wheat bran/sand mixture previously prepared by mixing wheat bran:sand:water (1:1:2, w/w) in a 50-ml glass beaker and autoclaving it for 1 hr on two successive days.

The infested wheat bran/sand mixture was incubated for 2–3 days at 20–25 C and then mixed thoroughly with approximately 2,000 cm^3 of soil infested with *R. solani*. The soil infested with *R. solani* was prepared by combining (in a twin-shell blender) inoculum of *R. solani*, prepared as previously described, with steamed mineral potting soil (soil:peat, 5:1, v/v) at a rate of 1:1,000, inoculum:soil (v/v). The final mixture of potential antagonist, wheat bran/sand, inoculum of *R. solani*, and soil was placed in four 10-cm-diameter pots per isolate in which 32 radish (cultivar Scarlet Knight) seeds were planted. Steamed soil with and without the addition of uninfested wheat bran/sand mixture were used as controls. The treatments were randomized on benches within four replicate blocks. Plants were harvested after 2 wk of growth in the greenhouse at 18–24 C under a 12-hr photoperiod. All seedlings were uprooted, and hypocotyls were evaluated for infection by *R. solani* on a scale from 1 to 4: (1 = symptomless, 2 = small lesions with no hypocotyl constriction, 3 = large lesions with some hypocotyl constriction, and 4 = hypocotyl girdled). These ratings were converted to a disease index (DI) value for each pot by using the formula

$$DI = 32(1A + 2B + 3C + 4D)/N \quad (1)$$

in which A, B, C, and D represent the number of seedlings rated as 1, 2, 3 or 4, respectively; 32 = the number of seeds planted; and N = the number of seedlings rated after 2 wk. Possible disease indices ranged from 32 to 128. These data were analyzed by ANOVA and LSD mean separation tests.

Growth of antagonists on various gels. After several promising antagonists were identified in greenhouse tests, the suitability of several methods for delivering inocula of these organisms into field soil were investigated. Tests were conducted to ascertain the ability of *R. solani* and *T. hamatum* to grow on a variety of gels used in fluid-drilling systems (15). Selected gels (4% Natrosol, 2% Laponite 508, and 1% Viterra 332) were prepared with sterile distilled water in petri plates in concentrations that would approximate the consistency of 2% WA. Laponite 508 (Laporte [USA] Inc.; 411 Hackensack Ave., Hackensack, NJ) is a synthetic magnesium silicate; Natrosol (Hercules Inc.; 910 Market St., Wilmington, DE) is a hydroxyethyl cellulose; and Viterra 332 (Nepera Inc.; Rt 17, Harriman, NY) is a potassium propenoate-propenamide copolymer. Water agar (2%) was used as a control. *Rhizoctonia solani* and *T. hamatum* (isolate 44) were grown 2 wk on MA at 20–25 C under continuous fluorescent light. Mycelial disks (5-mm diameter) were removed from plates and placed on the gels and on the WA control. Each treatment was replicated five times. Plates were incubated at 20–25 C. Colony radii were recorded after 1, 3, 4, 5, 6, and 10 days of growth.

Seed coating study. To investigate the viability over time of conidia of *Trichoderma* coated onto radish seed with methylcellulose, *T. hamatum* (isolates 4, 8, 12, 17, and 44), *T. harzianum* (isolate WT-6), and *T. koningii* Oud. (isolate T8) were grown on MA for 2 wk at 20–25 C under continuous fluorescent light. Conidial suspensions (2.9×10^8 conidia per milliliter) were prepared as previously described and conidia of each isolate were bound to seed by coating 10 g of radish seed with a mixture of 1 ml of the conidial suspension and 1 ml of 2% methylcellulose. Final concentration of conidia per seed was approximately 2.7×10^6. Methylcellulose-coated seed without *Trichoderma* and uncoated seeds were used as controls. Coated seeds were air-dried overnight at 20–25 C, and then stored in the dark in envelopes at 20–25 C. Thirty seeds per treatment were placed on MA plates (10 seeds per plate) every week for 12 wk. Plates were observed after 1 wk and each treatment was evaluated for recovery of *Trichoderma*.

Field studies in 1983. During the summer of 1983, two field tests were conducted in organic soil on commercial vegetable farms located at Celeryville and Hartville, OH. Tests were designed to evaluate seven isolates of *Trichoderma* spp. that had been selected as potential biocontrol agents of *R. solani* in greenhouse bioassays. Five of these were *T. hamatum* (isolates 4, 8, 12, 17 and 44) obtained from the rhizosphere of radish in organic soil. The others were *T. harzianum* (isolate WT-6, originally isolated from soil in southern Georgia) and *T. koningii* (isolate T8, originally isolated from soil in New York) obtained from J. A. Lewis (USDA, ARS, Soilborne Diseases Laboratory, Beltsville, MD) and G. E. Harman (New York State Agric. Exp. Stn., Geneva), respectively. A second goal of these field studies was to evaluate the usefulness of three antagonist delivery systems: fluid drilling seed and 5-day-old liquid cultures produced on a shake table containing conidia, hyphal fragments, and chlamydospores in molasses yeast broth (20 g of molasses, 5 g of dried brewer's yeast, and 1 L of water) (MYB); application of pregerminated conidia in a moistened wheat bran/sand mixture to soil at the time of planting; and coating seed with ungerminated conidia 24 hr before planting. Radish cultivar Scarlet Knight was used in all field tests.

Inocula for field studies. Inocula of *Trichoderma* used in fluid drilling were produced by growing each isolate on MYA at 20–25 C under continuous fluorescent light for approximately 2 wk. Five to six 5-mm-diameter plugs of mycelia and conidia from MYA plates of each isolate were used to infest 1 L of MYB. Cultures were incubated on a shake table for 5 days at 25 C under constant fluorescent light. On the day of planting, each culture was poured into a 19-L bucket, and the final volume was brought up to 7.6 L with tap water. One liter of sterile MYB and 1 L of distilled water were each treated in the same manner as controls.

Into each 7.6-L of solution, 76 g of Natrosol gel was added to form a 1% gel. Just before fluid drilling, seeds were added to each gel treatment and mixed thoroughly. At Hartville, approximately 300 g of seeds were added to 7.6 L, giving approximately 80 seeds sown per meter of row. At Celeryville, this amount was reduced to 225 g per 7.6 L, resulting in approximately 60 seeds per meter of row. Gel treatments were sown with a tractor-drawn fluid drill pulled at 3–5 km/hr. The fluid drill used a peristaltic pump to extrude the gel matrix from a reservoir through flexible tubing into a shallow furrow in the soil. The gel seeder was flushed thoroughly with water between treatments.

Wheat bran/sand mixtures were prepared similarly to those used in greenhouse studies by combining 200 g of wheat bran, 200 g of sand, and 320 ml of distilled water. After mixing, wheat bran/sand was placed in a 1,000-ml beaker and autoclaved 1 hr on each of 2 successive days. Isolates of *Trichoderma* were grown on MYA for approximately 2 wk at 20–25 C under continuous fluorescent light

and conidial suspensions were prepared as before and adjusted to 3.4×10^6 (Hartville) or 2.8×10^6 conidia per milliliter (Celeryville). Eighty milliliters of each conidial suspension were used to infest a 720-g wheat bran/sand preparation. As a control, 80 ml of sterile distilled water were added to a 720-g wheat bran/sand mixture. These cultures were incubated for 2–3 days at 20–25 C, then each was added to 4 L of steam-disinfested mineral potting soil (soil plus peat, 5:1, v/v) and mixed thoroughly to increase the bulk amount of inoculum.

Seed furrows were opened by hand to a depth of 2–3 cm, and each wheat bran/sand soil mixture was evenly distributed by hand over 30 m of open furrow (258 kg/ha). Untreated radish seed were then sown over the wheat bran/sand preparations with a hand-pushed seed drill.

Using techniques described previously, radish seeds were coated with methylcellulose and ungerminated conidia 24 hr before planting, Concentrations of conidial suspensions were 1.7×10^8 (Hartville) and 2.6×10^8 conidia per milliliter (Celeryville). Seeds coated with a 1% methycellulose solution and uncoated seeds were used as controls. Seeds were sown with a hand-pushed seed drill.

Experimental design. Because these plots were placed in commercial radish fields, where space was limited to long narrow strips, and due to physical constraints of the tractor-drawn gel seeder, all treatments consisted of single rows 30 m long planted in random order. Rows were spaced 15 cm apart for a total plot width of approximately 4 m. Rows were subdivided into four 7.6-m sections, and portions of each section served as replications for each treatment. The field trial at Hartville was planted the week of 25 July, and the one at Celeryville the week of 15 August.

The first 30 plants were uprooted from each replication 2 wk after planting, rated on a scale from 1 to 4 as previously described, and disease indices were calculated by using the formula:

$$DI = 1A + 2B + 3C + 4D \quad (2)$$

in which A, B, C, and D represent the number of seedlings rated as 1, 2, 3, or 4, respectively. Possible disease indices ranged from 30 to 120. All data were statistically analyzed by using a one-way ANOVA and means were separated with LSD tests. A linear contrast comparison test was done on disease indices to compare the three application methods.

Field studies in 1984. Two field tests were conducted in 1984 at Celeryville (Celeryville I and II) and two at Hartville (Hartville I and II) on the same farms as before. In these studies, three isolates of *Trichoderma* spp. and three application methods were field-tested in organic soil. Of the three isolates of *T. hamatum* used, two (isolates 17 and 44) had been used in 1983, and the other (isolate 199, isolated directly from organic soil) appeared to be a very promising antagonist in greenhouse bioassays the previous winter. Application methods used in the 1984 field studies were similar to those used in 1983, except that culture nutrients were removed from preparations of antagonists before use. Delivery systems used were: fluid drilling of seed and washed liquid cultures produced on a shake table; drench application of washed liquid culture produced on a shake table to the seed furrow prior to planting; and coating radish seed with ungerminated conidia in methylcellulose 5 days before planting.

Each isolate was grown on MYA at 20–25 C under continuous fluorescent light for approximately 2 wk before use. Antagonist substrate for fluid-drilling and drench application were prepared from a conidial suspension (2×10^6 conidia per milliliter) as previously described. Five milliliters of this conidial suspension were transferred to 500 ml of MYB. Three 2-L flasks, each containing 500 ml of MYB, were used for each isolate. Cultures were grown under continuous fluorescent light at 25 C on a shake table for 5–6 days. Cultures of each isolate were fragmented in a blender for 1 min, resulting in a suspension containing conidia, hyphal fragments, and chlamydospores in MYB. Suspensions were centrifuged at 10,000 g for 5 min, the supernatent decanted and the pellet resuspended in deionized water. This mixture was blended again and washed, and the final pellet resuspended in 550 ml of deionized water. Three 1-ml samples of each suspension were dried in a drying oven at 72 C for 1–2 wk, and then weighed to insure that the relative amount of antagonist suspension added was similar in each case. Four hundred milliliters of suspension was added to 7.2 L of tap water, to give a total volume of 7.6 L. When applied to the field with a fluid drill, this gave a rate of approximately 100 ml of suspension per 30 m row (35.8 L/ha). To obtain a 1% gel solution, 76 g of Natrosol were added to each 7.6 L of mixture. As a control, 7.6 L of a 1% Natrosol solution were made by adding 400 ml of deionized water in place of the antagonist suspension.

For drench application of liquid suspension into the seed furrow, a hose with a double valve system was attached to a 4-L metal container fitted onto a hand-pushed seed drill. The delivery rate of suspension diluted with water was 1.9 L/30 m of row in the first Hartville test, and 1.3 L/30 m of row in the latter three field trials. In each, approximately 100 ml of actual antagonist suspension was distributed over 30 m of row (35.8 L/ha). As a control, water was applied as a drench in the same manner.

The same conidial suspensions that were used to prepare MYB suspensions for the gel and drench treatments also were used to coat radish seed. Radish seeds were coated, using techniques previously described, with ungerminated conidia in 1% methylcellulose at 2×10^5 conidia per seed 5–6 days before sowing. On the day of planting, 50 seeds from each methocellulose-coated treatment were placed on MA (10 seeds/plate) to check viability of the conidia of *Trichoderma* spp. Seed coated with 1% methylcellulose and uncoated seed were used as controls.

PCNB (75% WP) was used as a fungicide control for comparison in all four tests. The fungicide was diluted with water and drenched over the previously-seeded row with a sprinkling can in a 5–8 cm band at a rate of 15 g a.i./30 m of row (5.4 kg a.i./ha).

Because of the same limitations encountered in commercial radish fields in 1983, all treatments were again single rows 30 m long and 15 cm apart planted in random order. Rows were subdivided into five, 6.1-m sections, and portions of each section served as replications for each treatment. Planting dates of field trials were: Hartville I, 7 May; Celeryville I, 11 May; Celeryville II, 17 May; and Hartville II, 22 May.

Plants were drenched with diazinon (0.3 g a.i./L) when the first true leaves developed and 1 wk after for root maggot (*Hylemya brassicae* Wiedemann) control. Plants also were sprayed with carbaryl (2 g a.i./L) when needed for flea beetle (*Phyllotreta* spp.) control.

Sampling plots for natural populations of *R. solani* and *Trichoderma* spp. Just before planting, a grid pattern 6.1 m × 1.1 m was laid out at each field plot location. At the intersection of each grid line, a soil sample was taken (800–1,000 cm^3) from the upper 5–8 cm of soil. Twenty-four soil samples taken from each field were assayed to determine the spatial pattern of natural populations of *R. solani* and *Trichoderma* spp. within the plot area.

A modification of a beet (*Beta vulgaris* L.) seed colonization technique (29) was used to determine the relative concentration of propagules of *R. solani* in each soil sample. Fifty autoclaved beet seeds each were recovered from 100-g-soil samples incubated for 2 days at 21 C and plated on WA amended with metalaxyl at 50 μl/ml. Chi-square analysis of the variance-to-mean ratio of the number of colonized seeds in each sample was used as an index of dispersion to determine if the spatial pattern of *R. solani* was random throughout the field plot. Only typical colonies of *R. solani* were counted. A nuclear staining procedure (21) was used to determine the nuclear condition of several isolates of *Rhizoctonia* obtained from radish hypocotyls and organic soil.

To assay soil samples for naturally occurring populations of *Trichoderma*, 10 g (fresh weight) of soil were added to 100 ml of 0.1% WA. This was agitated, diluted 1:10 with 0.1% WA, and 0.5 ml of the suspension were plated (0.1 ml/plate) on each of five plates containing the medium selective for *Trichoderma* spp. Cultures were incubated at 20–25 C for 1 wk in the dark, and then for 1 wk in continuous fluorescent light. Following incubation, plates were observed and colonies of *Trichoderma* spp. were counted and recorded. Chi-square analysis of the variance-to-mean ratio of the colony counts from each sample was used to determine

if the spatial pattern of *Trichoderma* spp. was random throughout the field plot.

At Hartville, a recording thermometer with remote probes was used in 1984 to record soil temperatures at 5- and 13-cm depths during both tests.

For each field trial in 1984, data were collected after 2 wk. The first 15 plants from each treatment replication were uprooted and rated on a scale from 1 to 5: (1 = symptomless; 2 = small lesions, no constriction of hypocotyl; 3 = larger lesions, no hypocotyl constriction; 4 = large lesions with hypocotyl constriction; and 5 = hypocotyl girdled). Disease indices were calculated by using the formula:

$$DI = 1A + 2B + 3C + 4D + 5E \quad (3)$$

in which A, B, C, D, and E represent the number of seedlings rated as 1, 2, 3, 4, or 5, respectively. Possible disease indices ranged from 15 to 75. All data were statistically analyzed by using a one-way ANOVA and LSD mean separation tests. A linear contrast comparison test was done on disease index values to compare the three application methods.

RESULTS

Screening potential antagonists. Of the 225 isolates of *Trichoderma* tested in the greenhouse as potential antagonists of *R. solani*, only 15% effectively controlled Rhizoctonia damping-off of radish. Of these, 25 isolates were obtained directly from organic soil; six and one isolate(s) came from rhizosphere isolations from organic and mineral soils, respectively; and two isolates were obtained from other laboratories. The baiting technique did not yield any highly effective isolates. Among the most effective antagonists found, 30 isolates of *T. hamatum* were selected from 142 isolates tested; two isolates of *T. harzianum* from 52 tested; and two isolates of *T. koningii* from 22 tested. Nine isolates were of other species.

T. hamatum isolate 44 grew well on Natrosol gel, and less well on Laponite 508 and Viterra 332 (Fig. 1). Growth of *R. solani* on Natrosol was as good as its growth on the WA control, but it grew much more slowly on Viterra 332, and Laponite 508 had a fungistatic effect (Fig. 1).

Two weeks after radish seed were coated with conidial suspensions, all coated seed showed abundant growth of *Trichoderma* when placed on MA. After 2 wk, the survival of *Trichoderma* declined, with considerable variability among isolates (Fig. 2). *T. koningii* isolate T8 survived in a radish seed coating up to 12 wk. *T. hamatum* (isolate 4), on the other hand, showed little or no viability after only 2 wk. No recovery of *Trichoderma* was observed from seed coated with methylcellulose alone or from untreated controls.

Field studies in 1983. The best treatment in 1983 at Hartville 2 wk after planting was *T. hamatum* isolate 17 applied with the fluid drilling technique (Fig. 3A). The disease index in this treatment was 42, compared with 77 in the fluid drilling control, and 59 in the untreated control. This was the only treatment that performed significantly better than the untreated control. The fluid drilling control, that received both gel and broth, was the worst treatment, probably indicating a stimulation of the pathogen with added nutrients.

At Celeryville, several treatments resulted in less Rhizoctonia damping-off than the untreated control (Fig. 3-B). Isolates added to soil by fluid drilling controlled the disease significantly better than other application methods. The best three treatments (*T. hamatum* isolates 44, T8, and 17) applied with the fluid drilling technique had disease indices of 34, 37, and 43, respectively, compared with 82 in the untreated control.

Comparison of 2-wk data with respect to application techniques at both field locations in 1983 showed that fluid drilling was significantly better than the other two application methods, and the coated seed was intermediate (Table 1).

Field studies in 1984. In 1984 field studies, it was confirmed, after oven drying a sample of antagonist suspension used in the gel and drench treatments, that approximately the same biomass of *Trichoderma* was added per isolate in each test. It was calculated that 100 ml of antagonist suspension per 30 m row was approximately 0.04 g dry matter per meter of row (14.3 kg/ha). In each field study, all seed coated with *T. hamatum* and tested on the day of planting had viable conidia present on the seed coats. None of the methylcellulose-coated controls tested had viable conidia of *Trichoderma*.

Naturally-occurring population densities of *Trichoderma* in 1984 field trials ranged from an average of 6.9 to 38.9 propagules per gram of oven-dried soil. Chi-square analysis ($P = 0.05$) of the variance-to-mean ratio indicated that populations of *Trichoderma* had a random spatial pattern throughout all field plot locations. Although population densities varied among fields, this did not affect results. Using a beet seed colonization technique (28),

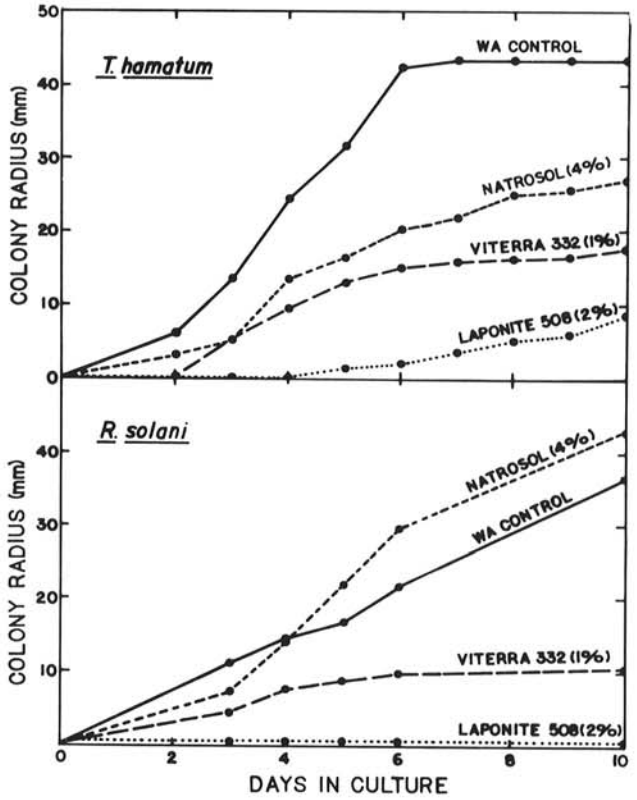

Fig. 1. Growth of *Trichoderma hamatum* (isolate 44) and *Rhizoctonia solani* (isolate 1) in vitro at 20–25 C on various gels used in fluid drilling and on water agar.

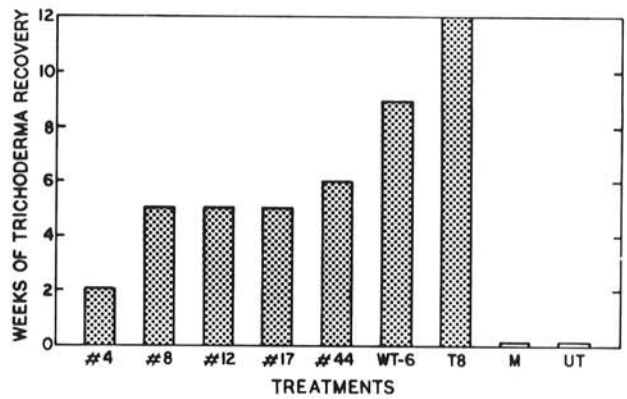

Fig. 2. Survival of conidia of *Trichoderma* coated onto radish seed with methylcellulose as indicated by weekly recovery on malt extract agar. Isolates 4, 8, 12, 17, and 44 = *T. hamatum*; WT-6 = *T. harzianum*; T8 = *T. koningii*; M = methylcellulose without *Trichoderma*; and UT = untreated seed.

numbers of seeds colonized by *R. solani* in soil samples from 1984 field trials ranged from an average of 38.7 to 44.0 per 50 seed. Chi-square analysis ($P = 0.05$) of the variance-to-mean ratio of these data indicated that populations of *R. solani* also had a random spatial pattern throughout all field plot locations. Several isolates of *R. solani* obtained from radish hypocotyls and organic soil were shown by using a nuclear staining procedure (21) to be multinucleate .

The first and second Hartville field trials were planted when soil temperatures were 10–13 C and 17–19 C, respectively. During the tests, there was a general warming trend in both cases.

In the first Hartville field trial, one isolate of *T. hamatum* (isolate 17) applied in a gel matrix was significantly better in control of *R. solani* than the untreated controls when evaluated 2 wk after planting (Fig. 3C). This treatment had a disease index of 24 as compared with 35, 40, and 40 in the untreated controls. All gel treatments were more effective than the PCNB control.

Isolates 44 and 199 of *T. hamatum* applied in a gel matrix were the only two treatments that resulted in significantly less disease than the untreated controls in the Hartville II field trial (Fig. 3-D). Several treatments were better than PCNB. Gel-seeded treatments of *T. hamatum* resulted in lower disease indices (21, 27, and 31) than any of the untreated controls (39, 39, and 49). Overall, the fluid drilling technique was the best application method in both Hartville field trials (Table 1).

None of the treatments in either of the 1984 Celeryville field trials provided significant control of Rhizoctonia damping-off when evaluated 2 wk after planting (Table 1).

DISCUSSION

Among species of *Trichoderma* recovered from various soils throughout this study, *T. hamatum* was most abundant in organic soil from radish rhizospheres, and in mineral soil. *T. harzianum* was isolated most frequently from mineral soil from radish rhizospheres and with the baiting technique. Others also have found *Trichoderma* spp. to vary in adaptability to different soil types (8,9).

The antagonistic ability of isolates of *Trichoderma* is highly variable (4,5), as was shown in this study in which only 15% of the isolates tested were effective in controlling Rhizoctonia damping-off in greenhouse bioassay tests. In greenhouse bioassays, more effective antagonists were identified as *T. hamatum* than as *T. harzianum*, and those isolates recovered from organic soil generally were superior to those from mineral soils.

The best isolates in the 1983 field trials were those obtained from the rhizospheres of radish in organic soil, especially isolates 17 and 44 of *T. hamatum*. *T. harzianum* isolate WT-6 and *T. koningii* isolate T8, originally isolated from mineral soil in Georgia and New York, respectively, were less effective. Antagonists often have been shown to perform more effectively if used in soil from which they were isolated (17). This was also the case in our studies.

The development of practical and efficient delivery systems for biocontrol agents is equally as important as the identification of superior antagonists. Delivery systems must be economical, easy to use, and adaptable to farm machinery. Coating seed with conidia has been widely tested as a potential delivery system for antagonists

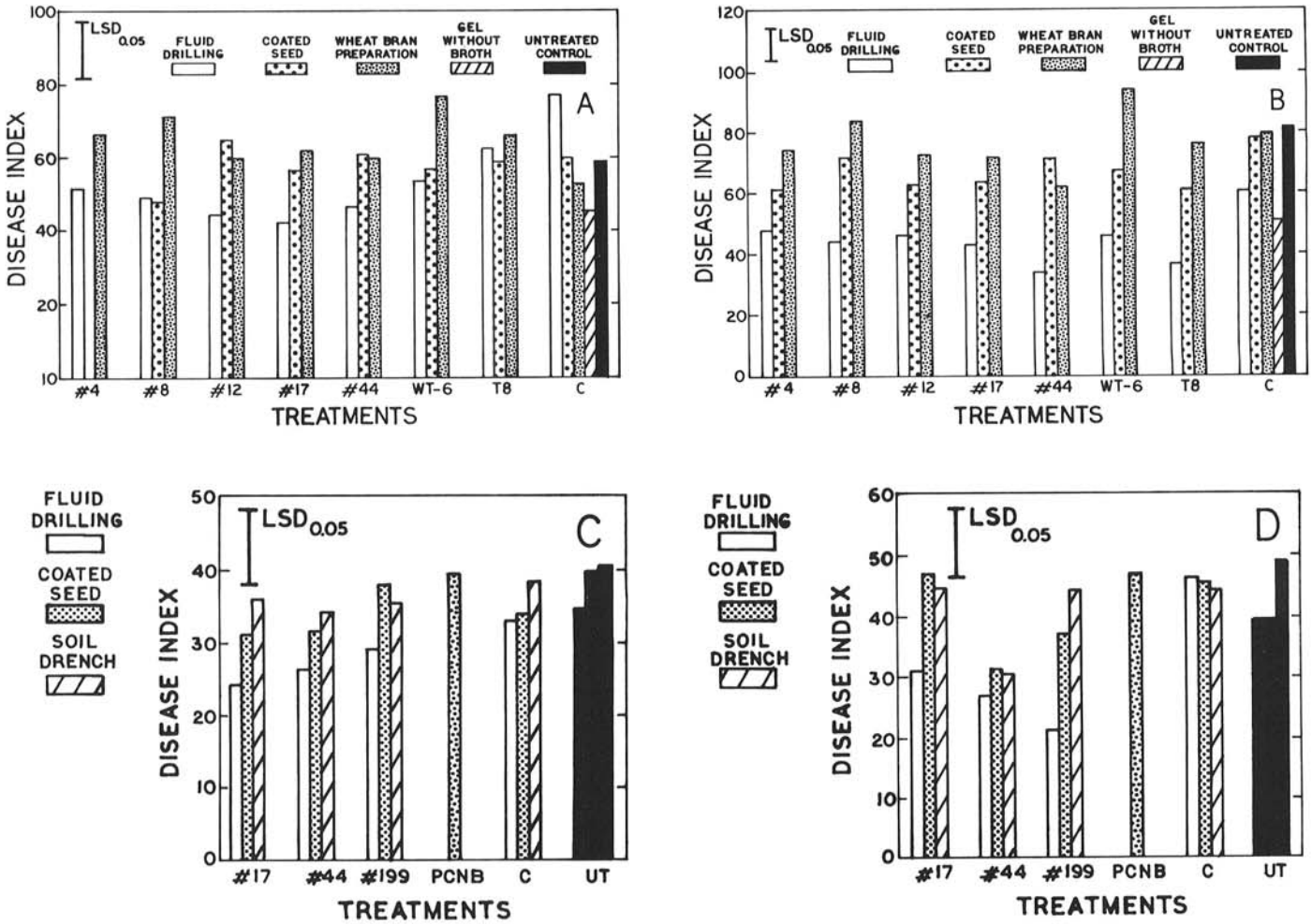

Fig. 3. Effects of isolates of *Trichoderma hamatum* applied in four different ways to organic soil on commercial vegetable farms at two Ohio locations to control Rhizoctonia damping-off of radish evaluated 2 wk after planting. **A,** Hartville 1983; **B,** Celeryville 1983; **C,** Hartville 1984, test 1; and **D,** Hartville 1984, test 2). Isolates 4, 8, 12, 17, 44 and 199 = *T. hamatum*; WT-6 = *T. harzianum*; T8 = *T. koningii*; PCNB = pentachloronitrobenzene; C = delivery systems without *Trichoderma*; and UT = untreated controls. See text for procedure for calculating disease index.

(5,11,18,19,31,34). In our study, survival of conidia coated onto radish seed varied among isolates of *Trichoderma* spp. Differences were probably due to natural isolate variation and not nutrition or numbers of conidia, because these variables were held constant. Variability in conidial survival among certain isolates suggests that this type of inoculum may not store well for longer than 2 wk. Further studies are needed in which various storage conditions and binding materials are compared with survival of conidia or other propagules coated onto seed.

Fluid drilling has several advantages as a means of applying biocontrol agents to soil (6,7,13,15,17). The volume of antagonist suspension used in fluid drilling is less than either in-furrow or broadcast treatments (17) and fluid drilling can establish a biological control agent directly in the infection court of the host (15). Another possible advantage of fluid drilling is that the gel itself may be a food source for the antagonists (17). Growth of *T. hamatum* and *R. solani* on Natrosol and Viterra 332 gels in this study indicates that they were able to utilize these substances as sole carbon sources. Suppressed growth of *T. hamatum* and *R. solani* on Laponite 508 was probably due to its alkaline nature (pH = 9.6).

In 1983 and 1984 field studies, fluid drilling was superior to other delivery systems tested where significant differences were noted. Germinated cultures were used in fluid drilling, and this form of inoculum has been shown to be more effective than ungerminated conidia (31). This may not be the only explanation for the success of this application method, however, for the antagonist used in the wheat bran preparation, an inferior delivery system, also was germinated. In 1984, the same form of antagonist was used for fluid drilling and drench applications. The latter was inferior, indicating that the gel itself may play an integral part in the success of fluid drilling. Gels applied without added antagonists often controlled Rhizoctonia damping-off comparably to some of the biological treatments. Hadar et al (17) also have observed gel treatments without antagonists to have less disease than their untreated controls. The gel may be acting as a barrier between the seed and the soil, adsorbing exudates of germination and rendering them less available to soilborne pathogens.

In 1983, fluid-drilled antagonists were added to soil with a considerable amount of uncolonized food base (MYB). Removal of this food base from the inoculum in 1984 resulted in better and more consistent control of Rhizoctonia damping-off. More research needs to be done on the use of food bases in association with biocontrol agents applied to soil. Papavizas et al (32) reported that *Trichoderma* spp. delivered to soil on a completely colonized food base provided better control than a conidial preparation applied without added nutrients. Delivery of antagonistic isolates of *Trichoderma* spp. on completely colonized food bases in this study may have been more effective than the washed antagonist suspension.

Application of 5.3 kg a.i./ha PCNB failed to control *R. solani* in 1984 tests. Many biological treatments tested consistently gave better control of Rhizoctonia damping-off than PCNB. In organic soils, where fungicides have generally been ineffective, biocontrol systems may be viable alternatives for control of soilborne pathogens.

Results of this research support the hypothesis that antagonistic isolates of *Trichoderma* may be useful in control of *R. solani* on radish seedlings. Although control of Rhizoctonia damping-off with *Trichoderma* in the field was variable, this was expected, for biological entities are influenced by many environmental, biological, and physical factors. Several delivery systems, not used in these studies, have been suggested for *Trichoderma* spp. and other antagonists. These, as well as fluid drilling, should be investigated more comprehensively under field conditions.

TABLE 1. Comparison of delivery systems used in 1983 and 1984 Ohio field studies for application of isolates of *Trichoderma* spp. as biocontrol agents of Rhizoctonia damping-off of radish in organic soils

Field test	Application method[w]	Average disease index 2 wk after planting[x]	
		Celeryville	Hartville
1983	Fluid drilling	42.5 a[y]	49.8 a
	Coated seed	65.5 b	57.5 b
	Wheat bran/sand	76.4 c	65.9 c
	Untreated control	81.7[z]	58.7
1984-Test 1			
	Fluid drilling	32.8 a	26.5 a
	Soil drench	36.9 a	35.1 b
	Coated seed	31.9 a	33.6 b
	Untreated control	36.3 a	38.3 b
1984-Test 2			
	Fluid drilling	38.3 a	26.3 a
	Soil drench	41.4 a	39.8 b
	Coated seed	37.0 a	38.5 b
	Untreated control	48.6 b	42.2 b

[w] *Fluid drilling*-Radish seed and 5-day-old culture of *Trichoderma* spp. extruded into soil in gel matrix; *Coated seed*-conidia of *Trichoderma* spp. coated onto radish seed with methylcellulose before planting; *Wheat bran/sand*-conidia of *Trichoderma* spp. pregerminated in a wheat/bran sand culture and applied to planting furrow before planting; *Soil drench*-Five-day-old culture of *Trichoderma* spp. drenched into planting furrow before planting.

[x] Disease indices were calculated as follows. In 1983, the disease index was based on a scale from 1 to 4 (1 = symptomless; 2 = small lesions with no hypocotyl constriction; 3 = large lesions with some hypocotyl constriction; 4 = hypocotyl girdled) and disease indices were calculated with the formula in equation 2 (see text). Possible disease indices ranged from 30 to 120. In 1984, the disease index was based on a scale from 1 to 5; (1 = symptomless; 2 = small lesions, no constriction of hypocotyl; 3 = larger lesions, no hypocotyl constriction; 4 = large lesions with hypocotyl constriction; and 5 = hypocotyl girdled). Disease indices were calculated with the formula in equation 3 (see text). Possible disease indices ranged from 15 to 75.

[y] Application methods within a field study followed by the same letter are not significantly different ($P = 0.05$) by a linear contrast comparison test.

[z] The differences between the untreated control and the *Trichoderma* application methods were not tested in 1983 because each application method comprised data from seven isolates, whereas there was only one control.

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