A New Approach to Stimulate Population Proliferation of *Trichoderma* species and Other Potential Biocontrol Fungi Introduced into Natural Soils

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ABSTRACT

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Population densities of isolates of *Trichoderma viride* (T-1-R4) and *T. harzianum* (WT-6-24) increased about 10^4- and 10^3-fold, respectively, in natural soil during the first 3 wk of incubation when the antagonists were added as a mycelial preparation (sterile bran-sand-water, [1:1:2, w/w/v] inoculated with conidia and allowed to incubate 1–3 days before addition to soil). The preparation applied consisted of 1.0% bran and 10^3–10^4 young, actively growing propagules per gram of soil. Populations did not increase when conidia were added to soil with or without bran. An 8-day-old preparation was less effective in stimulating a population increase and a 40-day-old preparation was entirely ineffective. The number of colony-forming units (cfu) gradually decreased over 18–36 wk and stabilized at 10^5–10^6/g of soil. Addition to soil of a mycelial preparation to provide as little as 0.01% bran and 10^1–10^2 propagules per gram resulted in a 10^5- to 10^6-fold increase in population. Proliferation in soil resulted only when hyphae were in intimate contact with or in possession of the substrate (bran). Mycelium of T-1-R4 and WT-6-24 in bran resulted in population densities of about 10^8/g of soil in several soil types (sandy loam, loamy sand, loam, silty clay loam) of various organic matter content. Proliferation of populations of *Trichoderma* in soil as a result of addition of mycelial preparations was accompanied by an increase in metabolic activity as determined by CO_2 evolution and by growth of the antagonist from a food base to pieces of organic matter in soil. A wide variety of isolates of *T. viride*, *T. harzianum*, and *T. hamatum* as well as other antagonists (*Talaromyces flavus, Gliocladium virens, G. roseum, G. catenulatum,* and *Aspergillus ochraceus*) added to soil as mycelial preparations gave results similar to those obtained with T-1-R4 and WT-6-24. Population densities increased up to 10^6-fold within the first 3 wk of incubation, with the rate of increase greatest in the first week.

For effective biological control of soilborne plant pathogens, a major consideration is antagonist proliferation after introduction into the soil or rhizosphere. Among the desirable attributes of a successful antagonist are its ability to produce inoculum in excess and to survive, grow, and proliferate in soil and the rhizosphere (2). Ecologically, this has been difficult because of the general inability of introduced alien microorganisms to survive in soils for a variety of reasons (8). Despite this problem, research in the area of biological control of soilborne plant pathogens with introduced antagonists has increased significantly.

More than half of the antagonists applied to seedlings and soil are hyphomycetes, and about half of these belong to the genera *Trichoderma, Gliocladium*, and *Penicillium* (11). In general, antagonistic fungi (including *Trichoderma*) applied to seed did not establish or proliferate in the rhizosphere (11,20). There have been very few studies on the survival or growth of antagonistic fungi introduced into soil. *Sporidesmium sclerotivorum*, a mycoparasite of *Sclerotinia* spp., became established in field soils and population densities increased 10^2-fold during a 12-wk period in the laboratory (1). In contrast, populations of conidia of various *Trichoderma* spp. did not survive when introduced into soil and less than 50% of the added numbers were retrieved after 35 days (20).

In several biological control studies with *T. harzianum*, population densities of the antagonist added to pathogen-infested soils steadily declined despite the control achieved (6,7,19). However, the phenomenon of antagonist establishment and proliferation in soil in relation to biological control may be important and deserves consideration.

This report describes a new approach for inoculum preparation that results in the proliferation and establishment of several isolates of various *Trichoderma* spp. and other potential biocontrol fungi introduced into different natural soils. A preliminary report has been presented (12).

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MATERIALS AND METHODS

Fungal cultures and soils. For most of the experiments, an isolate of *T. viride* Pers. ex Gray designated T-1-R4 was used. Other antagonists used were *T. viride* (TS-1-3), *T. harzianum* Rifai (Th-23, WT-6-24), and *T. hamatum* (Bonord.) Bain (Tm-1, Tm-4). The two benomyl-resistant isolates T-1-R4 and WT-6-24 were developed by ultraviolet irradiation (22,23) of the wild strains T-1 and WT-6 provided by H. D. Wells, Tifton, GA. The benomyl-resistant biotype, TS-1-3, was developed from a naturally occurring isolate of *T. viride* (TS-1) by chemical mutation with *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (Dunn and Papavizas, *unpublished*). We also used *Gliocladium virens* (Gl-21) Miller, Giddens & Foster, *G. roseum* Bain., *G. catenulatum* Gilm. & Abbott, *Talaromyces flavus* (Tf-1) (Klöcker) Stolk & Sampson, and *Aspergillus ochraceus* Wilhelm. All these antagonists were identified as effective biocontrol agents against several diseases (18,19,21–23).

Cultures were maintained on V-8 juice agar (200 ml V-8 juice®, 800 ml water, 1 g glucose, 20 g agar, 6.0 ml 1.0 N NaOH) in the light, and for all fungi (except *T. flavus*) conidial suspensions for introduction into various media were prepared from 9-day-old colonies growing on this medium. Conidia of *T. flavus* were produced after 3 wk of growth on molasses/corn steep medium (g/L of water: molasses, 50; corn steep liquor, 5; glucose, 25; NaCl,

25; agar, 20) (26) under continuous light. Ascospores were produced by growing *T. flavus* in the dark on potato-dextrose agar for 3 wk.

The soils used included a loamy sand (pH 6.4, 0.4% organic matter [O.M.]), a sandy loam (pH 7.3, 12.0% O.M.), a loam (pH 6.2, 5.0% O.M.), and a silty clay loam (pH 6.3, 1.4% O.M.), from Beltsville, MD, and a loamy sand (pH 6.0, 0.3% O.M.), from Salisbury, MD. Soils were passed through a 2-mm screen and maintained at approximately −0.3 to −1.0 bars matric potential for 3 wk before use.

Preparation of spore and mycelium inocula and their addition to soil. Portions of wheat bran (100 g) passed through a 1-mm sieve were mixed with 100 g of quartz sand and 200 ml of water and autoclaved in 1-L Erlenmeyer flasks for 1 hr. Ten milliliters of a spore suspension, counted with a hemacytometer, were added to each flask after cooling and the cultures were incubated at 25 C. Depending upon each experiment, spores were added to provide from 10^5 to 10^8 per flask with incubation from 0 to 40 days before preparations were added to soils. Preparations of moistened sand-bran containing young hyphae (1–3 days after inoculation) are hereafter referred to as mycelial preparations (MP). Sand-bran containing spores and immediately added to soil is referred to as a spore preparation (SP). In one experiment, conidia of T-1-R4 were germinated in potato-dextrose broth (1 g/L) in shake flasks to obtain 2-day-old mycelium which was washed free of nutrients before addition to soil with or without bran. After various incubation periods, MP and SP were mixed with 4-kg (dry weight equivalent) of soil to provide 0.01, 0.10, or 1.0% bran. The amended soils were mixed well, divided into four portions, and placed in 18 × 12 × 6.5-cm plastic flats. The flats were randomized in the greenhouse under natural light at 20–23 C and watered daily to approximately −0.7 bars matric potential. Each experiment was performed twice with four replicates.

Assay for colony-forming units (cfu) of *Trichoderma* spp. and other biocontrol fungi. Soil samples from flats were withdrawn immediately after amendment and periodically thereafter with a 1-cm-diameter cork borer from the top 3 cm. Serial dilutions were prepared immediately and 1-ml aliquots were spread on the appropriate medium (five plates per replicate per dilution). For biotypes T-1-R4, WT-6-24, and TS-1-3 of *Trichoderma* the benomyl-amended, semiselective medium (TME) of Papavizas and Lumsden (24) was used. Biotype T-1-R4 was chosen for use in most of the experiments because of its readily identified morphological characteristics on TME + benomyl, its ability to tolerate benomyl, and because it is isolated and detected easily on this medium.

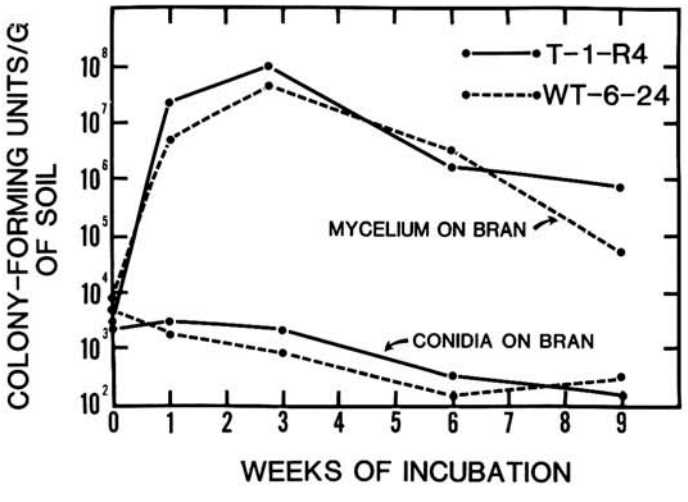

Fig. 1. Colony-forming units of *Trichoderma viride* (T-1-R4) and *T. harzianum* (WT-6-24) over a 9-wk period in a loamy sand after amendment of the soil with a bran-sand-water preparation (1:1:2, w/w/v) inoculated with 10^6 conidia per gram of bran 3 days before (mycelium on bran) and at the time of addition (conidia on bran) to soil. Preparations were added to provide 1.0% bran and about 10^4 propagules per gram of soil.

Population densities (cfu) of *Trichoderma* Tm-1, Tm-4, Th-23; *G. virens* Gl-21; and *A. ochraceus* were determined on TME medium without benomyl and those of *G. roseum* and *G. catenulatum* on TME without fungicides. Population densities of *T. flavus* Tf-1 were assayed on Papavizas' selective medium for this fungus (17). Fungal colonies were counted on the agar plates 7–10 days after incubation at 25–28 C under continuous fluorescent light and populations were reported as colony-forming units per gram (cfu/g) of soil.

Metabolic activity and growth of *Trichoderma* spp. in soil. Carbon dioxide (CO_2) evolution from growth of fungi added to natural loamy sand as MP was determined with a modification of the alkaline trap method (25). Portions of moist soil (100 g) amended with SP or 3-day-old MP (to provide 0.1% bran and 10^3 propagules per gram) of T-1-R4 and WT-6-24 were placed in 7 × 7-cm capped glass jars along with 5 ml of standard 0.1 N NaOH in a beaker. Periodically, over 24 hr, residual NaOH was titrated with standard 0.1 N HCl after precipitation of Na_2CO_3 with $BaCl_2$. Evolution of CO_2 is reported as micrograms per gram of soil per hour (μg/g/hr) for four replicates of each treatment. Other treatments consisted of nonamended soil and soil amended with bran alone.

Saprophytic growth of T-1-R4 and WT-6-24 was assayed by placing a layer of loamy sand (50 g) in a 9-cm-diameter petri plate. The soil was passed through a 2-mm screen and maintained at −0.3 bar (8% moisture) for 3 wk before use. A 3-mm-diameter core of soil was removed from the center of the plate and replaced with 100 mg of MP or SP covered with soil. Six sterilized table beet seeds (*Beta vulgaris* L.) were placed in a concentric ring 1.5 cm from the inoculum. Plates were covered and incubated 5 days at which time seeds were withdrawn from the soil, washed, surface-disinfested, and comminuted in a blender. One-milliliter aliquots were spread

Fig. 2. Three-day-old mycelial preparation of *Trichoderma viride* (T-1-R4) showing young hyphae developed from a germinated conidium (arrow) on surface of a bran particle and ramifying along bran surface. Bar = 15 μm. Hyphae are stained with lactophenol-trypan blue.

on TME with benomyl and T-1-R4 and WT-6-24 cfu/g of dry weight of beet seed were determined after incubation.

RESULTS

Proliferation of *Trichoderma* spp. introduced into natural soil. SP and 3-day-old MP of T-1-R4 and WT-6-24 were added to loamy sand to provide 1% bran and $\sim 10^4$ propagules per gram of soil. After MP were incubated in soil for 1 wk, 5×10^6 and 2×10^7 cfu/g of soil of WT-6-24 and T-1-R4 were recovered, respectively (Fig. 1). Three weeks after incubation, 5×10^7 and 10^8 cfu/g of soil of WT-6-24 and T-1-R4, respectively, were recovered, a 10^4-fold increase in cfu compared to the amounts initially introduced into the soil. By 6 wk, the cfu of both fungi decreased with T-1-R4 stabilizing at 10^6/g and WT-6-24 at 10^4/g over an 18–36 wk period. When conidia were added to soil alone or with wheat bran, fungal populations did not increase. Numbers steadily declined and by the end of 9 wk, populations of both T-1-R4 and WT-6-24 approached 10^2 cfu/g of soil. Attempts to increase populations of *Trichoderma* spp. more than fivefold in natural soil by using conidia of several isolates with various types of organic matter were unsuccessful and data are not reported in this paper.

In addition to determining the effect of 3-day-old MP on proliferation in soil, we also studied how 1-, 8-, 15-, and 40-day-old preparations of T-1-R4 contributed to population increases. Only 1- and 3-day-old inocula were considered MP because the young hyphae enmeshed with the bran appeared vigorous, and there was little evidence of lysis and no formation of conidia or chlamydospores (Fig. 2). MP added to soil to provide 1% bran and about 10^4 cfu/g of soil dramatically increased the population of T-1-R4 (Table 1). At 3 wk, there were $\sim 10^8$ cfu/g of soil, more than a 10^4-fold increase over the initial population level of both 1- and 3-day-old MP. In contrast, the population of T-1-R4 in soil just after amendment with 8-, 15-, and 40-day-old preparations were 8×10^6, 4×10^7, and 7×10^8 cfu/g of soil, respectively. These high numbers were due to the increases in conidia and chlamydospores that occurred in the preparations during the incubation period (8–40 days) before their addition to soil. Although the population density increased to 4×10^8 cfu/g of soil within 3 wk of amendment with an 8-day-old preparation, this was less than a 10^2-fold increase compared to the original population density. Populations never increased in soil amended with 15- or 40-day-old preparations. With all preparations, populations gradually declined in soil with further incubation.

To determine the minimum amount of bran and propagules of T-1-R4 necessary for a 3-day-old MP to induce proliferation, soils were amended with MP to provide 0.01, 0.1, and 1.0% bran and 10^2, 10^3, and 10^4 cfu/g of soil, respectively. All preparations were equally effective in inducing high population proliferation during the first week of incubation (Fig. 3). After 3 wk, MP providing 0.1 and 1.0% bran and 10^3 and 10^4 cfu/g, respectively, resulted in almost 10^9 cfu/g of soil. This represented a 10^5- to 10^6-fold increase.

The ability of mycelium of T-1-R4 (10^3 cfu/g of soil) to proliferate when added to soil without bran, added to bran just before amendment to soil, and added to soil and mixed before bran amendment was also investigated. As previously observed, a MP resulted in about 10^8 cfu/g of soil after 1 wk of incubation (Table 2). When preformed hyphae (from potato-dextrose broth) were mixed with bran and this preparation was added to soil, the population reached about 10^6 cfu/g of soil. Mycelium first mixed with soil followed by bran addition resulted in about 10^5 cfu/g of soil. Population densities did not increase when mycelium was added to soil alone without any food base. In all instances, populations decreased with continued incubation.

Proliferation of populations was also observed when 3-day-old MP of T-1-R4 were added to several soil types. Populations increased in loamy sand used in most experiments, silty clay loam, loam, sandy loam, and another loamy sand of various organic

TABLE 1. Colony-forming units (cfu) of *Trichoderma viride* (T-1-R4) over a 6-wk period in a loamy sand field soil amended with a 1% bran-fungus preparation of various ages

Preparation age[a] (days)	*T. viride* (cfu/g) in soil after incubation for:[b]			
	0 wk	1 wk	3 wk	6 wk
0	1×10^4 x	4×10^3 y	4×10^2 y	2×10^2 y
1	8×10^3 z	1×10^6 y	8×10^7 x	3×10^6 y
3	2×10^4 z	8×10^6 y	2×10^8 x	5×10^6 y
8	8×10^6 y	2×10^7 y	4×10^8 x	4×10^5 z
15	4×10^7 x	2×10^6 yz	3×10^6	3×10^5 z
40	7×10^8 x	3×10^6 y	1×10^6 y	5×10^5 y

[a]Bran-sand-water preparation (1:1:2, w/w/v) was inoculated with 10^6 conidia per gram of bran at indicated days before addition to soil.
[b]Each value is the average of four replications (five plates per replication). Numbers in each line followed by same letter do not significantly differ from each other according to Duncan's multiple range test, $P = 0.05$.

TABLE 2. Colony-forming units (cfu) of *Trichoderma viride* (T-1-R4) over a 3-wk period in a loamy sand field soil amended with mycelium without bran and in several states of contact with bran

Soil amendment[a]	*T. viride* (cfu/g) in soil after incubation for:[b]		
	0 wk	1 wk	3 wk
A. Mycelium without bran	1×10^3 x	5×10^2 x	4×10^2 x
B. Mycelium followed by bran	9×10^2 y	8×10^4 x	3×10^3 y
C. Mycelium mixed with bran	3×10^3 y	7×10^5 x	9×10^3 y
D. Mycelium grown on bran	8×10^2 z	4×10^8 x	7×10^6 y

[a]A = Mycelium of T-1-R4 produced on potato-dextrose broth (PDB) for 2 days and added to soil at about 10^3/g. B = Mycelium of T-1-R4 produced on PDB for 2 days and added to soil at about 10^3/g followed by bran at 1%. C = Mycelium of T-1-R4 produced on PDB for 2 days, mixed with bran, and the preparation added to soil to provide about 10^3/g and 1% bran. D = Two-day-old mycelium preparation developed on bran and added to soil at about 10^3/g and 1% bran.
[b]Each value is the average of four replications (five plates per replication). Numbers in each line followed by same letter do not significantly differ from each other according to Duncan's multiple range test, $P = 0.05$.

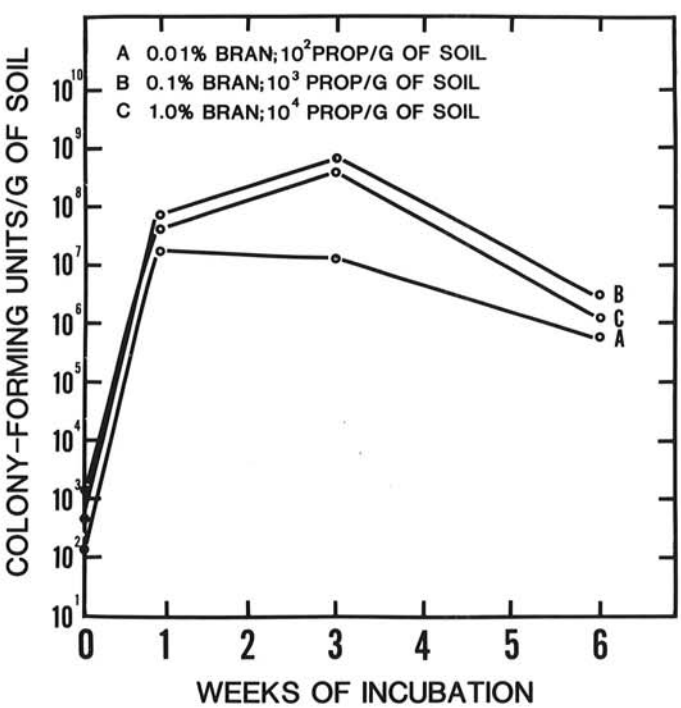

Fig. 3. Colony-forming units of *Trichoderma viride* (T-1-R4) over a 6-wk period in a loamy sand amended with various concentrations of a bran-sand-water preparation (1:1:2, w/w/v) inoculated with 10^6 conidia per gram of bran 3 days before addition to soil. Preparations were added to provide initially 0.01% bran with about 10^2 propagules per gram of soil (curve A), 0.1% bran with about 10^3 propagules per gram of soil (curve B), and 1.0% bran with 10^4 propagules per gram of soil (curve C).

matter contents. Over a 3-wk period, about 10^8 cfu/g of soil was reached in all soils studied, although the rate of proliferation was higher in the loamy sand than in the other soil types.

Proliferation was also observed using MP of other isolates of *Trichoderma* (Fig. 4). Population levels of *T. viride* TS-1-3, *T. harzianum* Th-23, and two isolates of *T. hamatum* (Tm-1, Tm-4) reached 10^8–10^9 cfu/g within 3 wk of incubation. These increases ranged from 10^4- to 10^6-fold compared to the initial inoculum added. Populations stabilized at about 10^4–10^5 cfu/g after 9 wk and were maintained until the end of the test (36 wk). As in previous tests, SP did not proliferate in soil. In general, population densities of all isolates using SP declined 10^2-fold over 9 wk compared to the initial inoculum.

Proliferation of other antagonistic fungi introduced into soil. Population densities of other fungi also increased after incorporation of MP into a loamy sand (Fig. 5). With the addition of 3-day-old MP to provide 0.1% bran and 10^3 cfu/g of soil populations of *G. virens, G. catenulatum, G. roseum*, and *T. flavus* were 4×10^9, 6×10^8, 10^8, and 2×10^7 cfu/g of soil, respectively, after the first week of incubation. This represented 10^5- to 10^6-fold increases in populations. Preparations of *T. flavus* with hyphae formed from conidia or ascospores gave similar results. After 1 wk, the population of *A. ochraceus* increased 10^4-fold to 8×10^6 cfu/g when the MP added to soil was only 1 day old because sporulation rapidly occurred in 3-day-old preparations. With these fungi, as with *Trichoderma* spp., propagule numbers gradually declined and stabilized in soil with further incubation.

Metabolic activity and growth of *Trichoderma* spp. in soil. Population proliferation was associated with increased metabolic activity of the MP in soil and growth of the antagonists. Within the first 24 hr of incubation, the amount of CO_2 evolved from bran added to soil (34.8 μg/g/hr) or from SP of T-1-R4 or WT-6-24 added to soil (30.3 and 35.1 μg/g/hr, respectively) did not differ statistically from that evolved from nonamended soil (29.1 μg/g/hr). When MP were added to soil to provide 0.1% bran and 10^3 cfu/g about three times more CO_2 (85.3 and 80.6 μg/g/hr from T-1-R4 and WT-6-24, respectively) evolved than from the SP. This indicated a high metabolic activity from MP but not from SP. Growth of both fungi occurred in soil infested with MP but not SP. Both T-1-R4 and WT-6-24 grew through soil up to 1.5 cm from the MP food base to an organic substrate (beet seed). After 5 days, seed was colonized with T-1-R4 and WT-6-24 and yielded 5×10^3 and 2×10^3 cfu/g dry weight of seed, respectively. With SP, there were 30 cfu/g of beet seed with T-1-R4 and no colonization with WT-6-24.

DISCUSSION

The results show that *Trichoderma* spp. as well as other potential biocontrol fungi proliferate abundantly in various natural soils in the greenhouse when added as young mycelium on bran but not as conidia. To our knowledge, this is the only example in which populations of introduced fungi increased to such a magnitude in soils that were not steamed, fumigated, or treated with other biocides. In addition, establishment of the biocontrol agents in soil was suggested since population levels remained nearly constant from 9–36 wk after inoculum was introduced. Populations were maintained at least 10^2-fold greater than the original populations even after 36 wk. Proliferation (up to 10^6-fold) and subsequent establishment of biocontrol fungi in soil depended on inoculum age and how inoculum was added in relation to the food base. Numbers of all fungi studied increased dramatically when inoculum was added to soil with wheat bran preparations upon which young hyphae had developed for 1–3 days. In addition, relatively small amounts of these MP (0.01%) were necessary to induce increases in soil population densities by as much as 10^5-fold.

The age of the inoculum was critical for maximum proliferation in soil. A 1- and 3-day-old MP resulted in almost a 10^3-fold increase in cfu/g after 1 wk of incubation and 10^4-fold increase to about 10^8 cfu/g at 3 wk of incubation. Even though a 40-day-old preparation of T-1-R4 gave 7×10^8 cfu/g of soil at time of amendment, populations never increased but decreased 10^3-fold over a 6-wk period. Mature 15- and 40-day-old preparations contained conidia and chlamydospores with few viable hyphae (13). Regardless of the nature of the initial inoculum, whether young or old bran preparations, population densities generally were similar after 6 wk of incubation and there appeared to be little difference in long-term survival levels. The results indicated that conidia added to soil fresh from culture without or with bran (0-day-old preparation) or those

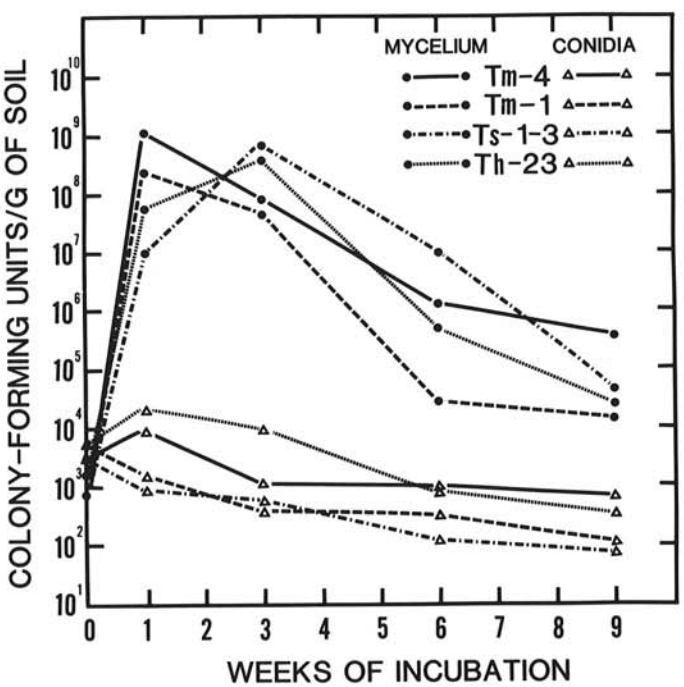

Fig. 4. Colony-forming units of *Trichoderma hamatum* (Tm-1, Tm-4), *T. viride* (TS-1-3), and *T. harzianum* (Th-23) over a 9-wk period in a loamy sand after amendment of the soil with a bran-sand-water preparation (1:1:2, w/w/v) inoculated with 10^6 conidia per gram of bran 3 days before (mycelium on bran) and at the time of addition (conidia on bran) to soil. Preparations were added to provide initially 0.1% bran and about 10^3 propagules per gram of soil.

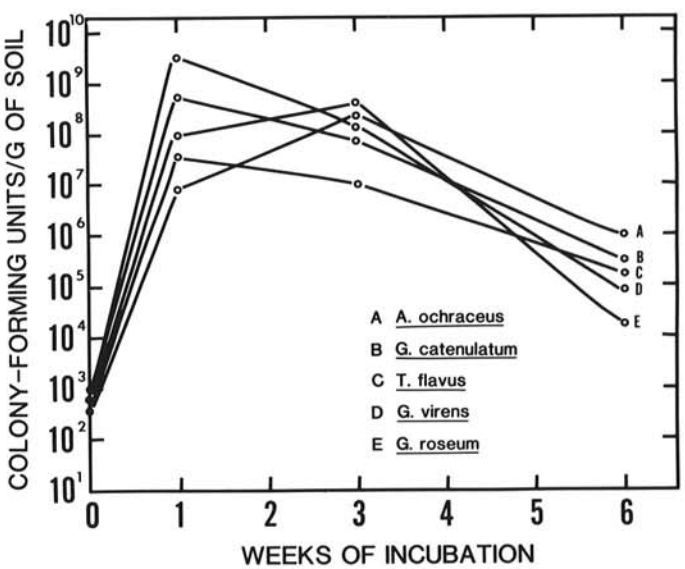

Fig. 5. Colony-forming units of *Gliocladium virens, G. catenulatum, G. roseum, Talaromyces flavus*, and *Aspergillus ochraceus* over a 6-wk period in a loamy sand after amendment of the soil with a bran-sand-water preparation (1:1:2, w/w/v) inoculated with 10^6 conidia per gram of bran. Soils were amended with 3-day-old mycelial preparations of the first four fungi and a 1-day-old preparation of *A. ochraceus* to provide initially 0.1% bran and about 10^3 propagules per gram of soil.

which form in older preparations, were ineffective in stimulating increases in antagonist populations. This is consistent with previous results in which populations of various *Trichoderma* spp. did not even increase significantly when inoculum as conidia was added to fumigated soils (15).

The unique ability of young hyphae of *Trichoderma* and other antagonists, but not conidia, to proliferate may be due, in part, to their resistance to fungistasis (16). Conidia of *Trichoderma* spp. and other Fungi Imperfecti are subject to this phenomenon. Failure of ungerminated conidia on bran to germinate in soil may be due to rapid colonization of the bran by other microbiota (3). In contrast, hyphae already occupying the food base do not appear to be subject to fungistasis. Bruehl's (3) substrate possession principle may explain the phenomenon of proliferation in our studies.

Hyphae grown on and enmeshed within the bran proliferated in soil much better than preformed hyphae added to soil with bran in any other combination. This intimate contact between mycelium and food base enabled the fungus to grow relatively unimpeded through the soil. We suspect that the cfu assayed throughout the period of population proliferation were conidia and chlamydospores that were abundantly produced as a result of growth regardless of the hostile environment into which the MP were introduced. The rapid formation of both conidia and chlamydospores during growth of *Trichoderma* spp. in natural organic matter in soil has been observed previously (14). The gradual decline in populations of all fungi studied which occurred by the sixth week of incubation may be due to lysis and disintegration of conidia, since numbers of even freshly added conidia declined when added to soil. The relatively stable population densities of *Trichoderma* that remained in soil after 9–36 wk of incubation may be due to survival of chlamydospores.

It is not known whether population proliferation of an introduced antagonist is directly related to biological control. Few studies have established such a relationship. For example, the exact amount and nature of antagonist inoculum or its subsequent survival was not indicated in any of the 13 examples of successful field biological control of soilborne pathogens performed in various parts of the world from 1971 to 1980 in which antagonists were amended to soils (21). Recently, we observed that MP, but not SP, of several isolates of *Trichoderma* reduced fruit rot of tomatoes and damping-off of cotton caused by *Rhizoctonia solani* in the greenhouse; this was accompanied by a reduction in the inoculum density of the pathogen and an increase in antagonist population density of 10^3-fold (*unpublished*). In a study with indigenous *Trichoderma* spp. and continuous radish monoculture, Rhizoctonia damping-off was gradually reduced accompanied by a 10^4-fold increase in cfu of the native soil *Trichoderma* spp. (9).

The biological activities associated with antagonist proliferation in soil are probably more critical than the proliferation itself. It is recognized that growth and metabolism of antagonists must be active for successful biological control. We suggest that what may occur during the first several weeks of population proliferation is rapid growth and development of the biocontrol agent from the hyphae on the food base into the soil. These highly metabolically active hyphae may colonize new organic matter and also attack and destroy pathogen propagules in their vicinity. The end result is an abundant antagonist population as well as a decrease in pathogen inoculum. *Trichoderma* spp. and other fungi were shown to attack sclerotia of various pathogens in soil (5,10). During active growth, *Trichoderma* spp. were also reported to produce large quantities of extracellular β-(1,3)-glucanase and chitinase, which dissolved pathogen cell walls (4).

Addition to soil of inoculum in the form of mycelium may also be necessary to enhance the activity of biocontrol agents other than those of the genus *Trichoderma*. The substantial increases in population of *Gliocladium, Talaromyces*, and *Aspergillus* with MP suggest the feasibility of using this approach to increase numbers and possibly the biocontrol ability of a wide variety of potential antagonists.

The potential for using preparations containing young hyphae in achieving biological control is an intriguing possibility that deserves continued investigation.

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