Combining Effective Strains of *Trichoderma harzianum* and Solid Matrix Priming to Improve Biological Seed Treatments

G. E. HARMAN, Professor, A. G. TAYLOR, Associate Professor, and T. E. STASZ, Research Associate, Department of Horticultural Sciences, Cornell University, New York State Agricultural Experiment Station, Geneva 14456

ABSTRACT

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Two strains of *Trichoderma harzianum* (T12 and T95) were fused to give an array of progeny strains, two of which (22 and 106) were selected for further study. Seeds of several crops were treated with the parental or progeny strains with or without solid matrix priming. For comparison, some seeds were treated only with a sticker (Pelgel) used in all treatments or with a standard fungicide, usually thiram, again with or without solid matrix priming. Seeds of cotton (*Gossypium* spp.), cucumber (*Cucumis sativus*), pea (*Pisum sativum*), snap bean (*Phaseolus vulgaris*), sweet corn (*Zea mays*), and wheat (*Triticum aestivum*) were planted in soil infested with *Pythium ultimum*. Wheat seeds were also planted in soil containing *Fusarium graminearum*, cucumber and snap bean seeds in soil containing *Sclerotium rolfsii*, and radish (*Raphanus sativus*) and cucumber seeds in soil infested with *Rhizoctonia solani*. In all crop-pathogen combinations, *Trichoderma* strains increased stands relative to the untreated control and were as effective as the chemical fungicides, even in the absence of solid matrix priming. Priming of seeds treated with *Trichoderma* strains increased plant stands in soils infested with *F. graminearum* and *P. ultimum* but not in soils infested with *R. solani* or *S. rolfsii*. In soils infested with *P. ultimum*, strain T95 generally gave the poorest results, T12 was intermediate, and the progeny strains (22 and 106) gave improved stands. All four strains gave similar levels of protection against the other pathogens. In *Pythium*-infested soils, cucumber seedlings from seeds treated with strain 22 or 106 were more robust and had greater root volumes than those from seeds treated with T12 or T95. In field trials, stands of peas were not significantly enhanced by seed treatment with *Trichoderma* strains in the absence of priming but were improved by *Trichoderma* plus priming. The progeny strains were more effective than the parental strains in colonizing roots of mature pea plants. In two field trials with sweet corn, strain T12 increased plant stand, reduced seedling mortality, and increased plant growth relative to no treatment. The increased plant growth was evident for the entire duration (98 days) of the longest trial.

Additional keywords: protoplast fusion, soilborne pathogens, biological control

Biological control differs fundamentally from conventional chemical control of plant pathogens. Biocontrol with beneficial microbes manipulates the environment around a crop plant to favor organisms that contribute to plant health and vigor, rather than simply applying pesticides to destroy a range of microorganisms including the target pathogen. Thus, biocontrol is less disruptive to ecosystems than are chemical pesticides (6). Biocontrol agents have the potential to augment or replace chemical pesticides. Moreover, they offer other advantages in plant health management not possible with chemical pesticides. For example, bioprotectants can grow on and colonize plant parts such as root systems, a trait referred to as rhizosphere competence. Such colonization can protect the entire root system from soilborne plant pathogens. Further, some biological agents can induce increased growth responses, resulting in higher yields in addition to the increase resulting from direct disease control (2,13).

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Despite these advantages, beneficial microbes are rarely used to control plant disease, primarily because biocontrol agents have been both less effective and more variable than competitive chemical pesticides. These generalizations are consistent with our own research findings (12). Recognizing this problem, we shifted the focus of our research with *Trichoderma*. We decided to genetically modify the strains to produce superior biocontrol strains and to also change the environment to enhance the efficacy of the biocontrol agent. Through protoplast fusion we produced strains that differed from the parental strains (14). We also employed solid matrix priming, a process conceived by J. Eastin (Kamterter Inc., Lincoln, NE), to enhance both seedling emergence rate (17) and activity of biocontrol agents (9).

In the present study, we examined the efficacy of parental and progeny strains of *T. harzianum* Rifai, the influence of seed treatment (conventional slurry, solid matrix priming), and the interaction of biocontrol strain and seed treatment type. We tested our biological seed treatment systems on a range of crops and pathogens.

MATERIALS AND METHODS

Strains. We used *T. harzianum* strains T12 (ATCC 56678) and T95 (ATCC 60850) as parental strains. Strain T12 possesses good biocontrol ability against *Pythium* and *Alternaria* spp. and competes well with the soil microflora (7,10,18). Strain T95 also possesses good activity against *Pythium* spp. and is rhizosphere-competent (1,2).

Details of preparation, fusion of protoplasts, and the genetic nature of progeny obtained are described elsewhere (14). Briefly, complementary auxotrophs of the two strains were prepared, and protoplasts were produced from these and fused in the presence of polyethylene glycol. Fusion mixtures were plated on a glucose-nutrient salts medium, and prototrophic colonies were collected. The resulting progeny initially grew very slowly. They were weakly prototrophic and unstable; numerous more rapidly growing sectors appeared as the initial progeny grew vegetatively. Progeny varied markedly in morphology but were similar to one or the other parent in isozyme patterns.

Progeny strains with various morphotypes and isozyme patterns were tested for their ability to protect cucumber seed against *Pythium* spp. using the procedures described below. Most were much poorer bioprotectants than the parental strains, but one class of progeny was superior to both parents. Several strains were tested more extensively, and two were selected for the work described in this paper. They were designated strain 1295-22 (ATCC 20847) and strain 1295-106 (ATCC 20873), hereinafter referred to as strains 22 and 106.

These strains were fully prototrophic and were identical to parental strain T12 in isozyme pattern. They grew more rapidly than either parental strain on potato-dextrose agar (PDA). For example, strains T95, T12, 22, and 106 grew 17, 20, 22, and 23 mm/day, respectively, at 24 C and 3.1, 6.2, 6.7, and 6.5 mm/day, respectively, at 11 C. None grew at 7 C.

Although strains 22 and 106 were isozymically identical to parental strain T12, the two progeny strains could readily be distinguished from the two parental strains by their appearance on

PDA amended with 75 mg/L cycloheximide, 20 μg/ml nystatin, 100 μg/ml streptomycin sulfate, 100 mg/L chlortetracycline, and 1 ml/L Igepal CO-630 (Applied Science Labs, Deerfield, IL) (4). On this medium, hereinafter designated CCNSI, strain T12 initially forms a tan colony with brown pigment in the medium, and bluish spore masses are produced with little diurnal variation. In contrast, the progeny strains initially form whitish colonies with little diffusible pigment, and spore masses are green with diurnal zones of heavy sporulation.

Crops and pathogens. Crops tested were cucumber (*Cucumis sativus* L. 'Slicemaster'), wheat (*Triticum aestivum* L. 'Houser'), sweet corn with the *su* gene and the *sh*-2 gene (*Zea mays* L. 'Jubilee' and 'Florida Staysweet,' respectively), radish (*Raphanus sativus* L. 'Early Scarlet Globe'), snap bean (*Phaseolus vulgaris* L. 'Bush Blue Lake 47'), pea (*Pisum sativum* L. 'Venus'), and cotton (*Gossypium* spp. 'Acala SJ-2' and 'Stoneville 112'). Pathogens tested were *Pythium ultimum* Trow (all crops), *Rhizoctonia solani* Kühn (cucumber and radish), *Fusarium graminearum* Schwabe (wheat), and *Sclerotium rolfsii* Sacc. (cucumber and snap bean).

Seed treatments. *T. harzianum* conidiospores were scraped from cultures grown in petri plates on PDA and were suspended in a 10% (w/v) aqueous suspension of Pelgel (Nitragin Co., Milwaukee, WI) at a concentration of 10^7–10^8 conidia per milliliter (8). Seed surfaces were fully covered with these spore suspensions. For wheat, radish, and cucumber, 1 ml of suspension was used to treat 4 g of seeds; for Jubilee sweet corn, 1 ml was used to treat 10 g of seeds; for Florida Staysweet corn and peas, 1 ml treated 6 g of seeds; and for cotton, 1 ml treated 7.8 g of seeds.

For comparison, some seeds were treated with Pelgel alone or with fungicides. Vitavax 200 (17% carboxin and 17% thiram) (Gustafson, Inc., Dallas, TX) was used at the rate of 420 μg of active ingredient of each component per gram of wheat seeds, while thiram alone was used for all other crops. Thiram was added at the rate of 1.35 mg a.i./g of seed for cucumbers, cotton, and radish and 700 μg a.i./g of seed for all other crops. These are the recommended rates on the label for these fungicide-seed combinations.

Solid matrix priming was performed as described earlier (9,17). Seeds treated as described above were mixed with 1.5 parts by weight of a finely ground Leonardite shale (Agro-Lig) (American Colloid Co., Agronomic Div., Arlington Heights, IL) for all crops except peas and beans, where two parts of Agro-Lig were used. Sufficient water was added to be just below the threshold required for seed sprouting (9,17). Percentage moisture contents were as follows (based on the dry weight of Agro-Lig): wheat seeds, 55%; cucumber, sweet corn, and radish, 60%; snap bean and cotton, 75%; and pea, 80%.

Seeds of all crops except cotton were then incubated for 4 days at 20 C. Cotton was incubated at 25 C to avoid chilling damage; however, this incubation regime permitted noticeable saprophytic growth, so experiments were conducted to determine whether moisture content or incubation time could be reduced. We found that incubation time could be reduced to 1 day with no adverse effects on biocontrol ability; Acala SJ-2 was incubated 4 days, and Stoneville 112 was incubated 1 day. After incubation, seeds were sieved to remove excess Agro-Lig and were then planted.

Seedling assays. Seedlings were assayed as described earlier (9). Seeds treated as described above were planted in 300 g of Arkport sandy loam soil in plastic boxes measuring 10 × 10 × 5 cm. Five boxes were planted of each treatment, and each box was considered a replicate. For radishes, 30 seeds were planted per box; for cucumber, 10 seeds; for cotton and wheat, eight seeds; and for pea and snap bean, five seeds. The boxes were placed in larger clear plastic boxes with lids, which obviated the need for irrigation during the experimental period (7–14 days). Experiments were conducted at 22–27 C, with a 12-hr photoperiod provided by cool white fluorescent lights.

In all experiments, soils were infested with one of the selected pathogenic fungi. Soils were infested with *P. ultimum* as described earlier (9). *R. solani, S. rolfsii,* and *F. graminearum* were grown on an autoclaved mixture of 20 g of wheat and 30 ml of water in petri dishes. When this substrate was completely colonized (i.e., the entire contents of the plate fused into a single mass with hyphae), the petri dish lids were removed, and the cultures were dried in a sterile airstream provided from a laminar flow transfer hood. The dried mixture was ground in a Waring Blendor and kept at 4 C until use. Preliminary experiments were conducted to deter-

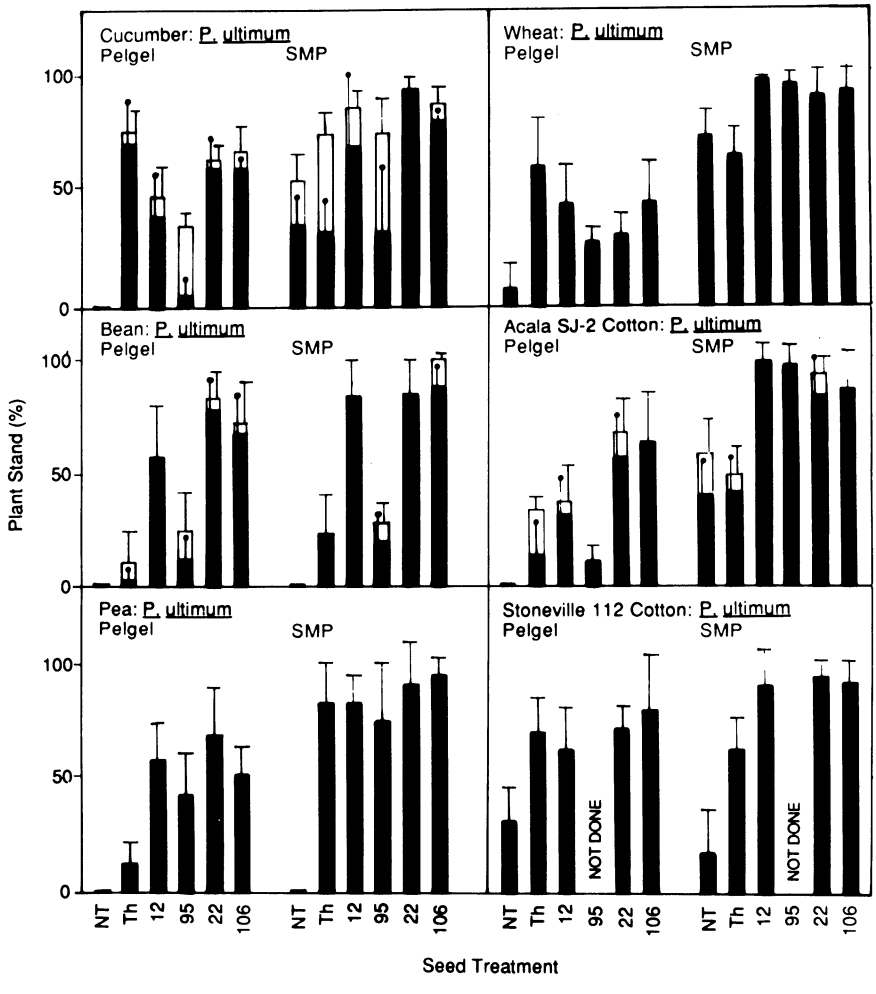

Fig. 1. Maximum (□) and final (■) stands of various crops from seeds treated with Pelgel sticker only (NT), thiram (Th) (wheat was treated with Vitavax), or *Trichoderma harzianum* strain T12, T95, 22, or 106, with or without solid matrix priming (SMP). Seeds were planted in soil infested with *Pythium ultimum*. The difference between the maximum and final stands equals the amount of postemergence damping-off. The bars represent the standard deviations of the maximum (T) and final (i) stands.

mine minimum levels of each pathogen that permitted no (or very few) seeds to survive for 8 days. This dosage was 50, 300, and 16,000 mg of autoclaved wheat preparation per kilogram of soil for *R. solani, S. rolfsii,* and *F. graminearum,* respectively.

Field trials with peas. In 1987, peas were planted on 23 April and 29 May. Soil temperatures in the first trial were cold, with daily lows frequently at 7 C or less. Soil temperatures in the second trial were warmer, not dropping below 20 C. Trials were planted in a Lima silt loam. Two separate trials were conducted for each planting date. In one (row trials), seeds were not treated, were treated with metalaxyl at the rate of 30 μg/g of seeds, or were treated with strain T12, T95, 22, or 106. Pelgel was used as a sticker with all seeds. All treatments were done with and without priming. Fifty seeds of each treatment were planted in rows 4 m long with 0.45 m between rows. Five rows (replications) were planted per treatment, and the entire plot was arranged in a randomized complete block design. For the second trial (block plots) at each date, seeds were planted untreated, treated with metalaxyl without priming, treated with metalaxyl with priming, or treated with strain T12, 22, or 106 with priming. One hundred seeds were planted in plots measuring 1 × 1 m to simulate commercial plant spacing. Plots were separated by 0.3 m.

P. ultimum was added to row trials to augment the natural population of this pathogen in the soil. The organism was produced as described earlier (9), fragmented in a Waring Blendor, and diluted in water. This mixture was applied at planting. A tractor-mounted system delivered the additional inoculum at a rate of 3×10^5 sporangia per row directly into the seed furrow and onto the planted seed immediately after sowing. The soil in the planting furrow was sampled after seedlings emerged; the *Pythium* numbers, as determined by plating on a selective medium (9), were 400–500 colony-forming units per gram of soil.

In addition to row and block trials, we conducted trials (rhizosphere competence trials) specifically to measure the ability of the four strains to colonize root surfaces. Fifty pea seeds were treated with each of strains T12, T95, 22, and 106 in the presence and absence of priming. Plants produced from dissimilarly treated seeds were separated by at least 5 m. Seeds were planted in a Lima silt loam, and the plants were allowed to grow until they were at the midbloom stage. Five plants representing each seed treatment were carefully dug, and the soil was washed from their roots. Segments 1 cm long were taken from each taproot at the cotyledonary attachment, middle, and tip. Lateral roots were similarly sampled at the midway point and at the tip. All lateral roots were sampled, providing 18–20 root samples from each root system.

Segments were plated on a medium highly selective for *Trichoderma* spp. (9). Colonies that developed on the selective medium were transferred to CCNSI for strains T12, 22, and 106, where strains were easily distinguished based on their morphologies. Colonies derived from roots grown from seeds treated with strain T95 were plated on PDA amended with 40 mg/L benomyl. The identification of colonies that developed on both media was spot-checked using isozyme electrophoresis, which readily distinguishes strains of *Trichoderma* (14,15).

Sweet corn field trials. Sweet corn trials were conducted in 1987 with strain T12 only. Trials with progeny from protoplast fusion required prior approval of the U.S. Environmental Protection Agency, and we did not have approval for trials on this crop in 1987. We compared seeds treated with strain T12 with seeds treated only with Pelgel, with and without priming. Two cultivars were studied in separate experiments within each trial; one (Florida Staysweet) contained the *sh*-2 gene, and the other (Jubilee) contained the *su* gene. Cultivars with the *sh*-2 gene are increasing in commercial importance because the kernels retain sweetness longer than those with the *su* gene. However, seeds of cultivars with the *sh*-2 gene are low in vigor and are severely affected by seed rots (16). Moreover, a large percentage of seedlings produced from these seeds are affected by "five-leaf dieback." Plants affected by this malady, which is assumed to be of microbial origin, grow slowly and frequently die at about the five-leaf stage.

In the first trial, 50 seeds were planted per replicate of each treatment in rows 3 m long, to study seedling emergence and growth. *Pythium* was added as described above. In a second trial, rows 5 m long contained 25 seeds to give commercial seed spacing. The second trial comprised two separate experiments; in one but not in the other, *Pythium* was applied in the planting furrow. Both were randomized complete block designs. Soils at the first planting date (22 May) were moderately warm; the minimum soil temperature was 13 C, the maximum temperature was 29 C. For the second planting date (23 July), the minimum was 23 C and the maximum

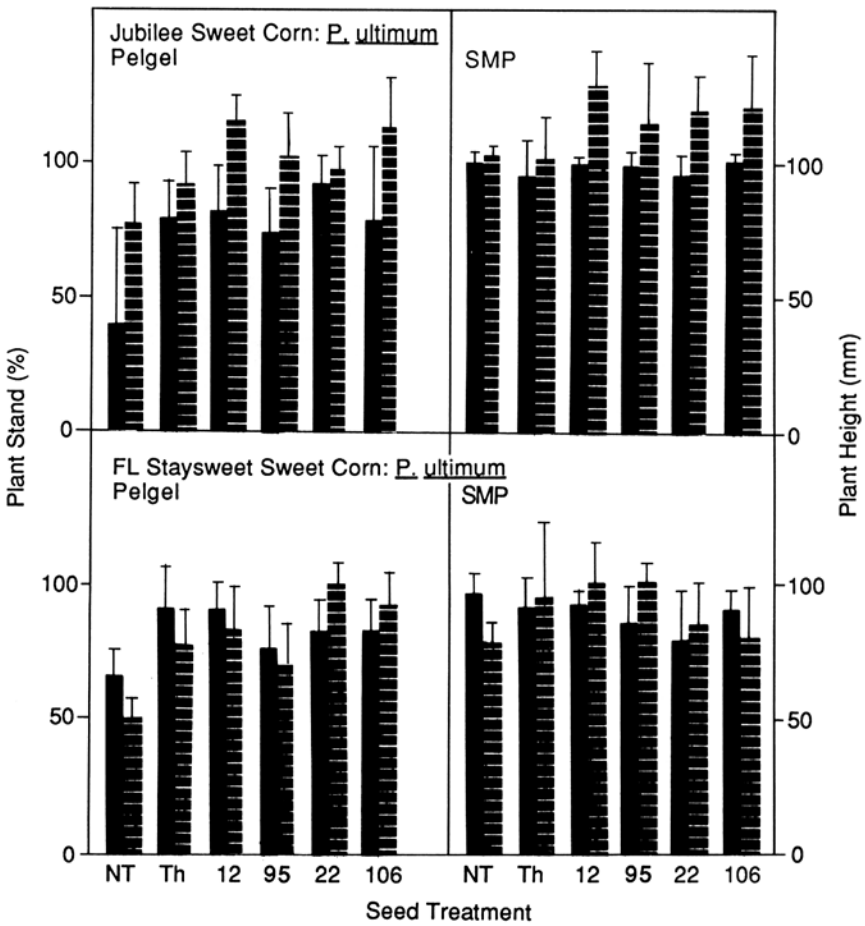

Fig. 2. Stands (solid bars) and plant heights (striped bars) of Jubilee and Florida Staysweet sweet corn from seeds treated with Pelgel sticker only (NT), thiram (Th), or *Trichoderma harzianum* strain T12, T95, 22, or 106, with or without solid matrix priming (SMP). Seeds were planted in soil infested with *Pythium ultimum.* The bars represent the standard deviations.

was 34 C.

Data analysis. All seedling assays were conducted at least twice with similar results. Replications of field trials are given in the tables and elsewhere in the text. Data are presented as mean values plus or minus standard deviations (Minitab Inc.). Data from field trials were analyzed using Waller and Duncan's test (SAS Institute Inc., Cary, NC).

RESULTS

Seedling assays with *P. ultimum*. The biocontrol strains and the chemical fungicides provided significant protection for all crops against *P. ultimum*. With some crops, more seedlings emerged and survived from primed seeds than from nonprimed seeds (Fig. 1). While results with most seed treatments were consistent among experiments, priming alone gave highly variable results. For example, with cucumber, maximum stands ranged from about 3% to 60% (*data not shown*).

Differences among strains in biocontrol ability were apparent. In general, strain T95 was the least effective bioprotectant tested. With most crops, strains T12, 22, and 106 gave equivalent levels of protection. However, in a few cases strains 22 and 106 gave better results than T12. In cucumber with priming, postemergence damping-off was considerably less with strains 22 and 106 than with any other treatment. In the absence of priming, stands of cucumbers, both cultivars of cotton, and beans were 15–20% greater with strains 22 and 106 than with strain T12. Wheat stands from seeds treated with strain T12, however, were greater than those from seeds treated with strain 22.

In all cases, the *Trichoderma* strains without priming were at least equivalent to the standard fungicides. The best results in all crops except sweet corn were obtained with strains T12, 22, or 106 plus priming. Stands ranged from nearly 0 with untreated seeds to 80% or better with these *Trichoderma* strains plus seed priming (Fig. 1).

The incidence of seed rot caused by *P. ultimum* was lower in sweet corn than in other crops, but plant height (and general plant robustness) differed markedly with this crop. The smallest sweet corn plants were produced from untreated seeds. Treatment with thiram or priming alone increased both plant stands and plant heights. With Jubilee, the best plant performance (stands and plant heights considered together) was obtained with priming plus *Trichoderma* strains. With Florida Staysweet, similar improved stands and plant heights were obtained with thiram or strains T12, 22, or 106 with or without priming (Fig. 2).

Differences in plant growth also were noted with cucumber in soil infested with *Pythium*. Seeds planted in soils with high potential for seed rot produced seedlings that varied in appearance (Fig. 3B). Most of the seedlings succumbed to postemergence damping-off with many of the treatments, but many survived following treatment with strains T12, 22, or 106 with priming. The seedlings from seeds treated with strain T12 or T95 lacked vigor and had cupped cotyledons, while those from seeds treated with strain 22 or 106 were normal in appearance. When plants were removed from soil, the roots of the plants from seeds treated with strain 22 or 106 were larger than those from other treatments (Fig. 3A). These differences were quantified by measuring the volume of water displaced by roots. The mean root volumes per plant (plus or minus the standard deviations) were as follows: thiram, 106 ± 18 µl; thiram plus priming, 216 ± 18 µl; strain T12 plus priming, 213 ± 61 µl; strain 22 plus priming, 301 ± 27 µl; and strain 106 plus priming, 257 ± 13 µl. Thus, treatment with strain 22 or 106 resulted in seedlings with root volumes 41 and 21% greater, respectively, than those in a comparable treatment with strain T12.

Seedling assays with other pathogens. The *Trichoderma* strains and the chemical pesticides all protected cucumber and radish seeds from attack by *R. solani* and wheat seeds from *F. graminearum*. Thiram and all *Trichoderma* strains gave similar protection against *R. solani*. Radish seedlings were more prone to damping-off with priming than without. A similar but nonsignificant trend was observed with cucumber and *R. solani* (Fig. 4).

Trichoderma strains protected wheat seeds against *F. graminearum* better than Vitavax. Stands were greatest when *Trichoderma* strains were combined with priming (Fig. 4).

When cucumbers or snap beans were planted in soil infested with *S. rolfsii*, thiram was largely ineffective as a seed treatment. All *Trichoderma* strains protected seeds and seedlings similarly, although strain T95 was poorer than the other strains in some trials. Priming had little effect on the efficacy of *Trichoderma* against this pathogen (Fig. 4).

Pea field trials. In row trials, treatment with *Trichoderma* strains had no effect on plant stands in the absence of priming but resulted in better stands with

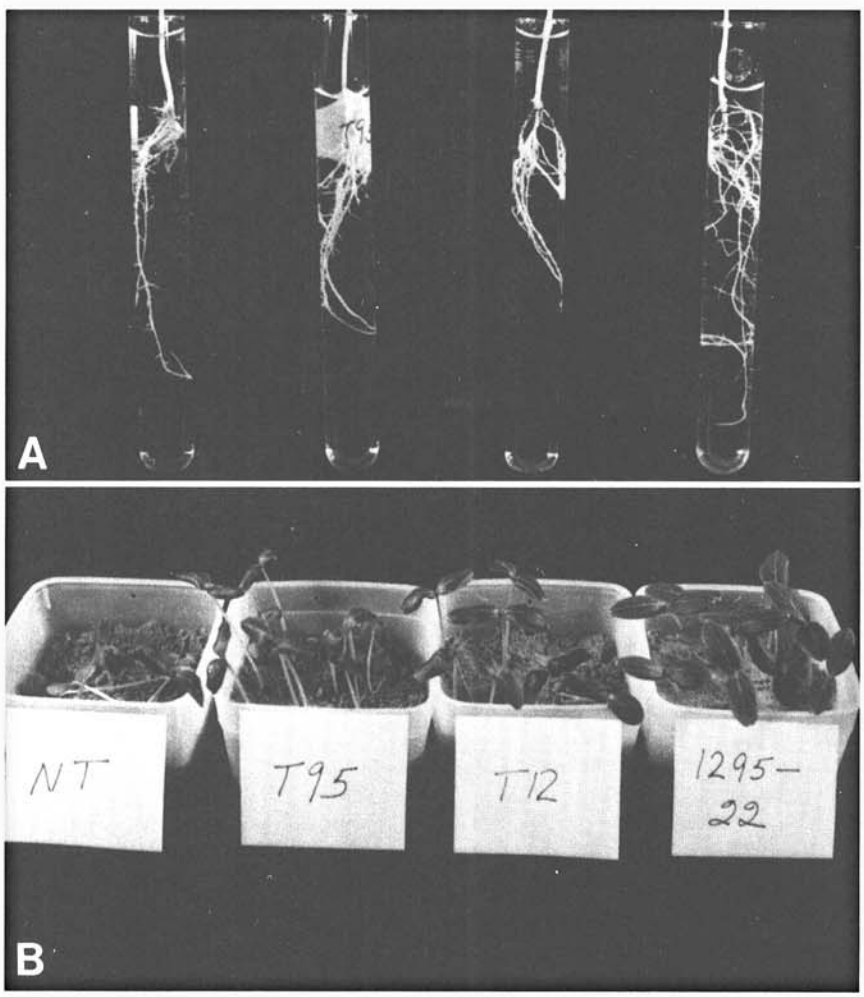

Fig. 3. Roots **(A)** and aerial portions **(B)** of cucumbers 9 days after planting seeds treated with Pelgel alone (NT) or with *Trichoderma harzianum* strain T95, T12, or 22, all after solid matrix priming.

priming. Metalaxyl increased plant stands both with and without priming (Table 1).

The amount of root colonization by the *Trichoderma* strains varied greatly. In the block trials, these strains colonized roots of plants in adjacent plots as well as roots of plants in plots treated with strain 22 or 106, so root colonization could not be accurately assessed. More accurate comparisons were possible in the rhizosphere competence trials, where rows representing different treatments were widely separated. Seed treatment with strains 22 and 106 resulted in colonization of nearly all (95% or more) root segments from mature plants, whether or not priming was used. Strains T12 and T95 colonized many fewer segments (only 37 and 13%, respectively); priming raised the percentage colonized to 67 and 37%, respectively (Table 1).

Sweet corn field trials. Treatment of sweet corn seeds with strain T12 improved plant stands and performance compared with no seed treatment. In the first trial with Jubilee in moderately cool soil, treatment with strain T12 without priming did not change plant stand significantly relative to the untreated control, whereas treatment with priming alone or with strain T12 plus priming resulted in significant increases in stand establishment (Table 2). With Florida Staysweet, treatment with strain T12 alone or with priming increased stands, but priming alone resulted in lower plant stands. Differences were also evident in plant growth. Strain T12 plus priming produced the largest plants; plant dry weights were 25 and 81% greater for treated Jubilee and Florida Staysweet plants, respectively, than for plants produced from untreated seeds (Table 2).

The second trial attempted to compare the effects of adding *P. ultimum* to the planting furrow with planting in natural field soil. In the hot soils in which these trials were planted, however, adding the pathogen had no significant effects, and data presented are from plots in which *Pythium* was not applied. Priming similarly had no effect on any parameter measured, so only data on treatment with strain T12 versus no treatment are presented.

Treatment with strain T12 had no effect on seedling emergence in Jubilee but resulted in better stands of Florida Staysweet (Table 3). Seed treatment with strain T12 also reduced postemergence mortality (five-leaf dieback) in both cultivars. The combination of better emergence and improved survival resulted in twice as many plants (64% versus 32%) per row from treated Florida Staysweet seeds as from untreated seeds 98 days after planting. Plant weights per plot from seeds of both cultivars treated with strain T12 were double those from untreated seeds (Table 3). Plant dry weights of Jubilee plants produced from

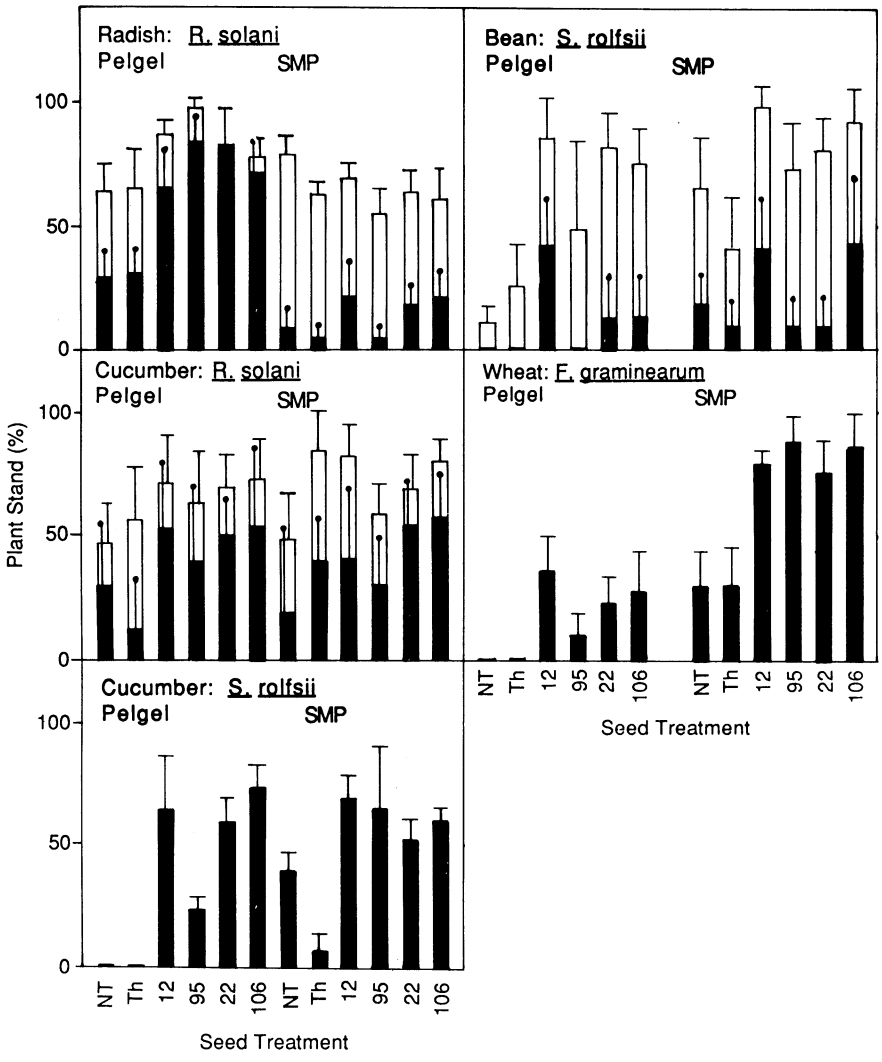

Fig. 4. Maximum (□) and final (■) stands of various crops from seeds treated with Pelgel sticker only (NT), thiram (Th), or *Trichoderma harzianum* strain T12, T95, 22, or 106, with or without solid matrix priming (SMP). Seeds were planted in soil infested with *Rhizoctonia solani, Sclerotium rolfsii,* or *Fusarium graminearum.* The difference between the maximum and final stands equals the amount of postemergence damping-off. The bars represent the standard deviations of the maximum (T) and final (i) stands.

Table 1. Stands and root colonization of peas (cv. Venus) grown in field soils amended with *Pythium ultimum* from seeds treated with metalaxyl or strains of *Trichoderma harzianum* with or without solid matrix priming (SMP)

Treatment	Stand[y] (%)		Root colonization[z] (%)
	Trial 1	Trial 2	
None	52 fg	34 e	—
Metalaxyl	86 ab	84 a	—
T12	52 fg	44 cd	37
T95	50 g	36 de	13
22	57 efg	36 de	95
106	44 g	26 e	97
SMP	61 defg	38 de	—
SMP + metalaxyl	87 a	88 a	—
SMP + T12	76 abc	68 b	67
SMP + T95	66 cdef	56 bc	37
SMP + 22	68 cde	62 b	97
SMP + 106	72 bcd	60 b	95

[y] Numbers followed by dissimilar letters are significantly different according to Waller and Duncan's test.

[z] Root colonization was determined after plants reached the midbloom stage. Five plants in each treatment group were then dug; their roots were washed; and 1-cm segments were taken just below the cotyledonary attachment, midway down, and at the tip of the taproot and from the middle and terminal portions of lateral roots. In this way, 18–20 root segments were sampled per plant. The segments were plated on selective and differential media, and colonizing strains were identified by morphology and isozyme electrophoresis.

treated seeds were greater after 98 days than those of plants from untreated seeds. Similar differences were observed throughout the growing season with Florida Staysweet; at the end of the growing season, however, the increased competition among the greater number of plants precluded greater dry weights from plants produced from treated seeds compared with those from untreated seeds (Table 3).

DISCUSSION

Seed companies want effective biological seed treatments. They are particularly interested in broad-spectrum seed protectants to replace captan or thiram. The future of captan is questionable because of unresolved questions concerning its long-term health effects. Moreover, restrictions in several states on the application of these chemicals and the disposal of unused treated seeds make use of these materials difficult.

For biological seed treatments to replace the more standard chemicals, they must be consistently effective against a range of pathogens. In the past, biological seed treatments have been both more variable and less effective than chemical fungicide treatments (11,12). Moreover, individual strains of *Trichoderma* have been effective against only one or two pathogens. A wide spectrum of activity is not usually associated with specific strains, although the entire genus, together with *Gliocladium,* controls many pathogens. In this study, all strains of *T. harzianum* tested controlled *P. ultimum, R. solani, F. graminearum,* and *S. rolfsii.* We have shown previously that strain T12 controls *Alternaria raphani* and *A. brassicicola* (18). Thus, these strains appear to have the wide spectrum of activity desirable for a general-purpose seed treatment or for other applications.

The range of pathogens controlled by these strains appears to depend strongly on the delivery system. For example, we found low levels of activity of the various strains against *F. graminearum* with conventional slurry seed treatments. However, when strains were applied in the presence of priming, their activities approximately tripled. These results suggest that the ability of a strain to control a specific pathogen may be more strongly related to the quantity, quality, and location of the bioprotectant than to its specific genetic ability to antagonize the pathogen per se.

Solid matrix priming markedly enhanced the ability of the *Trichoderma* strains to control *Pythium* spp. In the seedling assays, protection of nearly all crops was greater with priming than without. In field trials, protection of peas by the strains was evident only when seeds were primed. The effects of priming on stand establishment were less pronounced with sweet corn; in the first trial with Florida Staysweet, emergence was reduced by priming alone, although priming was not detrimental when combined with strain T12. Field trials were consistent with the seedling assays, which demonstrated that *P. ultimum* had less effect on stands of sweet corn than on other crops tested. Priming had little effect on the ability of the biocontrol strains to protect against *S. rolfsii* and appeared to reduce the effectiveness of the *Trichoderma* strains against *R. solani.*

Peas in the field appeared to derive only modest protection from the biological seed treatments. However, the conditions were quite unfavorable for the biocontrol strains, and for them to show any activity is encouraging. In the first trials, soil temperatures were at or below the minimum for growth of these strains. Furthermore, the pathogen was added to the planting furrow directly onto the seeds. Under these conditions, a toxicant such as metalaxyl would appear to be more effective than a biocontrol strain whose protective ability may depend on subtle mechanisms (5).

The strains differed in their ability to protect seeds against the various pathogens. In general, strain T95 was the least effective. Its relatively poor performance may reflect an intrinsic inability to control the pathogens in question or an inability to proliferate in the environment into which it was introduced. We showed earlier that the *Trichoderma* strain from which T95 was derived by mutation is poorly adapted to compete in New York soils (10).

The progeny strains were somewhat better seed protectants than either parental strain in some tests with *Pythium,* but the differences were small. It is difficult to see improvement with most tests with *Pythium,* however, especially when biocontrol strains are combined with priming, because there is little room for improvement. The stands plus the standard deviations frequently exceed 100%, even when the disease potential is quite high. Thus, one goal in developing improved strains is to devise tests that allow detection of improved strains when existing strains and systems already are quite effective.

The ability of biocontrol strains to colonize and protect roots and to otherwise benefit the crop is at least as important as seed protection from pathogens. The protoplast fusion progeny appeared to outperform the parental strains in this regard. They protected cucumber roots in the seedling assays, which could have resulted from better root-colonizing ability. Evidence of better root colonization was obtained in the field trials with peas; strains 22 and 106 colonized nearly all pea root segments tested, even when the plants were mature, unlike the parental strains T12 and T95, which colonized fewer segments. Our tests did not differentiate between growth of the strains directly on the root surface or in the surrounding soil (i.e., rhizoplane or rhizosphere competence) and transfer of strains by mass movement of propagules in water

Table 2. The effect of seed treatment with *Trichoderma harzianum* strain T12 with or without solid matrix priming (SMP) on seedling emergence and subsequent growth of sweet corn cultivars Jubilee and Florida Staysweet (trial 1)

Seed treatment	Seedling emergence[y] (%)		Dry weight per plant[y,z] (mg)	
	Jubilee	Florida Staysweet	Jubilee	Florida Staysweet
None	51 a	60 b	513 ab	380 b
SMP	71 bc	31 a	389 a	266 a
T12	61 ab	73 c	508 ab	480 b
T12 + SMP	77 c	77 c	639 b	689 c

[y] Numbers in a column followed by dissimilar letters are significantly different according to Waller and Duncan's test.
[z] Assessed 32 days after planting.

Table 3. The effect of seed treatment with *Trichoderma harzianum* strain T12 on seedling emergence, survival, and subsequent growth of sweet corn cultivars Jubilee (J) and Florida Staysweet (FS) (trial 2)[z]

Seed treatment	Seedling emergence (%)		Plant mortality after 98 days (%)		Dry weight per plant (g)		Dry weight per plot (kg)	
	J	FS	J	FS	J	FS	J	FS
None	69 a	55 a	33 b	37 a	184 a	271 a	2.1 a	2.1 a
T12	63 a	75 b	12 a	14 b	298 b	279 a	4.2 b	4.4 b

[z] Trial 2 included experiments with and without addition of *Pythium ultimum* and with and without priming. Neither *Pythium* nor priming had a significant effect on any parameter measured. Values in this table are means of experiments conducted without added *Pythium* and in the absence of priming. Numbers in a column followed by dissimilar letters are significantly different according to Waller and Duncan's test.

percolating through the soil. Movement by the latter method apparently occurred, as indicated by colonization of roots by strains 22 and 106 in block trials of untreated peas adjacent to peas treated with these fungi.

The increased growth of sweet corn that persisted through the life of the crop was dramatic. We have sought evidence of increased growth responses as a consequence of seed treatments across a range of crops but have never seen a consistent improvement except with sweet corn. Increases in growth of various crops as a consequence of *Trichoderma,* including T12, have been noted (2,3); however, these increases frequently have been associated with soil infestation with relatively high levels of *Trichoderma* (e.g., $> 10^4$ conidia per gram of soil) (2,3). Ahmad and Baker (2), however, have seen increased growth responses as a consequence of seed treatment of cucumber, pea, tomato, and radish with strains T95 and T12. The reasons for the discrepancy between their results and those reported in this paper are not clear, but they may involve the level of conduciveness to *T. harzianum* of the soil in which seeds were planted.

The marked increase in growth rate of sweet corn was unexpected. It was visually apparent from the time the plants emerged and continued throughout the life of the crop. We do not know the nature of this response, but it apparently is not the result of control of *Pythium* spp. Similar responses were noted in both trials, regardless of whether *Pythium* was added to the seed furrow. These responses may be caused by a direct effect on the plant or by control of some undiagnosed plant pathogen(s).

Taken together, the data suggest that the seed treatment systems we have developed—that is, the improved seed treatment procedures together with improved strains—are attractive candidates to augment or replace existing broad-spectrum fungicides (i.e., thiram or captan). They protect against a range of pathogens and are consistently as effective as thiram, at least in seedling trials. In addition, they offer benefits not obtainable with chemical seed protectants, especially the ability to colonize and protect roots. Strain T12 has the additional ability to enhance growth of sweet corn and to limit the incidence of five-leaf dieback. Trials now under way will test the ability of the progeny strains to enhance sweet corn performance and will test these strains and seed treatment systems on a range of crops in diverse geographic locations.

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