Ecology and Epidemiology

Rhizosphere Competence of *Trichoderma harzianum*

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ABSTRACT

Ahmad, J. S., and Baker, R. 1987. Rhizosphere competence of *Trichoderma harzianum*. Phytopathology 77:182-189.

Rhizosphere competence of *Trichoderma harzianum* was measured for roots of bean, cucumber, maize, radish, and tomato. When seeds were coated with conidia of the wild type and germinated in soil under constant matric potential with no additional water added, the fungus was not detected in the rhizospheres of roots from 1- to 8-cm depths after 8 days. Mutants tolerant to benomyl, however, were rhizosphere-competent when 10 μg of benomyl was added per gram of soil. These mutants were also rhizosphere-competent when benomyl was not added. The degree of rhizosphere colonization by mutants of *T. harzianum* was influenced by benomyl, soil pH, and temperature. Evidence provided herein indicates that a rhizosphere-incompetent biological control agent was induced by mutation to become rhizosphere-competent.

Trichoderma harzianum Rifai is a biological control agent effective against *Pythium* spp. (9,19,28), *Rhizoctonia solani* Kühn (12–17,19,26,31), and *Sclerotium* spp. (1,4,9,13,26,36). It also increased seed germination and enhanced plant growth (7,37).

Whereas seed treatment with *Trichoderma* spp. reduced preemergence damping-off (20), later protection of the mature plant has not been realized mainly because *Trichoderma* spp. were not rhizosphere-competent (8,26). Therefore, in discussion sessions and workshops (e.g., 5), the need is frequently expressed for inducing such antagonists to colonize not only the spermosphere but also the developing root.

The report by Mendez-Castro and Alexander (23) suggested a method for establishing previously rhizosphere-incompetent microorganisms on roots. Based on this approach, fungicide-tolerant mutants could become rhizosphere-competent by addition of fungicide to the soil. The chemical would adversely affect resident rhizosphere colonists thereby giving the fungicide-tolerant agent a competitive advantage. The objective of the research reported here was to investigate the possibility of inducing *T. harzianum* to become rhizosphere competent by use of this strategy. A preliminary report has been published (2).

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MATERIALS AND METHODS

Soil. Nunn sandy loam was collected near Nunn, CO, and passed through a 4-mm-mesh screen. Water contents of 6-kg portions were adjusted to −0.3 bar and the soil was incubated in plastic bags for 48 hr before use. The soil had the following characteristics: pH 7.0, conductivity 0.4 mmhos, lime low, organic matter 1.4%, NO_3-N 1 μg/g, P 9 μg/g, K 198 μg/g, Zn 0.5 μg/g, Fe 3.2 μg/g.

Measurement and adjustment of soil pH values. Soil pH was measured by the $CaCl_2$ method (34). To adjust soil pH, 10% (v/w)

0.1N H_2SO_4 was added to a 1-kg portion of soil. The soil was mixed thoroughly, allowed to dry, and ground with a mortar and pestle. By this method, soil pH was reduced from 7.0 to 2.5. Portions of this soil were added to field soil to adjust pH values from 7.0 to 5.0 and 6.0. No change in pH was observed during the course of experiments.

***Trichoderma* spp. and strains.** *T. harzianum* (designated hereafter as WT) originally was isolated from a soil in Colombia, SA (10,11). *T. harzianum* (T-95), a benomyl-tolerant mutant, was derived from *T. harzianum* WT (7). *T. harzianum* (T-12) and *T. koningii* (T-8), isolated from a soil in New York, were provided by G. E. Harman (New York State Agricultural Experiment Station, Geneva, NY). *T. viride* (T-S-1) was provided by M. T. Dunn (Mycogen Corporation, San Diego, CA). *T. harzianum* (T-12B) was a benomyl-tolerant mutant derived from *T. harzianum* (T-12) by the method of Chang et al (7). Conidia of T-12 were exposed to 100 μg/ml of *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (Tredom Chemical, Inc., Hauppauge, NY) for 30 min. The conidia were centrifuged at 2,500 *g* for 15 min and resuspended in sterile water three times. Conidia were plated on *Trichoderma*-selective medium (14) containing 100 g a.i. of benomyl per milliliter and tolerant strains were recovered.

Seed treatment. Strains of *Trichoderma* spp. were grown on potato-dextrose agar (PDA). Mutants tolerant to benomyl were grown on PDA containing 10 g a.i. of benomyl per milliliter. Plates were incubated for 8 days at 25 C, flooded with sterile distilled water and conidia were gently freed from the culture surface with a brush. The suspension was sieved through four layers of cheesecloth, centrifuged at 2,500 *g* for 15 min and resuspended in sterile distilled water three times. Density of conidia was adjusted to 10^8 per milliliter with the aid of a hemacytometer.

Seeds of cucumber (*Cucumis sativus* L. 'Straight Eight'), radish (*Raphanus sativus* L. 'Early Scarlet Globe'), tomato (*Lycopersicum esculentum* L. 'Burpee's Big Boy'), beans (*Phaseolus vulgaris* L. 'Olathe'), and maize (*Zea mays* L. T. E. 6998) were surface-disinfested for 10 min in 1.1% sodium hypochlorite solution and 5% ethanol, washed in distilled water, and air-dried. Seeds were treated with conidial suspensions of *Trichoderma* spp. in water containing 2% (v/w) Pelgel (The Nitragen Co., Milwaukee, WI) as a spreader or sticker. Conidial density was adjusted to 10^6 per seed. Controls were treated with Pelgel alone.

To determine the conidium density per seed, seeds were suspended in water and conidium density in the wash water was determined by use of a hemacytometer and by serial dilutions on *Trichoderma*-selective medium (14).

In experiments where glass beads (12.5 mm diameter) were used, the same procedure was used for application of conidia.

Rhizosphere-competence assay. Polypropylene centrifuge tubes (28.6 by 103.6 mm) were sliced longitudinally into two halves. Each half was filled with moistened soil (−0.3 bars) and preincubated for 48 hr in plastic bags. When benomyl was added to soil, the fungicide was added (10 μg a.i. per gram of soil) and mixed thoroughly just before the tubes were filled. One treated seed was placed on the half-tube 1 cm below the rim. The unseeded half-tube was placed on the first half and secured with rubber bands. Tubes were completely randomized and lots in portions of six each were placed vertically in 10-cm-diameter plastic pots. Soil, previously moistened to −0.3 bars and of the same pH as in the tubes, was added to the pots so that the length of the tube was surrounded by the soil, with the top 1 cm of each tube uncovered (Fig. 1A). No water was added to the tubes or the pots after seeds were sown. Pots were covered with plastic bags to maintain constant matric potential leaving enough space above the tubes for the plants to grow. Pots were placed under constant illumination supplied by 10 white, 40W, 120-cm-long fluorescent lamps (approximately 5,000 lx), at desired temperatures.

After 8 days, or as required by the experiment, tubes were removed from the pots. After the unseeded half of a tube was carefully lifted (Fig. 1B), the roots in the seeded half, starting from the crown, were excised in 1-cm segments with a sterile scalpel (Fig. 1C). The scalpel was flamed between cuts. After loosely adhering soil was shaken off root segments with their adhering rhizosphere soil were air-dried under a 100W lamp for 30 min. Each unit was weighed and transferred to a 20-ml glass vial containing 1 ml of sterile distilled water. The contents of the vial were stirred vigorously with a sterile spatula. The colony-forming units (cfu) of *Trichoderma* contained in the rhizosphere soil at each centimeter of root were determined by plating a series of 10-fold dilutions from the vial on *Trichoderma*-selective medium (14). Root segments were removed from the dilution flask, blotted on a paper towel, and weighed to determine the dry weight of rhizosphere soil removed through washing. In experiments where sand was substituted for soil and glass beads were coated with conidia, sand was sampled from different depths after 8 days and treated as above.

Plates were incubated at 25 C for 5 days. Counts of *Trichoderma* colony-forming units per milligram of rhizosphere soil for each root segment were made with six replicates per treatment. All experiments were repeated twice.

Microscopic observation of roots. Root segments with rhizosphere soil were placed in multi-well tissue culture plates. One-half milliliter of an aqueous 0.3% Calcofluor solution (Calcofluor white M2R, Polysciences, Inc., Warrington, PA) was added to each well. The plate was covered with aluminum foil and incubated at 25 C for 20 hr. Root segments were transferred to a microscope slide. After addition of a drop of water and a cover slip, the root segment was viewed with an Olympus BH microscope (Olympus Optical Co., Tokyo, Japan), with a blue exciter filter (BG-12) providing 400 nm light supplied by an epifluorescent illuminator and with a barrier filter (530 nm).

Each root segment was viewed and the total length of hyphae per root centimeter was measured with the aid of an ocular micrometer. The experiment was repeated twice.

Statistical analysis. The data were transformed to log (cfu count +1), subjected to polynomial contrasts, and two-way analyses of

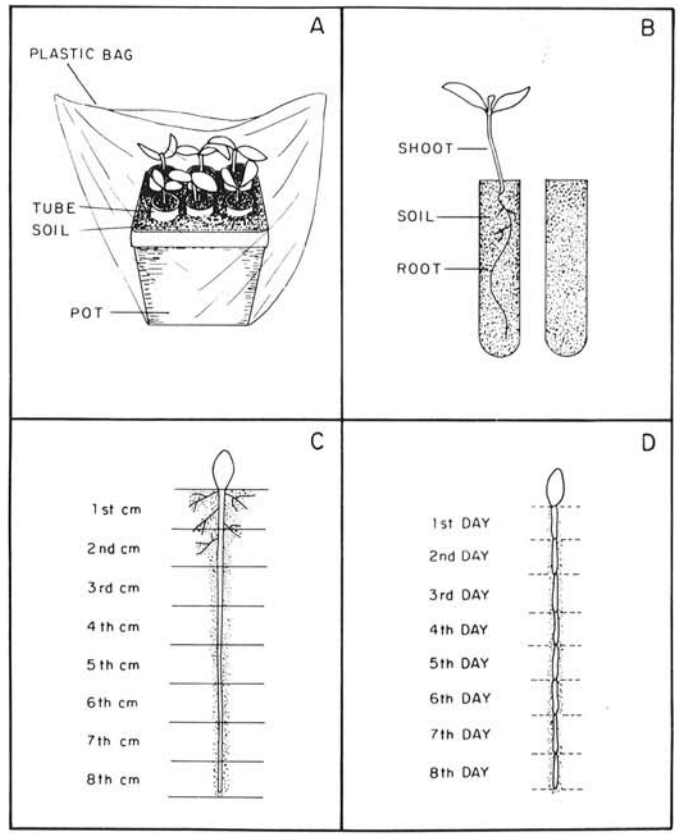

Fig. 1. The rhizosphere competence assay. **A,** Six tubes placed in soil (moistened to same matric potential as soil in tubes, −0.3 bars) in plastic pot and covered with a plastic bag. **B,** Half tube lifted after 8 days to sample rhizosphere. **C,** Sectioning of roots and rhizosphere 8 days after planting seed. **D,** Sectioning of last centimeter of cucumber roots each day for 8 days.

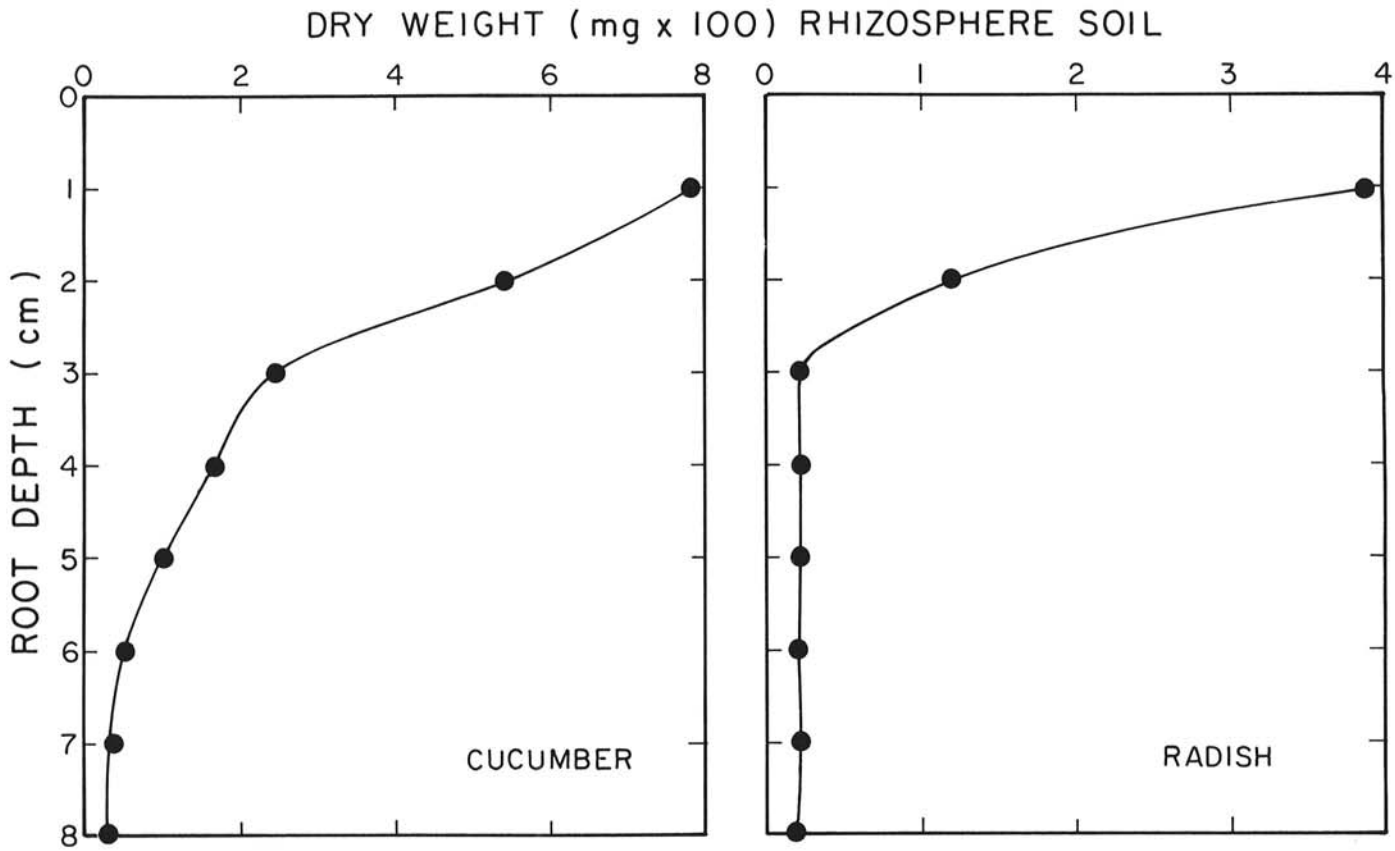

Fig. 2. Air-dried weight of rhizosphere soil sampled in cucumber and radish roots.

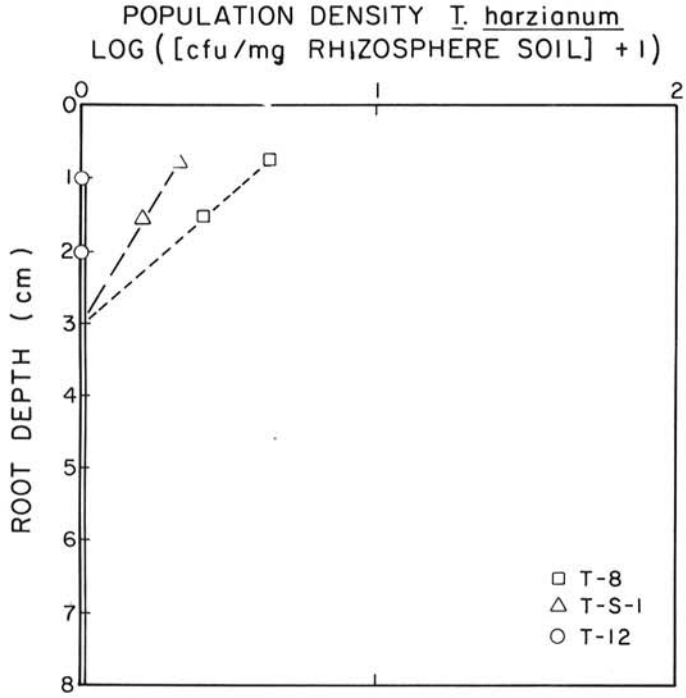

Fig. 3. Population densities of *Trichoderma* spp. from rhizosphere soil of cucumber plants.

variance were performed. The adjacent means within a treatment and means at a given depth among the treatments were separated with an FLSD ($P < 0.05$) (22).

RESULTS

Isolation of *Trichoderma* spp. from rhizosphere soil. Greater amounts of adhering rhizosphere soil was associated with the upper 2 or 3 cm of roots (Fig 2).

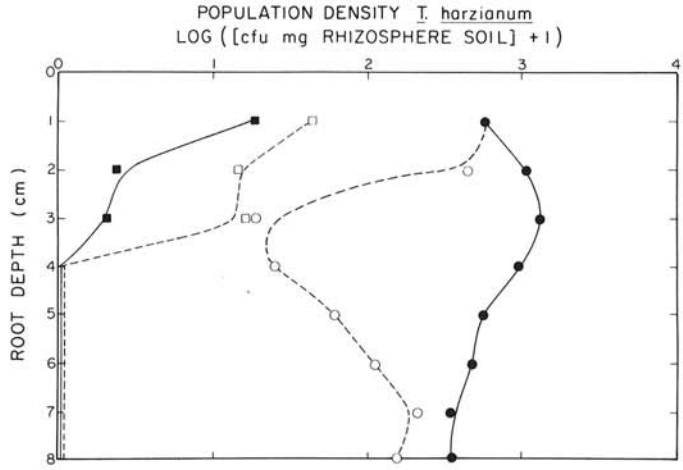

Fig. 4. Population densities of isolates WT and T-95 of *Trichoderma harzianum* in rhizosphere soil of cucumber plants, ■—■ isolate WT with benomyl in the soil, □---□ isolate WT without benomyl, •—• isolate T-95 with benomyl, o---o T-95 without benomyl.

When cucumber seeds were treated with conidia and grown in pH 7.0 soil at 26 C, *T. harzianum* (T-12) was not detected in the rhizosphere soil at any depth. *T. koningii* (T-8) and *T. viride* (T-S-1) were isolated from the rhizosphere soil adhering to the first 2 cm of seedling root (Fig. 3). *T. harzianum* (WT) was isolated from only 1–3 cm of root (Fig. 4). When plants were grown in soil with benomyl, this isolate was recovered from 1–3 cm of root depth, but the population density was significantly reduced as compared with nonbenomyl controls. *T. harzianum* (T-95) was isolated from the rhizosphere soil at all depths whether benomyl was or was not added to soil. However, when benomyl was added, population densities of T-95 in the rhizosphere were significantly higher at root depths from 2–8 cm. *T. harzianum* (T-12B) was isolated from all segments of the rhizosphere (Fig. 5) of cucumber

grown in soil at two pH levels with or without addition of benomyl to the soil.

T. harzianum (T-95) was isolated from rhizosphere soil at 1–8-cm root depth of bean, maize, tomato, and radish without the addition of benomyl to the soil (Figs. 6–8).

When conidia of T-95 were applied to seed coats of cucumber and germination occurred in washed sand, the mutant was detected in every root segment, whereas the *T. harzianum* (WT) was recovered only in the first 1–4 cm of root below the seed (Fig. 9). When glass beads were coated with conidia of WT and T-95, neither isolate was detected in the sand below the beads.

Microscopic observation of roots. Total length of hyphae per root segment is presented in Fig. 10. When benomyl was added to the soil and seeds were treated with T-95 conidia, more hyphae were observed than in controls without seed treatment. In soil where benomyl was not added, roots from seeds treated with T-95 had more hyphae than when seeds were not treated.

Effect of benomyl on rhizosphere competence of *T. harzianum*. *T. harzianum* (T-12B) was isolated from the rhizosphere of cucumber plants grown in pH 5.0 and 7.0 soils (Fig. 5). Compared

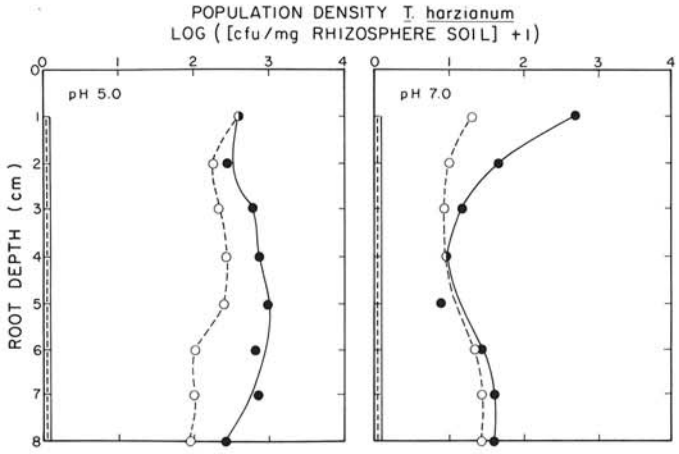

Fig. 5. Population densities of isolates T-12 and T-12B of *Trichoderma harzianum* in the rhizosphere soil (at pH 5 or 7) of cucumber plants, — (far left) T-12 with benomyl in the soil, ---- (far left) T-12 without benomyl, o---o T-12B with benomyl, •—• T-12B without benomyl. Strain T-12 was not recovered at any depth in the rhizosphere.

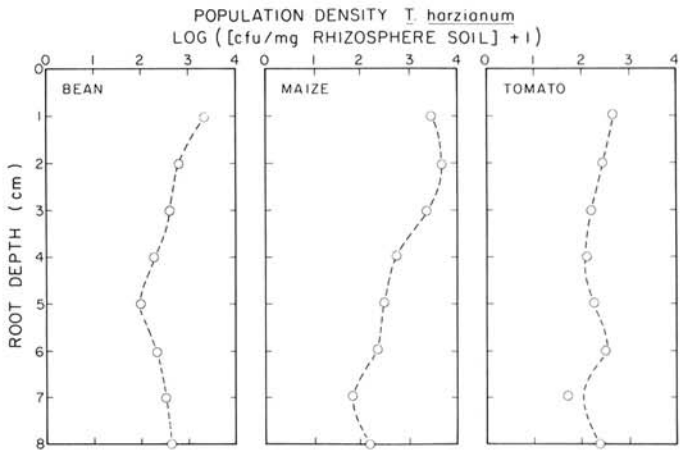

Fig. 6. Population densities of *Trichoderma harzianum* (T-95) in rhizosphere soil of three hosts, without the addition of benomyl to the soil.

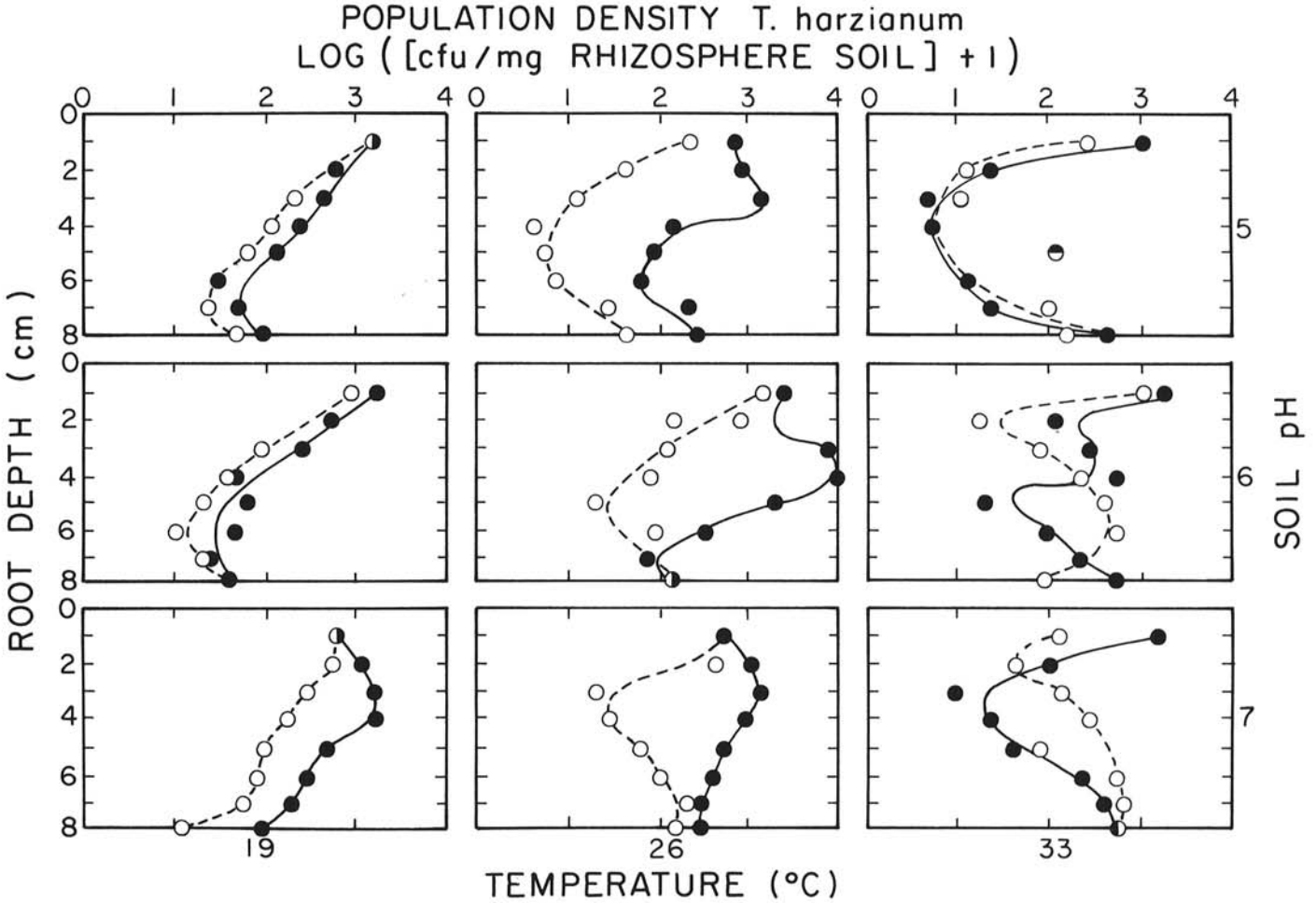

Fig. 7. Population densities of *Trichoderma harzianum* T-95 in rhizosphere soil of cucumber plants grown at three pH levels and three temperatures: •—• with benomyl in the soil, o---o without benomyl.

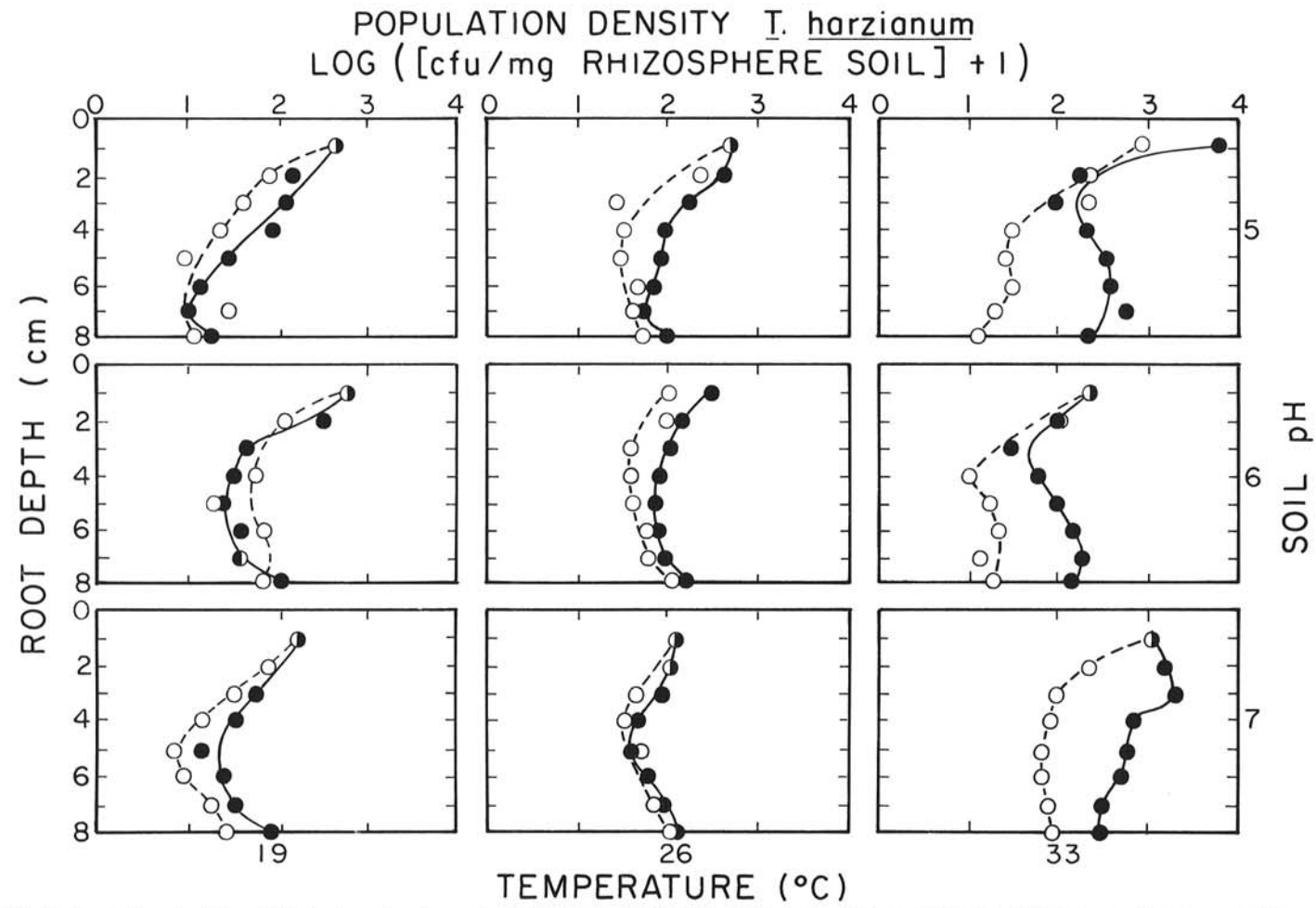

Fig. 8. Population densities of *Trichoderma harzianum* in rhizosphere soil of radish plants grown at three pH levels and three temperatures, ●—● with benomyl in the soil, o---o without benomyl.

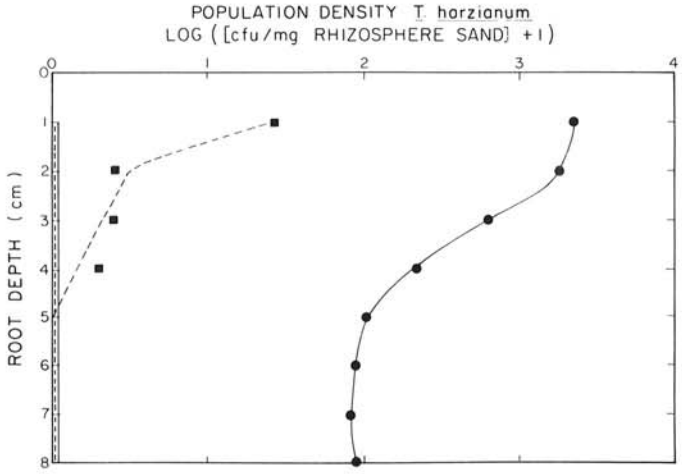

Fig. 9. Population densities of *Trichoderma harzianum* (T-95) in the rhizosphere of cucumber plants growing in sand, ●—● seeds treated with T-95, ■—■ seeds with WT, — (far left) treated with T-95, ---- (far left) beads treated with WT. Strain T-95 was not isolated from the sand at any depth when coated on glass beads.

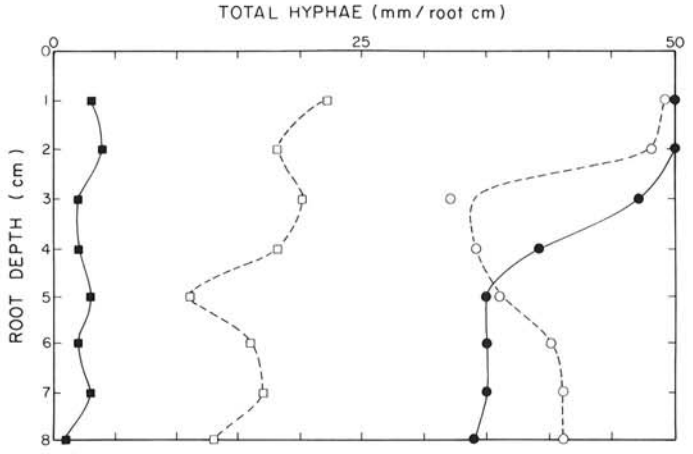

Fig. 10. Total hyphae observed per root centimeter depth of cucumber plants from treated (*Trichoderma harzianum*, T-95) and untreated seeds, ■—■ untreated seeds with benomyl in the soil, □---□ untreated seeds without benomyl, ●—● strain T-95 treated seeds with benomyl, o---o T-95 treated seeds without benomyl.

with controls without benomyl, population densities in benomyl-treated soils were significantly higher from 3–8 cm at pH 5.0 and from 1–3 cm at pH 7.0.

When cucumber seeds were treated with conidia of T-95, the isolate could be detected in rhizosphere soils (with or without benomyl) of pH 5.0, 6.0, and 7.0, at 19, 26, and 33 C (Fig. 7). In soils of pH 5.0, 6.0, and 7.0 incubated at 19 C, population densities were significantly higher in soil amended with benomyl compared with nontreated soil at depths of 3–8; 1, 3, 5, 6; and 2–8 cm, respectively. At pH 5, 6, and 7, incubated at 26 C, population densities were significantly higher in soils amended with benomyl than without at depths of 1–8, 1–6, and 2–8 cm, respectively. In soil pH 5, 6, and 7, incubated at 33 C, population densities were significantly higher in soils amended with benomyl than without at depths of 1, 2, and 8; 1–4 and 8; and 1 and 2 cm, respectively. When radish seeds were treated with conidia of T-95, the isolate was detected in the

rhizosphere soil of pH 5, 6, and 7 at 19, 26, and 33 C in soils with and without benomyl (Fig. 8). At pH 5, 6, and 7, incubated at 19 C, population densities were significantly higher in soil amended with benomyl than without at root depths 2–6 and 8; 2 and 8; and 3–8 cm, respectively. At pH 5, 6, and 7, incubated at 26 C, population densities were significantly higher in soil amended with benomyl than without at root depths 2–6 and 8; 1–5, 7, and 8; and 3, 4, and 7 cm, respectively. At pH 5, 6, and 7, incubated at 33 C, population densities were significantly higher in soil amended with benomyl than without at root depths 1, 4–8; 4–8; and 2–8 cm, respectively.

Rhizosphere competence of plants grown without light. When cucumber seeds were treated with conidia of T-95 and grown at 26 C in the dark, population densities were significantly higher in soils amended with benomyl at root depths 2–7 cm than in soils where benomyl was not added (Fig. 11). When plants were grown at 33 C, population densities were significantly higher in soils amended with benomyl at root depths 1, 2, and 4 cm than in soils where benomyl was not added. At 26 C, the root grew 7 cm, and at 33 C it grew only 5 cm in 8 days in the dark.

Plants grown in the dark in the benomyl-treated soil at 26 C had significantly lower population densities of T-95 than those grown under light at root depths of 4–7 cm (Fig. 11). At the 3-cm depth, population densities of T-95 in plants grown in the dark were significantly higher than of those grown under light. In rhizosphere soil not treated with benomyl, the population densities were significantly higher at root depths of 4–7 cm in plants grown under light than plants grown in the dark.

Plants grown in light at 33 C in soil amended with benomyl had significantly higher population densities of T-95 at root depths of 4 and 5 cm than those grown in the dark (Fig. 11). At 3-cm depth, the population density was significantly higher in plants grown in the dark than of those grown under light. In soils not supplied with benomyl, the population densities at the 2–5 cm root depth, were significantly higher in plants grown under light than those grown in dark.

Daily sampling of roots. Cucumber seeds were treated with conidia of T-95, and the last centimeter of root from different individual plants was sampled daily (Fig. 1D). Root growth rate was approximately 1 cm/day. The data collected by daily sampling of root tips was compared with that collected at each centimeter depth on an entire root. Strain T-95 was isolated from every root segment sampled from 1 to 8 days (Fig. 12). In soils of pH 5.0 and 7.0, population densities of T-95 in soils amended with benomyl were significantly higher than in soil to which no fungicide was added.

In pH 5.0 soil, population densities were significantly higher in soil amended with benomyl at root depths 1 and 4–6 cm when root tips were sampled daily than when entire roots were sampled after 8 days. There were no significant differences among population densities of T-95 in rhizosphere soil adjacent to root tips during the 8-day period except at the 1-cm depth.

In pH 7 soil, population densities were significantly higher in soils amended with benomyl at root depths 2–5 cm when roots were sampled after 8 days as compared with roots sampled daily. In soil not amended with benomyl, roots sampled daily had significantly higher population densities at root depths of 3–6 cm than those sampled after 8 days. Again, no significant differences in population densities of rhizosphere soil around root tip was observed during the 8 days except at the 1-cm depth.

Effect of soil pH on rhizosphere-competence of *T. harzianum*. Cucumber and radish seeds were treated with conidia of T-95 and grown in soils of pH 5.0, 6.0, and 7.0. T-95 was rhizosphere competent at all hydrogen-ion concentrations in soil (Figs. 7 and 8). Population densities decreased in the middle portions of the root forming a C-shaped curve. This curve was not as apparent in pH 5.0 soil. When cucumber seeds were treated with conidia of T-12B, population densities were higher in pH 5.0 than in pH 7.0 soil (Fig. 5). When the last centimeter of root was sampled daily there were no apparent differences in population densities in pH 5.0 and 7.0 soils (Fig. 12).

Effect of temperature on rhizosphere competence of *T. harzianum*. Cucumber and radish seeds were treated with conidia of T-95 and grown at 19, 26, and 33 C. T-95 was rhizosphere competent at all three temperatures (Figs. 7 and 8).

Population densities decreased in the middle portions of the root, forming a C-shaped curve. This curve was not as apparent at 19 C. When cucumber plants were grown in the dark at 26 C, the roots grew 7 cm in 8 days. Plants grown under light at the same temperature had significantly higher population densities at depths of 4–7 cm than plants grown in dark. At 33 C, plants grown in dark had rooted to a depth of 5 cm only. Population densities were significantly lower than in plants grown under light (Fig. 11).

DISCUSSION

Schmidt (33) employed the term "rhizosphere competence" to describe an attribute of rhizobia characterized by their consistent association with legume root nodules. Here, we use the term to describe the ability of a microorganism to grow and function in the developing rhizosphere.

Various methods have been employed to test rhizosphere competence (6,8,23–25,32,36). These were primarily based on a comparison of the numbers of microorganisms in the soil associated with roots to population densities in nonrhizosphere soil. The rhizosphere competence assay used in this research effort was developed to improve measurement in time and space of the activity of potential rhizosphere inhabitants. Certain criteria were demanded by the experimental questions to be examined.

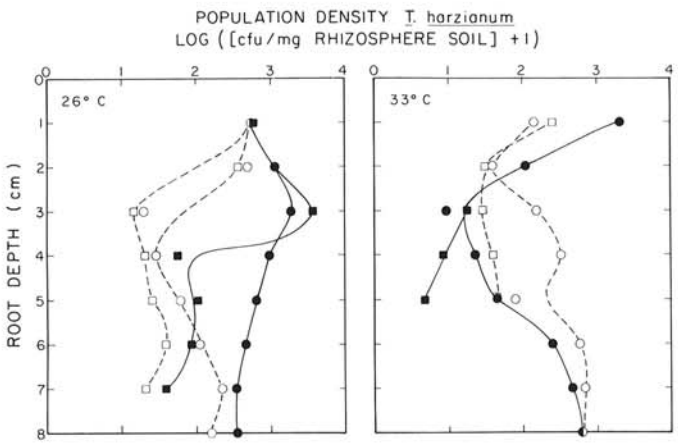

Fig. 11. Population densities of *Trichoderma harzianum* strain T-95 in rhizosphere soil of cucumber plants grown at two temperatures in the light and dark, •—• with benomyl in the soil in light, ■—■ with benomyl in dark, o---o without benomyl in light, □---□ without benomyl in dark.

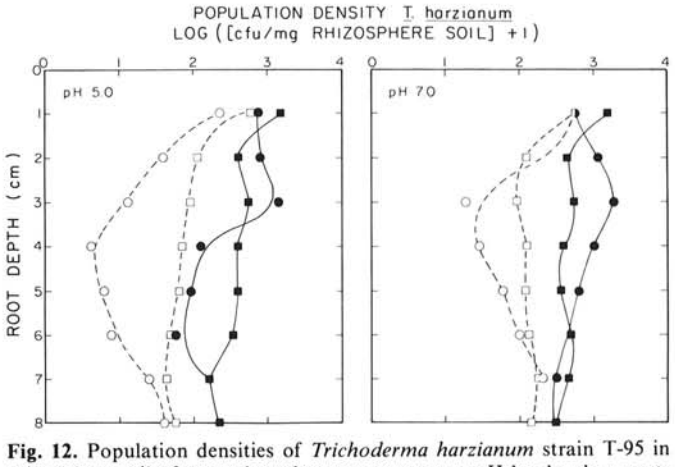

Fig. 12. Population densities of *Trichoderma harzianum* strain T-95 in rhizosphere soil of cucumber plants grown at two pH levels when roots were sampled daily or after 8 days, •—• sampled after 8 days with benomyl in soil, o---o sampled after 8 days without benomyl in soil, ■—■ sampled daily with benomyl in soil, □---□ sampled daily without benomyl in soil.

Quantitative analysis of population densities at each depth of root was necessary. No water was added during incubation obviating the possibility of propagules being washed into the rhizosphere. To test whether the agent introduced from a seed into the rhizosphere could compete under typical ecological conditions, raw soil was used. Therefore, the system allowed rhizosphere competence to be measured on the basis of population density of *Trichoderma* spp. as a function of root depth.

The nature and quantity of root exduates have been analyzed in the past in axenic systems by use of perfusion and filter paper absorption techniques (35). Because such analyses often are obtained under gnotobiotic conditions, it is difficult to extrapolate such findings into the ecological conditions present in the rhizospheres of plants growing in raw soil (18). To overcome this objection, bioassays relating relative magnitudes of microbial population densities in the rhizosphere compared with nonrhizosphere soil were developed, the so called R/S ratio. Such analyses are subjected to many variables (18) and, at best, provide only a relative gross assay of the activity of the total biomass about the root. The rhizosphere competence assay provided a quantitative measurement of a specific rhizosphere-competent microorganism at the root tip where exudates are in relatively high concentration (30). In more mature portions of the root, however, interpretations based on population densities are confounded by maturation of the agent resulting in propagule production, various interactions leading to auto- or heterolysis, or changes in characteristics of substrates provided by senile tissues of the root. Nevertheless, the rhizosphere competence assay provides the best bioassay yet developed for the rhizosphere nutrient status at root tips. It has potential for use in a wide variety of experimental problems related to ecological and nutritional interactions in the rhizosphere.

Several species of *Trichoderma* were tested for rhizosphere competence by coating the seed with each isolate and following population densities of the fungus to a root depth of 8 cm. No species grew to greater depth than 2 cm (Fig. 3). This confirms the conclusions of Papavizas (27) that *Trichoderma* spp. are not rhizosphere-competent.

Unlike the wild types of *Trichoderma* spp., mutants tolerant to benomyl colonized all sections of the rhizosphere to the root tips when benomyl was mixed into the soil (Figs. 4 and 5), a successful application of the strategy of Mendez-Castro and Alexander (23). Unexpectedly, however, our benomyl-tolerant mutants were rhizosphere-competent even when the fungicide was not added to the soil. At the root tips, population densities of the mutants were commonly up to 10^6 cfu per gram of rhizosphere soil. This suggests that biocontrol agents can be induced by mutation to colonize rhizospheres.

Additional evidence for rhizosphere competence of strain T-95 was obtained by microscopic observations comparing the length of hyphae on roots originating from seeds coated with or without conidia of the fungus (Fig. 10). Of course, it was not possible to identify with certainty the hyphae of T-95; however, the total length of hyphae observed was relatively similar to population densities obtained by use of the rhizosphere competence assay (Fig. 4).

Roots were essential for colonization below the site (seed) where the strains of *Trichoderma* were applied. The wild type was recovered at low densities to a 4-cm depth; the mutant was recovered at all depths of rhizosphere sand when applied to seed (Fig. 9). Neither the wild type nor the mutant was recovered below the glass beads.

Rhizosphere competence of the mutants with or without benomyl in the soil was demonstrated for cucumber, bean, maize, radish, and tomato (Figs. 5–8), at soil reactions of pH 5.0, 6.0, and 7.0 and temperatures of 19, 26, and 33 C (Figs. 5–8). At 19 C, significantly higher population densities of T-95 were observed in soils of pH 7.0 than 5.0 and 6.0. Because activity of *Trichoderma* spp. is favored by acid conditions (21), such a phenomenon may reflect measurements of proportionate densities of various morphological units of the thalli—that is, favorable soil pH may increase the period in which hyphae are present before sporulation occurs. Thus, measurements of colony-forming units could include a high proportion of hyphae that would not break up into multiple reproductive units such as that occurring with clumps of conidia and/or chlamydospores.

Another anomaly was apparent when population densities of T-95 were compared during various temperature regimes. Predictably, population densities were significantly higher at 26 C, near optimum for growth of such fungi, than at 19 C; however, the significantly higher densities at 33 compared with 19 C are not so readily explained. At such higher temperatures, activity of bacterial antagonists should be higher than at 19 C (3). A possible explanation is that more root exudates could be produced at the higher temperature (29).

Especially in treatments of seed with rhizosphere-competent mutants without benomyl in the soil, a curve in the form of a 'C' was often obtained when the number of colony-forming units per centimeter section as a function of depth was plotted (e.g., Figs. 7 and 8). Such differences among population densities were statistically significant. The high population densities immediately below the seed are readily explained because exudates from the seed provide more substrate than those from roots at lower depths (30). Exudates are relatively abundant at root tips (35); however, this cannot explain the lower counts in midportions of the root because such sections were root tips a few days previously.

Two hypotheses were tested to explain the C-shaped curve. First, population density increase below 6–8 cm in comparison with those at the midportion rhizosphere depth could be due to the 'flush' of exudates resulting from photosynthates as green cotyledons and shoots emerged below the soil level. Even so, elements of the C-shaped curve appeared to be present when the experiment was performed in the dark (Fig. 11). A more definitive test was performed that tested the constancy of exudates from the root tips during root development (Fig. 12). Instead of sampling sections of the root after it had developed for 8 days, 1-cm sections of root tips were sampled each day for 8 days with illumination. There were no significant differences among counts of colony-forming units below the 2-cm rhizosphere depth suggesting that quantity of root exudations was constant from the tips and that there was no increase when shoots emerged and photosynthesis was initiated. We conclude that an alternative hypothesis is more likely; population densities of maturing thalli (4 days old) of the mutant strains were lower in the longitudinal midportions of the rhizosphere than elsewhere because of auto- and/or heterolysis at these sites.

Although some biological control agents may protect seeds from soilborne pathogens, they do not proliferate in the rhizoplane and rhizosphere (25). Evidence herein provided indicates that strains of a rhizosphere incompetent biological control agent (*T. harzianum*) were induced to become rhizosphere competent. Rhizosphere-competent biological control agents are potentially more effective because they protect not only the seed but also the seedling root. They potentially eliminate the problem of adding large amounts of thalli to induce suppressiveness because substrates provided by the host can support their activity.

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