Biological Control of Fusarium Wilt of Greenhouse-Grown Chrysanthemums

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ABSTRACT

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Twenty-two isolates and biotypes of soil fungi representing three genera and five species were tested in the greenhouse against *Fusarium oxysporum* f. sp. *chrysanthemi*, causal agent of Fusarium wilt of chrysanthemum (*Chrysanthemum morifolium*). The four biocontrol agents that reduced disease incidence the most were tested alone and in several combinations in a greenhouse stock plant production system using the susceptible cultivar Yellow Delaware. A wild-type isolate of *Trichoderma viride* (T-1) and a benomyl-resistant biotype (T-1-R9), alone or in combination with *Aspergillus ochraceus*, reduced disease by at least 50% in vegetatively maintained plants. T-1-R9 was evaluated in a cut flower production system using the susceptible cultivar Cirbronze. Use of T-1-R9 with as few as two benomyl drenches provided control equal to a commercial integrated control procedure.

Additional key word: biorational

Fusarium wilt of florists' chrysanthemum (*Chrysanthemum morifolium* Ramat.), caused by *Fusarium oxysporum* (Schlecht.) f. sp. *chrysanthemi* Litt., Armst., & Armst., is one of the most widespread and destructive diseases of this major horticultural crop and is most severe in warm climates. The disease may become increasingly important in the production areas of the southeastern states where most of the foundation stock for cutting propagation is grown. Susceptibility of chrysanthemum cultivars to Fusarium wilt varies (3,4), and susceptible cultivars are grown in specific fields or blocks where special production procedures are employed. The threat of spread of this pathogen is of concern because infected, nonsymptomatic vegetative cuttings can be produced and distributed (1). A goal of the commercial chrysanthemum propagator is to produce 100% pathogen-free cuttings. To accomplish this, extensive preventive procedures are employed to maintain healthy stock plants. Culture indexing, certified stock block multiplication, and integrated control practices in field production are employed to keep the distribution of infected cuttings to a minimum. Field control of the disease is currently achieved with an integrated lime-nitrate-fungicide regime (2). Soil pH is raised with $Ca(OH)_2$ before planting and an all-NO_3-nitrogen fertilization program is employed that is supplemented by periodic benomyl soil drenches. These practices have been shown to have an additive effect on reducing Fusarium wilt.

The objective of this research was to evaluate the use of biological control against Fusarium wilt in chrysanthemum production. This approach involved the introduction of selected microorganisms (biorationals) into a steam-treated soil mix before planting rooted chrysanthemum cuttings and subsequently challenging the system with the pathogen. The goal of this approach was to develop an inexpensive management practice for effective control of this disease that would offer an alternative control procedure to the integrated regime currently in use.

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MATERIALS AND METHODS

Bench-plot design. All tests were conducted in standard, raised transite benches that were subdivided into plots separated by a 30-cm gap. Individual plots were filled with a mix (pH 6.4) consisting of composted loamy soil, peat moss, and perlite (3:3:1, v/v). The entire bench-plot system was tarped and steam-treated (82 C for 2 hr) before each test. Test biocontrol organisms were grown on potato-dextrose agar (PDA) plate cultures for 1 wk and applied as conidia in aqueous suspension (10^4 conidia per square centimeter) to the surface of the cooled mix immediately after untarping. Infested plots were incubated for 48–72 hr to enable the organisms to colonize the mix. In some tests, population levels of the benomyl-tolerant *Trichoderma viride* Pers. ex S. F. Gray (biotype T-1-R9 [7]) were monitored after introduction. Commercially produced (Yoder Brothers, Barberton, OH) cuttings of the *Fusarium*-susceptible cultivars Yellow Delaware (for screening and vegetative tests) and Cirbronze (for flowering tests) were planted in the colonized mix with a spacing of 15 × 15 cm unless noted otherwise. The number of cuttings per plot was noted for each test. Cuttings were allowed to establish their root systems for 7–14 days, then challenged with an aqueous application (500 propagules per square meter) of *F. oxysporum* f. sp. *chrysanthemi* propagules (microconidia, macroconidia, and chlamydospores) representing three isolates obtained from infested field soil in Florida. Inoculum was washed into the mix by watering and plants were left undisturbed without injury to the roots. Greenhouse air temperature was maintained at 24–27 C at night throughout all tests, and summer day temperatures rose to 30–33 C during peak hours of radiation. Soil temperature in the plots was maintained at 27 C with buried heat cables. Fertilization consisted of a combination of urea, nitrate, and ammoniacal forms of nitrogen (Peters 20-20-20, W. R. Grace & Co., Fogelsville, PA) applied through a Gewa injector (H. A. Wirth, Sayville, NY) once a week unless noted otherwise.

Initial screening. Twenty-two isolates of soil fungi, mainly strains of *Trichoderma*, were evaluated for their ability to control Fusarium wilt in vegetative stock plants of the highly susceptible cultivar Yellow Delaware. Isolates tested included six UV-induced biotypes (T-1-R1, T-1-R2, T-1-R3, T-1-R4, T-1-R5, and T-1-R9) of a single wild-type isolate of *T. viride* (T-1) that had been selected for their tolerance to benomyl (7). The chrysanthemum plants were maintained vegetatively with 10 ft-c of supplemental incandescent lighting from 10 p.m. to 2 a.m. The terminal 5–8 cm of each developing shoot was removed regularly from the plants to allow continued development of new growth. This practice simulated procedures for commercial cutting production, and cumulative counts of these cuttings were maintained as a measure of yield. Diseased plants were tagged as symptoms were observed during the 16-wk test. At the end of the test, all plants were evaluated as healthy or diseased on the basis of visual symptoms and examination of the vascular tissue for discoloration.

Soil colonization by biotype T-1-R9. The soil colonization potential of the

benomyl-tolerant biotype (T-1-R9) was determined using a PDA-Tergitol medium containing chlorotetracycline and benomyl (39 g of Difco PDA; 1 ml of Tergitol NP-10, 1:10.5 Nonylphenol-Ethylene Oxide Condensate [J. T. Baker Chemical Co.]; 30 mg of chlorotetracycline HCl [ICN Nutritional Biochemicals]; and 5 mg a.i. of benomyl per liter). Steamed and nonsteamed soil mix in 8-in. clay pots was either infested directly or after a benomyl drench equivalent to 0.5 lb a.i./100 gal/800 ft^2. Rooted Yellow Delaware cuttings were planted in each pot to provide the normal rhizosphere influence. Soil samples (about 5 g) were removed weekly from the top 2 cm of each pot for dilution-plate analysis. A 1-g soil subsample from each pot was suspended in 99 ml of water and aliquot dilution samples (10^{-5} and 10^{-6}) were pipetted into five petri plates, PDA-Tergitol medium cooled to 45 C was added, and the plates were swirled before the medium solidified. All plates were incubated at room temperature for 7 days before counting the T-1-R9 colonies. This count multiplied by the dilution factor gave the number of colony-forming units per gram of soil.

Vegetative plant system. The wild-type isolate of *T. viride* (T-1), two of the most effective biotypes from the initial screening (T-1-R4 and T-1-R9), and *Aspergillus ochraceus* Wilhelm (FL-5) were evaluated as control agents alone and in two combinations in three replicated tests (four replicate plots per test). Each plot (45 × 90 cm) was plated with 18 Yellow Delaware rooted cuttings. Each test was performed as described previously. Colonization of the soil mix by the benomyl-tolerant biotypes (T-1-R4 and T-1-R9) was monitored using the PDA-Tergitol medium throughout the tests, and the *Fusarium* population was monitored periodically using Komada's *Fusarium*-selective medium (5). After 16 wk, all plants were rated as healthy or diseased on the basis of visual symptoms.

Flowering plant system. Biotype T-1-R9 was tested in a cut flower production system using rooted cuttings of 9-wk-old cultivar Cirbronze planted in plots 90 × 90 cm, each containing 48 plants on a spacing of 15 × 12.5 cm. Biotype T-1-R9 was used alone and in combination with benomyl drenches at 14-, 28-, or 42-day intervals compared with an integrated control procedure (2). Plots receiving the commercially used integrated control were treated with 75 g $Ca(OH)_2$ per plot (about 2 g/kg mix) before pasteurization. The resulting pH of these plots was 7.2 compared with 6.4 for the untreated plots. Only NO_3-nitrogen fertilization was used on these plots, as described by Woltz and Engelhard (9). Soil temperature was maintained at 27 C. For the first 3 wk after planting, plants were maintained vegetatively with incandescent light during the dark period as described previously. Flowering was initiated by covering the benches with black cloth to create a 16-hr dark period followed by 8 hr of light. The short-day condition was maintained for the 9-wk period until harvest. Population levels of the biotype T-1-R9 and *Fusarium* were monitored throughout the tests.

Table 1. Incidence of Fusarium wilt and cutting yield from *Chrysanthemum morifolium* 'Yellow Delaware' grown in bench plots containing *Aspergillus* or *Trichoderma* in addition to *Fusarium oxysporum* f. sp. *chrysanthemi*[a]

Treatment	Diseased (%)	No. of cuttings
Control (uninoculated)[b]	0	580
Control (inoculated)	76	508
Aspergillus ochraceus (FL-5)	0	556
Trichoderma viride (T-1)	0	563
(T-1-R4)	0	576
(T-1-R9)	14	590

[a] Based on 18 plants per treatment over a period of 16 wk.
[b] No *Fusarium* applied.

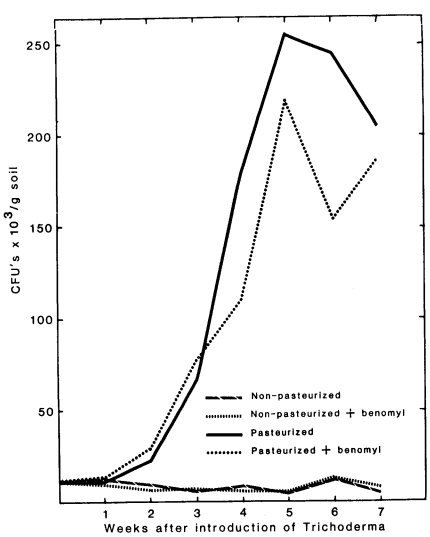

Fig. 1. Soil colonization of a benomyl-tolerant isolate of *Trichoderma viride* (T-1-R9) in pasteurized and nonpasteurized soil with and without benomyl treatment.

Table 2. Incidence of Fusarium wilt in *Chrysanthemum morifolium* 'Yellow Delaware' grown in bench plots containing *Aspergillus* and/or *Trichoderma* in addition to *Fusarium oxysporum* f. sp. *chrysanthemi*[y]

Treatment	Diseased (%)
Control	65 a[z]
Trichoderma viride (T-1)	17 c
(T-1-R9)	33 bc
(T-1-R4)	45 b
Aspergillus ochraceus (FL-5)	61 a
T. viride (T-1) + *A. ochraceus* (FL-5)	18 c
T. viride (T-1-R9) + *A. ochraceus* (FL-5)	20 c

[y] Disease is based on average of three tests with four to 18 plant replicates per treatment.
[z] Numbers followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

RESULTS

Initial screening. Wilt symptoms first appeared 6 wk after introduction of the pathogen. Symptoms appeared initially as temporary wilting followed by chlorosis, permanent wilting, veinal necrosis, and vascular discoloration that ultimately resulted in plant death about 2 wk after initial wilting. At the end of the 16-wk test, *T. viride* T-1, biotypes T-1-R4 and T-1-R9, and *A. ochraceus* reduced the number of symptomatic plants by more than 80% over the control (Table 1). The other biotypes tested provided less control or no control and were not evaluated further. Treatment with T-1-R9 resulted in as many cuttings being produced as in the uninoculated control even though there were fewer productive plants at the end of the test. Isolate T-1, biotypes T-1-R4 and T-1-R9, and *A. ochraceus* FL-5 were subsequently tested alone and in combination in replicated plots to verify their control potential.

Soil colonization by biotype T-1-R9. Soil colonization by biotype T-1-R9 was limited to the steam-treated soil mix (Fig. 1). The T-1-R9 population increased rapidly during the first 35 days after introduction, then leveled off. Application of a benomyl drench to either steamed or nonsteamed soil mix had no apparent effect on soil colonization by T-1-R9. In separate tests (J. Locke, *unpublished*), T-1-R9 was shown to preferentially colonize certain high-organic-matter mixes including those containing a processed bark component.

Vegetative plant system. As in the initial screening, Fusarium wilt symptoms first appeared 6 wk after the pathogen was introduced. The average disease reductions achieved in all tests with the individual isolates or biotypes and various combinations appear in Table 2. Isolate T-1, as well as the combinations T-1 + FL-5 and T-1-R9 + FL-5, all gave disease reductions of at least 70% over the control. When used alone, biotype T-1-R4 and isolate FL-5 were ineffective and biotype T-1-R9 reduced disease by 50%. Disease progress during the 16-wk tests (Fig. 2) was rapid between 10 and 14 wk after introduction of the pathogen in the untreated control and in the ineffective treatments (T-1-R4 and T-1-R9) but was gradual during this period in the effective treatments (T-1, T-1 + FL-5, and T-1-R9 + FL-5). Colonization of the soil by biotype T-1-R9 followed the same pattern as described earlier, and the final *Fusarium* population in the T-1-R9-amended soil was about 70% of the population in the control soil.

Flowering plant system. The initial test demonstrated that use of T-1-R9 with as few as two supplemental benomyl drenches (42-day interval) was as effective as the integrated control

procedure in reducing Fusarium wilt (Table 3). More than two applications of benomyl did not increase disease control. Disease progress curves for these treatments (Fig. 3) show that symptoms began to appear 4–6 wk after introduction of the pathogen. Disease in the control and T-1-R9 treatments increased rapidly through weeks 6–8, then began to level off. Final disease evaluation indicated that the combination of T-1-R9 with supplemented benomyl drenches at 42-day intervals provided control equal to the integrated commercial procedure (2).

Table 3. Incidence of Fusarium wilt in *Chrysanthemum morifolium* 'Cirbronze' grown in bench plots treated with *Trichoderma viride* (T-1-R9) alone and in combination with benomyl applications compared with a commercial integrated control program[w]

Treatment	Diseased at harvest[x] (%)
Control	47 a[y]
Integrated control program[z]	9 c
Trichoderma viride (T-1-R9)	29 b
T. viride (T-1-R9) + benomyl	
14-Day interval	8 c
28-Day interval	9 c
42-Day interval	7 c

[w] Based on 96 plants per treatment, grown on a commercial schedule to flowering.
[x] Indicates percentage of flower sprays rated as unsaleable because of *Fusarium*.
[y] Numbers followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.
[z] Consisted of preplant pH adjustment using $Ca(OH)_2$, an all-NO_3-nitrogen fertilization program, and benomyl application at a 14-day interval.

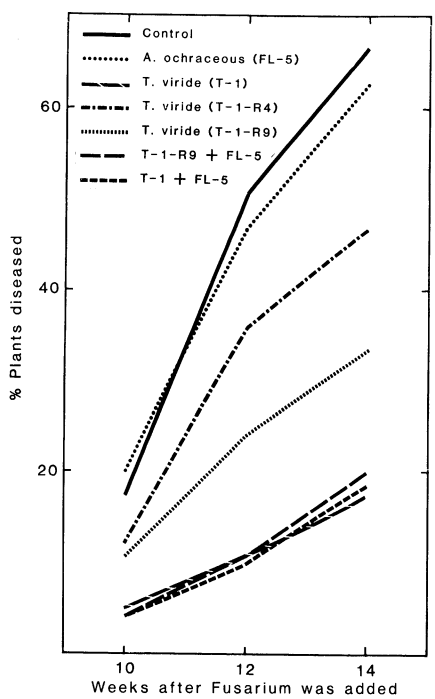

Fig. 2. Comparison of control of Fusarium wilt of vegetative Yellow Delaware chrysanthemums with *Aspergillus* and *Trichoderma* and with a combination of the two biorationals.

Benomyl and T-1-R9 used independently provided an average of 72 and 20% disease reduction, respectively. Population dynamics of T-1-R9, alone and in combination with benomyl applications, were not substantially different during these tests. In both cases, initial increases in colony-forming units were very rapid and the subsequent application of benomyl had no effect on the T-1-R9 population. Once populations reached the 10^6 level, a plateau was achieved followed by a gradual decline.

DISCUSSION

The benomyl-tolerant *T. viride* biotype T-1-R9 was shown to be a potential biocontrol agent for reducing Fusarium wilt in vegetative chrysanthemums. When combined with a minimal number of benomyl drenches, it was also effective in a flowering chrysanthemum system. Because in these two systems we used the highly susceptible cultivars Yellow Delaware and Cirbronze, respectively, disease control probably would have been more dramatic with less susceptible cultivars. This is the first report of the successful use of a UV light-induced biotype of *Trichoderma* to control Fusarium wilt or any soilborne disease. *T. viride* conidia (T-1-R9) rapidly colonized the steamed soil mix but were unable to colonize a nonsteamed soil mix even when added after benomyl treatment. Fungistasis may be operative in the nonsteamed soil, thus preventing germination of the conidial inoculum. Rapid colonization by the biorational is apparently achieved by its ability to utilize nutrients released by steam treatment as well as organic matter in the mix. However, the mechanism of disease control has not yet been determined.

The ability of the biorational to reach high populations quickly and maintain them may be enhanced further by adding certain types of organic matter. Whether competition is the mechanism involved in suppression of *Fusarium* as suggested in other systems (6), or whether other mechanisms are responsible, is not clear from this work. The beneficial effect of biotype T-1-R9 can apparently be overcome under high disease pressure, and thus the system requires supplemental benomyl applications to be effective. The amount of fungicide required, however, is much less than that used in the integrated control system (2). The host during flowering may possibly be more susceptible and thus disease suppression can be appreciable with the additive effect of the biorational and the fungicide. When combined with the biorational, however, the frequency of benomyl applications can be greatly reduced from that in the integrated control protocol (2).

If successful when tested under actual commercial conditions, this biorational could provide an alternative means of control to an industry that depends heavily on conventional chemical pesticides. Development of fungicide-tolerant biorationals such as the biotype T-1-R9 will allow industry the freedom to use fungicides as sprays or occasional applications as drenches without interfering with the efficacy of the biorational. Further modification of biorationals could even enhance their efficacy and incorporate tolerance to other commonly used agricultural chemicals (insecticides, herbicides, fungicides, and fertilizers) as has been reported (8). Combinations of biorationals could possibly be used to give broad-spectrum protection against a soilborne disease complex.

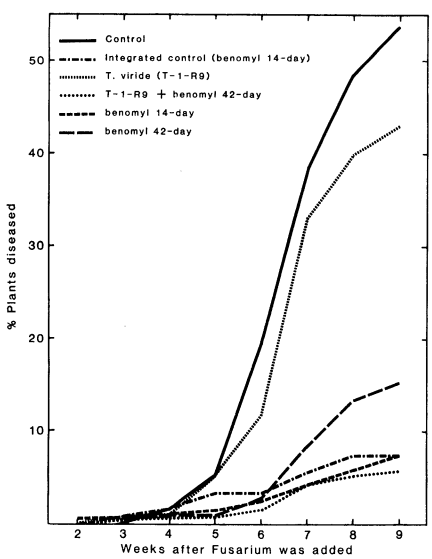

Fig. 3. Comparison of integrated and biological control procedures against Fusarium wilt of Cirbronze chrysanthemums grown on a commercial flowering schedule.

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