Improved Seedling Performance by Integration of Biological Control Agents at Favorable pH Levels with Solid Matrix Priming

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ABSTRACT

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Solid matrix priming (matrix priming) is a process in which moistened seeds are mixed with an organic carrier and the moisture content of the mixture brought to a level just below that required for seed sprouting. Untreated seeds or seeds treated with *Enterobacter cloacae* without matrix priming of either cucumber or tomato produced few or no seedlings when planted in infested soil. Treatment of cucumber seeds with *Trichoderma* strains without matrix priming resulted in about 30% of seeds producing seedlings, but most succumbed to postemergence damping-off. About 90% of the cucumber seeds treated with thiram alone gave rise to seedlings, all of which died within 8 days of planting. Treatment of tomato seeds with *Trichoderma* strains or thiram without matrix priming resulted in 70–95% stands, with a low level of damping-off. Combining matrix priming, in Agro-Lig (pH 4.1), with any of the biological control agents, improved seedling performance markedly relative to seeds treated with the organism alone. All organisms, when combined with matrix priming on cucumber seeds, gave 80–96% initial seedling emergence, and with *Trichoderma*-treated seeds, postemergence damping-off was less than when seeds were treated with thiram. With tomato seeds, combining matrix priming with *Trichoderma* or thiram resulted in very good stands and little damping-off. *E. cloacae* worked poorly on tomato seeds primed in Agro-Lig. Substitution of sphagnum or a pH 6.6 bituminous coal for Agro-Lig in matrix priming altered the results; *Trichoderma* strains worked most effectively in sphagnum or the lignite material, whereas the performance of *E. cloacae* was markedly improved in the coal carrier. On tomato, *E. cloacae* did not survive matrix priming in Agro-Lig, whereas 10^7 propagules per seed were recovered from matrix priming plus *E. cloacae* in the coal carrier. On cucumber, 10^8 propagules of the bacterium were recovered per seed in the coal carrier, but only 10^5 in Agro-Lig. Seeds could be dried after matrix priming with retention of enhanced microbial activity, but levels of *E. cloacae* on coal-primed tomato seeds declined from 10^7–10^8 to 10^5 propagules per seed on drying, and *Trichoderma* numbers decreased four- to sixfold. A series of treatments were conducted to determine whether the carrier, priming of the seed, priming of the biocontrol agent, or some combination of these was responsible for the beneficial results noted. The best results were obtained with matrix priming when *T. harzianum* was primed on the seeds. Priming of seeds plus *T. harzianum* in a conventional liquid priming system using polyethylene glycol as the osmoticant resulted in no recovery of the organism and poorer performance in infested soil than when matrix priming was combined with this fungus. Matrix priming plus *Trichoderma* strains resulted in more rapid emergence from *Pythium*-infested soil and more rapid seedling growth than seeds given matrix priming alone or matrix priming plus thiram. In uninfested soil, seeds treated with matrix priming alone or matrix priming plus thiram emerged and grew at the same rapid rate as seeds treated with matrix priming plus *Trichoderma*. Addition of 0.5 N HCl to cucumber seeds (making the seed pH at 3.7) treated with *T. harzianum* markedly improved stands when planted in *Pythium*-infected soils relative to seeds treated only with *T. harzianum*.

Additional key words: *Cucumis sativus*, *Lycopersicon esculentum*, *Pythium ultimum*.

Biological control (biocontrol) of plant pathogens is becoming an important component of plant disease management practices. Biocontrol potentially offers answers to many persistent problems in agriculture, including problems of resource limitations, nonsustainable agricultural systems, and over-reliance on pesticides (6). Seed treatment is an attractive delivery system for either fungal or bacterial bioprotectants. Bioprotectants applied to seeds may not only protect seeds (9,10,19) but also may colonize and protect roots (1,5,13) and increase plant growth (3,4). However, biological agents have tended to be somewhat less effective and more variable than chemical seed treatments (9,18). Thus, seed treatment systems that will enhance efficacy of biological agents are needed.

Various physiological seed treatments have been described; one of the most promising of these is seed priming (2). In this process, seeds are placed in an aerated osmotic solution of known water potential under controlled environmental conditions. Seeds imbibe and complete early metabolic processes of germination. However, the solutions provide insufficient water for the completion of germination (radicle emergence) (2). Primed seeds consistently emerge more rapidly than untreated seeds and provide better stands under adverse environmental conditions than do untreated seeds. Priming seeds in osmotically adjusted solutions requires relatively large volumes of solutions, temperature control, and aeration, and is therefore restricted to seeds of very high economic value.

Solid matrix priming (matrix priming) has been developed as an alternative to priming seeds in osmotic solutions (Taylor, *unpublished*). In this procedure, seeds are moistened and mixed with a finely ground lignite or coal substance, sufficient additional water is added to achieve the appropriate moisture potential for priming, and then incubated for a given duration at a constant temperature. Seeds so primed have all the advantages of seeds treated with a liquid priming system, but the procedure is simpler and more economical (Taylor, *unpublished*).

This solid matrix system seems ideal for integration with biological control agents. Organic carriers have been shown to enhance the efficacy of *Trichoderma* spp. (11), and the biological agents may themselves be primed or proliferate during the procedure. If so, they should be more active and/or have colonized the seed coat surface thoroughly before planting.

The objectives of the work reported in this paper were to evaluate whether integration of priming and various biological agents were more effective than either treatment alone, whether various carriers might be used, and to determine which component(s) of the system (e.g., organic carriers, the priming process itself, the biological agents) were providing beneficial effects. The influence of pH of seed treatments in relation to their ability to protect seeds also was determined.

MATERIALS AND METHODS

Crops and microorganisms. Seeds used in the study were cucumber (*Cucumis sativus* L. 'Slicemaster') and tomato (*Lycopersicon esculentum* Mill. 'New Yorker'). Tomato seeds were extracted from the fruit by the supplier with HCl. The seeds were strongly acidic, and when 4 g of seeds was leached in 100 ml of water for 6 hr, the pH of the extract was 2.8.

Biological control agents included *Trichoderma harzianum* Rifai strain T95 (ATCC 60850), obtained from R. Baker, Colorado State University, *T. harzianum* strain T12B, which is a benomyl-resistant (1) strain of T12 (ATCC 20737) (9), and *T. koningii* Oud. T8 (ATCC 20736) (9). The bacterial agent used was *Enterobacter cloacae* ((Jordan) Hormlache and Edwards) strain E6 (ATCC 39978) (10). All strains used were chosen because of their ability to control seed rots (9,10,18).

Seeds were shaken in aqueous media and washings plated to determine levels of microbial colonization, and soil was sampled at the end of experiments for the same purpose. These seeds were placed in 4.5 ml of water (for seeds treated with *Trichoderma*) or in 4.5 ml of 0.87% NaCl (for seeds treated with E6), mixed vigorously on a vortex mixer for 2 min, and serial dilutions prepared. *Trichoderma* spp. were enumerated by plating dilutions on the selective medium of Davet (7) modified by the addition of 10 mg/ml of captan to eliminate growth of *Fusarium* and other interfering fungi, and 0.1% w/v Igepel Co-630 (Applied Science, State College, PA) to restrict colony size (16). E6 was enumerated by plating dilutions on tripticase soy agar (BBL Microbiology Systems, Cockeysville, MD) amended with 5 mg/ml of pimaricin (Sigma Chemical Co., St. Louis, MO) to eliminate fungal growth. This latter medium is nonselective among bacteria, and while useful for enumerating E6 on seeds following treatment, could not be used reliably for enumeration of E6 in soil.

The pathogen screened against was *Pythium ultimum* Trow; in all experiments a mixture of equal amounts of strain P4 (17), which primarily produced sporangia, and 220 (20), which primarily produced oospores, were used.

Soil. The soil used was an Arkport sandy loam whose characteristics have been described earlier (12). This soil was used without modification or after addition of *P. ultimum*. Sporangia of strain P4 and oospores of strain 220 were produced and harvested as described earlier and added in approximately equal numbers to the soil (20). After addition, the soil was incubated for at least 3 wk to permit loss of constitutive dormancy of oospores. *Pythium* levels were then determined by plating dilutions onto a modification (16) of Mircetich's (15) selective medium. Soil was adjusted to give 200–250 propagules per gram of soil.

Seed treatments. Trichoderma strains were grown on potato-dextrose agar (Difco Laboratories, Detroit, MI) at 25 C in light until a heavy growth of conidia was evident. Conidia were harvested by scraping the surface of the colonies with a spatula and were transferred to water to give a spore concentration of 10^7–10^8 per milliliter. *E. cloacae* was grown overnight in 250 ml of tripticase soy broth, and cells were harvested by centrifugation. Bacteria were resuspended in 0.85% w/v NaCl and adjusted to give 10^{10} cells per milliliter.

One-milliliter suspensions of individual strains of microorganisms or, for comparison, 0.2% (w/v) thiram (tetramethylthiuram disulfide) or water, were added to 4 g of seeds. The mixture was shaken until the water was absorbed by the seeds.

The combination of matrix priming with biological agents was performed as follows: 4 g of seeds was moistened with 1 ml of water containing microorganisms or pesticides and was then mixed with 6 g of an organic carrier. The seeds and organic carriers were again mixed, and sufficient water was added to provide final moisture contents of 60% for cucumber and 90% for the tomato. The moisture content is based on the organic carrier and was determined to be just slightly below that required for sprouting of these two seed kinds. The mixes were then incubated for 4 days at 20 C, and then planted.

Three different organic carriers were used; these were a Leonardite shale (Agro-Lig, ultrafine grade, American Colloids Co, Skokie, IL), a bituminous coal, and sphagnum moss. The chemical composition of the Agro-Lig and coal are given in Table 1. The pH of the materials was determined from a water solution (8). Organic and inorganic constituents were determined by standard methodology (14).

Seedling assays. Ten seeds were planted in 300 g of soil contained in 10- × 10- × 5-cm plastic boxes. Five boxes were planted of each treatment, and each was considered a replicate. Soils were adjusted to a moisture content of −70 kPa at the beginning of the experiment and watered as required. Experiments were conducted at 22–25 C with a 12-hr photoperiod provided by cool-white fluorescent lights.

Experiments conducted. In an initial experiment, two separate samples of seeds were treated with each of the biocontrol agents, thiram, or water. Some of these received matrix priming, while similar samples did not. Comparisons of seedling performance were made in *Pythium*-infested and uninfested soil.

In a second experiment, cucumber seeds were treated with various organisms, water, or thiram, and subjected to matrix priming with either Agro-Lig, bituminous coal, or sphagnum moss as the carrier.

In a similar experiment, tomato and cucumber seeds were treated with E6, T12B, or with nothing, and given matrix priming with either coal or Agro-Lig. Half the seeds of each treatment were planted immediately after treatment, while the other half were dried for 24 hr at 25 C and approximately 30% relative humidity before planting.

Other experiments were designed to study the influence of carrier, priming of the seed, priming of the biocontrol agent, or some combination of these, on the beneficial effects measured in experiments 1 and 2. *T. harzianum* T12B was the test organism in all cases. In the first treatment (A), seeds treated with T12B were primed in Agro-Lig as previously described. Treatment B consisted of seeds treated with T12B and mixed with Agro-Lig carrier and sown immediately (i.e., no priming treatment). The carrier and T12B were mixed without seeds in treatment C and incubated at 40% moisture content. Seeds received matrix priming without T12B, and the carrier was removed in treatment D. Strain T12B and fresh Agro-Lig at the standard moisture content were then remixed and planted immediately. Treatment E was similar to treatment A, but seeds were soaked before priming to remove seed exudates. Treatment F repeated A except that the carrier was completely washed away before planting. In treatment G, seeds were treated with T12B and placed in an aerated water column with 30% (w/v) polyethylene glycol (PEG) (MW 8,000, VWR, Rochester, NY). This compared matrix priming with a standard liquid priming system. An untreated, nonprimed check also was included.

The experiments above indicated that pH of seed treatments was an important factor, and that low pH levels, similar to those of the HCl-treated tomato seeds, favored *Trichoderma* strains. Therefore, an experiment was conducted in which cucumber seeds

TABLE 1. Chemical composition of the Agro-Lig and bituminous coal used as carriers[a]

Material	pH	N	P	K	Na	Ca	Mg	Fe	Zn	Mn	Organic matter (%)
Agro-Lig lignite	4.1	8,600	3.4	128	1,868	10,200	1,836	57	0.8	84	84
Bituminous coal	6.6	7,300	2.8	130	4,900	10,720	1,793	4.3	0.4	52	90

[a] All values except pH and percent organic matter are in μg/g.

were treated with T12B in a 1% Methocel slurry, as described elsewhere (9). HCl was added to some slurries at concentrations ranging from 0.01 to 1 N. These were compared with seeds that were treated with Methocel alone or HCl plus Methocel in the absence of T12B. These treatments were evaluated both in *Pythium*-infested soil and by germination between moist rolled towels at 25 C.

Statistical analyses. All experiments were repeated at least once. Data were analyzed using the SAS ANOVA procedure (SAS Institute Inc., Cary, NC) with mean separations by the Waller and Duncan *k*-ratio test. Values for any given day were statistically analyzed independently of values at any other day.

RESULTS

Quantities of *Pythium* present in infested soil were sufficient to cause death of all water-treated tomato and cucumber seeds before emergence (Fig. 1A and C). Seeds treated with thiram without matrix priming resulted in initial stands of 90–95% with both crops, but with cucumbers, postemergence damping-off reduced stands to 0% 8 days after planting. Treating tomato seeds with E6 increased stands to about 30%, and 10% of these subsequently damped-off. Conversely, all cucumber seeds treated with E6 were killed before emergence. Treatment with strains of *Trichoderma* allowed about 20% of the cucumber seedlings to emerge, and most of these eventually damped-off. Conversely, about 80% of Trichoderma-treated tomato seedlings emerged and most of these appeared healthy 9 days after planting (Fig. 1A and C).

Matrix priming improved cucumber seedling performance. The matrix priming treatment with no pesticides or microorganisms resulted in greater numbers of seedlings of both tomato and cucumber than occurred from untreated seeds (Fig. 1). Combining E6 with matrix priming resulted in improved initial cucumber, but not tomato, emergence. With cucumber, nearly perfect stands were initially obtained, and the majority survived through the end of the experiment. With tomato seeds, initial stands ranged from 84 to 94%, and most of the seedlings survived through the end of the experiment. Combining thiram with matrix priming gave less protection to cucumber seeds and seedlings than combining *Trichoderma* and matrix priming. However, these treatments did not differ significantly in tomato (Fig. 1B and D).

Matrix priming alone provided more rapid seedling emergence than that obtained with nonprimed seeds. (Figs. 1, 2, and 3). In *Pythium*-infested, but not in uninfested soil, combining matrix

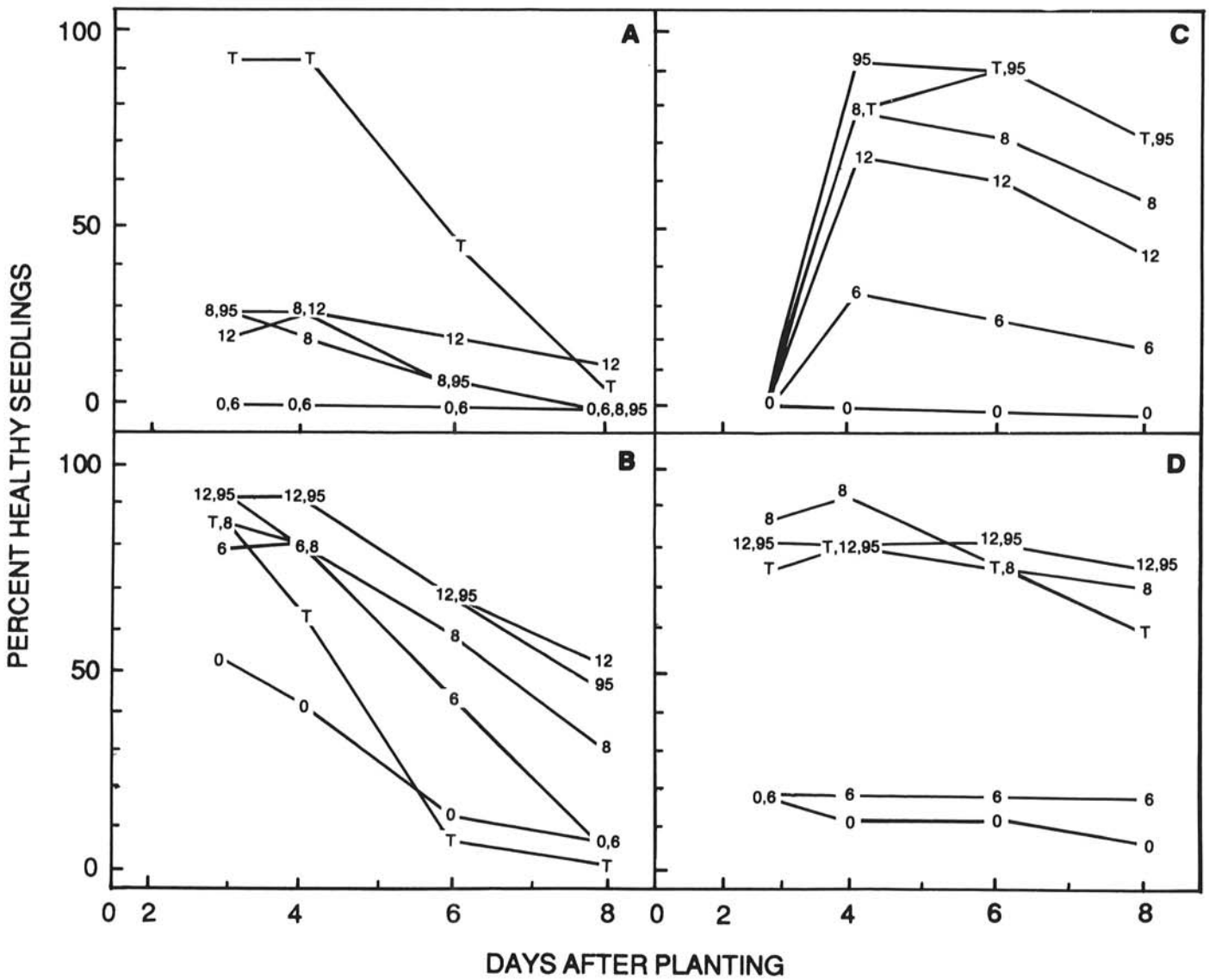

Fig. 1. Percent healthy cucumber, **A and B,** and tomato, **C and D,** at various times after planting in *Pythium*-infested soil. A and C provide percentages of seedlings that grew from nonprimed seeds, and B and D give data from matrix priming seeds. Symbols on graphs are for seeds that were not treated (0), thiram-treated (T), treated with E6 (6), treated with T8 (8), treated with T12B (12), and treated with T95 (95) before planting or priming. The minimum significant difference (MSD) for A and B is 11, 14, 24, and 26 for data at days 3, 4, 6, and 8 respectively; for C and D, the MSD values are 11, 13, 16, and 22 for days 3, 4, 6, and 8, respectively.

priming with *Trichoderma* strains (T12B was most effective) gave rise to more rapidly growing seedlings than seeds treated otherwise, including ones treated with matrix priming plus thiram (Fig. 2).

Numbers of colony-forming units (cfu) of *Trichoderma* increased on seeds during the matrix priming treatment in Agro-Lig by about 10-fold. Numbers of *Trichoderma* on matrix priming plus *Trichoderma*-treated seeds were 10^4 cfu per seed, while on seeds treated with *Trichoderma* alone there were about 10^3. Numbers of E6 propagules on primed or nonprimed cucumber seeds were 10^5 cfu per seed. Conversely, E6 did not survive matrix priming when applied to tomato seeds.

The greater activity of *Trichoderma* strains on seeds was reflected in numbers of colony-forming units of *Trichoderma* in soil at the termination of the experiments. Soils planted with nontreated or thiram-treated seeds contained less than 100 cfu/g of soil at the end of experiments, soils planted to cucumber seeds treated with *Trichoderma* alone contained about 10^4 cfu/g, while soils planted to seeds treated with *Trichoderma* plus matrix priming contained about 10^5 cfu/g of soil.

Efficacy of the various organisms when conditioned in different carriers indicated that materials ranging from sphagnum to bituminous coal could all be used (Fig. 4). However, the *Trichoderma* strains gave numerically better protection in Agro-Lig or sphagnum than in coal, although differences were not significant. E6 protected best in coal, followed by Agro-Lig, and performed very poorly in sphagnum, while thiram protected best in sphagnum. The sphagnum mix, due to its fluffy nature, was very difficult to work with.

The enhanced biocontrol ability of E6 when combined with matrix priming in coal was reflected in numbers of propagules on seeds. With tomato, 10^7 propagules per seed were recovered from seeds primed with coal, while none could be detected when seeds were primed in Agro-Lig. Similarly, with cucumber, 10^8 propagules per seed were detected on coal-primed seeds, but only 2×10^5 were recovered from Agro-Lig-primed seeds. Drying seeds reduced numbers on coal-primed tomato or cucumber seeds to 2 or 7×10^5 propagules per seed, respectively. On Agro-Lig-primed seeds, numbers were about 8×10^4. Drying seeds treated with T12B after priming reduced numbers on seeds by four- to sixfold.

Experiments designed to indicate which component(s) of the matrix priming were required for best seedling protection indicated that the best seedling performance was obtained when the entire mixture (A) was conditioned together (Fig. 3). Priming T12B in Agro-Lig separately from the seed (C) gave the poorest results, with other treatments giving intermediate results. Differences were more pronounced with cucumber than with tomato. Soaking seeds to remove seed exudates gave no reduction in efficacy of the treatment. Treating seeds with T12B and Agro-Lig, and then sowing immediately, gave poor results with cucumbers, but good results with tomatoes. Tomato or cucumber seeds treated with T12B and primed in PEG performed relatively poorly when planted in *Pythium*-infested soil relative to those given matrix priming. No *Trichoderma* could be detected on PEG-treated seeds.

Experiments in which various levels of HCl were added to seed treatments with T12B in the absence of matrix priming indicated that highly acidic environments strongly enhance activity of *Trichoderma*. The pH of cucumber seeds treated only with Methocel or with Methocel plus 0.01 N HCl was 5.3, while pH levels were 4.6, 3.7, and 3.1 on seeds treated with 0.1, 0.5, or 1 N HCl Methocel, respectively. HCl at concentrations of 0.1 N or less

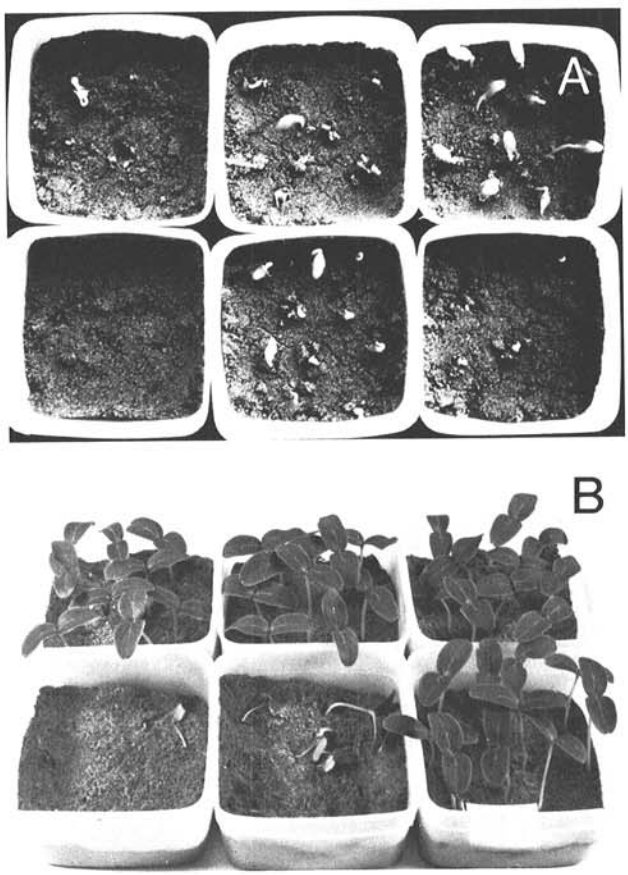

Fig. 2. Growth of cucumber seedlings after various treatments in infested or uninfested soil. **A,** Seedlings 3 days after planting all in infested soil. Seeds were given matrix priming with no other treatment (upper left), matrix priming and thiram (upper center), or matrix priming and T12B (upper right); growth of seedlings with no treatment (lower left), thiram alone (lower center), or T12B alone (lower right). **B,** Seedlings 8 days after planting when planted in uninfested (upper row) or *Pythium*-infested (lower row) soils. Seeds were treated with matrix priming (left), matrix priming and thiram (center), and matrix priming and T12B (right).

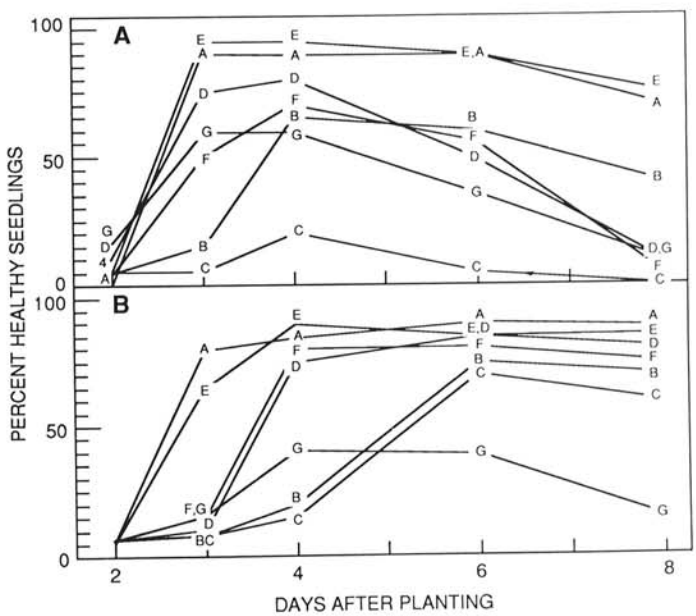

Fig. 3. Effects of variations on matrix priming treatment of seeds of cucumber, **A,** and tomato, **B,** in combination with *Trichoderma harzianum* strain T12B. Seeds were given the standard matrix priming treatment and T12B (A), as in A, except the 4-day incubation period was omitted (B), T12B was added to Agro-Lig, the mixture incubated 4 days at 20 C, and then added to seeds (C), as in A, except that the Agro-Lig was removed just before sowing, and replaced with fresh Agro-Lig and T12B (D), as in A, except that seeds soaked for 6 hr before matrix priming (E), as in (A), but carrier was rinsed off and seeds were blotted dry before planting (F). Seeds treated with T12B also were primed in aerated water column containing polyethylene glycol before planting (G). The minimum significant difference (MSD) for cucumbers was 10, 14, 21, and 23 at days 3, 4, 6, and 8, respectively; while for tomatoes it was 9, 12, 14, and 15 for days 3, 4, 6, and 8, respectively.

gave similar results to seeds treated only with Methocel when planted in *Pythium*-infested soil. However, when seeds were treated with 0.5 or 1 N HCl together with T12B, much improved stands were obtained relative to seeds treated only with T12B. No seeds treated either with HCl plus Methocel or Methocel alone emerged from infested soil (Fig. 5). When seeds were germinated immediately after treating, no toxic effects of this treatment were noted, but after storage for more than 3 days, germination was reduced markedly by the HCl or HCl plus T12B treatments.

DISCUSSION

These results clearly demonstrate that efficacy of *Trichoderma* and *Enterobacter* strains can be increased by combining with matrix priming. When matrix priming and *Trichoderma* were combined, seeds were better protected and grew more rapidly in *Pythium*-infested soil than if they were treated similarly with thiram. This is the first case in our experience where these organisms were superior to an effective chemical fungicide.

Differences in efficacy between matrix priming plus *Trichoderma* and *Trichoderma* treatment alone were greater with cucumbers than with tomatoes. *Trichoderma* strains protected tomato seeds much more effectively than cucumber seeds in the absence of matrix priming. This difference may be due to the highly acidic nature of the tomato seeds, although other differences (e.g., seed size or quantity of exudates) cannot be excluded.

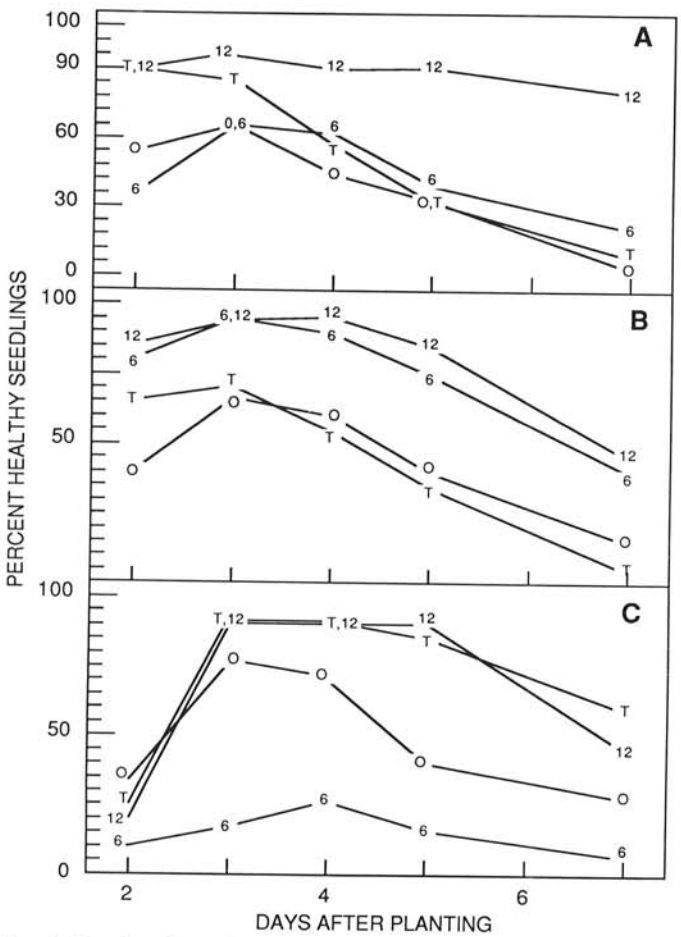

Fig. 4. Results of matrix priming of cucumber using Agro-Lig, **A,** bituminous coal, **B,** or sphagnum peat, **C,** as the carrier. Symbols on graphs are for seeds that were not treated (0), treated with *Trichoderma harzianum* strain T12B (12), treated with *Enterobacter cloacae* strain E6 (6), or thiram (T), in addition to matrix priming in the carrier indicated. Seeds were also treated with *T. harzianum* strain T95 and *T. koningii* strain T8; data with these treatments were similar to that with T12B, and are not shown to improve clarity of presentation. The minimum significant difference (MSD) was 17, 11, 15, 19, and 36 for days 2, 3, 4, 5, and 7, respectively.

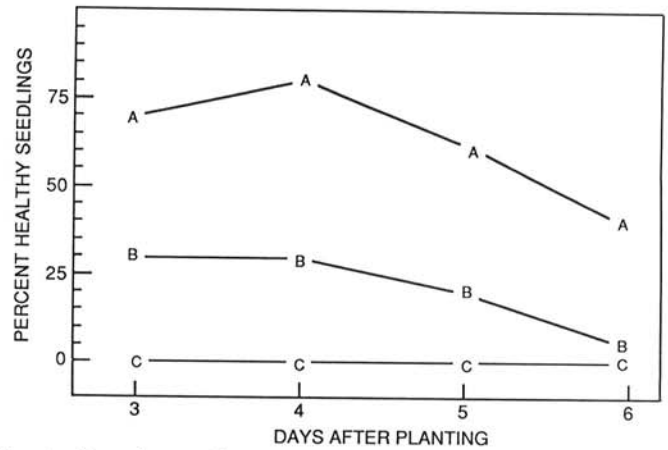

Fig. 5. Cucumber seedling emergence in *Pythium*-infested soil after treatment with T12B and 0.5 N HCl and Methocel (A), T12B and Methocel (B), or with either Methocel alone or 0.5 N HCl and Methocel (C). All implied comparisons at each day are significantly different.

Trichoderma spp., including ones used in this study, grow well at pH 3 (E. B. Nelson and G. E. Harman, *unpublished*). Such a pH should be inhibitory to most other microorganisms, and, thus, *Trichoderma* strains should have a competitive advantage over other microorganisms in the spermosphere on tomato, but not on cucumber. Thus, on cucumber seeds the initiation of growth and of proliferation that occurs during matrix priming is required for effective protection of cucumbers, but not for tomatoes. Conversely, *E. cloacae* did not survive the matrix priming treatment in Agro-Lig on the acidic tomato seeds, and therefore performed poorly.

Matrix priming with Agro-Lig was more effective in protecting seedlings when *Trichoderma* was the organism used, but matrix priming with coal was more effective with E6. A major difference between these two materials is the pH, with Agro-Lig being considerably more acidic than coal. As noted above, *Trichoderma* strains are favored by acidic environments, while E6 is favored by conditions approaching neutrality. At least a portion of the carrier effect on the different biocontrol agents probably is due to this pH difference. However, Agro-Lig contained higher levels of iron than the coal, and low levels of iron adversely affect the ability of some strains of *Trichoderma* to compete with other soil microflora (12).

Further evidence for the role of pH, and for enhancement of activity of *Trichoderma* by highly acidic pH levels was provided by experiments in which HCl was added to Methocel seed treatments on cucumber. Addition of 0.5 or 1 N HCl to T12B, making the pH of the seed 3.7 or 3.1, respectively, markedly improved its ability to control seed rots. Even though this treatment was phytotoxic after a few days of storage, these results indicate that other acidic *Trichoderma* seed treatments may give good seed protection. Such a system would be advantageous for seeds that, for economic or other reasons, are not given matrix priming.

There was a marked increase in the growth rate and in the earliness of emergence in seeds treated with matrix priming plus *Trichoderma* (Figs. 2 and 3). This increase was seen only when seeds were planted in *Pythium*-infested soil and was more pronounced on seeds treated with T12B than with seeds treated with T8 or T95. Similar responses have been noted by others (4,21), but in these reports, effects were seen in both infested and uninfested soil.

Seeds treated with *Trichoderma* plus matrix priming emerged and grew at a rapid rate regardless of whether the soil was infested with *P. ultimum*. Untreated or thiram plus matrix priming seeds emerged and grew at the same rapid rate when they were planted in uninfested soil. However, even though thiram protected seeds against preemergence damping-off and seedlings initially seemed healthy, seedlings from thiram plus matrix priming-treated seeds emerged and grew more slowly than seeds treated with matrix priming plus *Trichoderma* in infested soil. These data suggest that

P. ultimum even in the absence of obvious disease, slowed seedling growth and emergence. *Trichoderma* plus matrix priming was able to overcome this reduction in growth.

Matrix priming alone allows seeds to emerge rapidly under a variety of stress conditions, including sub- and supra-optimal temperatures (A. G. Taylor, *unpublished*). Addition of biological control agents results in highly effective seed treatments that protect and enhance seedling growth in the presence of *P. ultimum*. The choice of carrier has a large effect on efficacy of microorganisms. *Trichoderma* strains performed better in sphagnum or in Agro-Lig than in coal, while E6 performed and survived much better in coal than in Agro-Lig. These differences probably are related to the different chemical composition, including pH, of these substances.

LITERATURE CITED

1. Ahmad, J. S., and Baker, R. 1987. Rhizosphere competence of *Trichoderma harzianum*. Phytopathology 77:182-189.
2. Bradford, K. J. 1986. Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. HortScience 21:1105-1112.
3. Burr, T. J., Schroth, M. N., and Suslow, T. 1978. Increased potato yields by treatment of seed pieces with specific strains of *Pseudomonas fluorescens* and *P. putida*. Phytopathology 68:1377-1383.
4. Chang, Y., Chang, Y., Baker, R., Kleifeld, O., and Chet, I. 1986. Increased growth of plants in the presence of the biological control agent *Trichoderma harzianum*. Plant Dis. 70:145-148.
5. Chao, W. L., Nelson, E. B., Harman, G. E., and Hoch, H. C. 1986. Colonization of the rhizosphere by biological control agents applied to seeds. Phytopathology 76:60-65.
6. Cook, R. J., and Baker, K. F. 1983. The Nature and Practice of Biological Control of Plant Pathogens. American Phytopathological Society, St. Paul, MN. 539 pp.
7. Davet, P. 1979. Technique pour l'analyse des populations de *Trichoderma* et de *Gliocladium virens* dans le sol. Ann. Phytopathol. 11:529-533.
8. Greweling, T., and Peech, M. 1965. Chemical Soil Tests. Cornell University Bull. 960. NY Agric. Exp. Stn., Ithaca.
9. Hadar, Y., Harman, G. E., and Taylor, A. G. 1984. Evaluation of *Trichoderma koningii* and *T. harzianum* from New York soils for biological control of seed rot caused by *Pythium* spp. Phytopathology 74:106-110.
10. Hadar, Y., Harman, G. E., Taylor, A. G., and Norton, J. M. 1983. Effects of pregermination of pea and cucumber seeds and of seed treatment with *Enterobacter cloacae* on rots caused by *Pythium* spp. Phytopathology 73:1322-1325.
11. Harman, G. E., Chet, I., and Baker, R. 1981. Factors affecting *Trichoderma hamatum* applied to seeds as a biocontrol agent. Phytopathology 71:569-572.
12. Hubbard, J. P., Harman, G. E., and Hadar, Y. 1983. Effect of soilborne *Pseudomonas* spp. on the biological control agent, *Trichoderma hamatum*, on pea seeds. Phytopathology 73:655-659.
13. Kloepper, J. W. 1983. Effect of seed piece inoculation with plant growth-promoting rhizobacteria on populations of *Erwinia caratovora* on potato roots and in daughter tubers. Phytopathology 73:217-219.
14. MacDonald G. E., Peck, N. H., and Vittum, M. T. 1978. Relationships between ammonium acetate-acetic acid and Morgan's solution for determining extractable P, K, Ca and Mg in soil derived from calcareous glacial till. Comm. Soil Sci. Plant Anal. 9:717-728.
15. Mircetich, S. M. 1971. The role of *Pythium* in feeder roots of diseased and symptomless peach trees and in orchard soils in peach tree decline. Phytopathology 61:357-360.
16. Norton, J. M., and Harman, G. E. 1985. Responses of soil microorganisms to volatile exudates from germinating pea seeds. Can. J. Bot. 63:1040-1045.
17. Pieczarka, D. J., and Abawi, G. S. 1978. Population and biology of *Pythium* species associated with snapbean roots and soils in New York. Phytopathology 68:409-416.
18. Ruppel, E. G., Baker, R., Harman, G. E., Hubbard, J. P., Hecker, R. J., and Chet, I. 1983. Field tests of *Trichoderma harzianum* Rifai aggr. as a biocontrol agent of seedling disease in several crops and Rhizoctonia root rot of sugar beet. Crop Prot. 2:399-408.
19. Sivan, A., and Chet, I. 1986. Biological control of *Fusarium* spp. in cotton, wheat and muskmelon by *Trichoderma harzianum*. J. Phytopathol. 116:39-47.
20. Stasz, T. T., and Harman, G. E. 1980. Interactions of *Pythium ultimum* with germinating resistant or susceptible pea seeds. Phytopathology 70:27-31.
21. Windham, M. T., Elad, Y., and Baker, R. 1986. A mechanism for increased plant growth induced by *Trichoderma* spp. Phytopathology 76:518-521.