Comparative Control of *Sclerotium rolfsii* on Golf Greens in Northern California with Fungicides, Inorganic Salts, and *Trichoderma* spp.

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ABSTRACT

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Sclerotium rolfsii blight on bentgrass-annual bluegrass golf greens in Sacramento, CA, was controlled with 2,6-dichloro-4-nitroaniline with cycloheximide, carboxin, and PCNB with fertilizer applied at 14-day intervals over a 4-mo period starting 1 May 1981. Captan and furmecyclox (OAC 3890) also significantly reduced disease severity. Carboxin applied at reduced rates in combination with reduced amounts of either captan or ammonium bicarbonate provided better disease control than carboxin or captan applied alone at higher rates. Applications of calcium nitrate or hydrated lime did not give satisfactory control of disease. Similarly, applications in 1979 and 1980 of *Trichoderma* inoculum grown on diatomaceous earth granules impregnated with a 10% molasses solution did not reduce disease severity in either year, although there was a significant increase in the level of *Trichoderma* in treated plots in comparison with control plots.

Additional key words: bicarbonate, biocontrol, chemical control, southern blight

Previous reports indicate that disease caused by *Sclerotium rolfsii* on various crops can be controlled with the fungicides PCNB (1,3,7), carboxin (3,13), triphenyltin hydroxide (3), and chloroneb (13). Disease was also reduced following applications of anhydrous ammonia (9,10), ammonium sulfate $(NH_4)_2SO_4$ (10), ammonium nitrate (12), and calcium nitrate $[Ca(NO_3)_2 \cdot H_2O]$ (10,17). Non-chemical methods that have significantly reduced disease are few, including deep ploughing to turn under the organic matter and possibly sclerotia (6) and applications of biological control agents, such as species of *Trichoderma* (2,5,19).

We reported previously (15) that *S. rolfsii* blight on bentgrass-annual bluegrass golf greens in northern California was controlled with bimonthly applications of fungicides such as 2,6-dichloro-4-nitroaniline (DCNA) with cycloheximide, captan, carboxin, and PCNB with fertilizer. Inorganic salts such as $(NH_4)_2SO_4$ and ammonium bicarbonate (NH_4HCO_3) also reduced disease severity on turf (15). We report here results of continued efforts to control *S. rolfsii* on golf greens with previously used fungicides (15) applied at reduced rates separately or in combination; with inorganic salts, such as $Ca(NO_3)_2 \cdot H_2O$ and hydrated lime $[Ca(OH)_2]$; and with *Trichoderma* spp. Preliminary results from this study have been published (14).

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MATERIALS AND METHODS

Field application of fungicides and inorganic salts. The location of the plots and the experimental design were the same as reported previously (15). However, the plots were 7.6 m instead of 8.4 m long; in addition, for four of the chemicals, two replicates were used instead of four because of insufficient space available. Six fungicides and three inorganic salts were applied either separately or in various combinations at 14-day intervals starting on 1 May 1981. Eight applications of each material or combination were made during the season using a hand-operated spray boom (15). The materials and rates used are indicated in Table 1. Disease severity was assessed on 27 July and 24 August 1981 using the same methods as in a previous study (15).

Attempts to control disease with *Trichoderma* spp. *Isolation of* Trichoderma. *Trichoderma* isolates were obtained from field soil collected in July 1978 from the rhizosphere of sugar beet plants in two fields in Sutter County, CA. The following methods were used to isolate *Trichoderma* from soil: (a) 0.1–0.2 g of soil was mixed with 20 ml of either cooled, autoclaved peptone-dextrose rose bengal agar (PDRB) (11) or potato-dextrose agar (PDA) containing streptomycin sulfate at 35 μg/ml and poured into plastic petri dishes (100 × 25 mm)

Table 1. Efficacy of six fungicides and two inorganic salts for control of *Sclerotium rolfsii* blight on golf greens in northern California

Chemical and rate (kg a.i./93 m²)[v]	Total number of diseased spots[w]		Average diameter of spots (cm)[x]		Total diseased area (%)	
	27 July	24 August	27 July	24 August	27 July	24 August
DCNA-cycloheximide, 0.14–0.04	0 d[y]	0 d	0 e	0 f	0 d	0 c
Captan, 0.68	2 d	6 c	29.7 b	22.3 c	1.1 d	1.6 c
Mancozeb, 0.88	5 c	14 b	42.6 a	31.5 b	3.5 c	8.7 b
PCNB, 0.17, with fertilizer	0 d	0 d	0 e	0 f	0 d	0 c
Carboxin, 0.15	1 d	1 d	7.6 d	5.4 e	0.07 d	0.05 c
Carboxin, 0.1 + NH_4HCO_3, 0.085[z]	0 d	0 d	0 e	0 f	0 d	0 c
Carboxin, 0.1 + captan, 0.34	0 d	0 d	0 e	0 f	0 d	0 c
Furmecyclox (OAC 3890), 0.057	1 d	3 d	22.8 c	16.9 d	0.5 d	0.6 c
$Ca(NO_3)_2 \cdot H_2$, 0.14[z]	11 b	14 b	49.5 a	46.1 a	11.9 b	12.8 a
$Ca(OH)_2$, 1.8[z]	16 a	18 a	44.4 a	44.8 a	13.1 b	14.3 a
Control, 0	17 a	15 b	47.2 a	43.1 a	16.8 a	15.4 a

[v] Chemicals were applied every 14 days starting 1 May 1981; eight applications were made.

[w] Data are the means of four replicates, with the exception of plots treated with DCNA-cycloheximide, mancozeb, carboxin + captan, and furmecyclox, in which two replicates were used.

[x] Diameters are of entire spots including the apparently healthy green centers and are the means of all spots present in the plot.

[y] Means in a column followed by the same letter are not significantly different ($P = 0.01$) according to Duncan's multiple range test.

[z] Amounts of ammonium bicarbonate (NH_4HCO_3), calcium nitrate $[Ca(NO_3)_2 \cdot H_2]$, and lime $[Ca(OH)_2]$ are kilograms of salt used per 93 m².

(modification of the soil-plate method of Warcup [18]); (b) 0.4–0.6 g of soil was added to 9.0 ml of sterile distilled water, and 1.0 ml of this suspension (or more dilute suspensions) was mixed with 20 ml of PDRB before being poured into petri dishes; (c) 0.2 g of soil was placed directly on PDRB and streaked across the plate with a transfer loop; and (d) sclerotia were sieved out of soil using the method of Leach (8) and plated onto either PDA containing lactic acid at 0.1% and streptomycin sulfate at 35 μg/ml or onto 1% Bacto water agar. All dishes were incubated in the dark at 25 ± 2 C for 5–7 days.

Screening of isolates of Trichoderma *in vitro*. Fifty-one isolates of *Trichoderma* from soil in Sutter County and one (1970-3A) from Georgia (courtesy D. K. Bell, University of Georgia, Tifton 31794) were screened for ability to inhibit mycelial growth and sclerotial production of *S. rolfsii* on PDA using the paired-culture technique, in which mycelial plugs from *Trichoderma* and *S. rolfsii* cultures were placed about 80 mm apart at the periphery of a petri dish. Inhibition of growth and sclerotial production of *S. rolfsii* by each *Trichoderma* isolate was rated after 6 days of incubation at 25 ± 2 C.

Three isolates of *Trichoderma* were selected from the above tests, and tolerance to each of the following fungicides was induced separately in each isolate by growing and subculturing the fungus on increasing concentrations of the fungicide: chlorothalonil (Bravo 75 W), PCNB (Terraclor 75 W), Terrazole (5 G), thiram (Tersan 75 W), and cycloheximide (Acti-dione TGF 2.1 W)-thiram (Tersan 75 W) mixture. These fungicides were selected because they are used routinely on golf greens for control of other fungal diseases. Five replicate plates were used for each fungicide concentration. Initially, a single, 1-cm-diameter mycelial plug was taken from a 5-day-old PDA culture of the *Trichoderma* isolate and transferred to PDA containing low levels of the fungicide; the initial fungicide concentrations tested ranged from 5 to 20 μg/ml, depending on the fungicide. Following incubation for 2–3 wk in the laboratory, 1-cm-diameter mycelial plugs were removed from that part of the colony exhibiting vigorous growth and transferred to PDA plates with higher concentrations of the fungicide. This method of subculturing was continued for four or five transfers. The final fungicide tolerance level achieved ranged from 50 to 200 μg/ml, depending on the fungicide.

Production of inoculum and field application. Two *Trichoderma* isolates (1970-3A and F-1) most antagonistic to *S. rolfsii* in vitro were grown on autoclaved diatomaceous earth (DE) granules (Celatom diatomite, Eagle-Picher Industries Inc., Cincinnati, OH 45201) impregnated with a 10% molasses solution containing 3 g each of monobasic potassium phosphate and potassium nitrate per liter (2). These two isolates were previously identified as *T. harzianum* (19) (courtesy D. K. Bell, University of Georgia; ATCC 24274) and *T. viride* (isolated from a sugar beet field in California), respectively, according to Rifai (16). The requisite amount of DE was measured out into autoclavable plastic bags (20 × 25 cm) according to the calculated amount (kg) required for each plot (30 m^2); approximately 110 ml of molasses solution was added for each 150-ml quantity (about 50 g) of DE. The bags were sealed and autoclaved for 30 min at 121 C and 1.05 kg/cm^2 pressure.

The medium was inoculated twice at 14-day intervals with eight to ten 1-cm-diameter mycelial plugs taken from 5-day-old cultures. Both *Trichoderma* isolates, tolerant to the highest concentration of each fungicide, were added to each bag. The bags were incubated at 25 ± 2 C under fluorescent lights for 6 wk and occasionally shaken by hand. Prior to field application, autoclaved and uninoculated, molasses-impregnated DE was added to the medium at a rate of one part uninoculated to three parts inoculated. An uninoculated DE treatment was also included as a control.

For field application, the contents of each bag were mixed separately in 3.8 L of water to form a slurry and sprayed onto each plot in 26.5 L of water using a spray boom (0.56 kg/cm^2 [8 psi] pressure). Four passes were made over each plot. The plots were 1.8 m wide by 16.7 m long and arranged in a completely randomized design, with four replicates per treatment. The DE was applied at rates of 140 and 110 kg/ha; the controls included untreated areas in addition to plots treated with DE without *Trichoderma*. Two applications were made in 1979 on 6 June and 17 July; an additional application was made on 11 May 1980. The density of *Trichoderma* spores applied at the 140 kg/ha rate was approximately 2 × 10^5 spores per milliliter of water. Following each application, the DE on each plot was washed into the soil with about 75 L of water. Disease ratings were taken on 15 August 1979 and on 9 June, 7 July, and 4 August 1980 using the methods described previously (15). At the first application date (6 June 1979), there was no disease visible on any of the experimental plots.

Monitoring Trichoderma *levels in soil.* To determine levels of *Trichoderma* in soil in treated and untreated plots, soil cores (1.3 cm wide by 2.5 cm deep) were taken at weekly intervals starting on 30 May 1979 and continuing until 7 August 1980. Two cores were taken at random from each of two untreated plots and plots receiving *Trichoderma* inoculum at 140 kg/ha. The soil from the cores from each plot was mixed, and 0.5 g was streaked across PDA containing rose bengal at 54 μg/ml and streptomycin sulfate at 35 μg/ml (both added after autoclaving). Four plates were used for each plot sampled. The average number of *Trichoderma* colonies was recorded after 4 days of incubation at 25 ± 2 C.

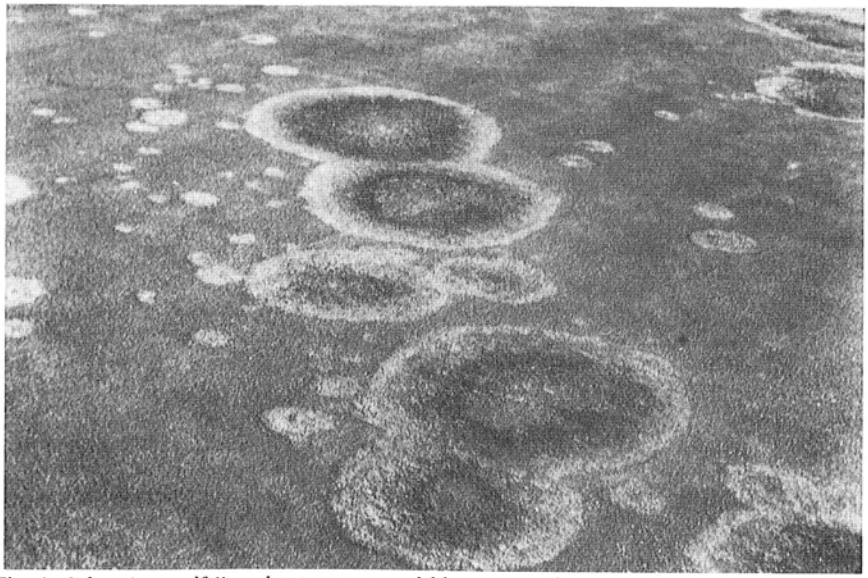

Fig. 1. *Sclerotium rolfsii* on bentgrass-annual bluegrass golf green, showing circular areas of diseased grass on control plots.

RESULTS

Field application of fungicides and inorganic salts. Disease severity ratings at the two assessment dates are in Table 1. Approximately 17% of the area of control plots was diseased by 27 July 1981 (Fig. 1). All materials tested reduced the total number of diseased spots and the percentage of area diseased at this date. However, the level of disease in plots receiving $Ca(NO_3)_2 \cdot H_2O$ and $Ca(OH)_2$ was fairly high. With the exception of plots treated with $Ca(NO_3)_2 \cdot H_2O$, the average diameter of the spots was also

reduced in all treated plots. There was no disease in plots receiving DCNA-cycloheximide, PCNB with fertilizer, carboxin + NH_4HCO_3, or carboxin + captan (Table 1). The disease severity indexes for 24 August were not much greater than those recorded on 27 July. The average diameter of the spots in general was reduced because of an increase in the number of new and smaller spots. In addition, spots on control plots were filling in with healthy bentgrass, and this reduced the total percentage of area diseased (Table 1). The most effective chemicals were carboxin applied in combination with either NH_4HCO_3 or captan. Although disease was also controlled with DCNA-cycloheximide and PCNB, some phytotoxicity was observed on plots treated with these chemicals.

Attempts to control disease with *Trichoderma* spp. *Isolation of* Trichoderma. The most numerous *Trichoderma* colonies were obtained using the methods in which soil was either streaked directly onto PDRB or following dilution and incorporation into cooled agar. Numerous colonies of *Trichoderma* from soil taken from both fields in Sutter County were observed on PDRB after 4 days of incubation at 25 C.

Screening of isolates of Trichoderma *in vitro.* Of the 52 *Trichoderma* isolates tested in paired-culture with *S. rolfsii*, 19 grew less vigorously than the pathogen and were overgrown, 14 were equal in growth rate, and 19 were inhibitory. The most effective isolates produced marked zones of inhibition against *S. rolfsii*, and sclerotia were usually colonized.

Tolerance to five fungicides routinely used on the golf greens for control of other fungal pathogens was induced in three isolates of *Trichoderma* to levels approximately tenfold that of the initial tolerances. The isolates were found to be most sensitive to chlorothalonil and least sensitive to Terrazole.

Production of inoculum and field application. The method of producing inoculum in plastic bags on molasses-impregnated diatomaceous earth granules was quite satisfactory; abundant growth and sporulation of the *Trichoderma* spp. were observed after 6 wk of incubation. The material was easily applied in water if large enough nozzle openings were used to prevent clogging.

Monitoring Trichoderma *levels in soil.* Following the first application on 6 June 1979, the level of *Trichoderma* spp. in soil was not significantly higher than that in control plots (Fig. 2). However, after a second application 4 wk later, a significantly higher population level was observed in treated plots. This difference was apparent over a 12-mo period. A third application of *Trichoderma* on 11 May 1980 did not increase the population level in soil further (Fig. 2). However, application of *Trichoderma* inoculum at either 110 or 140 kg/ha did not reduce the level of disease in 1979 or 1980 from that in control plots, although a slight reduction in percentage of area diseased was apparent in treated plots in 1980 (Table 2). Total disease was greater in 1980 than in 1979.

DISCUSSION

The results from chemical control trials in the present study and those obtained in 1980 (15) show that PCNB and carboxin controlled disease in both years. However, in 1981 effective control was achieved using lower amounts of these fungicides. DCNA-cycloheximide and mancozeb provided better control in 1981 than in 1980 (15), whereas captan was slightly less effective. The percentage of diseased area in control plots was slightly greater in 1980 than in 1981, possibly because onset of disease in 1981 was later,

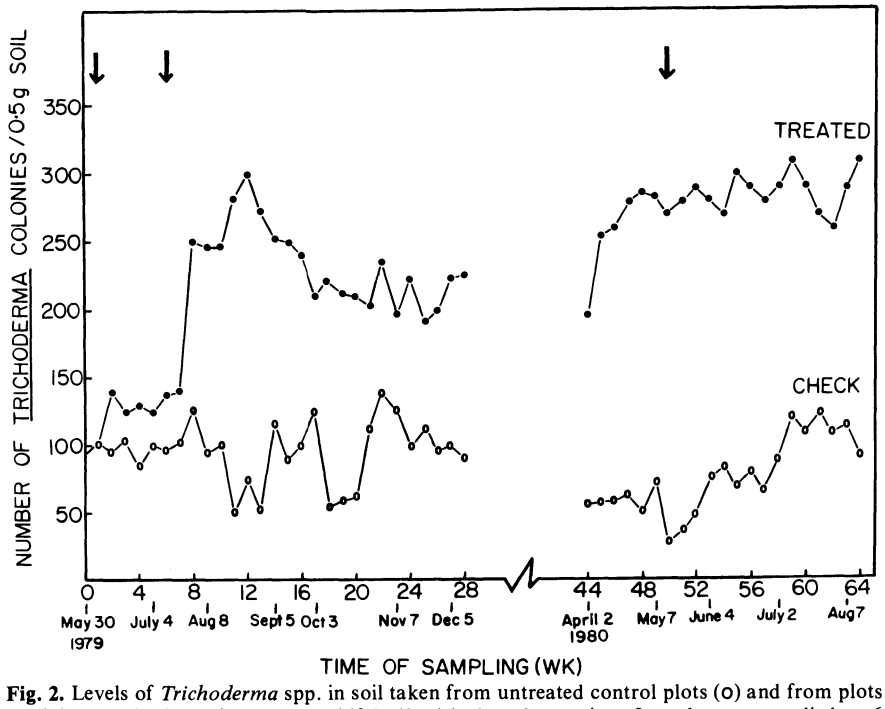

Fig. 2. Levels of *Trichoderma* spp. in soil taken from untreated control plots (o) and from plots receiving *Trichoderma* inoculum at 140 kg/ha (•) plotted over time. Inoculum was applied on 6 June and 17 July 1979 and on 11 May 1980 (arrows indicate times of application). Soil samples were taken at weekly intervals, starting on 30 May 1979, until 7 August 1980; 0.5 g of soil was streaked across potato-dextrose agar containing rose bengal at 54 μg/ml and streptomycin sulfate at 35 μg/ml. The number of colonies was recorded after 4 days of incubation at 27 C.

Table 2. Efficacy of *Trichoderma* spp. for control of *Sclerotium rolfsii* on golf greens in northern California

		Treatment[x]		
	Untreated control	Diatomaceous earth control	*Trichoderma* at 140 kg/ha	*Trichoderma* at 110 kg/ha
Total number of diseased spots[y]				
15 August 1979	21	19	20	19
9 June 1980	6	4	5	7
7 July 1980	21	19	19	20
4 August 1980	27	24	25	27
Average diameter of spots (cm)[z]				
15 August 1979	20.9	28.3	28.1	28.6
9 June 1980	6.1	6.9	5.8	5.0
7 July 1980	17.8	16.1	17.1	16.9
4 August 1980	30.4	30.2	28.0	28.8
Total diseased area (%)				
15 August 1979	16.1	14.6	15.8	15.2
9 June 1980	1.3	1.1	0.8	1.0
7 July 1980	11.8	10.7	9.8	10.3
4 August 1980	18.7	16.6	15.9	16.3

[x] Materials were applied on 6 June and 17 July 1979 and on 11 May 1980.
[y] Disease severity ratings were taken at four different times. Data are the means of four replicates.
[z] Diameters are of entire spots including the apparently healthy green centers and are the means of all spots present in the plot.

with initial symptoms appearing around 12 June.

In comparing disease control achieved with chemicals applied in combination at a reduced rate with either chemical applied alone at a higher rate, it was apparent that carboxin applied in combination with captan in 1981 at one-half the rate used singly in 1980 provided better control than either chemical applied separately. Also, carboxin applied together with low levels of NH_4HCO_3 gave better disease control than carboxin alone at higher rates.

Applications of *Trichoderma* inoculum produced on molasses-impregnated diatomaceous earth granules did not reduce disease, although the population levels of *Trichoderma* spp. in treated plots were significantly higher. These levels (Fig. 2) represent total levels of *Trichoderma* present in soil; we could not monitor the populations of the two introduced *Trichoderma* species because of the absence of specific markers. Levels of naturally occurring or resident *Trichoderma* spp. in control plots were fairly high; however, considerable variations were noted in the populations over time, possibly because of temperature and moisture fluctuations and fungicide applications. Because the introduced *Trichoderma* species were tolerant of various fungicides commonly used for control of other fungal diseases, this could, in part, explain why we were successful in establishing higher populations in soil. However, we did not test the feasibility of applying other fungicide-tolerant *Trichoderma* isolates together with reduced rates of fungicides effective against *S. rolfsii*, such as carboxin or PCNB.

The first application of *Trichoderma* did not alter the initial population level, possibly because the existing soil microflora exerted a buffering influence. The third application also had no effect on the population levels; this may, however, have resulted from problems in distinguishing individual colonies on PDRB at high population densities. Perhaps the medium recently developed by Elad et al (4) may be more useful for this purpose; their results suggest, however, that the medium is poor for recovery of indigenous *Trichoderma* spp. in natural soils unless population levels are high.

The amount of disease in plots receiving *Trichoderma* inoculum was almost the same as that in control plots, although higher population levels of *Trichoderma* spp. were present in the upper 2.5 cm of soil in treated plots. This suggests either that growth of *S. rolfsii* in soil was not inhibited by the *Trichoderma* or, more likely, that colonization of organic matter in the thatch above the soil surface by *S. rolfsii* occurred; therefore, disease could have been initiated from residual or introduced inoculum in the thatch that was not subjected to antagonism by the *Trichoderma* in the underlying soil. Other workers have reported significant reduction in disease caused by *S. rolfsii* on tomatoes (19), peanuts (2), and beans (5) following applications of *T. harzianum* inoculum produced on moistened annual ryegrass/soil mixture, molasses-impregnated diatomaceous earth granules, and moistened wheat bran/sawdust mixture, respectively. In all instances, the biocontrol inoculum was applied to the soil surface, and control of disease probably resulted from a reduction of the primary inoculum in the soil. The quite different ecologic conditions in turfgrass may have contributed in part to the lack of disease control observed in this study following applications of *Trichoderma* spp. In none of the previous studies, however, were attempts made to monitor the level of introduced *Trichoderma* or of survival of *S. rolfsii* over time. Thus, the mechanism of biocontrol is unknown.

At present, bimonthly applications (starting in early May) of captan, PCNB, and DCNA-cycloheximide used in rotation are recommended for control of *S. rolfsii* blight on golf greens in northern California. These materials may also be used in combination. All three materials are registered for use on turf, whereas carboxin and furmecyclox, which also provided good disease control in experimental plots, are not. High rates of $(NH_4)_2SO_4$ or NH_4HCO_3 cannot be used exclusively to control *S. rolfsii* because the additional nitrogen stimulates excessive growth of the turf, resulting in increased maintenance costs and reduction in the quality of the putting surface.

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