Chemical Control of *Sclerotium rolfsii* on Golf Greens in Northern California

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ABSTRACT

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Eruptive and hyphal germination of dried sclerotia of two isolates of *Sclerotium rolfsii* on 1% Noble and Bacto water agar was totally inhibited by carboxin, cycloheximide, oxycarboxin, and experimental fungicide CGA-64251 in the agar at rates of 100 and 200 μg a.i./ml. None of the compounds, however, was fungicidal. Cadmium succinate, captan, chlorothalonil, thiophanate-methyl with zinc ion and manganese ethylenebisdithiocarbamate, mancozeb, pentachloronitrobenzene alone or with fertilizer, and furmecyclox in agar reduced germination of sclerotia by 80–95%. Benomyl, 2,6-dichloro-4-nitroaniline, thiram, triphenyltin hydroxide, vinclozolin, and experimental fungicide MF-647 had no significant effect on sclerotial germination. Applications of 2,6-dichloro-4-nitroaniline and cycloheximide in combination, captan, carboxin, pentachloronitrobenzene with fertilizer, ammonium bicarbonate, and ammonium sulfate controlled *S. rolfsii* under natural conditions when applied at 14-day intervals over a 3.5-mo period. Fungicides and nitrogenous compounds such as ammonium bicarbonate used separately or in combination may control *S. rolfsii* on turf.

Additional key words: fungicide screening, southern blight

The soilborne fungus *Sclerotium rolfsii* Sacc. was first reported on bentgrass (*Agrostis palustris* Huds.) golf greens in 1975 in North Carolina (12) and in southern California in 1977 (16). Although the disease on turf is relatively new and uncommon, the fungus causes damage to a wide variety of crops, and extensive efforts have been directed at control of this pathogen (3). Fungicides reported to prevent mycelial growth of *S. rolfsii* in vitro include cycloheximide (17), carboxin (4,15), triphenyltin hydroxide (TPTH) (4), chloroneb (15), captan (3,9), thiram (9), and mancozeb (9). Some of these fungicides have also been reported to inhibit germination of sclerotia (1–4,7,17), but only carboxin was fungicidal when used at high concentrations (2). Disease severity in the field, however, has been significantly reduced on a variety of crops with pentachloronitrobenzene (PCNB) (3,5), carboxin (4,15), TPTH (4), and chloroneb (15). In addition to these fungicides, inorganic salts such as calcium nitrate (10,20), ammonium sulfate (10), and ammonium nitrate (13) have also provided partial control.

S. rolfsii was a major problem on golf greens in Sacramento, CA, when this study was initiated in 1978. By the summer of 1980, 12 golf courses in northern and southern California had reported the occurrence of *S. rolfsii* on some greens. The fungus also caused damage on fairways and roughs comprised of annual bluegrass (*Poa annua* L.), Manhattan ryegrass (*Lolium multiflorum* Lam.), and bermudagrass (*Cynodon dactylon* (L.) Pers.). On the Sacramento golf course where the field trials were conducted, *S. rolfsii* was detected on 16 of the 18 greens of the Del Paso Country Club during the summer of 1979. Symptoms of the disease have been adequately described by Lucas (12). There are no fungicides currently registered for the control of *S. rolfsii* on golf greens in California. Therefore, the objectives of this study were to screen fungicides for ability to inhibit germination of sclerotia of *S. rolfsii* in vitro and to test the most effective of these chemicals for control of the pathogen on golf greens under natural conditions. Preliminary results from this study have been published (19).

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MATERIALS AND METHODS

Screening of fungicides in vitro. Isolates 1126 and 2672 of *S. rolfsii* from bentgrass (*A. tenuis* Sibth.) and annual bluegrass golf greens in Sacramento were used in this study. Sclerotia used for in vitro screening of fungicides were obtained from 2-mo-old oat cultures and dried for 20 hr over calcium chloride in a desiccator to induce eruptive germination (18). This form of germination was characterized by plugs of mycelium erupting through the sclerotial rind, utilization of internal stored materials to leave an empty sclerotial rind, and production of secondary sclerotia; it was observed only on substrates devoid of nutrients (18).

Twenty-two fungicides were tested for their ability to inhibit both eruptive and hyphal germination (characterized by growth of individual hyphal strands from the surface of the sclerotium, and always observed on nutrient-rich substrates) of sclerotia at concentrations of 50, 100, and 200 μg a.i./ml (Table 1). The desired quantities of each fungicide at the three concentrations were each suspended in 50 ml of sterile distilled water, mixed, then added to 50 ml of cooled, autoclaved 2% Bacto or 2% Noble water agar (Difco Laboratories, Detroit, MI 48232) and poured into plastic petri dishes measuring 60 × 15 mm. Four replicate plates, each with 25 sclerotia, were used for each test.

Sclerotial germination was determined after 3 days of incubation in the dark at 27 C, and the experiment was repeated twice. Contamination with bacteria was not a problem using this method of screening fungicides. To determine whether fungicides were fungistatic or fungicidal, ungerminated sclerotia on 1% water agar plates containing the fungicides were removed, washed in sterile distilled water for 5 min, blotted dry, and plated onto potato-dextrose agar. Mycelial growth from the sclero-

tium after 3 days at 27 C was used as an indication of a fungistatic effect.

Field trials. The experimental plots were set up on a large practice green, on which the pathogen was found to be uniformly distributed in 1979. Plots were 1.7 m wide × 8.4 m long and arranged in a completely randomized design, with four replicates per treatment. Routine fertilization and management practices were maintained on the green throughout the experiment. Five fungicides selected from the in vitro screening tests and two inorganic salts screened previously (19) were applied at 14-day intervals starting on 5 May 1980.

Seven applications of each chemical were made during the season. Four of the chemicals—2,6-dichloro-4-nitroaniline (DCNA) and cycloheximide in combination, mancozeb, carboxin, and ammonium bicarbonate (NH_4HCO_3)—were applied in water using a hand-operated spray boom (pressure of 2.1 kg/cm^2, or 30 psi) that applied 7.5 L/14.3 m^2 in two passes over each plot. The other three chemicals—captan, PCNB with fertilizer, and ammonium sulfate (NH_4SO_4)—were broadcast as a dust or granular formulation. After each application, the chemical on each plot was washed into the soil with approximately 38 L of water. Fungicide rates (Table 2) were two to three times higher than those recommended for control of other fungal pathogens on turf because these fungicides at recommended label rates gave inadequate control of *S. rolfsii* in earlier trials.

Assessing disease severity. Disease ratings were taken for each plot on 9 June, 7 July, and 4 August 1980 after 3, 5, and 7 applications of the chemicals, respectively. Most diseased areas were almost circular, with a small inner green area of apparently healthy grass surrounded by a larger outer circular area of dead grass (Fig. 1A). Two measures were used to assess disease severity: total number of diseased spots of all sizes in each plot and percentage of diseased area in each plot. This was done by measuring the diameters of the inner green healthy portion and of the entire spot for all spots in each plot. By subtracting the area of healthy grass from the total area of the spot, the diseased area of each spot was obtained. Finally, by summation of the areas of diseased spots in each plot, the percentage of diseased area in each plot was calculated.

RESULTS

Screening of fungicides in vitro. Most of the 22 fungicides at 50 μg a.i./ml had no effect on sclerotial germination, and only a few inhibited germination at 100 μg a.i./ml. Thus, only the data from tests at 200 μg a.i./ml are shown in Figure 2. The effect of fungicides on both eruptive germination of sclerotia on 1% Noble water agar and on hyphal germination of sclerotia on 1% Bacto water agar was similar, and differences between the two isolates tested were not significant ($P = 0.05$, according to Duncan's multiple range test). Carboxin, cycloheximide, oxycarboxin, and CGA-64251 (Ciba-Geigy Corp., experimental compound) totally inhibited germination at 100 and 200 μg a.i./ml. Germination of sclerotia was reduced to 5–15% in the presence of 200 μg a.i./ml of cadmium succinate, captan, chlorothalonil, thiophanate-methyl with zinc ion and manganese ethylenebisdithiocarbamate (Duosan),

Table 1. Fungicides tested against germination of sclerotia of *Sclerotium rolfsii* in vitro[a]

Fungicide	Chemical composition
Anilazine (Dyrene 50 W)	2,4-Dichloro-6-(2-chloroanilino)-1,3,5-triazine
Benomyl (Benlate 50 W)	Methyl 1-(butylcarbamoyl)benzimidazol-2-ylcarbamate
Cadmium succinate (Cadminate 60 W)	Cadmium succinate 60%, inert 40%
Captan (Captan 50 W)	*N*-(Trichloromethane sulphenyl)-cyclohex-4-ene-1, 2-dicarboximide
Carboxin (Vitavax 75 W)	2,3-Dihydro-6-methyl-5-phenylcarbomoyl-1, 4-oxathiin
Chloroneb (Demosan 65 W)	1,4-Dichloro-2,5-dimethoxybenzene
Chlorothalonil (Bravo 75 W)	Tetrachloroisophthalonitrile
Cycloheximide (Acti-dione TGF 2.1 W)	3[2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-glutarimide
DCNA (Botran 75 W)	2,6-Dichloro-4-nitroaniline
Fenaminosulf (Dexon 35 W)	Sodium 4-dimethylamino benzenediazo sulphonate
Furmecyclox (OAG 3890 40 W)	*N*-Cyclohexyl-*N*-methoxy-2,5-dimethyl-furan-3-carboxylic acid-amide
Mancozeb (Dithane M-45 80 W)	Complex of zinc ion and maneb (manganese ethylenebisdithiocarbamate) containing 2.5% zinc and 20% manganese
Oxycarboxin (Plantvax 75 W)	2,3-Dihydro-6-methyl-5-phenylcarbomoyl-1, 4-oxathiin-4,4-dioxide
PCNB (Terraclor 75 W)	Pentachloronitrobenzene
PCNB with fertilizer (FF-II)	PCNB (15.4%) + N (14%), P_2O_5 (3%), K_3O (3%), and inert (64.6%)
Terrazole (Terrazole 5 G)	Ethoxytrichloromethylthiadiazole
Thiophanate-methyl with zinc ion and manganese ethylenebisdithiocarbamate (Duosan 75 W)	Dimethyl[(1,2-phenylene)bis(iminocarbonothioyl)] bis(carbamate) (15%), zinc ion (1.5%), manganese ethylenebisdithiocarbamate (58.5%), and inert (25%)
Thiram (Tersan 75 W)	Tetramethylthiuram disulphide
TPTH (Du-Ter 47.5 W)	Triphenyltin hydroxide
Vinclozolin (Ronilan 50 W)	3-(3,5-Dichlorophenyl)-5-methyl-5-vinyl-1, 3-oxazolidine-2,4-dione
Experimental fungicide (MF-647 50 W)	Chemical name not known
Experimental fungicide (CGA-64251 10 W)	(1-[[2-(2,4-Dichlorophenyl)-4-ethyl-1,3-dioxolan-2-yl]methyl]-1*H*-1,2,4,-triazole)

[a] Fungicides were tested against two isolates at concentrations of 50, 100, and 200 μg a.i./ml in 1% Bacto and Noble water agars. Four replicate plates, each with 25 sclerotia, were used for each test. Sclerotial germination was determined after 3 days of incubation in the dark at 27 C, and the experiment was repeated twice.

Table 2. Efficacy of five fungicides and two inorganic salts for control of *Sclerotium rolfsii* blight on golf greens in northern California

Chemical and rate (kg a.i./93 m^2)[v]	Total number of diseased spots[w]		Average diameter of spots (cm)[x]		Total diseased area (%)	
	7 July	4 August	7 July	4 August	7 July	4 August
DCNA-cycloheximide, 0.14–0.04	1 c[y]	3 c	14.9 d	24.1 d	0.2 c	0.9 c
Captan, 0.73	1 c	2 c	8.6 e	9.9 e	0.1 c	0.2 c
Mancozeb, 0.88	5 b	31 a	26.1 b	23.6 d	2.1 b	13.4 b
PCNB, 0.2; with fertilizer	0 c	0 c	0 f	0 f	0 c	0 c
Carboxin, 0.2	0 c	3 c	0 f	10.4 e	0 c	0.2 c
NH_4HCO_3, 0.18[z]	1 c	2 c	19.1 c	27.0 c	0.4 c	0.8 c
NH_4SO_4, 0.23[z]	2 c	2 c	26.9 b	41.5 a	0.7 c	2.3 c
Control, 0	11 a	20 b	29.7 a	37.0 b	6.2 a	17.9 a

[v] Chemicals were applied every 14 days starting on 5 May 1980; a total of seven applications were made.

[w] Disease severity ratings are for the last two assessment dates (7 July and 4 August 1980). Data are the means of four replicates.

[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 NH_4HCO_3 and NH_4SO_4 are kilograms of salt used per 93 m^2.

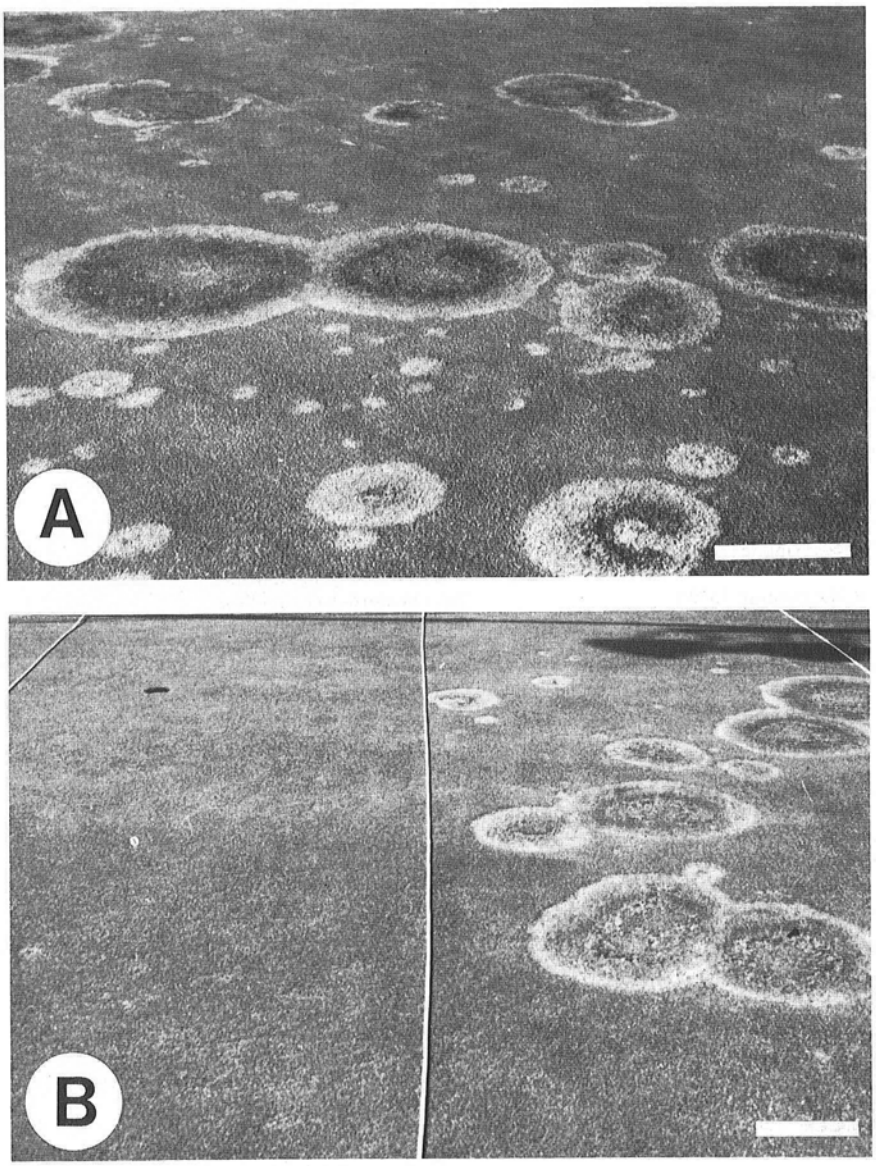

Fig. 1. *Sclerotium rolfsii* on bentgrass-annual bluegrass golf green. **(A)** Diseased spots on control plots showing circular areas of killed grass of varying sizes. **(B)** Comparison of plot treated with PCNB plus fertilizer **(left)** with control plot **(right)**. Both photographs were taken on 4 August 1980. Scale bar = 0.4 m.

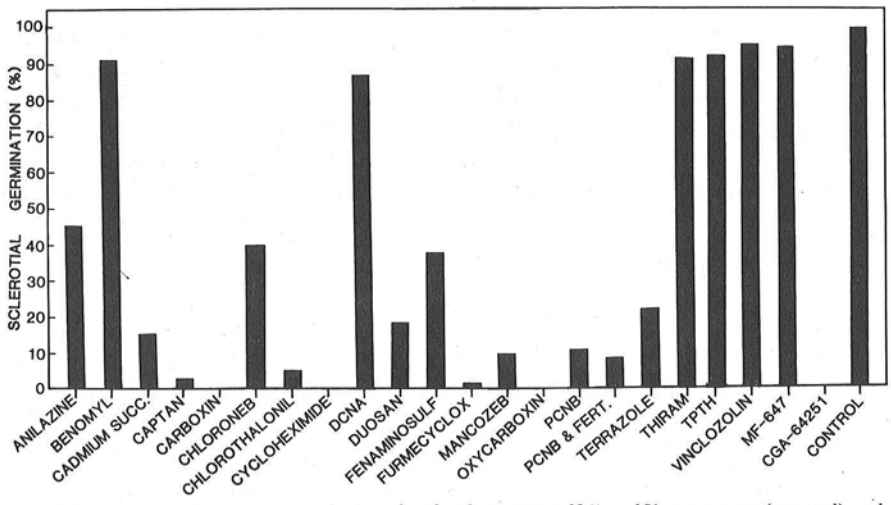

Fig. 2. Percentage of germination of sclerotia of *Sclerotium rolfsii* on 1% water agar (control) and 1% water agar containing the fungicides listed at 200 μg a.i./ml each. Germination was rated after 3 days of incubation in the dark at 27 C. Data are the means of four replicates, and the experiment was repeated twice.

mancozeb, PCNB with fertilizer, PCNB, and furmecyclox (Fig. 2). However, none of the above chemicals was fungicidal, and all sclerotia were viable and germinated hyphally when transferred to potato-dextrose agar. The fungicides benomyl, DCNA, TPTH, thiram, vinclozolin, and MF-647 (Mallinckrodt Inc., experimental compound) had no significant effect on sclerotial germination.

Field trials. The total number and average diameter of diseased spots and the total percentage of diseased area in the plots for seven chemical treatments at the last two assessment dates (7 July and 4 August) are given in Table 2. Disease severity ratings for 9 June are not included, as there was virtually no disease in control plots because of unusually cool weather during the early part of the season. Diseased spots were first observed on control plots on 24 May, and by 4 August approximately 18% of the total area of the control plot was diseased (Fig. 1A). All chemicals tested except mancozeb significantly reduced the total number of diseased spots on 7 July and 4 August. The average diameter of the spots was also reduced in all treated plots at both assessment dates with the exception of the plots treated with NH_4SO_4, in which larger but fewer spots were apparent on 4 August (Table 2).

All diseased spots on the plots increased in diameter as the season progressed, although this increase was not apparent in the plots treated with mancozeb because a large increase in the number of small spots reduced the average diameter. The apparently healthy green centers of the spots also increased in diameter, but to a lesser extent than that of the outer ring of diseased area. All chemicals significantly ($P=0.01$) reduced the percentage of diseased area, although mancozeb was the least effective. Plots treated with captan, carboxin, and PCNB with fertilizer were virtually free of disease (Fig. 1B). Plots receiving NH_4HCO_3 and NH_4SO_4 were much darker green and also had significantly less total diseased area than control plots (Table 2).

DISCUSSION

Occurrence of *S. rolfsii* on golf greens in California was not reported until 1977 (16), and since then 12 golf courses have reported this disease on some greens. Sclerotia may be the primary but perhaps not the only means by which the fungus is spread; all 18 isolates of *S. rolfsii* from turf in California have produced the basidial state (*Athelia rolfsii* (Curzi) Tu and Kimbrough) (21) in culture. It is possible that basidiospores may have a role in the spread of the pathogen, although their ability to infect has not yet been established (authors, *unpublished*).

Fungicides were screened for their ability to inhibit eruptive and hyphal germination of sclerotia rather than for

inhibition of radial growth on nutrient agar because preliminary studies had shown that sclerotia were much less likely to be affected by fungicides than exposed mycelium. It was hoped that these in vitro results might indicate which fungicides could be used to prevent germination of sclerotia and occurrence of disease in the field. A good correlation between effectiveness in inhibiting sclerotial germination in vitro and performance in the field was, in fact, obtained for five of the seven chemicals applied in the field. However, although NH_4SO_4 had no effect on sclerotial germination in vitro (authors, *unpublished*), it significantly reduced the amount of disease, whereas mancozeb, which inhibited sclerotial germination in vitro, reduced disease in the field by only 25% when compared with control plots.

Effective control of *S. rolfsii* in the greenhouse or in the field has been reported for carboxin (4), captan (1,9), PCNB (3), and NH_4SO_4 (10). We also obtained effective control of this pathogen on turf using these chemicals. In addition, we achieved good control using DCNA and cycloheximide in combination, PCNB with fertilizer, and NH_4HCO_3. Although others have shown that benomyl (9), chloroneb (15), TPTH (4), and thiram (9) inhibit mycelial growth of *S. rolfsii*, our results showed that these chemicals were not effective in inhibiting sclerotial germination even at 200 μg a.i./ml (Fig. 2).

None of the 22 fungicides tested in vitro was fungicidal to sclerotia, confirming results from other studies (1,3,4,7,17). However, NH_4HCO_3 at a 30 or 50 mM concentration was fungicidal to sclerotia of *S. rolfsii* in vitro (authors, *unpublished*). The rate of 0.18 kg/93 m^2 of NH_4HCO_3 used in this study applied in 50 L of water gives a concentration of 46 mM; with the further application of 38 L of water per plot, the final concentration of NH_4HCO_3 in the soil would be about 1.2 mM. Percentage of diseased area was reduced from about 18% in control plots to 0.8% in plots receiving seven applications of NH_4HCO_3. This is the first report of the use of NH_4HCO_3 in the field for the control of a fungal disease on turf.

The decrease in percentage of diseased area observed in plots receiving either NH_4SO_4 or NH_4HCO_3 may be the result in part of increased growth rate of the turf with greater capacity for regeneration with the higher nitrogen regimes. The percentages of nitrogen in NH_4HCO_3 and NH_4SO_4 are 17.7 and 21.2%, respectively; at the rates used in this study, about 3.4 and 5.2 kg N/ha, respectively, were applied to the plots every 14 days. The pH of the soil following these applications was not significantly altered from the pH of 6.1 in control plots. Nitrogenous compounds may also have had an effect on the pathogen by altering the soil microbial population in the vicinity of sclerotia (6), thereby increasing the antagonistic activity; a similar phenomenon has been observed after addition of plant tissues or residues to soil (8,11,14). This possibility was not investigated in this study. The major drawback to increased application of nitrogen alone as either NH_4SO_4 or NH_4HCO_3 for the control of *S. rolfsii* on turf is that the lush growth makes undesirable conditions for putting; in addition, more frequent mowing was required, and damage from *Pythium aphanidermatum* (Edson) Fitzpatrick on turf was increased.

Of the chemicals that gave the highest level of disease control, DCNA and cycloheximide in combination, carboxin, and NH_4HCO_3 were slightly phytotoxic. Most of the injury was apparent after three or four applications in the season or when temperatures exceeded 30 C following application. However, the rates used were probably higher than necessary for effective control of the pathogen in the field; further studies on optimal rates and times of application are required. Combinations of low levels of nitrogenous compounds (such as NH_4HCO_3) with reduced rates of fungicides (such as captan or carboxin) may be the most effective treatments for control of *S. rolfsii* on turf grass.

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LITERATURE CITED

1. Abeygunawardena, D. V. W., and Wood, R. K. S. 1957. Effect of certain fungicides on *Sclerotium rolfsii* in the soil. Phytopathology 47:607-609.
2. Anilkumar, T. B., Sastry, M. N. L., and Patil Kulkarni, B. G. 1979. Effect of fungicides on the viability of sclerotia of *Sclerotium rolfsii*. Phytopathol. Z. 95:1-5.
3. Aycock, R. 1966. Stem rot and other diseases caused by *Sclerotium rolfsii*. N.C. Agric. Exp. Stn. Tech. Bull. 174. 202 pp.
4. Diomande, M., and Beute, M. K. 1977. Comparison of soil plate fungicide screening and field efficacy in control of *Sclerotium rolfsii* on peanuts. Plant Dis. Rep. 61:408-412.
5. Harrison, A. L. 1961. Control of *Sclerotium rolfsii* with chemicals. Phytopathology 51:124-128.
6. Henis, Y., and Chet, I. 1968. The effect of nitrogenous amendments on the germinability of sclerotia of *Sclerotium rolfsii* and on their accompanying microflora. Phytopathology 58:209-211.
7. Indulkar, A. S., and Grewal, J. S. 1970. Studies on chemical control of *Sclerotium rolfsii*. Indian Phytopathol. 23:455-458.
8. Johnson, S. P. 1953. Some factors in the control of the southern blight organism, *Sclerotium rolfsii*. Phytopathology 43:363-368.
9. Khare, M. N., Agrawal, S. C., Kushwaha, L. S., and Tomar, K. S. 1974. Evaluation of fungicides for the control of wilt of lentil caused by *Sclerotium rolfsii*. Indian Phytopathol. 27:364-366.
10. Leach, L. D., and Davey, A. E. 1942. Reducing southern Sclerotium rot of sugarbeets with nitrogenous fertilizers. J. Agric. Res. 64:1-18.
11. Linderman, R. G., and Gilbert, R. G. 1973. Influence of volatile compounds from alfalfa hay on microbial activity in soil in relation to growth of *Sclerotium rolfsii*. Phytopathology 63:359-362.
12. Lucas, L. T. 1976. *Sclerotium rolfsii* on bentgrass in North Carolina. Plant Dis. Rep. 60:820-822.
13. McClellan, W. D. 1947. Efficacy of certain soil fumigants and fertilizers against crown rot in annual larkspur caused by *Sclerotium rolfsii*. Phytopathology 37:198-200.
14. Mixon, A. C., and Curl, E. A. 1967. Influence of plant residues on *Sclerotium rolfsii* and inhibitory soil microorganisms. Crop Sci. 7:641-644.
15. Mukhopadhyay, A. N., and Thakur, R. O. 1971. Control of Sclerotium root rot of sugarbeet with systemic fungicides. Plant Dis. Rep. 55:630-634.
16. Ohr, H. O., Humphrey, W. A., and Henry, M. J. 1977. *Sclerotium rolfsii* on turf in California. Pages 12-13 in: V. Gibeault, ed. Proc. Turf Landscape Inst., Anaheim, CA. Riverside Publication, University of California.
17. Porter, F. M., and Cox, C. E. 1958. Some effects of certain antibiotics and other organic chemicals on the growth of *Sclerotium rolfsii* in the laboratory. (Abstr.) Phytopathology 48:463.
18. Punja, Z. K., and Grogan, R. G. 1981. Eruptive germination of sclerotia of *Sclerotium rolfsii*. Phytopathology 71:1092-1099.
19. Punja, Z. K., Grogan, R. G., and Unruh, T. 1981. *Sclerotium rolfsii* on turf in California: Attempted biological and chemical control. (Abstr.) Phytopathology 71:251.
20. Sitterly, W. R. 1962. Calcium nitrate for field control of tomato southern blight in South Carolina. Plant Dis. Rep. 46:492-494.
21. Tu, C. C., and Kimbrough, J. W. 1978. Systematics and phylogeny of fungi in the *Rhizoctonia* complex. Bot. Gaz. Chicago 139:454-466.