Population Dynamics of *Trichoderma viride* in Steamed Plant Growth Medium

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ABSTRACT

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Some factors affecting the saprophytic proliferation of T-1-R9, a benomyl-tolerant isolate of *Trichoderma viride* that controls Fusarium wilt of chrysanthemum, were investigated in a freshly steamed plant growth medium. A reduction in the proliferation of T-1-R9 was observed when the medium was diluted by 75% with silicate sand. The addition of T-1-R9 increased the total population density of *T. viride* in the medium, which suggests that instraspecific competition was not an important limiting factor in colonization of the medium by *T. viride*. The addition of T-1-R9 to freshly steamed growth medium resulted in increased CO_2 evolution during the first 12 days which indicated an increased level of nutrient utilization. This was further correlated with a reduction in the final population density of *Fusarium oxysporum* f. sp. *chrysanthemi* and in disease incidence.

The development of biological control systems for soilborne plant pathogens is difficult because of differences in climate, soil type, and crop production methods. These differences are reduced in the greenhouse floral industry due to the strict growing procedures that require a controlled environment. The constraints of quality, quantity, and timing of crops are such that steamed plant growth media, ambient temperatures, fertilizer and watering regimes, and propagation material are monitored and controlled to a much greater extent than in most agricultural production systems. Because of the high production costs and cash value of these crops, the use of chemicals to control plant pests is intensive. In an attempt to reduce the industry's dependency on chemicals, several organisms were tested for their potential to reduce Fusarium wilt of chrysanthemum (*Chrysanthemum morifolium* Ramat.) caused by *Fusarium oxysporum* Schlecht. f. sp. *chrysanthemi* Litt., Armst. and Armst. It was found that T-1-R9, a mutant isolate of *Trichoderma viride* Pers. ex. S. F. Gray in which tolerance to benomyl had been induced with ultraviolet light (13), in combination with two benomyl drenches controlled Fusarium wilt of chrysanthemum (10) as effectively as the present practice of integrating an all-nitrate nitrogen fertilizer, benomyl soil drenches, and lime (5).

It was hypothesized that the success of the biological control procedure depended upon the ability of T-1-R9 to proliferate in a freshly steamed plant growth medium and to utilize nutrients that would otherwise be available to the pathogen (10). This concept of controlled recolonization was utilized for the control of soilborne plant pathogens in steamed (2,12,15,16) or fumigated soils (11).

The objectives of this study were to determine if the saprophytic proliferation of the biocontrol agent T-1-R9 in treated plant growth medium was affected by dilutions with sand, if the initial population density of T-1-R9 affected the subsequent inoculum density of *F. oxysporum* f. sp. *chysanthemi*, and if the application of T-1-R9 to a freshly steamed plant growth medium affected the rate of CO_2 evolution from the medium. The distribution of T-1-R9 within the soil depths of the bench production system was also determined.

MATERIALS AND METHODS

This study consisted of four separate experiments, each repeated at least three times. Experiment 1 compared the population densities of T-1-R9 that developed in a plant growth medium diluted to four levels with sand. Each treatment contained three replicate bench plots in a randomized complete block design. Experiment 2 compared total population densities of *T. viride* in plots with and without added T-1-R9; three replicates of the two treatments were arranged in a completely randomized design. Experiment 3 compared population densities of *F. oxysporum* and incidence of Fusarium wilt in chrysanthemum in plots sprayed with T-1-R9, T-1-R4 (another isolate in which benomyl tolerance was induced with ultraviolet light), or with no antagonists; three replicate plots of each of the three treatments were arranged in a randomized complete block design. Experiment 4 compared

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population densities of T-1-R9 at three depths in three replicate plots that were all sprayed with T-1-R9. Evolution of CO_2 was also determined in these plots and in three additional unsprayed plots, all of which were arranged in a completely random design.

All tests were conducted in standard raised transite benches which were subdivided into 45 × 90 × 15-cm plots. The growth medium used consisted of Canadian sphagnum peat moss, coarse-grade Perlite, and composted sandy loam with a pH of 6.4–6.5 and a bulk density of 1.67. The entire bench-plot system was steamed for 2 hr at 82 C before each test. A conidial suspension of *Trichoderma viride*, biotype T-1-R9 or T-1-R4, was obtained from 2-wk-old cultures grown on potato-dextrose agar (PDA) (Difco, MI) at 25 C and was applied to the steamed medium at 10^4 conidia per cubic centimeter immediately after the medium had cooled and the tarp was removed. The spores were applied by spraying the surface of each treated plot with 430 ml/m^2 of a suspension that contained 2.3×10^5 conidia per milliliter. Controls consisted of plots sprayed with similar amounts of water.

Eighteen rooted cuttings of the Fusarium wilt-susceptible cultivar Yellow Delaware chrysanthemum were planted on a 15 × 15-cm spacing in each plot after spraying with or without conidia of T-1-R9. After 7–14 days, spores of *Fusarium oxysporum* f. sp. *chrysanthemi* were sprayed on the surface of all plots at a rate of 500 spores per square centimeter. The suspension of microconidia, macroconidia, and chlamydospores washed from cultures grown on PDA for 2 wk at 25 C was a blend of three isolates obtained from an infested field in Florida.

Komada's medium (9) and TME medium (14) were used to isolate *F. oxysporum* and *Trichoderma* sp., respectively. Colonies of *T. viride* were identified by the presence of echinulate spores 4–5 μm in diameter. Colonies of T-1-R9 were readily differentiated from indigenous colonies of *T. viride* by the addition of 5 mg (active ingredient) of benomyl (50% W.P.; E.I. duPont de Nemours & Co., Wilmington, DE) per liter of TME. Dilutions were done on a weight per volume basis. The growth medium was sampled every 4–7 days by making a composite of five 5-g subsamples at 0–5-cm depth plot, except for the depth experiment. To determine the population densities of T-1-R9 at different depths, five subsamples were collected at each of 0–5, 5–10, and 10–15-cm-deep intervals before composites were made. The effect of diluting the medium on proliferation of T-1-R9 was determined by mixing growth medium with fine silica sand in ratios of 1:0, 4:1, 3:2, 2:3, and 1:4 (v/v) before steaming.

The amount of CO_2 evolved from growth medium, treated or not treated with T-1-R9, was monitored with an alkaline trap method (4) to determine how the biological activity of the medium was affected by T-1-R9. A 10-g sample consisting of 10 1-g subsamples was removed from each bench plot and immediately placed in a 473-ml canning jar. A 10-ml beaker was placed in the center of the jar and 1 ml of 0.02 N NaOH was added to the beaker. The jar was immediately sealed for 1 hr at 25 C. The jars were then placed on a rotary shaker at 100 revolutions per minute for 1 hr to aid in absorption of the CO_2. The NaOH was titrated to neutrality with 0.01 N HCl using phenolphthalein as the indicator. The amount of CO_2 evolved per gram of medium in 1 hr was calculated in milliequivalents (meq).

RESULTS

Effect of diluting the medium on population densities of T-1-R9. The final population density of T-1-R9 (54 days after treatment) was inversely correlated ($y = -15{,}000 + x(603)$, $r = 0.975$) with the percentage of sand used (Fig. 1). There was no consistent differences in the population density of T-1-R9 in the early stages of colonization, regardless of the amount of sand used.

Population densities of T-1-R9 at different depths in plots. The population density of T-1-R9 was always significantly ($P = 0.01$) greater at the 0–5-cm depth and increased from 2.7×10^2 to a level of about 10^5 colony-forming units (cfu) per gram (Fig. 2). In the 5–10 and 10–15-cm depths, the population density of T-1-R9 was not significantly different. The highest populations in the lower depths developed 6–7 wk after infestation and were estimated at $1.1–1.7 \times 10^4$ cfu/g. The population densities in the lower depths

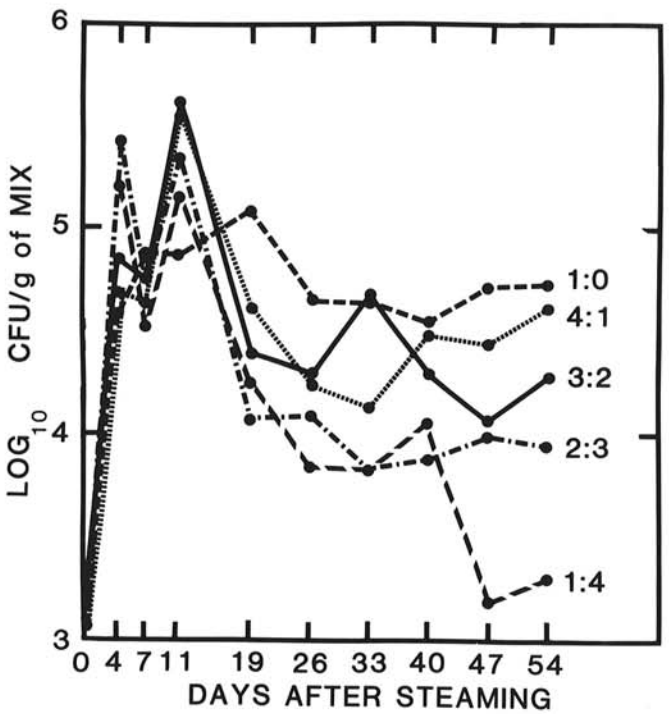

Fig. 1. Population density of *Trichoderma viride* isolate T-1-R9 over time in plant growth medium that was amended with proportions of silica sand, medium:sand, on a volume basis. Data are means of three replicates from each of three experiments.

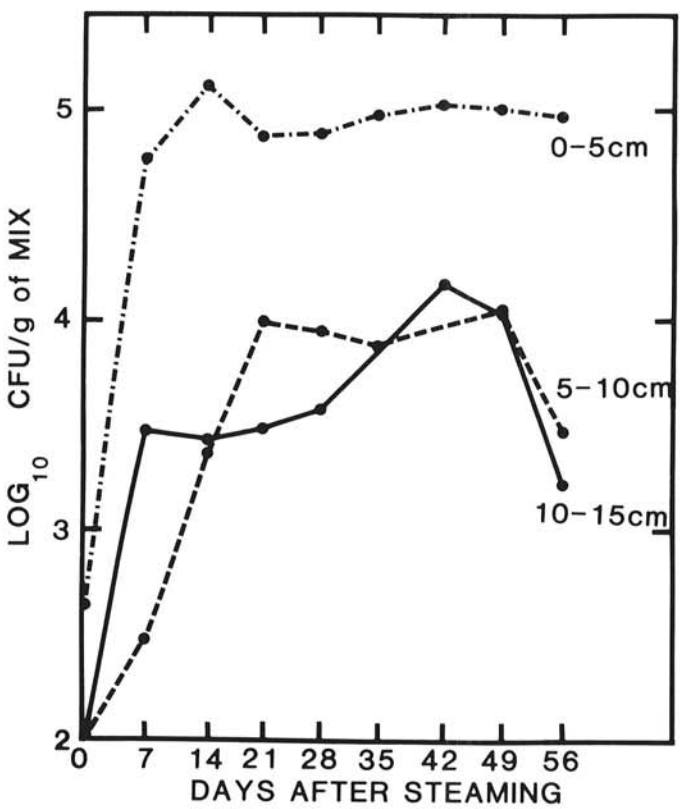

Fig. 2. Population density of *Trichoderma viride* isolate T-1-R9 at 0–5, 5–10, and 10–15-cm depths of plant growth medium over time. Means from 0–5-cm depth were always significantly greater than from the other depths, as determined by Duncan's multiple range test ($P = 0.05$). Data are means of three replicates from each of three experiments.

increased to maximum 2–3 wk later and declined earlier than did the population densities at the 0–5-cm depth.

Population density of T-1-R9 and of total *T. viride*. When benomyl was omitted from the TME medium, the total populations of *T. viride* could be determined. In all experiments, the final population densities of *T. viride* were greater in the T-1-R9-infested medium than in the control medium (Table 1). The increase varied from 27 to 57%, depending upon the particular experiment. The least significant difference (LSD) analyses indicated that treatment with T-1-R9 significantly increased the final population of *T. viride* ($P = 0.05$).

Effects of T-1-R9 on disease incidence and pathogen population density. When the antagonist T-1-R9 was added to the plant growth medium, the population density of *Fusarium oxysporum* f. sp. *chrysanthemi* after 10 wk was estimated at 380 propagules per gram compared to 610 propagules per gram in the nontreated control (Table 2). A decreased pathogen population density also occurred when another benomyl-tolerant isolate, T-1-R4, was added to the growth medium. There was also a significant ($P = 0.05$) reduction in disease incidence of 33 or 46% when the antagonists T-1-R4 or T-1-R9 were added, respectively.

CO_2 evolution. The evolution of CO_2 was monitored in T-1-R9-amended and unamended plant growth medium. The amount of CO_2 produced was greater in the amended medium as compared to the unamended control at 2, 5, and 12 days after steaming, but not after 19 days ($P = 0.05$) (Fig. 3). The major difference occurred during the first week following addition of the T-1-R9 isolate. After 19 days, the rate of CO_2 evolution was the same in both treatments. The highest rate of CO_2 evolution was 4.45 meq of CO_2/g of medium per hr and occurred 2 days after amendment with T-1-R9.

DISCUSSION

The potential for use of biological control methods in greenhouse production systems is great because broad-spectrum biocides are used in soil treatment, environmental conditions are consistently controlled, and the crops have a high cash value. Ferguson (6) reviewed controlled recolonization as a biological method to reduce plant disease in greenhouse production systems. The basic concepts were investigated by Vaartaja (18) and Olson and Baker (12). Underlying this research was the concept that after treatment of a growth medium with a broad-spectrum biocide (eg, steam), the competition in the medium is nearly eliminated. If a pathogen depends upon this low level of competition for saprophytic proliferation to incite an epidemic, then any increase in competition in the medium should result in a reduced pathogen inoculum potential.

The importance of growth medium content was investigated by diluting the growth medium with silicate sand. In these experiments, the dilution with sand at 20, 40, and 60% did not affect the proliferation of T-1-R9. Only a dilution with 80% sand resulted in a reduced population density of T-1-R9, and even then it was only apparent after 40 days.

The population of T-1-R9 was highest in the upper 0–5 cm of medium. Populations were observed in the lower soil depths, but these decreased as the depth of soil increased. This phenomenon may be extremely important in the development of biological control programs for soilborne plant pathogens. The distribution of a control agent is critical for it to be effective since interaction with a pathogen requires a close spatial relationship. Recolonization of steamed soils in the greenhouse by *Fusarium oxypsorum* may be due to airborne propagules (16). In these experiments, the pathogen was applied to the medium surface to simulate natural recolonization. Therefore, the introduced pathogen enountered an established antagonist population of over 6×10^4 cfus per gram of medium.

The role of competition in community development is of great interest to ecologists. Usually, however, plant pathologists working with biological control only consider the interactions between the control agent and the pathogen. With the labeled isolate T-1-R9, it

TABLE 1. Total population density of *Trichoderma viride* in steamed plant growth medium after 84 days with and without addition of isolate T-1-R9

	T. viride colony forming units/g × 10^{2y}				
	Experiment number				
Treatment	1	2	3	4	5
With T-1-R9[z]	130	280	283	47	68.7
Without T-1-R9	95	119	196	37	33.8

[y] Each value is the mean of three replicates.
[z] Benomyl-tolerant isolate T-1-R9 of *T. viride* was added at 10^4 conidia per square centimeter after the medium was steamed. Addition of this isolate significantly ($P = 0.05$) increased population density of *T. viride* as analyzed by LSD in all five experiments.

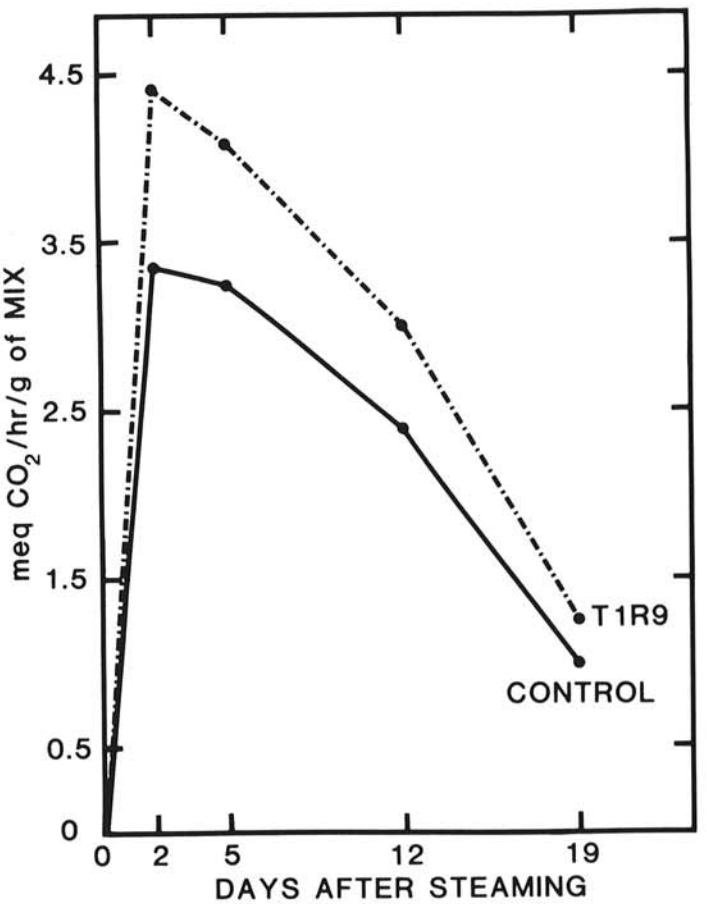

Fig. 3. Rate of CO_2 evolution in plant growth medium treated with T-1-R9, an isolate of *Trichoderma viride*, at 10^4 conidia per square centimeter. Data are means of three replicates from each of three experiments.

TABLE 2. Effect of benomyl-tolerant isolates T-1-R4 and T-1-R9 of *Trichoderma viride* on population densities of *Fusarium oxysporum* f. sp. *chrysanthemi* and on incidence of Fusarium wilt of chrysanthemum[w]

Treatment[x]	CFU/g of mix[y]	Disease incidence (%)[z]
T-1-R9	380 a	46 a
T-1-R4	441 a	57 a
Control	610 b	85 b

[w] Data are means of three replicates from each of three experiments. Means followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple-range test.
[x] The surface of plant growth medium was sprayed with 10^4 conidia of isolate T-1-R9, T-1-R4, or water (control) per square centimeter 24 hr after steam was applied.
[y] *F. oxysporum* f. sp. *chrysanthemi* was applied to plots after treatments and population densities were determined after 84 days by soil dilution plating with Komada's selective medium. CFU = colony-forming units.
[z] Percentage of plants showing symptoms of Fusarium wilt after 12 wk. Each value is based on observations of 162 plants.

was possible to determine the effect that the addition of an organism has on natural recolonization by other propagules of the same species. The T-1-R9 amendment resulted in an overall higher population density of *T. viride* in the medium. This suggests that intraspecific competition between T-1-R9 and naturally occurring populations was not a limiting factor in recolonization of treated media. However, the reduction in the proliferation of *F. oxysporum* f. sp. *chrysanthemi* when introduced following the application of T-1-R9 suggests that competition between the antagonist and the pathogen was an important factor.

Inoculum density-disease incidence relationships have not been reported for Fusarium wilt of chrysanthemum; however, they have been described for some other diseases caused by *Fusarium* spp. (1,3,7,17). It is apparent that a reduction in the inoculum density of the pathogen can result in decreased disease if the population level is in the range of inoculum density in which disease incidence can be affected. In these experiments, a reduction in pathogen inoculum density was associated with a reduction in disease.

The rate of CO_2 evolution was increased in the T-1-R9-amended plant growth medium during the first 12 days of recolonization. The more rapid utilization of substrate when one saprophytic species is added was also observed by Wicklow and Yocom (19). The level of soil respiration was correlated positively with the level of soil suppression (8) and the rate of soil organic matter utilization (20). Soil respiration is probably the most commonly measured factor in ecological studies to determine the rate of nutrient flow in terrestrial ecosystems. In the steamed medium, the increased rate of respiration suggests that nutrients available to the pathogen for saprophytic proliferation were instead utilized by the control agent.

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