# The Possible Role of Competition between *Trichoderma harzianum* and *Fusarium oxysporum* on Rhizosphere Colonization

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## ABSTRACT

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Soil was enriched with chlamydospores of *Fusarium oxysporum* f. sp. *vasinfectum* and *F. oxysporum* f. sp. *melonis* and amended with increasing concentrations of glucose and asparagine. Maximal germination of chlamydospores was obtained in soil amended with 0.4 mg of of glucose and 0.08 mg of asparagine per gram of soil. Addition of conidia of the biocontrol agent *Trichoderma harzianum* (T-35) significantly ($P = 0.05$) reduced the chlamydospore germination rate of both Fusaria. However, in soils amended with concentrations higher than 0.3 and 0.06 mg/g of soil of glucose and asparagine, respectively, the inhibition was nullified. Chlamydospore germination of *F. o. melonis* and *F. o. vasinfectum* in melon and cotton rhizosphere soil were significantly inhibited after soil or seed application with T-35. As in the case of the glucose and asparagine, addition of an excess of seedling exudates increased the germination rate and eliminated the inhibition. Moreover, a continuous application of germinating cotton seed exudates to a soil infested with *F. o. vasinfectum* planted with cotton and treated with T-35 significantly reduced the disease control capability of the antagonist. A seed treatment with T-35 in a constantly humid soil resulted in high population densities of the antagonist on the developing rhizosphere. Plants grown from seeds treated with T-35 had roots with lower levels of *Fusarium* spp. in their rhizosphere than roots from plants from untreated seeds. The greatest density and the largest reduction in levels of *Fusarium* were detected on the lower 4 cm of the roots. Numbers of *Fusarium* in the rhizosphere were inversely proportional to the number of conidia of T-35 applied to soil. On the other hand, as the concentration of the pathogen in soil increased, T-35 counts on root segments decreased. *Trichoderma* had little effect on the survival of *Fusarium* spp. in nonrhizosphere soil. Inhibition of germination may therefore have resulted from competition.

Plant roots growing in soils are a major source of carbon and energy to microorganisms in the form of root exudates, cells detached from old parts of the root, or the root itself after plant death (7). Barber and Martin (3) estimated that 3–9% of assimilated carbon by wheat and barley is exuded by root by-products. Whipps and Lynch (25) in a similar experiment showed that the total carbon released from cereals was 25–30% of the total fixed carbon.

Competition for nutrients, primarily carbon, nitrogen, and iron, may result in biological control of soilborne plant pathogens (4,18). In many cases where the amount of these nutrients become limiting for the pathogen due to intensive microbial activity, the soil becomes suppressive (7).

Cook and Schroth (8) identified carbon and nitrogen compounds required for chlamydospore germination of *F. solani* f. sp. *phaseoli*. Similarly, Sneh et al (23) demonstrated the role of glucose and asparagine in stimulating chlamydospore germination of *F. oxysporum* f. sp. *lini*. Recently, Elad and Baker (9) and Elad and Chet (10) reported that carbon sources, either provided by synthetic substances or excreted by the plant rhizosphere, might be involved in the chlamydospore and oospore germination of *F. oxysporum* and *Pythium aphanidermatum*, respectively. Proliferation along the developing rhizosphere is one of the most important traits for antagonists applied to seeds (7).

Most studies in this field have been done with antagonistic rhizobacteria (5,12,24), and there is relatively little information involving fungi. Recently, Ordentlich et al (15) showed the potential of chitinolytic *Serratia marcescens* in colonizing bean rhizosphere after its application to soil surrounding the germinating seed.

Chao et al (6) reported the unsuccessful establishment of *T. harzianum* in pea rhizosphere. The failure of *Trichoderma* to establish in rhizosphere of pea and bean was also reported by Papavizas (16). Recently, Ahmad and Baker (1) demonstrated that a benomyl-resistant strain of *T. harzianum* colonized the rhizosphere of several crops when applied to seeds. Wild types failed to establish in the rhizosphere.

The objectives of the present study were to evaluate the role of competition in the biological control of Fusarium wilt of melon and cotton and Fusarium crown rot of tomato by *T. harzianum* strain T-35 (20,22). We investigated the potential of this strain to inhibit chlamydospore germination in nutrient-amended soils. We also examined the rhizosphere colonization of T-35 and its effect on rhizosphere populations of *F. oxysporum*.

## MATERIALS AND METHODS

**Fungal isolates.** *F. oxysporum* f. sp. *melonis* Snyd. and Hans., *F. oxysporum* f. sp. *vasinfectum* (Atk.) Snyd. and Hans., and *F. oxysporum* f. sp. *radicis-lycopersici* Jarvis and Shoemaker were isolated from infected plants on the selective medium for *Fusarium* of Nash and Snyder (13). After isolation, these fungi were cultured at 27 C on a yeast extract-glucose medium (YM) containing 5 g of yeast extract (Difco Laboratories, Detroit, MI), 5 g of peptone (Difco), 10 g of glucose, and 20 g of agar (Difco) per liter. An isolate of *T. harzianum*, obtained from a cotton plant wilted by *Fusarium* and designated T-35 (20), was cultured on potato-dextrose agar (PDA; Difco) or in a liquid synthetic medium (SM) (14). Isolation and properties of T-35 have been described elsewhere (20).

**Greenhouse experiments.** Experiments were carried out in artificially infested sandy loam soil (pH 7.1) consisting of 82.3% sand, 2.3% silt, 15% clay, 0.3% organic matter, 0.02% nitrogen, 0.067% potassium, 0.01% phosphorus, and 0.003% extractable iron. Soil moisture at field capacity was 12.2% (w/w). Soil was infested with *F. oxysporum* with a microconidial suspension (20). Erlenmeyer flasks (250 ml), each containing 50 ml of liquid YM, were seeded with mycelial disks from 72-hr-old cultures of the pathogen. Flasks were incubated at 27 C in a rotary shaker at

120 rpm for 4 days. Microconidia were then separated from the mycelium by filtration through eight layers of cheesecloth. The conidia were washed three times by centrifugation at 9,150 *g* for 30 min at 4 C. Ten ml of this suspension, adjusted to $2 \times 10^7$ microconidia per milliliter, was mixed with sandy loam soil in an electrical soil mixer.

The test plants were cotton (*Gossypium barbardense* L. 'Pima') and melon (*Cucumis melo* L. '56').

Experiments, comprising six replicates, were set up with plastic pots ($9 \times 4 \times 10$ cm) each containing 0.5 kg of soil sown with 10 seeds of the test plant. Seeds were not subjected to any chemical treatment. *T. harzianum* (T-35) was applied as a conidial suspension, seed coating, or in a wheat-bran peat preparation. A conidial suspension was prepared from conidia collected from cultures grown in Erlenmeyer flasks containing 20 ml of PDA. The suspension was washed three times in sterile tap water by centrifugation (15,000 *g*) and adjusted to $1 \times 10^6$ conidia per milliliter. A seed coating was prepared from conidia collected as described above, adjusted to $5 \times 10^9$ conidia per milliliter, and supplemented with 0.015% (v/v) of Nu-film-17 (Miller Chemicals, Hanover, PA) as an adhesive. Four ml of this suspension was used to coat 20 g of melon and cotton seeds or 10 g of tomato seeds. The number of populations of *Trichoderma* on seed surfaces was counted by shaking 5 g of seeds in 50 ml of sterile tap water for 1 hr in 250-ml Erlenmeyer flasks in a rotary shaker at 200 rpm. Serial dilutions of the suspension were plated on a selective medium for *Trichoderma* (TSM) (11). A wheat-bran peat preparation (1:1, v/v) (21) at 10% moisture was amended with 40 ml of tap water per 100 grams of mixture. The mixture was then autoclaved in polyethylene bags ($50 \times 50$ cm) for 1 hr at 121 C on three successive days. Homogenized mycelium from 48-hr-old cultures of *T. harzianum* grown in liquid SM was transferred to the wheat-bran peat mixture and incubated in an illuminated chamber for 14 days at 30 C. This preparation, containing $5 \times 10^9$ colony-forming units (cfu) per gram, was mixed with soil at a concentration of 5 g/kg of soil.

Soil enrichment with chlamydospores of *Fusarium* was performed according to Sneh et al (23). Mycelial mats of *F. o. melonis* and *F. o. vasinfectum* were grown in liquid YM, in Roux bottles at 27 C. After 7 days, mycelial mats were separated, placed in four layers of cheesecloth, and washed under running, distilled water for 5 min. Twelve mats of each fungus were blended for 1 min and centrifuged for 10 min at 3,000 *g*. The pellet was resuspended in 150 ml of water and added to 1 kg of soil. The soil was mixed, placed in glass bottles, and incubated at 27 C for 4 wk, then air-dried to 10% moisture, sieved through a 2-mm-mesh screen, mixed well, and stored at 4 C until use. The soil contained an average of $5 \times 10^4$ cfu/g of *F. oxysporum* as determined after soil dilutions and plating on selective medium for *Fusarium*.

Chlamydospore germination tests in soil were carried out according to Sneh et al (23). Samples of 1 g of chlamydospore-enriched soil were placed in 10-ml test tubes. Aliquots (0.1 ml) of glucose and asparagine (5:1, w/w) were added to reach the desired concentrations of nutrients at 15% water content. These compounds were often used to induce germination of *Fusarium* spp. chlamydospores (9,23). T-35 was added as a pregerminated conidial suspension (in water solution of 0.01% glucose). Soil was thoroughly mixed and incubated at 27 C for 22 hr. After the incubation, the soil in each test tube was stained with 1-ml aliquots of 0.3% Calcofluor New M2R (American Cyanamid Company, Bound Brook, NJ) solution according to Scher and Baker (19). After 5 min, the excess solution was removed and replaced with 1 ml of distilled water. Soil was suspended and a drop of the suspension taken for fluorescent observation under an epi-fluorescent light microscope under UV light (Olympus, Japan) at 400. Three test tubes were replicated for each treatment, and 100 chlamydospores were counted at each observation.

Chlamydospore germination in the rhizosphere was tested according to Sneh et al (23). Cotton and melon seeds were disinfested for 3 min in 4% sodium hypochlorite and placed between four layers of wet chromatography paper, which were then wrapped in plastic bags and incubated at 30 C for 24 hr. After germination, the seeds were transferred to 5 g of chlamydospore-enriched soil; some samples were also amended with T-35. The mixture was placed between two microscope glass slides, which were secured with two rubber bands. The slides were slightly slanted in moist soil in plastic pots ($9 \times 9 \times 10$ cm), wrapped in a plastic bag, and incubated at 27 C for the desired period. The slides were then recovered from soil; the germinated seedlings, with soil adhering to roots, were carefully removed and transferred to a 10-ml test tube for microscopic observation, as described above.

In both chlamydospore germination tests (e.g., rhizosphere and nonrhizosphere soil), T-35 was also applied as a seed coating. Three germinated seeds were replicated per treatment, and 100 chlamydospores were counted at each observation.

**Production and collection of plant exudates.** Cotton and melon seeds were disinfested with 4% sodium hypochlorite for 10 min. The efficacy of disinfestation was tested by placing samples of the treated seeds on PDA or nutrient agar (Difco) plates. Eight hundred seeds of each crop were placed in a glass column ($80 \times 7$ cm) containing 1 L of sterile tap water. The lower part of the column was connected to an air compressor, and air was forced inside through a sterile glass fiber filter at a pressure that thoroughly agitated the suspended seeds. At the upper part of the column an air outlet containing an additional glass fiber filter was mounted. The column with the germinating seeds was incubated at 27 C for 4 days. The resulting seed exudate mixture was then collected in a sterile container, frozen at −30 C, lyophilized, and kept at −30 C until use.

**Rhizosphere colonization tests.** Rhizosphere population dynamics of *T. harzianum* and *Fusarium* spp. were studied according to the method developed by Scher et al (19) as modified by Ahmad and Baker (1) and Elad and Chet (10). A seed of the test plant was placed at the upper part of two longitudinal halves of 50 ml polypropylene tubes measuring $11.5 \times 2.8$ cm (Falcon Div., Becton-Dickinson Co., Oxnard, CA) filled with sandy loam soil with 15% (w/w) moisture content. T-35 was applied to soil as a seed coating or as a conidial suspension, whereas *F. oxysporum* was introduced to soil as a microconidial suspension ($10^3$ microconidia per gram of soil). One seed of cotton, melon, or tomato (*Lycopersicon esculentum* L.) was placed on the half tube 1 cm below the rim. The unseeded half tube was carefully placed on the first half and secured with rubber bands. Tubes of all treatments were completely randomized and placed in plastic pots ($9 \times 9 \times 10$ cm) containing sandy loam soil with the same water content. The pots were covered with polyethylene bags to maintain the same soil water content and placed in an illuminated chamber at 28 C.

In an additional experiment, aimed at studying the population dynamics of T-35 in the rhizosphere of irrigated plants, the soil was irrigated daily with 3 ml of tap water. After 8 days of incubation, tubes were removed from the pots and their two halves carefully separated. The roots with adhering soil were cut, starting from the crown, into 2-cm segments. Corresponding segments from six plants were combined and shaken in a rotary shaker for 1 hr in 250-ml Erlenmeyer flasks each containing 30 ml of sterilized tap water. Serial dilutions were plated on TSM and Nash and Snyder medium for *Trichoderma* and *Fusarium* spp. counts, respectively. The counts of each fungus were expressed as cfu/g of root, including the adhering soil (dry weight).

Each colony of *Trichoderma* was transferred from TSM to PDA containing cycloheximide (100 mg/L) to distinguish between the resident soil population of *Trichoderma* and T-35, which is very resistant to cycloheximide (Péer and Chet, unpublished).

All tests were performed at least twice. Statistics were carried out by using Duncan's multiple-range test with a significance level of $P = 0.05$.

## RESULTS

**Chlamydospore germination in soil amended with glucose and asparagine.** Without the addition of nutrients to soil, 19–30% of added chlamydospores of *F. o. melonis* or *F. o. vasinfectum* germinated. The first germinating chlamydospores were observed after 18 hr, while maximal germination occurred after 22 hr of

incubation.

Addition of pregerminated conidia of T-35 to soils reduced germination of chlamydospores of *F. o. melonis* and *F. o. vasinfectum* (Fig. 1, Table 1). Levels of nutrients amended to soil were inversely related to the ability of T-35 to reduce germination. At 0.3 and 0.06 mg/g of soil of glucose and asparagine, respectively, T-35 had almost no effect (Fig. 1). T-35 had a similar effect when applied as a seed treatment (Table 1). However, when (in another experiment) T-35 was added as ungerminated conidia, the inhibitory effect was not significant.

**Chlamydospore germination in rhizosphere soil.** In rhizosphere soil of melon and cotton seedlings previously enriched with chlamydospores of *F. o. melonis* and *F. o. vasinfectum* and planted with melon and cotton seeds, respectively, the maximal germination rate of chlamydospores (45% and 56%, respectively) was observed after 60 hr of incubation (Fig. 2).

The inhibitory effect of T-35 treatments on chlamydospore germination in cotton or melon rhizosphere soil was nullified after the addition of an excess of germinating cotton and melon seed exudates (Table 2). Similarly, under greenhouse conditions, a continuous external addition of these exudates to soil infested with

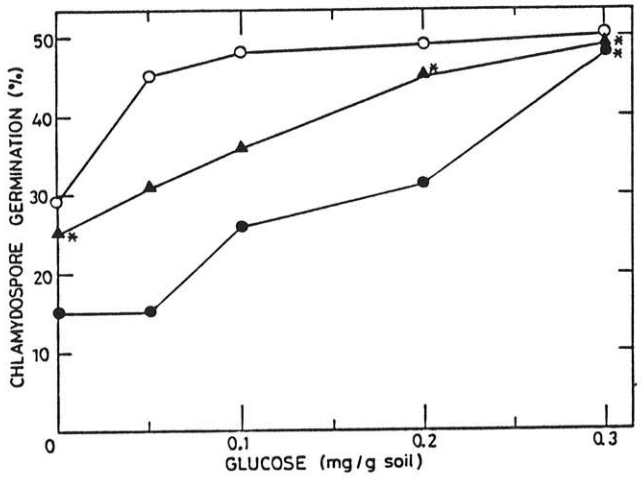


**Fig. 1.** The effect of pregerminated conidia of *Trichoderma harzianum* (T-35) on chlamydospore germination rate of *Fusarium oxysporum* f. sp. *melonis* after 22 hr of incubation in soil enriched with chlamydospores and amended with glucose and asparagine (5:1; w/w). Conidia of T-35 were applied at concentrations of $10^5$ (▲) and $10^6$ (●) conidia per gram of soil and compared with the untreated control (○). Germination values, at each level of nutrients marked with an asterisk, are not significantly different from the control ($P = 0.05$).

TABLE 1. Effect of addition of *Trichoderma harzianum* on chlamydospore germination of *Fusarium oxysporum* f. sp. *melonis* and *F. oxysporum* f. sp. *vasinfectum* in rhizosphere and nonrhizosphere soil

| | Chlamydospore germination (%)[u] | | | |
|---|---|---|---|---|
| | Rhizosphere soil[v] | | Nonrhizosphere soil[w] | |
| *Trichoderma* treatments | *F. o. melonis* | *F. o. vasinfectum* | *F. o. melonis* | *F. o. vasinfectum* |
| None | 43.5 a[x] | 50.3 a | 39.1 a | 25.1 a |
| Seed coating[y] | 25.3 b | 33.1 b | 19.3 b | 12.1 b |
| Conidial[z] suspension | 28.1 b | 34.5 b | 15.8 b | 7.5 c |

[u]Counted under UV light microscope by using Calcofluor.
[v]Melon and cotton seeds were germinated for 48 hr in soil enriched with chlamydospores of *F. o. melonis* and *F. o. vasinfectum*, respectively.
[w]Soil enriched with chlamydospores of *F. oxysporum* and amended with 0.05 mg of glucose and 0.01 mg of asparagine per gram of soil.
[x]Values of each column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple-range test.
[y]$7.5 \times 10^4$ and $5 \times 10^4$ conidia of T-35 per each cotton and melon seed, respectively.
[z]$1 \times 10^6$ pregerminated conidia of T-35 per gram of soil.

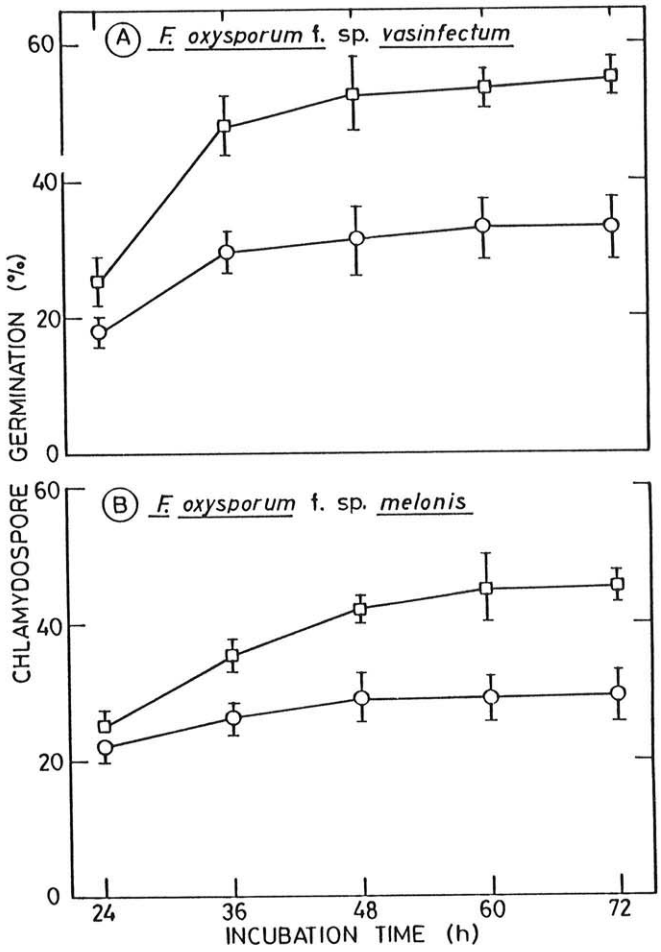


**Fig. 2.** Chlamydospore germination of *Fusarium oxysporum* f. sp. *vasinfectum* (**A**) and *F. oxysporum* f. sp. *melonis* (**B**) in cotton and melon rhizosphere soil (□), respectively, as compared with the germination rate in nonrhizosphere soil (○). Brackets indicate the standard error.

TABLE 2. Effect of germinating seed exudates of cotton and melon on the inhibitory effect of *Trichoderma harzianum* on chlamydospore germination of *Fusarium oxysporum* and on the disease control of Fusarium wilt of cotton

| | | Chlamydospore germination (%)[u] | | |
|---|---|---|---|---|
| *Trichoderma* treatments | Exudates[v] | *F. o. vasinfectum* | *F. o. melonis* | Wilted cotton plants[w] |
| None | Not added | 24.5 ab[x] | 23.8 ab | 73.8 ab |
| | Added | 29.8 a | 27.1 a | 85.9 a |
| Conidial[y] suspension | Not added | 12.4 c | 10.1 c | 33.6 c |
| | Added | 22.1 b | 19.3 b | 68.9 b |
| Seed coating[z] | Not added | 14.7 c | 13.1 c | 39.1 c |
| | Added | 23.4 b | 25.6 a | 75.1 ab |

[u]Counted under UV light microscope by using Calcofluor.
[v]Exudates were produced from 800 germinating seeds. The calculated amount of exudates produced by 10 seeds was added to each replicate (0.5 kg of soil sown with 10 seeds) at sowing date and once every 3 days, until the end of the experiment (20 days after sowing). Calculated amount of exudates produced by three cotton or melon seeds was mixed with 50 g of soil enriched with chlamydospores of *F. o. vasinfectum* or *F. o. melonis*.
[w]Under greenhouse conditions in soil infested with microconidia of *F. o. vasinfectum* ($2 \times 10^8$/kg of soil).
[x]Values of each column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple-range test.
[y]$1 \times 10^6$ conidia of T-35 per gram of soil.
[z]$7.5 \times 10^4$ and $5 \times 10^4$ conidia of T-35 per each cotton or melon seed, respectively.

*F. o. vasinfectum* and planted with cotton reduced the disease control obtained by T-35 after its application as either seed coating or a conidial suspension (Table 2).

**Rhizosphere colonization by *T. harzianum* and *F. oxysporum*.** After its application as a seed treatment, T-35 successfully colonized the rhizospheres of developing melon, cotton, and tomato roots up to 12 cm from the crown. In irrigated soil the rhizosphere population of *T. harzianum*, resistant to 100 mg/L of cycloheximide (e.g., T-35), was much higher than that counted in nonirrigated soil (Fig. 3). In both cases the maximal counts of T-35 were monitored on root segments that included the crown and the root tip.

In soil infested with *F. o. vasinfectum* ($10^3$ microconidia per gram of soil), the colonization of cotton rhizosphere by T-35 resulted in a decline in *Fusarium* spp. cfu on the respective root segments (Fig. 4). A reduction of more than one order of magnitude was recorded on root segments at distances of 8–12 cm from the crown. A similar effect was obtained in the rhizosphere of melon as a consequence of addition of T-35.

The amount of colonization with T-35 of melon rhizosphere was dependent on the inoculum concentration of *F. o. melonis*. Soil infested with increasing numbers of microconidia of either *F. o. melonis* or *F. o. vasinfectum* reduced the establishment of T-35 in the rhizosphere by up to two orders of magnitude on the root tip (Fig. 5). Similarly, establishment of *F. o. melonis* in melon rhizosphere was affected by the number of conidia of T-35 introduced in the soil. In untreated soil the counts of *Fusarium* spp. in the rhizosphere ranged between $8.8 \times 10^4$ and $1.5 \times 10^5$ cfu/g of root and rhizosphere soil. However, T-35 treatment with $10^6$ conidia/g of soil reduced the establishment of the pathogen up to $6.3 \times 10^2$ cfu/g of melon root and rhizosphere soil (Fig. 6).

To determine the colonization of rhizosphere and nonrhizosphere soils infested with *F. o. melonis* and treated with T-35, serial dilutions from these soils were carried out (Table 3). After a seed coating treatment, *T. harzianum* effectively colonized the rhizosphere soil but failed to establish in nonrhizosphere soil. Application of T-35 as a conidial suspension or a wheat-bran peat preparation resulted in high colonization of both rhizosphere and nonrhizosphere soils. All treatments of *Trichoderma* had little effect on the recovery of *Fusarium* spp. from the nonrhizosphere soil. However, treatments of *Trichoderma* reduced (by up to three orders of magnitude) the numbers of Fusaria in rhizosphere soil (Table 3).

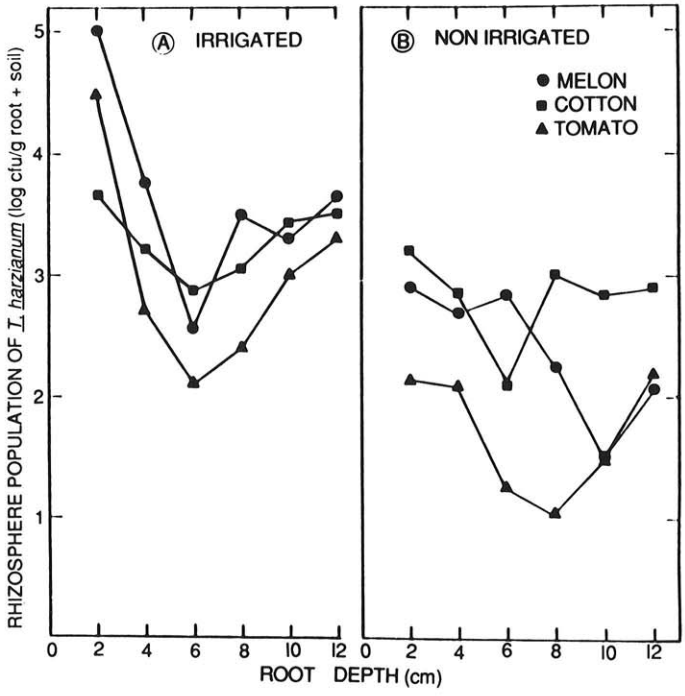


**Fig. 3.** Rhizosphere colonization of several crops by *Trichoderma harzianum* (T-35), applied as a seed coating, in irrigated (**A**) and nonirrigated (**B**) soil.

## DISCUSSION

Currently, there are two indirect approaches to test competition as a mechanism in biological control of soilborne plant pathogens. The first is based on the hypothesis that biological control resulting from competition has little or no effect on the viability of the pathogen (2). The second involves the use of limiting factors for

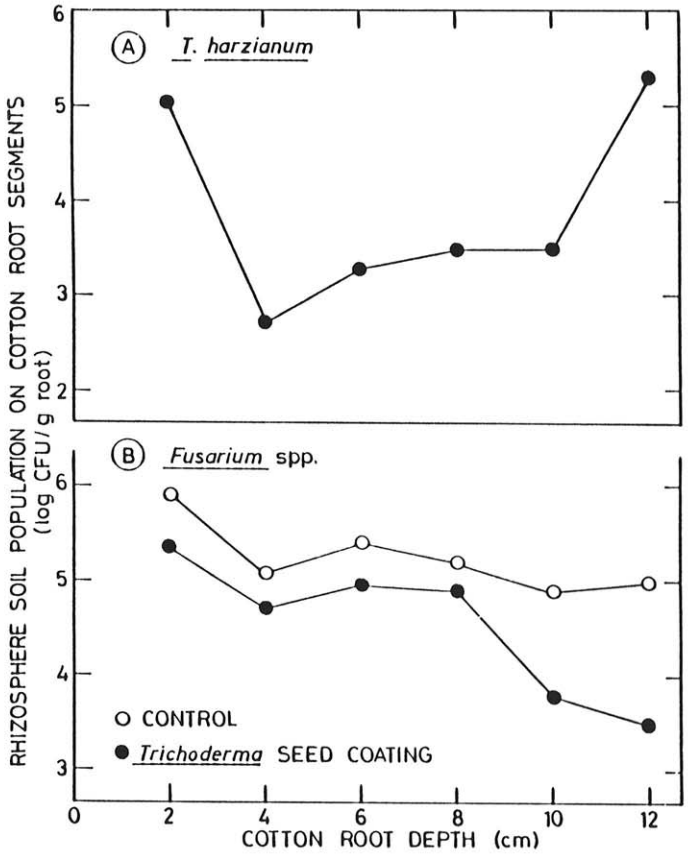


**Fig. 4.** The effect of cotton seed coating with *Trichoderma harzianum* (T-35) on rhizosphere soil population of T-35 (**A**) and *Fusarium* spp. (**B**) after 8 days of incubation in soil inoculated with microconidia of *F. oxysporum* f. sp. *vasinfectum* ($10^3$/g of soil).

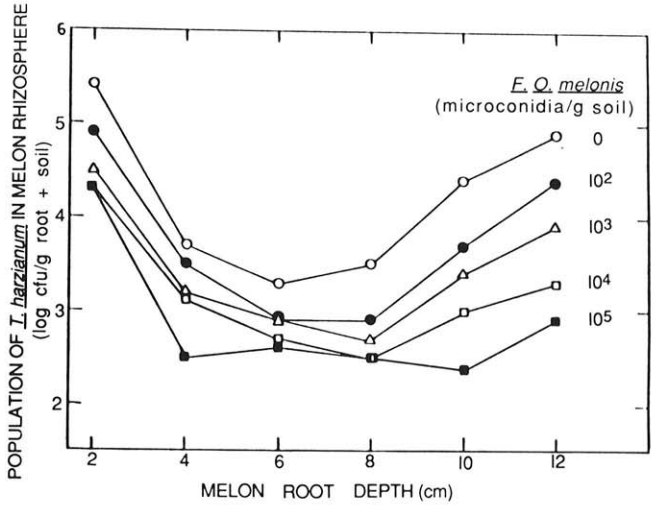


**Fig. 5.** The effect of microconidial concentration in soil of *Fusarium oxysporum* f. sp. *melonis* on colonization of melon rhizosphere by *Trichoderma harzianum* (T-35) after its application as a seed coating.

which (by definition) microorganisms may compete. Addition of an excess of the candidate factor should nullify the antagonistic effect. The present study using those approaches raises the question whether competition for nutrients and rhizosphere colonization are involved in the biocontrol of *F. oxysporum* obtained with T-35 (20,22).

One of the most sensitive stages for nutrient competition in the life cycle of *Fusarium* is chlamydospore germination (2). In soil infested with chlamydospores of *F. oxysporum*, the germination rate ranged between 20–30%. These levels of germination could result from reserve materials in the chlamydospore or might be due to residual organic matter in the raw soil. The major sources of nutrients for soil microorganisms are nutrients derived from seeds or roots (7). In our study we used glucose and asparagine to simulate the organic carbon and nitrogen components of plant exudates as stimulants of chlamydospore germination. Soil amendment with increasing amounts of glucose and asparagine enhanced the germination rate to 62%. However, the addition of higher concentrations of these nutrients did not result in further increase in germination. The failure of the remaining chlamydospores to germinate is probably a result of their continuous dormancy or may be due to utilization of most of the amended nutrients by the resident soil microflora (7).

Although the role of plant exudates as an important nutritional factor for rhizosphere microorganisms is well established, their role in biocontrol is still unclear. In the present study we evaluated the effect of plant exudates on the inhibition of chlamydospore germination in vitro and on the suppression of Fusarium wilt of cotton in vivo obtained with T-35. When soil was amended with low concentrations of glucose and asparagine (0.05 and 0.01 mg/g of soil, respectively) or with plant exudates, pregerminated conidia of T-35 were found to inhibit the germination of chlamydospores. However, at concentrations higher than 0.3 and 0.06 mg/g of soil of amended glucose and asparagine, respectively, or when an excess of exudates was added, the inhibition was nullified.

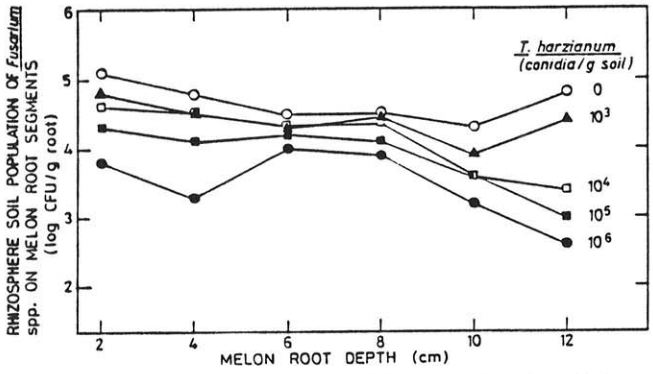


**Fig. 6.** The effect of concentration of conidial suspension of *Trichoderma harzianum* (T-35) on colonization of melon rhizosphere by *Fusarium oxysporum* f. sp. *melonis*.

TABLE 3. Effect of *Trichoderma harzianum* on *Fusarium* spp. population in melon rhizosphere and nonrhizosphere soil[r]

| | Colony-forming units per gram of soil × $10^2$ | | | |
|---|---|---|---|---|
| | *Trichoderma* spp.[s] | | *Fusarium* spp.[t] | |
| *Trichoderma* treatments | Rhizosphere[u] soil | Nonrhizosphere[v] soil | Rhizosphere soil | Nonrhizosphere soil |
| None | 4.2 c[w] | 1.5 d | 1,900 a | 920 a |
| Seed coating[x] | 480 b | 9 c | 35 b | 750 a |
| Conidial[y] suspension | 51,000 a | 20,000 b | 41 b | 30 b |
| Preparation[z] | 55,000 a | 55,000 a | 43 b | 35 b |

[r]The soil was infested with $2 \times 10^8$ microconidia per gram of soil planted with melon seeds and placed in the greenhouse for 30 days.
[s]Counted on TSM (11).
[t]Counted on selective medium for *Fusarium* (13) amended with 2 mg/L of benomyl.
[u]The plants were gently uprooted, and the soil adhering to the roots was collected.
[v]Combined soil samples from distances greater than 1 cm from the roots.
[w]Values of each column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple-range test.
[x]$7.5 \times 10^4$ conidia of T-35 per seed.
[y]$5 \times 10^6$ conidia of T-35 per gram of soil.
[z]Wheat-bran peat (1:1, v/v) preparation of T-35 consisting of $5 \times 10^6$ cfu/g (dry wt.) was introduced into soil at a rate of 5 g/kg of soil.

Similarly, in the presence of an excess of exudates, the reduction in Fusarium wilt of cotton by T-35 was completely neutralized. This indicates that competition for these or similar nutrients may occur. At low levels nutrients are limiting and competitive effects are evident, resulting in significant reduction in chlamydospore germination. Indeed, Rovira and his co-workers (17) have already mentioned that plant root exudates contain lower amounts of carbon and energy than those required by rhizosphere microflora. Similar phenomena have already been described (9,23) in the inhibition of chlamydospore germination of *F. oxysporum* f. sp. *cucumerinum* by pseudomonads. Chlamydospores of *F. o. vasinfectum* and *F. o. melonis* in the rhizosphere of cotton and melon, respectively, germinated at higher levels compared with the germination obtained in nonrhizosphere soil. The same also occurred with chlamydospores of *F. solani* f. sp. *phaseoli* and *F. oxysporum* f. sp. *cucumerinum* when exposed to bean and cucumber exudates (23). Inhibition of chlamydospore germination of *F. oxysporum* could be obtained even when the antagonist was applied as a seed coating. Apparently, effective utilization of root exudates may enable antagonists applied to seeds to proliferate along the developing rhizosphere and interfere with seed and root pathogens (7).

After application of T-35 as a seed treatment in soil without irrigation, T-35 was recovered from the rhizosphere, indicating the active proliferation of this strain along the developing roots. The greatest counts of T-35 in the rhizosphere were detected on root segments, which included the root base and tip. Because the population dynamics of T-35 were recorded from rhizosphere portions that included roots and soil, it is not clear whether T-35 colonized the rhizoplane, the rhizosphere soil, or both. The population of T-35 could be distinguished from the resident soil population of *Trichoderma* spp. by using the natural resistance of this strain to relatively high concentrations of cycloheximide. Similar findings were demonstrated by Ahmad and Baker (1) with a benomyl-resistant mutant of *T. harzianum*. The relatively high population levels of antagonists recovered from root tips were also demonstrated with bacteria (10,15,19).

Most of the exudates present in the rhizosphere are excreted from the root tips. Thus, colonization of this region in the rhizosphere might reduce infection by *Fusarium*-like pathotypes that penetrate the vascular system of their hosts through the undifferentiated xylem at the root tip (7). Rhizosphere colonization of cotton and melon by T-35 was accompanied by a simultaneous decline in *Fusarium* spp. Reduction in the pathogen population in the rhizosphere should lead to a decrease in the infection rate. The level of rhizosphere colonization by T-35 was affected by the inoculum density of the pathogen in soil, and counts decreased in soils infested with high inoculum levels of both Fusaria. On the other hand, increasing the conidia concentration of T-35 in soil resulted in a decrease in rhizosphere colonization by *F. oxysporum*.

In soil infested with *F. o. melonis*, treatments of *Trichoderma* had only a slight effect on the survival of *F. oxysporum* in nonrhizosphere soil. However, application of T-35 as a wheat-bran peat preparation, conidial suspension, or seed coating significantly reduced the pathogen counts in the rhizosphere soil of melon and cotton. The minimal effect of T-35 on population densities of *F. oxysporum* in nonrhizosphere soil further suggests a competitive mechanism in biocontrol (2). These findings are in correlation with the lack of mycoparasitic and antibiotic interactions between T-35 and *F. oxysporum* in vitro (Sivan and Chet, unpublished). The great reduction of the pathogen population densities in the

rhizosphere soil could be a result of a lower proliferation rate of the pathogen in a rhizosphere already colonized by the antagonist. However, this does not exclude other biocontrol mechanisms from being involved in the rhizosphere.

## LITERATURE CITED


1. Ahmad, J. S., and Baker, R. 1987. Rhizosphere competence of *Trichoderma harzianum*. Phytopathology 77:182-189.
2. Baker, R. 1981. Ecology of the fungus *Fusarium*: Competition. Pages 245-249 in: *Fusarium*: Diseases, Biology, and Taxonomy. P. E. Nelson, T. A. Tousson, and R. J. Cook, eds. Pennsylvania State University Press, University Park. 457 pp.
3. Barber, D. A., and Martin, J. K. 1976. The release of organic substances by cereal roots in soil. New Phytol. 76:69-80.
4. Benson, D. M., and Baker, R. 1970. Rhizosphere competition in model soil systems. Phytopathology 60:1058-1061.
5. Burr, T. J., Schroth, M. N., and Suslow, T. 1978. Increased potato yields by treatment of seedpieces with specific strains of *Pseudomonas fluorescens* and *P. putida*. Phytopathology 68:1377-1383.
6. 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.
7. 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.
8. Cook, R. J., and Schroth, M. N. 1965. Carbon and nitrogen compounds and germination of chlamydospores of *Fusarium solani* f. sp. *phaseoli*. Phytopathology 55:254-256.
9. Elad, Y., and Baker, R. 1985. The role of competition for iron and carbon in suppression of chlamydospore germination of *Fusarium* spp. by *Pseudomonas* spp. Phytopathology 75:1053-1059.
10. Elad, Y., and Chet, I. 1987. Possible role of competition for nutrients in biocontrol of Pythium damping-off by bacteria. Phytopathology 77:190-195.
11. Elad, Y., Chet, I., and Henis, Y. 1981. A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. Phytoparasitica 9:59-67.
12. Kloepper, J. W., Leong, J., Teintze, M., and Schroth, M. N. 1980. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature 286:885-886.
13. Nash, S. M., and Snyder, W. C. 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. Phytopathology 52:567-572.
14. Okon, Y., Chet, I., and Henis, Y. 1973. Effect of lactose, ethanol and cycloheximide on the translocation pattern of radioactive compounds and on sclerotium formation in *Sclerotium rolfsii*. J. Gen. Microbiol. 74:251-258.
15. Ordentlich, A., Elad, Y., and Chet, I. 1987. Rhizosphere colonization by *Serratia marcescens* for the control of *Sclerotium rolfsii*. Soil Biol. Biochem. 19:747-751.
16. Papavizas, G. C. 1982. Survival of *Trichoderma harzianum* in soil and in pea and bean rhizospheres. Phytopathology 72:121-125.
17. Rovira, A. D., Newman, E. I., Bowen, H. J., and Campbell, R. 1974. Quantitative assessment of the rhizoplane microflora by direct microscopy. Soil Biol. Biochem. 6:211-216.
18. Scher, F. M., and Baker, R. 1982. Effect of *Pseudomonas putida* and a synthetic iron chelator on induction of soil suppressiveness to Fusarium wilt pathogens. Phytopathology 72:1567-1573.
19. Scher, F. M., Ziegle, J. S., and Kloepper, J. W. 1984. A method for assessing the root colonization capacity of bacteria on maize. Can. J. Microbiol. 30:151-157.
20. Sivan, A., and Chet, I. 1986. Biological control of *Fusarium* spp. in cotton, wheat and muskmelon by *Trichoderma harzianum*. J. Phytopathol. 116:39-47.
21. Sivan, A., Elad, Y., and Chet, I. 1984. Biological control effects of a new isolate of *Trichoderma harzianum* on *Pythium aphanidermatum*. Phytopathology 74:498-501.
22. Sivan, A., Ucko, O., and Chet, I. 1987. Biological control of Fusarium crown rot of tomato by *Trichoderma harzianum* under field conditions. Plant Dis. 71:587-592.
23. Sneh, B., Dupler, M., Elad, Y., and Baker, R. 1984. Chlamydospore germination of *Fusarium oxysporum* f. sp. *cucumerinum* as affected by fluorescent and lytic bacteria from *Fusarium*-suppressive soil. Phytopathology 74:1115-1124.
24. Weller, D. M. 1983. Colonization of wheat roots by a fluorescent pseudomonad suppressive to take-all. Phytopathology 73:1548-1553.
25. Whipps, J. M., and Lynch, J. M. 1983. Substrate flow and utilization in the rhizosphere of cereals. New Phytol. 95:605-623.