Physiological and Biocontrol Characteristics of Stable Mutants of *Trichoderma viride* Resistant to MBC Fungicides

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ABSTRACT

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Aqueous suspensions of conidia of *Trichoderma viride* wild strain T-1 were placed on V-8 juice agar and exposed to ultraviolet (UV) radiation for 80 min. The 10 colonies (biotypes) that survived the irradiation tolerated high concentrations (up to 100 μg of active ingredient per milliliter) of benomyl, thiabendazole, and thiophanate-methyl, as indicated by growth in fungicide-amended potato-dextrose broth and conidial germination tests on benomyl-amended V-8 juice agar. The UV-induced biotypes differed from T-1 in appearance, sporulation habit, and ability to grow in a liquid medium at various pH values. The wild strain and all biotypes grew and sporulated better in a synthetic medium adjusted to pH 3.5 and 4.5 than at higher pH values. In dual cultures with eight soilborne plant-pathogenic fungi, biotypes R3 and R4 inhibited growth of five of the eight pathogens tested. Two biotypes (R1 and R2) inhibited growth of *Fusarium oxysporum* f. sp. *melonis* only. The wild strain and the remaining biotypes did not produce any zones of inhibition against the eight pathogens tested. Several UV-induced biotypes (R4 and R6) were more effective than the wild strain T-1 in suppressing damping-off (*Pythium ultimum*) of peas when their conidia were applied as seed treatments. Two UV-induced biotypes (R5 and R6) significantly suppressed the level of damping-off and blight on beans (*Sclerotium rolfsii*) compared to the level of the control, and suppression by the two biotypes was significantly better than that by the wild strain.

Previous work performed in this laboratory (11) led to the production of several mutant biotypes of *Trichoderma harzianum* (from the wild strain WT-6) that tolerated high concentrations (100–500 μg/ml) of the fungicide benomyl. The biotypes induced by ultraviolet (UV) light also differed considerably from the wild strain in appearance, growth habit, survival ability in soil, fungitoxic metabolite production, and ability to suppress damping-off (induced by *Pythium ultimum*) of peas, damping-off (induced by *Rhizoctonia solani*) of cotton and radish, and white rot (induced by *Sclerotium cepivorum*) of onion. Troutman and Matejka (12) also induced tolerance of *T. viride* to benomyl by gamma irradiation but did not report on the biological control capabilities of their benomyl-tolerant mutants. Improved yield and quantity of enzymes synthesized by *T. reesei* (7,8) and *T. viride* (6) were also obtained by mutation and selection.

The objectives of this study were to induce new biotypes of *T. viride* by UV irradiation, to isolate biotypes tolerant to fungicides of the methyl benzimidazole carbamate (MBC) group, and to test them for biocontrol capabilities against selected soilborne diseases. A preliminary report has been presented (10).

MATERIALS AND METHODS

Strain T-1 of *T. viride* Pers. ex S. F. Gray, provided by H. D. Wells, Tifton, GA, was used. Conidia of T-1 were produced by growing the isolate on V-8 juice agar (200 ml of V-8 juice, 800 ml of water, 1 g of glucose, 15 g of agar, and 6.0 ml of 1.0 N NaOH) for 7 days under continuous fluorescent light (~700 $\mu Ein/m^2 \cdot sec^{-1}$). Conidia were removed from the agar surface by pipetting 5 ml of sterile distilled water onto the surface and gently rubbing the surface with a sterile cotton-tipped applicator. Conidia were counted in a hemacytometer, and the suspensions were adjusted with water to provide the desired concentration of conidia in each test.

Five replications were made in all experiments, and each experiment was done twice. A randomized complete block design was used in all experiments.

Induction and isolation of mutants. One-milliliter aliquots of a conidial suspension (10^5 conidia per milliliter) were placed on 150 V-8 juice agar plates and exposed to UV radiation for 80 min. Irradiation was provided by two adjacent (7 cm apart) G-E Germicidal Lamps (G36T6, General Electric Co., Nela Park, Cleveland, OH 44112). The plates, with the lids removed, were placed so that the distance from the agar surface to the lamps was 25 cm. The irradiated plates were covered and incubated at 25 ± 2 C under fluorescent light. Ten colonies developed from 1.5×10^7 conidia irradiated. The colonies, isolated and grown on V-8 juice agar, were designated T-1-R1 to T-1-R10. For the sake of brevity, these are referred to as R1 to R10.

Tolerance of the mutants to MBC fungicides. Tolerance to fungicides in the MBC group was the criterion used to distinguish mutants from the wild strain. The fungicides benomyl (50% WP, E. I. du Pont de Nemours & Co., Wilmington, DE 19898), thiabendazole (TBZ) (42.28% F, Merck & Co., Chemical Division, Rahway, NJ 07065), and thiophanate-methyl (70% WP, Pennwalt Corp., Oak Brook, IL 60521) were suspended in sterile distilled water and were each added to potato-dextrose broth (PDB) (40 ml per 250-ml Erlenmeyer flask) at 0, 50, and 100 μg of active ingredient (a.i.) per milliliter. Disks (5 mm in diameter) of 7-day-old colonies of T-1 and of the 10 biotypes were transferred to flasks. Dry weights of mycelial mats of flask cultures were determined after 1 and 3 wk of incubation.

We also studied comparative toxicity of benomyl (added to V-8 juice agar at 0, 25, 50, and 100 μg a.i./ml) to spore germination of T-1 and of the 10 biotypes. One-milliliter aliquots of aqueous conidial suspensions (1×10^4 conidia per milliliter) were pipetted onto the surface of V-8 juice agar containing benomyl in petri plates and incubated at 25 ± 2 C under fluorescent light. After 24, 36, and 48 hr, we stained randomly selected areas in the plates with lacto-fuchsin and counted germinated and nongerminated conidia.

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Germinability readings were based on 100 conidia per replication and five replications per treatment; the data were expressed as percent inhibition of germination.

Effect of culture medium pH on growth. The gliotoxin fermentation medium (GFM) of Brian and Hemming (2), a chemically defined medium containing ammonium tartrate, glucose, and mineral salts, was adjusted to pH values of 3.5, 4.5, 5.5, 6.5, 7.5, and 8.5 with 1.0 N NaOH or HCl. The media at pH values of 6.5 or below were buffered with citrate-phosphate buffer (0.1 M citric acid and 0.2 M $Na_2HPO_4 \cdot 7H_2O$) and those at pH 7.5 and 8.5 with phosphate buffer. The media were sterilized by membrane filtration and dispensed into flasks (40 ml per 250-ml Erlenmeyer flask). Inocula of T-1 and of the 10 biotypes were 4-mm disks transferred to flasks from 7-day-old cultures grown on solid gliotoxin fermentation medium (pH 6.5). Dry weights of mycelial mats were determined after 1 and 3 wk. Five replications were used in this test, and the test was done twice.

Antibiosis in dual cultures. The ability of strain T-1 and of the 10 UV-induced biotypes to inhibit growth of eight soilborne plant pathogens (*Fusarium oxysporum* f. sp. *melonis, F. solani* f. sp. *phaseoli, Phytophthora capsici, Pythium ultimum, S. cepivorum, S. rolfsii, R. solani*, and *Verticillium dahliae*) in vitro on potato-dextrose agar (PDA) (22 ml per plate) was determined. Disks (with 4-mm diameters) from the edge of 4-day-old colonies of the *Trichoderma* cultures and of 7-day-old colonies of the pathogens, all grown on PDA, were paired on the medium, on opposite sides of 10 × 1.5-cm petri plates. Disks of *Trichoderma* were placed 3 cm from those of the pathogens. Inocula of *P. capsici, R. solani, S. cepivorum*, and *V. dahliae* were placed on the agar 48 hr before those of the antagonists. All other combinations were plated simultaneously. The cultures were incubated at 25 C under continuous fluorescent light as described earlier and examined for zones of inhibition after 7 days. Antagonism was scored arbitrarily as no inhibition and visible zone of inhibition.

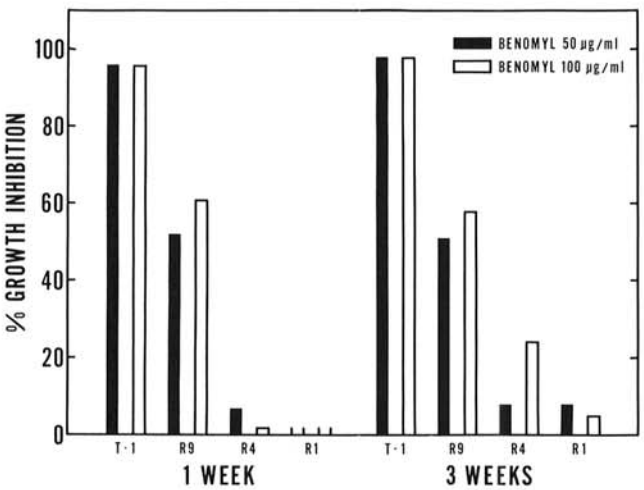

Fig. 1. Inhibition of mycelial dry weight of *Trichoderma viride* strain T-1 and of three biotypes induced by ultraviolet light, after 1 and 3 wk of incubation in potato-dextrose broth containing benomyl.

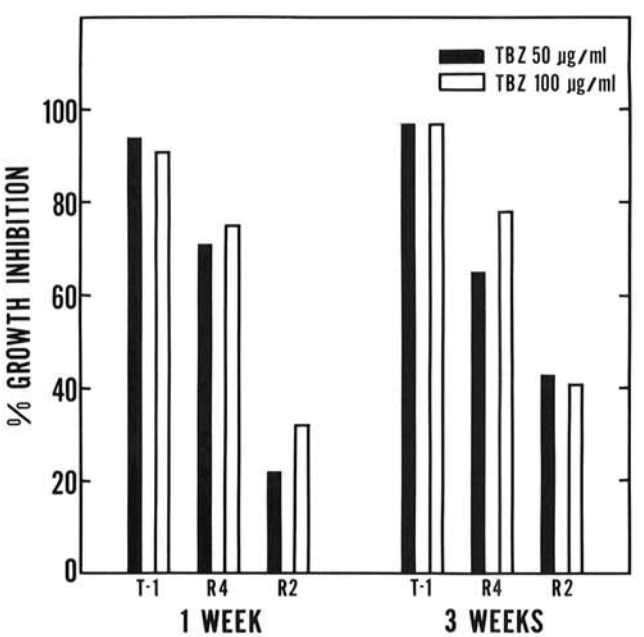

Fig. 2. Inhibition of mycelial dry weight of *Trichoderma viride* strain T-1 and of two biotypes induced by ultraviolet light, after 1 and 3 wk of incubation in potato-dextrose broth containing thiabendazole.

Lytic activity. Lytic activity of T-1 and of the 10 biotypes against *R. solani* hyphae was tested by a method described by Chet and Baker (3). Cultures of *R. solani* (isolate R35, AG-4) were grown for 2 wk in PDB in 200-ml Erlenmeyer flasks (30 ml per flask) on a reciprocal shaker, and mycelial mats were washed with sterile distilled water several times and transferred to a medium described by Okon et al (9) in flasks (40 ml per flask). Each flask was inoculated with 0.2 ml of a conidial suspension (5×10^4 conidia per milliliter) of T-1 or of one of the 10 biotypes, and the flasks were shaken continuously at 25 C. Aliquots of the *Trichoderma-R. solani* cultures were removed with a pipette every week for 6 wk (five replications) and examined with a compound microscope for the presence of lysis. Lysis was scored on a scale of zero to four: 0 = no lysis; 1 = 1–25% of mycelium lysed; 2 = 26–50% of mycelium lysed; 3 = 51–75% of mycelium lysed; and 4 = 76–100% of mycelium lysed. Fifty small samples of the *R. solani* mycelium (mycelial tufts) were also transferred to water agar (1.5% agar) containing streptomycin sulfate and chlorotetracycline HCl at 100 and 50 µg/ml (WAA medium), respectively, to test the viability of the pathogen. Mycelial viability was expressed as percent of mycelial tufts that yielded *R. solani* colonies on the WAA medium.

Disease suppression. Conidia of T-1 and of the 10 UV-induced biotypes were harvested from 8-day-old cultures growing on V-8 juice agar by rubbing (with a cotton tipped applicator) sporulating surfaces to which 2 ml of 4% methyl cellulose (MC) solution had been added. Dilute suspensions of conidia were counted in a hemacytometer and adjusted to contain 2.5×10^9 conidia per milliliter, of which 98% germinated. One milliliter of a spore-MC suspension was applied to 20 g of Perfected Freezer pea seed (*Pisum sativum* L.) in a 100-ml screw-top jar. The seeds were shaken by hand for 1 min and allowed to dry at room temperature. The 1 ml of conidial suspension theoretically added 1.25×10^8 conidia per gram of seed. Treated and untreated seeds and seeds

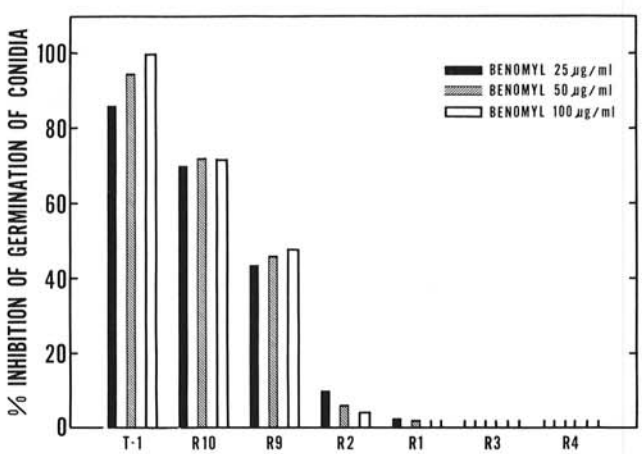

Fig. 3. Inhibition of conidial germination of *Trichoderma viride* strain T-1 and of six biotypes induced by ultraviolet light, after 36 hr of incubation in V-8 juice agar containing benomyl at 25, 50, and 100 µg/ml.

treated with thiram were planted in plastic pots 11 cm in diameter (10 seeds per pot) containing a Beltsville sandy loam naturally infested with *Pythium ultimum*. The pots were incubated at 18 C, and plant stands were determined 11 and 25 days after planting. In a second test, *S. rolfsii* sclerotia, produced on PDA, were added to a Beltsville sandy loam at 50 sclerotia per 100 g of soil and mixed thoroughly in a Hobart Mixer (Hobart Corp., Troy, OH 45374). The soil was divided into 10-kg portions to receive treatments. Aqueous suspensions of conidia from V-8 juice agar plates of T-1 and of each of the 10 UV-induced biotypes were added separately to 1-kg lots of *S. rolfsii*-infested soil to provide 6×10^5 colony-forming units (cfu) per gram of soil. One week after treatment, soil amended with each antagonist was subdivided into 1-kg batches, and these were placed in plastic pots 11 cm in diameter. The pots were planted with untreated bean seed (*Phaseolus vulgaris* L.) of cv. Blue Lake 274 (10 seeds per pot), and these were incubated in a greenhouse compartment at 27 ± 2 C. The plants were harvested and evaluated for disease 1.5 and 5 wk after planting.

RESULTS

Effect of MBC fungicides on growth and conidial germination. In the PDB-benomyl test, growth of wild strain T-1 was completely inhibited at 50 μg of benomyl per milliliter (Fig. 1). Four of the 10 biotypes tested in this experiment (R1–R4) grew as well at 50 μg of benomyl per milliliter of PDB as they did in the untreated control; the growth of the other six (R5–R10) was reduced by about 50% at that concentration of benomyl. Only representative isolates are shown in Fig. 1. Growth of T-1 and of the 10 biotypes in the PDB-thiophanate-methyl test was similar to that observed in the PDB-benomyl test, except that thiophanate-methyl was slightly less toxic to T-1 than benomyl was.

In the PDB-TBZ test, growth of wild strain T-1 was completely inhibited at 50 and 100 μg of TBZ per milliliter of medium (Fig. 2). TBZ, however, was more inhibitory to the UV-induced biotypes than benomyl was. Growth of three biotypes (R2, R3, and R5) was inhibited by about 25 and 40% at both concentrations of TBZ after 1 and 3 wk, respectively. Growth of the other biotypes was inhibited by almost 70%.

Benomyl prevented 85–100% of conidia of wild strain T-1 from germinating at 25, 50, and 100 μg a.i./ml (Fig. 3). In contrast, benomyl prevented only less than 10% of conidia of R1 and R2 from germinating, even at 100 μg/ml. Conidia of R3 and R4 germinated 100% even at 100 μg a.i./ml of benomyl. Benomyl prevented approximately 45–50% of the conidia of R9 and those of the remaining biotypes (R5–R8 and R10) from germinating.

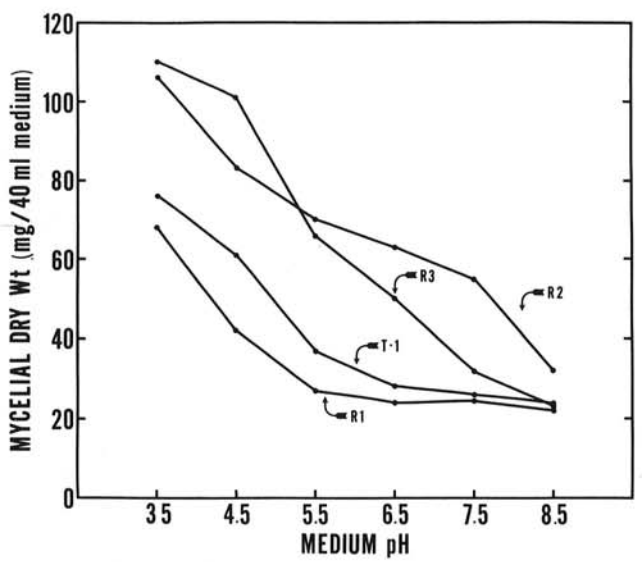

Fig. 4. Mycelial dry weight of *Trichoderma viride* strain T-1 and of three biotypes induced by ultraviolet light, as affected by the pH of a synthetic medium after 3 wk of incubation.

Effect of culture medium pH on growth. Although mycelial dry weights produced at various medium pH values differed among the 10 UV-induced biotypes, the best growth of T-1 and of all the biotypes was obtained at pH 3.5. Results for T-1 and three biotypes are shown in Fig. 4. Growth of all isolates decreased with increasing pH values, with the least amount of mycelial weight obtained at pH 7.5 and pH 8.5. Even at pH 4.5, growth was less than at 3.5. A few biotypes (eg, R3) grew profusely at pH 3.5 but were as strongly inhibited in their growth at high pH values as were other biotypes.

Antibiosis in dual cultures. T-1 and the 10 UV-induced biotypes showed different abilities to inhibit growth of the eight soilborne plant pathogens tested (Table 1). Wild strain T-1 and biotypes R5–R10 did not produce zones of inhibition against any of the pathogens. Biotypes R1 and R2 produced zones of inhibition against *F. oxysporum* f. sp. *melonis* only. Biotypes R3 and R4 were the only ones that exhibited a broader spectrum of in vitro antagonism, producing inhibitory zones when paired with five of the eight pathogens (*F. oxysporum* f. sp. *melonis, F. solani* f. sp. *phaseoli, P. capsici, S. cepivorum*, and *V. dahliae*). None of the biotypes inhibited growth of *P. ultimum, R. solani*, or *S. rolfsii* in dual cultures.

Lytic activity of *T. viride*. Very little or no lysis of *R. solani* mycelium occurred in the control medium and in the samples of the medium to which conidia of R2, R5, or R8 were added 5 wk before the final lysis assay (Table 2). *R. solani* mycelium survived well in these four samples, as indicated by plating out tufts of *R. solani* mycelia on WAA medium. Very little survival of *R. solani* mycelium was observed when conidia of R6 or R10 were added to the medium, and none survived when conidia of T-1 or the biotypes R1, R3, and R4 were added to the medium. The highest percent mycelial lysis was observed with T-1 and the biotypes R1, R3, and R4, and the lowest with R2, R5, and R8.

Disease suppression. Conidia of T-1 and the UV-induced

TABLE 1. Antibiosis against soilborne pathogens in vitro by *Trichoderma viride* strain T-1 and by 10 biotypes induced by ultraviolet light

Strain and biotype	Antibiosis[a] against pathogen[b]				
	Fom	Fsp	Pc	Sc	Vd
T-1	−	−	−	−	−
R1, R2	+	−	−	−	−
R3, R4	+	+	+	+	+
R5, R6, R7, R8, R9, R10	−	−	−	−	−

[a] Exhibited as zones of inhibition on potato-dextrose agar. − = no zone, + = zone.

[b] Fom = *Fusarium oxysporum* f. sp. *melonis*, Fsp = *F. solani* f. sp. *phaseoli*, Pc = *Phytopthora capsici*, Sc = *Sclerotium cepivorum*, Vd = *Verticillium dahliae*.

TABLE 2. Survival of *Rhizoctonia solani* mycelium in a synthetic medium to which conidia (5×10^4/ml) of *Trichoderma viride* strain T-1 and of eight biotypes induced by ultraviolet light were added 5 wk before assay

Strain or biotype	Survival of *R. solani* after 5 wk[a] (%)	Lysis index[b]
None	100 a	0
R2	100 a	1
R7, R8, R9	92 b	1
R5	78 c	1
R10	8 d	2
R6	7 d	2
T-1 (wild)	0 e	3
R1	0 e	3
R3	0 e	4
R4	0 e	4

[a] Values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

[b] 0 = no lysis, 4 = 76–100% of the mycelium lysed.

biotypes R1, R3–R6, R8, and R10, added to Perfected Freezer pea seed at equal population densities, resulted in significantly less preemergence damping-off (11-day postplant reading) than that obtained in the untreated control or the methyl cellulose control (Table 3). Twenty-five days after planting, T-1 and all biotype seed treatments, except R2, significantly suppressed seed rot and damping-off. The stand obtained at 11 days with seed treated with R1 and at 25 days with seed treated with R1 and R4 was significantly better than that obtained with T-1 and equal to that obtained with thiram.

In the *S. rolfsii* test, aqueous suspensions of conidia of T-1, R5, and R6, all added at 6×10^5 cfu per gram of soil, significantly suppressed bean damping-off and blight, as determined at the 1.5-wk postplant examination (Table 4). None of the UV-induced biotypes was significantly better at 1.5 wk than the wild strain. Five weeks after planting, only biotypes R5 and R6 significantly suppressed disease, compared to the control; disease suppression by R5 and R6 was significantly better than that brought about by wild strain T-1 and not significantly different from that of the uninfested control.

TABLE 3. Biological control of seed rot (*Pythium ultimum*) of Perfected Freezer peas with conidia of *Trichoderma viride*[a] strain T-1 and of 10 biotypes induced by ultraviolet light

Seed treatment	Healthy plant stand (%) at time of observation[b]	
	11 days	25 days
Methyl cellulose (control)	42 a	34 a
Untreated (control)	44 a	36 a
R7	52 ab	54 bc
R2	54 ab	48 ab
R9	60 abc	62 bcd
R3	68 bcd	70 cd
T-1 (wild)	68 bcd	62 bcd
R5	70 bcde	70 cd
R8	70 bcde	72 de
R10	72 bcde	70 cd
R6	80 cde	76 de
R4	82 de	78 e
Thiram (control)	82 de	78 e
R1	90 e	88 e

[a] For each isolate, 1 ml of conidial suspension in 4% methyl cellulose provided 1.25×10^8 conidia per gram of seed.

[b] Plant stand (percent of originally planted seed) was rated 11 and 25 days after planting. In each column, values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

TABLE 4. Biological control of snapbean blight (*Sclerotium rolfsii*) with conidia of *Trichoderma viride*[a] strain T-1 and of 10 biotypes induced by ultraviolet light

Strain or biotype	Healthy plant stand (%) at time of observation[b]	
	10 days	35 days
R9	16 ab	8 a
None (control)	12 a	12 ab
R1	28 abc	20 abc
R4	32 abcd	26 abc
R8	28 abc	26 abc
R7	40 bcd	28 abc
R2	40 bcd	34 abc
T-1 (wild)	48 bcde	36 abc
R10	42 bcd	36 abc
R3	44 bcde	40 bcd
R6	54 cde	46 cd
R5	60 de	60 d
Uninfested (control)	74 e	64 d

[a] Conidia from V-8 juice agar plates added to soil to provide 6×10^5 conidia per gram of soil.

[b] Plant stand (percent of originally planted seed) was rated 10 and 24 days after planting. In each column, values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

DISCUSSION

The evidence presented in this paper, coupled with evidence presented in two recent publications (1,11), suggests that it is feasible to induce new, stable biotypes of *Trichoderma* spp. with long exposure to UV irradiation or to various fungicides. As with the new biotypes of *T. harzianum* (11), the 10 new biotypes of *T. viride*, induced by irradiating wild strain T-1, differed from T-1 not only in their ability to tolerate fungicides of the MBC group (benomyl, TBZ, thiophanate-methyl), but also in their ability to suppress disease. Some biotypes were similar to or less effective than T-1 in suppressing disease; a few biotypes were more effective than T-1 (Table 4). One biotype of this series, R9, greatly suppressed Fusarium wilt of chrysanthemum in greenhouse culture (5) and is now being considered for experimental production and use by the ornamentals industry, with or without MBC fungicides.

The test on the in vitro antibiotic activity in dual cultures (Table 1) showed considerable differences among the 10 biotypes from T-1 in their ability to produce zones of inhibition against several soilborne plant pathogens; and this ability paralleled that for lytic activity against *R. solani* (Table 2). Comparison of the data on in vitro antibiosis with the data on the ability to suppress disease, however, indicates a lack of correlation between the two characteristics. For instance, none of the biotypes exhibited in vitro antibiosis against *P. ultimum*. Yet several biotypes suppressed damping-off of peas caused by *P. ultimum* (Table 3). Kommedahl and Windels (4) also found that laboratory or greenhouse screening tests for antagonists could not predict their performance in the field. Although in vitro antibiosis may be used to make a relevant point, such as to show any changes in genome manifested by the ability to produce metabolic products inhibitory to plant-pathogenic fungi on agar, it can not be used to select biotypes for disease control. In fact, R9 developed no inhibitory zones against *Fusarium* spp. (Table 1), yet it proved to be one of the best biotypes for control of Fusarium wilt of chrysanthemum (5).

Although our data on mycelial growth (Figs. 1 and 2) showed cross resistance of the UV-induced biotypes to benomyl, TBZ, and thiophanate-methyl, the extent of resistance to the three MBC fungicides was not equal. The biotypes tested were more sensitive to TBZ than to benomyl or thiophanate-methyl. Although our tests do not show a complete lack of cross resistance to benomyl and TBZ, similar to that observed by Van Tuyl et al (13) in *Aspergillus nidulans*, they do show the occurrence of biotypes that are less sensitive to benomyl than to TBZ, despite the fact that both fungicides have the same active ingredient. No explanation for this discrepancy can be offered at this time.

LITERATURE CITED

1. Abd-El Moity, T. H., Papavizas, G. C., and Lewis, J. A. 1982. Induction of new isolates of *Trichoderma harzianum* tolerant to fungicides and their experimental use for control of white rot of onion. Phytopathology 72:396-400.
2. Brian, P. W., and Hemming, H. G. 1945. Gliotoxin, a fungistatic metabolic product of *Trichoderma viride*. Ann. Appl. Biol. 32:214-220.
3. Chet, I., and Baker, R. 1980. Induction of suppressiveness to *Rhizoctonia* solani in soil. Phytopathology 70:994-998.
4. Kommedahl, T., and Windels, C. E. 1978. Evaluation of biological seed treatment for controlling root diseases of pea. Phytopathology 68:1087-1095.
5. Locke, J. C., Marois, J. J., and Papavizas, G. C. 1982. Biocontrol of Fusarium wilt of greenhouse grown chrysanthemums. (Abstr.) Phytopathology 72:709.
6. Mandels, M., Weber, J., and Parizek, R. 1971. Enhanced cellulase production by a mutant of *Trichoderma viride*. Appl. Microbiol. 21:152-154.
7. Montenecourt, B. S., and Eveleigh, D. E. 1977. Semiquantitative plate assay for determination of cellulase production by *Trichoderma viride*. Appl. Microbiol. 33:178-183.
8. Montenecourt, B. S., and Eveleigh, D. E. 1979. Production and

characterization of high yielding cellulase mutants of *Trichoderma reesei*. Pages 101-108 in: TAPPI Proc. Annu. Meet., 1979.
9. 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.
10. Papavizas, G. C., and Lewis, J. A. 1981. New biotypes of *Trichoderma viride* with tolerance to MBC fungicides. (Abstr.) Phytopathology 71:898.
11. Papavizas, G. C., Lewis, J. A., and Abd-El Moity, T. H. 1982. Evaluation of new biotypes of *Trichoderma harzianum* for tolerance to benomyl and enhanced biocontrol capabilities. Phytopathology 72:126-132.
12. Troutman, J. L., and Matejka, J. C. 1978. Induced tolerance of *Trichoderma viride* to benomyl. (Abstr.) Phytopathol. News 12:131.
13. Van Tuyl, J. M., Davidse, L. C., and Dekker, J. 1974. Lack of cross resistance to benomyl and thiabendazole in some strains of *Aspergillus nidulans*. Neth. J. Plant Pathol. 80:165-168.