Induction of New Isolates of *Trichoderma harzianum* Tolerant to Fungicides and Their Experimental Use for Control of White Rot of Onion

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ABSTRACT

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Prolonged exposure of mycelia and conidia of *Trichoderma harzianum* to the fungicide benomyl did not produce isolates tolerant to the fungicide. Exposure of four wild strains of *T. harzianum* to the fungicides chlorothalonil, procymidone, iprodione, and vinclozolin resulted in selection of several isolates tolerant to these fungicides. Some of the fungicide-tolerant isolates grew better radially on media containing the fungicides than their respective wild strains did. Other isolates lost their tolerance after being cultured on fungicide-free media. Conidia of certain isolates of the wild strains WT-6 and T, tolerant to chlorothalonil and iprodione, respectively, germinated better on media containing high concentrations of the fungicides than did conidia of their respective wild strains. Exposure of conidia of the wild strain T-14 to 0.1% (active ingredient) chlorothalonil for 4 wk reduced germination by 80% when the conidia were placed on a fungicide-free medium. Similar exposure of conidia of T-14(3M), a chlorothalonil-tolerant isolate, reduced germination by only 20%. An iprodione-tolerant isolate derived from the Egyptian strain T produced more toxin, as measured by inhibition of mycelial growth of *Sclerotium cepivorum,* than did the wild strain. One fungicide-tolerant isolate of strain Th-1 (Th-1 [procy-2M]) reduced white rot of onion caused by *S. cepivorum* more effectively than did Th-1 or other fungicide-tolerant isolates. The iprodione-tolerant isolate T(ipro-25M) and iprodione combined with T(ipro-25M) gave the best control of white rot of onion in the field in Egypt.

Additional key words: biological control.

Research done during the last 10–15 yr on resistance developed by plant-pathogenic fungi as a result of exposure to modern fungicides has been summarized in several review articles (5–9). Delp (7), who used the words "resistance" and "tolerance" interchangeably, suggested the following three mechanisms of resistance to fungitoxicants: nongenetic adaptation (training), which may be lost after a given pathogen is cultured on a fungicide-free medium; genetic adaptation, which may depend on chromosomal gene changes; and adaptation due to extra-chromosomal inheritance. Hastie (10) also discussed "the possible roles of genetically active fungicides in causing the formation of new fungal biotypes." According to him, the genetic effects of fungicides may include "gene mutation, chromosome breakage, mitotic nondisjunction and mitotic recombination."

Despite the abundance of work done on tolerance of plant pathogens to fungicides, virtually no research has been done on tolerance of biocontrol fungi to fungicides nor on purposeful induction of tolerance in biocontrol agents for use in integrated control of plant diseases. Recently, Papavizas et al (13) induced tolerance of *Trichoderma harzianum* Rifai to benomyl by ultraviolet light irradiation and selection. Tolerance was a stable characteristic of the new biotypes.

The objectives of the present study were to induce new isolates of *T. harzianum* tolerant to certain fungicides, using prolonged exposure to increasing concentrations of the toxicants, and to evaluate various characteristics of tolerant isolates, including their biocontrol ability against white rot of onion caused by *Sclerotium cepivorum* Berk., a serious disease of onion (*Allium cepa* L.) in Egypt and in the United States. A preliminary report of part of this work was presented (11).

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MATERIALS AND METHODS

Isolates of *T. harzianum*. Four strains of *T. harzianum* were used in this study. Strains T-14 and WT-6 were obtained from H. D. Wells, Tifton, GA. Strain T was isolated by the senior author in Egypt and Th-1 by the second author from a Beltsville field plot soil. Stock cultures of the strains were maintained at 5 C on potato-dextrose agar (PDA) in Egypt and on V-8 juice agar (200 ml of V-8 juice per liter of liquid, 20 g of agar, 1 g of glucose, 6.0 ml of 1.0 N NaOH) at Beltsville. Conidia of *T. harzianum* were obtained from 5-day-old cultures by adding a few milliliters of sterile distilled water to cultures and gently rubbing the surface with a sterile cotton-tipped applicator. Conidia were counted in a hemacytometer, and suspensions were adjusted so that they had the desired number of conidia for each experiment.

Fungicides. The following fungicides were used: 3-(3,5-dichlorophenyl)-*N*-(1-methylethyl)-2,4-dioxo-1-imidazolidinecarboxamide (iprodione, 50% wettable powder, Rohne-Poulenc, Lyon, France); tetrachloroisophthalonitrile (chlorothalonil, 75% wettable powder, Diamond Shamrock Corp., Painesville, OH 44077); 1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl, 50% wettable powder, E. I. du Pont de Nemours & Company, Wilmington, DE 19898); 3-(3,5-dichlorophenyl)-1,5-dimethyl-3-azalicyclo-[3.1.0]hexane-2,4-dione (procymidone, DPX 4424, 50% wettable powder, E. I. du Pont de Nemours & Company); and 3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione

(vinclozolin, BASF Wyandotte Corp., Parsippany, NJ 07054). The fungicides were suspended in sterile distilled water and added in appropriate amounts (w/v) to the autoclaved media before they were dispensed into petri dishes or flasks.

Development of tolerance to fungicides. The process of developing tolerance to each of the five fungicides was initiated at predetermined sublethal concentrations. Because benomyl completely inhibited growth at 1.0 μg of active ingredient (a.i.) per milliliter, the adaptation was begun at 0.5 μg/ml. For iprodione, procymidone, chlorothalonil, and vinclozolin, the process was begun at 5 μg/ml. One milliliter of aqueous suspension of conidia (1×10^4/ml) was spread on the surface of 12 dishes containing V-8 juice agar (at Beltsville) or PDA (in Egypt) supplemented with the fungicides. Conidia from vigorous surviving colonies were transferred to media containing higher concentrations of the fungicides. Further transfers of conidia were made in succession on media containing progressively higher concentrations up to 3,000 and 25,000 μg a.i./ml for chlorothalonil and iprodione, respectively. In addition to exposing conidia of *T. harzianum* to increasing concentrations of the fungicides, we transferred 5-mm disks from 5-day-old colonies to the media containing fungicides. After prolonged incubation, usually 2 mo, subculturing was repeated if growth occurred.

Effect of fungicides on radial growth and conidial germination. Strains T and Th-1, nontolerant to iprodione, and six isolates tolerant to the fungicide were transferred to V-8 juice agar containing iprodione at 0, 2,000, 5,000, and 25,000 μg a.i./ml. Colony radii were measured after 3, 7, and 14 days of incubation.

We studied comparative toxicity of iprodione (used at 0, 2,000, 5,000 and 25,000 μg a.i./ml) and chlorothalonil (used at 1, 1,000, and 3,000 μg a.i./ml) on spore germination of wild strain Th-1 and six isolates adapted to iprodione and of the wild strain WT-6 and six isolates adapted to chlorothalonil, respectively. One-milliliter aliquots of conidial suspensions (1×10^4 conidia per milliliter) were pipetted onto the surface of V-8 juice agar containing the fungicides in petri dishes and incubated at 25 C under fluorescent light. After 24 and 36 hr, we stained selected areas in the dishes with lacto-fuchsin and counted germinated and ungerminated spores. Germinability readings were based on 100 conidia per replication and four replications per treatment. The data were expressed as percent inhibition of germination.

In a separate test, aqueous suspensions of conidia (1×10^4/ml) of strains T-14 and WT-6 and of two isolates tolerant to 3,000 μg a.i./ml of chlorothalonil—designated T-14(3M) and WT-6(3M), respectively—were added separately to 250-ml Erlenmeyer flasks containing 30 ml of 0.1 and 0.2% (1 and 2 mg a.i./ml, respectively) suspensions of chlorothalonil (spore suspension:chlorothalonil suspension, 1:1, v/v). The conidia were thus exposed directly to 0.5 and 1 mg/ml of chlorothalonil. The flasks were placed on a platform shaker and shaken continuously for 4 wk in the dark at 5 C to prevent germination and growth. At 1 and 4 wk, drops containing conidia were placed on V-8 juice agar in petri dishes and incubated at 25 C under continuous fluorescent light. Percent germination was recorded after 24 and 36 hr.

Production of fungitoxic metabolites. Strain T, nontolerant to iprodione, and isolates T(ipro-5M) and T(ipro-25M), tolerant to the fungicide, were grown in 50 ml of the gliotoxin fermentation medium of Brian and Hemming (3) for 10 days in the dark. Culture filtrates were sterilized by passing through Millipore filters and were added at 10% (v/v) to PDA or potato-dextrose broth (PDB) after the media were autoclaved and cooled to about 50 C. PDA was dispensed into petri dishes (15 ml per dish) and PDB into Erlenmeyer flasks (50 ml per flask). The media without culture filtrates and media with 10% fermentation medium were used as controls. Disks 5 mm in diameter from 5-day-old colonies of *S. cepivorum* were transferred to the center of the dishes or to flasks. Colony radii of *S. cepivorum* on PDA were measured at 4 days, and dry weights were determined after 7 days of growth in the PDB medium. The effect of toxic metabolites was expressed as percent inhibition of growth of *S. cepivorum*. The term "fungitoxic metabolite" is used here in a general sense because no attempts were made to determine the identity of the inhibitory substances produced by *T. harzianum*.

White rot suppression in the greenhouse. Conidia of the wild strain Th-1 and of four isolates tolerant to procymidone and vinclozolin were harvested from 10-day-old cultures growing on V-8 juice agar by rubbing a cotton applicator over sporulating surfaces to which 5 ml of sterile distilled water had been added. Conidia were counted as before, and aqueous suspensions were added to 5-kg soil batches naturally infested with *S. cepivorum* at 4.4×10^5 conidia per gram of soil. The same five antagonists were also grown for 3 wk on a new medium (J. A. Lewis and G. C. Papavizas, *unpublished data*) containing the following ingredients: quartz sand, 1,200 g; cornmeal, 40 g; wheat bran, 40 g; and gliotoxin fermentation medium (3), 150 ml. The inocula on this medium were air-dried and mixed thoroughly. The numbers of colony-forming units (cfu) in the dry preparations were determined by the dilution-plate method on TME medium (12), and the preparations were added to soil at a rate sufficient to provide 4.4×10^5 cfu/g of soil. Autoclaved dry preparations were added to soil to equalize the amounts of organic matter added with the active antagonists.

Seven days after treatments, the soil portions were subdivided into five 1-kg batches and placed in plastic pots 11 cm in diameter. Six-week-old onion seedlings of cv. Yellow Globe Danvers were transplanted into the infested soil and grown in a chamber at 16 C with a 12-hr day length (about 800 μEin/m^2/sec) for 8 wk. Disease severity data were recorded as the percentage of plants infected with *S. cepivorum* in each pot. We used five replications and the experiment was done twice.

White rot suppression in the field. A field at Shandawil, Egypt, heavily infested with *S. cepivorum,* was used for the field test. Inoculum of strain T and of an isolate adapted to iprodione was grown on barley grain moistened with modified gliotoxin fermentation medium (half the concentration of glucose and twice the concentration of ammonium tartrate) and supplemented with onion extract (barley:fermentation medium:onion extract, 1:0.8:0.1, w/v/v). Onion extract was prepared by adding 200 g of onion bulb tissue in 1L of water, autoclaving the mixture at 121 C for 15 min, and filtering it through cheesecloth and cotton. Three-kilogram batches of this medium were placed in autoclavable plastic bags, autoclaved at 121 C for 30 min, and inoculated with *T. harzianum.* The bags were incubated at room temperature (25–28 C) for 20 days. Before use in the field, 25 g of iprodione was added to 1 kg of inoculum and mixed thoroughly. The inocula were added to the field in-furrow at 240 kg/ha (fresh weight). Sixty-day-old onion transplants (cv. Giza 6) were transplanted immediately after inoculum addition (300 transplants in each 2 × 2-m plot). Five plots (replications) were used per treatment. Four months after transplanting, all plants were uprooted and examined for visual symptoms of *S. cepivorum* infection. The experiment was done twice.

RESULTS

Tolerance to fungicides. After prolonged and repeated exposure of *T. harzianum* isolates T-14, WT-6, and Th-1 to benomyl, 0.5–10 μg a.i./ml, no isolates from these strains were tolerant to benomyl. Continuous growth of these three strains and of the Egyptian strain T on increased concentrations of the other four fungicides resulted in selection of several isolates tolerant to fungicides as follows: In Egypt, T(ipro-5M), T(ipro-25M), and T(ipro-40M); in Beltsville, T-14(chloro-3M), WT-6(chloro-3M), Th-1(procy-1M), Th-1(procy-2M), Th-1(vinclo-1M), Th-1(vinclo-2M), Th-1(ipro-2M), and Th-1(ipro-3M). The designations in parentheses indicate the fungicides to which tolerance was developed and the concentration to which the strain was tolerant (M = 1 mg a.i./ml). Some of these isolates lost their tolerance after repeated transfers on fungicide-free medium. For instance, the variants WT-6(chloro-3M-1), WT-6(chloro-3M-2), and WT-6(chloro-3M-3) lost their tolerance to chlorothalonil after five transfers.

The continuous exposure of wild strains of *T. harzianum* to fungicides resulted in some morphological changes in colony characters. For instance, one of the iprodione-tolerant isolates of

Th-1 changed from deep green to light green. A chlorothalonil-tolerant isolate of WT-6 became almost tan, whereas all others retained the white color characteristic of strain WT-6. All the chlorothalonil-tolerant isolates of strain WT-6, however, differed from WT-6 in the pattern of sporulation. Some became granular in appearance; others developed concentric rings or sporulated sporadically on the agar.

Radial growth. Radial growth (in millimeters) of strain Th-1 was less than that of isolates Th-1(ipro-2M-1), Th-1(ipro-2M-2), and Th-1(ipro-3M) on V-8 juice agar containing 2,000 μg a.i./ml of iprodione (Table 1). On the medium containing 5,000 μg/ml, only the latter two iprodione-tolerant isolates had greater radial growth than Th-1. The three iprodione-tolerant isolates grew more slowly on the iprodione medium than on the fungicide-free V-8 juice agar. Although the three isolates were obtained by prolonged exposure to iprodione, they also exhibited tolerance to vinclozolin.

TABLE 1. Radial growth (mm)[a] of a wild strain (Th-1) of *Trichoderma harzianum* and of three isolates tolerant to iprodione after 4 days on V-8 juice agar containing varied concentrations of iprodione or vinclozolin

Fungicide	Concentration (μg/ml)	Strain or isolate			
		Th-1	Th-1 (ipro-2M-1)	Th-1 (ipro-2M-2)	Th-1 (ipro-3M)
Iprodione	0	40 a	40 a	40 a	40 a
	2,000	2 c	15 b	18 b	19 b
	5,000	1 c	7 c	12 b	12 b
Vinclozolin	0	40 a	40 a	40 a	40 a
	2,000	5 c	11 b	14 b	17 b
	5,000	4 c	7 c	12 b	10 b

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

TABLE 2. Effect of two concentrations of chlorothalonil on inhibition of conidia germination of *Trichoderma harzianum* strain WT-6 and of six isolates of WT-6 in which tolerance to chlorothalonil was developed initially

Strain or isolate	Percent inhibition of conidial germination[a,b] on V-8 juice agar containing chlorothalonil (μg/ml)	
	1,000	3,000
WT-6	96 a	98 a
WT-6(chloro-3M-1)[c]	99 a	100 a
WT-6(chloro-3M-2)[c]	94 a	100 a
WT-6(chloro-3M-4)[c]	95 a	100 a
WT-6(chloro-3M-8)	48 b	63 ab
WT-6(chloro-3M-3)	20 c	44 b
WT-6(chloro-3M-11)	17 c	43 b

[a] Average percent germination based on readings at 24 and 36 hr at 25 C under continuous fluorescent light.

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

[c] No tolerance to chlorothalonil was found after five transfers on fungicide-free medium. The other three isolates maintained their tolerance despite five transfers.

TABLE 3. Effect of increasing concentrations of iprodione on germination inhibition of conidia of *Trichoderma harzianum* strains Th-1 (Beltsville isolate) and T (Egyptian isolate) and of six isolates that developed tolerance to iprodione

Strain or isolate	Percent inhibition of conidial germination[a,b] on V-8 juice agar containing iprodione (μg/ml)		
	2,000	5,000	25,000
Th-1	100 a	100 a	100 a
Th-1(ipro-2M-1)	26 c	100 a	100 a
Th-1(ipro-2M-2)	11 cd	100 a	100 a
Th-1(ipro-3M)	79 b	100 a	100 a
T	22 c	100 a	100 a
T(ipro-5M)	0 d	89 b	100 a
T(ipro-25M)	0 d	88 b	100 a
T(ipro-40M)	0 d	97 ab	100 a

[a] Average percent germination based on readings at 24 and 36 hr at 25 C under continuous fluorescent light.

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

Conidial germination. Conidia of strain WT-6 and of the six chlorothalonil-tolerant isolates germinated 100% on fungicide-free V-8 juice agar. At concentrations of 1,000 and 3,000 μg a.i./ml of V-8 juice agar, chlorothalonil prevented the germination of 94–100% of conidia of strain WT-6 and of three isolates originally developing tolerance to the fungicide (Table 2). In contrast, chlorothalonil at 1,000 μg a.i./ml prevented only about 17–48% of conidia of isolates WT-6(chloro-3M-11), WT-6(chloro-3M-3), and WT-6(chloro-3M-8) from germinating; and at 3,000 μg/ml, the fungicide prevented 43 and 44% of conidia of two isolates from germinating.

A second experiment compared germination of conidia of iprodione-tolerant isolates developed in Egypt with those obtained at Beltsville (Table 3). Conidia of Th-1 exposed to iprodione at 2,000 μg a.i./ml did not germinate, and only 22% germination of conidia of isolate T occurred. Germination of conidia of the iprodione-tolerant isolates from both strains was inhibited less than that of conidia of wild strains on the medium containing 2,000 μg a.i./ml of iprodione. With the exception of conidia of T(ipro-5M) and T(ipro-25M), germination of conidia of all strains and isolates was inhibited 100% by 5,000 and 25,000 μg a.i./ml of iprodione.

In a third experiment, exposure of conidia of the wild strain T-14 to 0.5 and 1 mg/ml of chlorothalonil in water at 5 C for 1–4 wk prevented about 80% of the conidia from germinating when they were transferred to the chlorothalonil-free V-8 juice agar for 36 hr (Fig. 1). In contrast, exposure of conidia of isolate T-14(chloro-3M) to the same two chlorothalonil suspensions prevented only about 20 and 10% of the conidia from germinating after 1 and 4 wk, respectively. Conidia of WT-6 were less sensitive to chlorothalonil than were those of T-14. No appreciable differences were found between germination of conidia of WT-6 and WT-6(chloro-3M) after exposures of 1 and 4 wk to both concentrations of the fungicide.

Fungitoxic metabolite production. The wild Egyptian strain T produced a toxic metabolite that inhibited 52% of the radial growth and 28% of the growth in liquid medium of the *S. cepivorum* mycelium (Table 4). Isolate T(ipro-5M) produced more toxin against radial growth than strain T but toxin equal to that

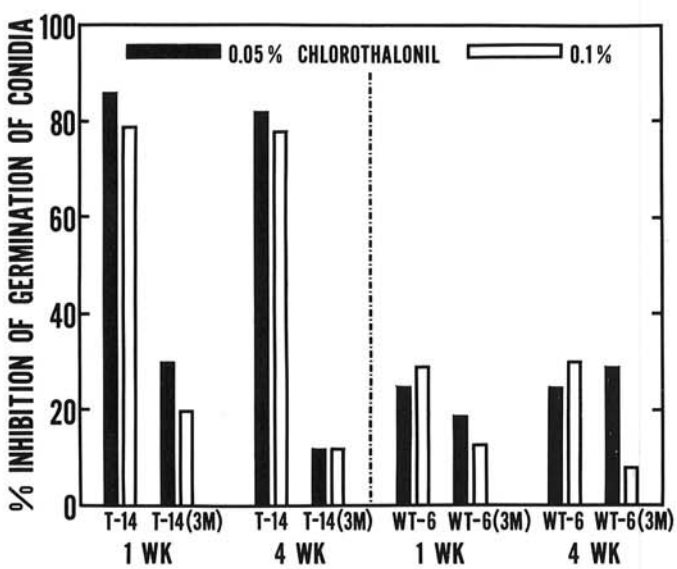

Fig. 1. Effect of exposure of conidia of *Trichoderma harzianum* strains T-14 and WT-6 and of isolates T-14(chloro-3M) and WT-6(chloro-3M) to chlorothalonil suspensions (0.5 and 1 mg active ingredient per milliliter) at 5 C for 4 wk on their subsequent germinability on chlorothalonil-free V-8 juice agar.

TABLE 4. The effect of fungitoxic metabolite produced in culture by *Trichoderma harzianum* strain T and by two iprodione-tolerant isolates on growth of *Sclerotium cepivorum*

Strain or isolate	Percent growth inhibition[a]	
	Radial growth[b]	Dry weight[c]
T	52 a	28 a
T(ipro-5M)	62 b	24 a
T(ipro-25M)	65 b	57 b

[a] In each column, values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.
[b] On potato-dextrose agar in petri plates.
[c] In potato-dextrose broth in Erlenmyer flasks.

produced by strain T when dry weight was used for the assay. The second iprodione-tolerant isolate, T(ipro-25M), produced more toxin than strain T irrespective of the kind of assay used to detect it.

White rot suppression in a growth chamber. Incidence of white rot in onion transplanted in soil enriched with the wild strain Th-1 or with the isolate Th-1(procy-1M) was not significantly lower than that in the control soil (Table 5). Infestation of the *S. cepivorum* soil with the isolate Th-1(procy-2M) or with the isolate Th-1(vinclo-2M) reduced the incidence of white rot appreciably irrespective of the kind of antagonist used, and the reduction differed significantly from that obtained with the wild strain Th-1. The fungicides procymidone and vinclozolin almost eliminated the disease at 2.5 mg a.i./kg of soil.

White rot suppression in the field. A combination of iprodione and the iprodione-tolerant isolate T(ipro-25M) gave significantly higher control of white rot of onion transplanted at Shandawil, Egypt, in a field naturally infested with *S. cepivorum* than did iprodione alone at 3 kg a.i./ha (Table 6). The wild strain T and the isolate T(ipro-25M) also suppressed white rot and were equally effective in doing so.

DISCUSSION

From the evidence presented here, the development of isolates of *T. harzianum* resistant or tolerant to fungicides by prolonged and repeated exposure to fungicides appears feasible. Tolerance in most cases may be due to nongenetic adaptation (training) (7) and may be acquired by certain plant-pathogenic fungi. Three of six isolates from strain WT-6 tolerant to chlorothalonil (up to 3,000 μg a.i./ml) lost their ability to tolerate chlorothalonil when spore germination was considered as the criterion of tolerance (Table 2). The concept of induction of permanent changes by fungicides is in agreement with Hastie's research (10), which suggested that the "genetic effects" of some fungicides may very well include gene mutation, chromosome breakages, and mitotic recombinations. From the practical standpoint, if fungicide-tolerant isolates of biocontrol agents are desired for integrated pest management programs involving fungicides, and tolerance is not a stable characteristic, tolerance can be "preserved" by maintaining cultures on media containing the appropriate fungicide.

The effects of fungicides on growth in liquid and solid media, on fungitoxic metabolite production, and on germination of conidia of wild strains and of fungicide-adapted isolates showed that changes in these characters actually occurred, that some fungicide-tolerant isolates differed substantially from their respective wild strains, and that conidia or mycelia of isolates tolerant to high concentrations of fungicides can be exposed to fungicides without appreciable loss of viability. Similar results were obtained with isolates of *T. harzianum* that were induced by ultraviolet light to become resistant to benomyl (13). Conidia and mycelia of such tolerant isolates could be used in conjunction with fungicides for seed treatments, foliar sprays, or soil applications. Experimental use of fungicides added to the seed with a solvent before addition of *T. harzianum* was suggested recently (12). For future integrated pest management systems, development of strains compatible with fungicides would be of high priority.

TABLE 5. White rot of onion caused by *Sclerotium cepivorum* as affected by soil treatment with *Trichoderma harzianum* strain Th-1 and four fungicide-tolerant isolates and by two fungicides

Treatment	Infected seedlings[a,b] (%) with indicated kind of antagonist preparation	
	Conidia[c]	Dry preparation[d]
None (control)	54 a	56 a
T. harzianum Th-1	52 ab	58 ab
Th-1(procy-1M)[e]	46 abc	50 abc
Th-1(vinclo-1M)[e]	38 bcd	34 bcd
Th-1(vinclo-2M)[e]	28 cd	26 cd
Th-1(procy-2M)[e]	18 de	14 de
Procymidone[f]	4 f	4 f
Vinclozolin[f]	4 f	4 f
Control (sterile dry preparation)	8 ef	8 ef
Control (uninfested)	4 f	4 f

[a] Disease determined 8 wk after transplanting onion seedlings in soil infested with *S. cepivorum*.
[b] Values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.
[c] Conidia from V-8 juice agar plates added to soil 1 wk before transplanting onions at 4.4×10^5 conidia per gram of soil.
[d] Antagonists were grown on sand-cornmeal-bran-modified gliotoxin fermentation medium for 3 wk at 25 C.
[e] New isolates of *T. harzianum* strain Th-1 developed by repeated culturing of Th-1 on increasing concentrations of procymidone or vinclozolin.
[f] Added at 2.5 mg a.i./kg of air-dry soil.

TABLE 6. White rot of onion caused by *Sclerotium cepivorum* in a naturally infested field in Egypt as affected by soil treatment with the fungicide iprodione and with *Triochoderma harzianum* strain T and an iprodione-tolerant isolate

Treatment	Concentration (kg/ha)	Infected plants[a,b] (%)
Medium only (control)	240	86 a
None (control)		74 b
Iprodione	3	51 c
T. harazianum strain T	240	18 d
T(ipro-25M)	240	13 de
Iprodione + T(ipro-25M)	3 + 240	6 e

[a] Disease determined 4 mo after transplanting; average of two experiments.
[b] Values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

The desirable effects of combining small amounts of chemicals with a biocontrol agent are shown in the field experiment at Shandawil (Table 6). The combination of iprodione with T(ipro-25M), an iprodione-tolerant isolate, gave white rot control significantly higher than that obtained by the fungicide alone. This experiment, however, may involve a more intricate system than the mere use of two components for disease control. The growth medium for the antagonist also included onion extract. If constituents of the onion extract were not metabolized by the antagonist, the extract may have stimulated the sclerotia of the pathogen to germinate and thus become vulnerable to the mycoparasitic (1,2) or antibiotic (3) action of *T. harzianum*. Onion extracts and onion root exudates are known to stimulate germination of sclerotia of *S. cepivorum* (4). All these, of course, are assumptions. Further research is needed to unravel the mechanism of the combined action of fungicide and *T. harzianum*, including the use of the stimulators of sclerotial germination.

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