Effects of Three Herbicides on Selected Pathogens and Diseases of Turfgrasses

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ABSTRACT

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The effects of the herbicides 2-ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranyl methanesulphonate (NC8438); 0, 0-diisopropyl phosphorodithioate S-ester with N-(2-mercaptoethyl) benzenesulfonamide (bensulide), and N-butyl-N-ethyl-α,α,α-trifluoro-2,6-dinitro-*p*-toluidine (benefin) on radial growth of *Drechslera cynodontis*, *Pythium aphanidermatum*, *Rhizoctonia solani*, and *Sclerotinia homoeocarpa* on artificial media were determined at 18, 26, and 35 C. Fungal growth was inhibited proportionally by most herbicide concentrations (1, 2, and 10× recommended field rates) at all temperatures. Growth of *R. solani* at 35 C was stimulated by the lowest rate of bensulide and the two lowest rates of NC8438. Pot-grown bermudagrass and annual ryegrass plants were sprayed with 1× and 3× the recommended field rates of the herbicides and inoculated with one of the four fungi. The 1× rate of benefin increased the severity of brown patch and dollar spot caused by *R. solani* and *S. homoeocarpa*, respectively. NC8438 reduced severity of these diseases and also Pythium blight caused by *P. aphanidermatum*.

Additional key words: nontarget organism, pest control, *Cynodon*, *Lolium*.

Pesticide usage increases annually, with the major part of the increase attributable to herbicides (1). Nontarget effects of herbicides on plant pathogens and diseases have received considerable attention and, depending on the herbicide, pathogen, and host involved, effects ranging from inhibition to stimulation of pathogen and/or disease symptoms have been observed (1,3–7,11–14,19).

While testing herbicides for turfgrass weed control the past several years, occasionally we noted that incidence and severity of some grass diseases were affected by herbicide treatments. Therefore, additional tests were conducted to substantiate these apparent nontarget effects. This paper reports the effects of three herbicides on the growth in culture of *Drechslera cynodontis* Nelson, *Pythium aphanidermatum* (Edson) Fitz., *Rhizoctonia solani* Kuhn, and *Sclerotinia homoeocarpa* F. T. Bennett, and on the turfgrass diseases caused by them (leaf blotch, Pythium blight, brown patch, and dollar spot, respectively [10]).

MATERIALS AND METHODS

Herbicides. The herbicides used were: benefin (N-butyl-N-ethyl-α,α,α-trifluoro-2,6-dinitro-*p*-toluidine, as the commercial preparation Balan, a liquid concentrate, 179.76 g/L (Elanco Products Company, Indianapolis, IN 46206); bensulide (0,0-diisopropyl phosphorodithioate S-ester with N-(2- mercaptoethyl) benzenesulfonamide), as the commercial preparation Prefar, an emulsifiable concentrate, 479.34 g/L (Stauffer Chemical Company, Mountain View, CA 94042); and NC8438 (2-ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranyl methanesulphonate), as the commercial preparation Nortron, an emulsifiable concentrate, 191.74 g/L (Fisons Corporation, Bedford, MA 01730). All three are used or are under test for weed control in established turf (20,21).

Fungi. The turfgrass pathogens and their sources were: *D. cynodontis*, isolated from bermudagrass collected in Harris County, Georgia; *P. aphanidermatum*, ATCC 16994; *R. solani*, ATCC 14006; and *S. homoeocarpa*, from J. L. Dale, University of Arkansas.

Fungal growth studies. The herbicides were dissolved in acetone and added to melted potato-dextrose agar (PDA) at rates equal to 1, 2, and 10× the recommended field rates as preemergence sprays, which are 3.36 kg AI/ha for benefin, 13.44 kg AI/ha for bensulide, and 3.36 kg AI/ha for NC8438. Final concentrations of herbicides in agar for the 1× rate were: benefin, 174 μg/ml; bensulide, 928 μg/ml; NC8438, 186 μg/ml. These were increased proportionately for the 2 and 10× rates. PDA containing equivalent amounts of acetone alone served as a control. After thorough mixing, 10 ml each of control and treated PDA was dispensed into petri plates (90 mm diameter). Thirty plates were prepared for each concentration of each herbicide and the control. About 3 hr after the plates were poured, a mycelial disk (7 mm diameter) taken from the periphery of a 2- to 3-day-old culture of *R. solani* growing on PDA was transferred to each plate. Ten plates for each treatment were placed in each of three incubators under continuous darkness at 18, 26, and 35 C, and the radial growth of the fungus was measured daily until control plates were overgrown or the experiment was otherwise terminated.

Effects of the herbicides on *S. homoeocarpa*, *D. cynodontis*, and *P. aphanidermatum* were determined using the procedures described above, except that lima bean agar was used with *P. aphanidermatum*.

Disease studies. Plugs (11.3 cm diameter) of bermudagrass, *Cynodon dactylon* (L.) Pers. 'Tifdwarf', were removed from an established turf with a golf cup cutter. Most of the adhering soil was washed from the roots with water and the plugs were transplanted into U.C. System soil mix C with fertilizer 1 (c) (17) in 454-ml plastic pots in the greenhouse. Two weeks later, the grass plugs were sprayed with 0, 1, and 3× the recommended field rates of benefin, bensulide, or NC8438; each treatment was replicated six times (six pots). The grass was placed in a controlled environment chamber programmed for 14 hr of light (17,216 lux) at 28–30 C and 10 hr of dark at 21–22 C. One week later, three mycelial disks (12 mm diameter) from a culture of *R. solani* were positioned

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equidistantly on the soil surface in each pot, and the pots were sealed in plastic bags to ensure high humidity. The daytime temperature in the bags was maintained at about 29 C by appropriate shading. The bags were removed after 4 days and disease development was rated 1 to 5 according to a scale in which 1 = no signs or symptoms apparent beyond the point of inoculation, 2 = area of diseased grass to 1.5 cm diameter, 3 = to 2.5 cm, 4 = 4.0 cm, and 5 = greater than 4.0 cm.

Similarly, additional plugs of healthy bermudagrass were inoculated with each of the other pathogens. With *S. homoeocarpa*, four mycelial disks were used in each pot. For the experiment with *P. aphanidermatum*, two disks were used to inoculate each grass plug, and the grass was incubated at 33–35 C daytime temperature. With *D. cynodontis*, the inoculum consisted of a spore-mycelial-agar suspension prepared by blending cultures of the fungus from 12 PDA plates with 1,200 ml of water for 15–20

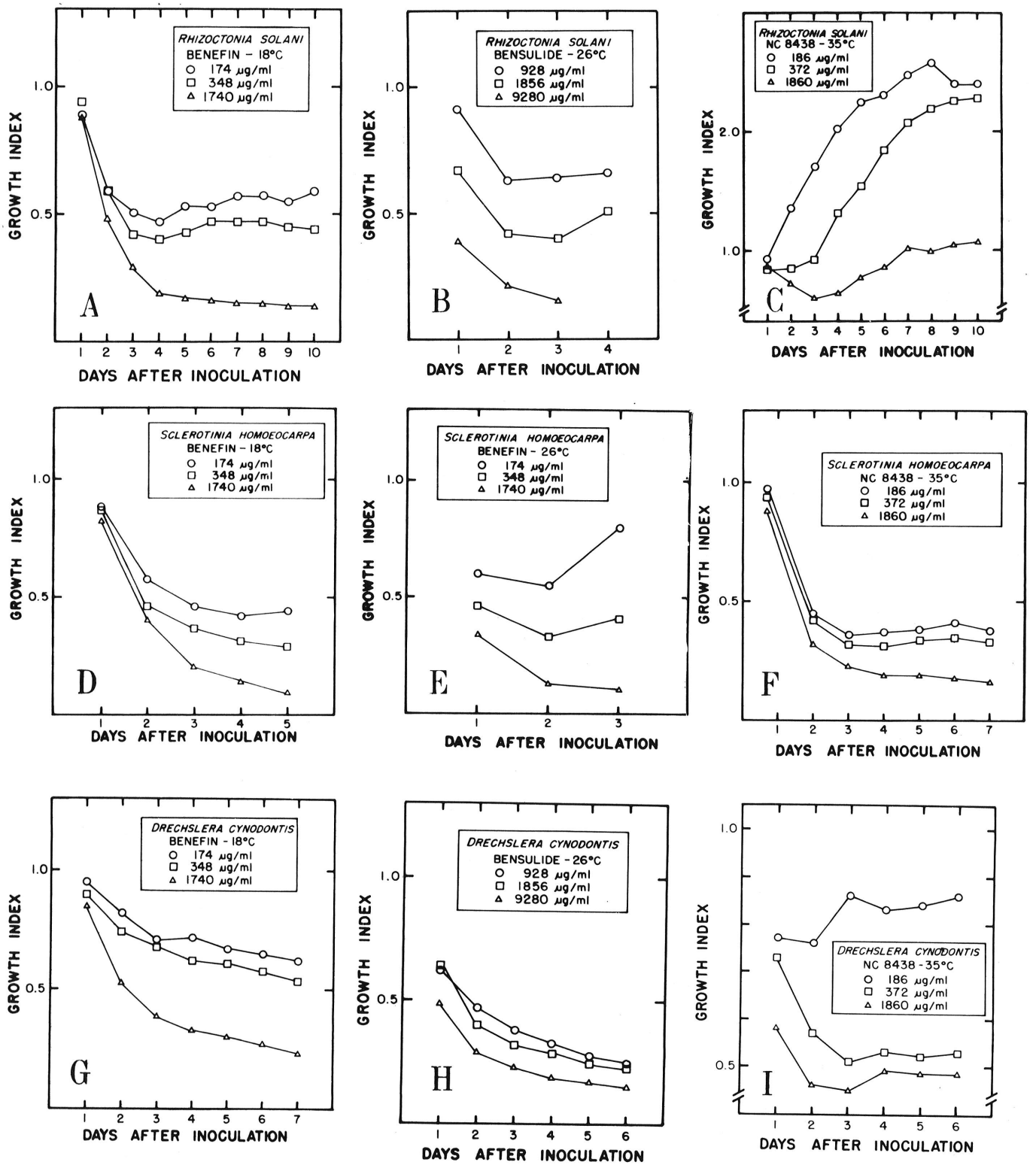

Fig. 1. Growth of *Rhizoctonia solani* (A-C), *Sclerotinia homoeocarpa* (D-F), and *Drechslera cynodontis* (G-I) on potato-dextrose agar containing three concentrations of the herbicides benefin, bensulide, or NC8438. Cultures were incubated at 18 C, 26 C, or 35 C as indicated. Data are given as a growth index obtained by dividing average diameter for the treatment by that for the corresponding control.

sec. The suspension was sprayed to runoff onto the grass in each pot, and pots were incubated at 33–35 C daytime temperature.

An additional test was conducted with *P. aphanidermatum* using annual ryegrass, *Lolium multiflorium* Lam., as the host. Procedures were as described above, except that the grass was grown from seed for 16–30 days and trimmed to about 6.4 cm in height prior to being sprayed with the herbicides. Also, only one mycelial disk was used to inoculate with *P. aphanidermatum*, and grass was incubated for 3–4 days.

Statistical analyses. Data were subjected to analysis of variance and tested for significance ($P = 0.05$) by the F-test; where applicable, mean separations were performed by using Duncan's new multiple range test (18). Disease ratings were transformed to normal scores prior to analysis of variance (16).

For graphical presentation of data from the growth studies, treatment effects are given by a growth index obtained by dividing average diameter growth for the treatment by that for the corresponding control.

RESULTS

Effect of herbicides on growth of fungi in agar. With few exceptions, each of the herbicides inhibited growth of the four fungi on PDA, and the degree of inhibition increased in proportion to concentration of herbicide. Growth data for three of the fungi are shown in Fig. 1; unless specified otherwise, each graph is typical of the growth pattern observed with all the herbicides at the given temperature. Growth of *R. solani* at 18 C (Fig. 1-A) and 26 C (Fig. 1-B) was inhibited significantly by all concentrations of the three herbicides, and at 35 C by the two highest concentrations of benefin and bensulide (not shown); significant stimulation of growth by the fungus occurred at 35 C in PDA containing the lowest rate of bensulide (not shown) and the two lowest rates of NC8438 (Fig. 1-C). At all temperatures, the mycelial mats on herbicide-treated PDA were thicker and produced fewer sclerotial initials than those on untreated medium.

Growth of *S. homoeocarpa* at the three temperatures was inhibited significantly by all concentrations of the herbicides (Fig. 1-D to F), except for the two lowest rates of bensulide which had no effect at 35 C. No differences in mycelial characteristics were apparent between cultures on herbicide-treated or herbicide-free PDA. Similarly, herbicide treatments did not affect the general appearance of *D. cynodontis* cultures at any temperature; at 26 C, however, onset of sporulation on any herbicide-treated medium was delayed 24 hr compared to untreated control cultures. Mycelial growth of the fungus on herbicide-treated PDA was significantly less than that on herbicide-free medium at all three temperatures (Fig. 1-G to I).

Growth of *P. aphanidermatum* at 18 C and 35 C was not affected by the two lowest rates of benefin; otherwise, all concentrations of the three herbicides significantly inhibited growth of the fungus at 18, 26, and 35 C (author's data, *not shown*) Cultures on PDA containing the highest rates of each herbicide produced less aerial mycelium than those on any other treated medium or on herbicide-free medium.

Effect of herbicides on diseases of turfgrass. In bermudagrass, both rates of NC8438 reduced severity of brown patch caused by *R. solani*, and the higher rate reduced severity of dollar spot and Pythium blight caused by *S. homoeocarpa* and *P. aphanidermatum*, respectively (Table 1). Severity of brown patch and dollar spot was increased in grass treated with the lower rate of benefin. None of the herbicide treatments affected development of Pythium blight in annual ryegrass (Table 1). The isolate of *D. cynodontis* used in this study was not consistently pathogenic, hence no data are given.

DISCUSSION

The general inhibitory effect of benefin, bensulide, and NC8438 on radial growth of the four fungi in vitro agrees with some results from other studies of herbicide effects on *R. solani*, *P. aphanidermatum*, and other fungi (1,3,4,6,7,11–13). An exception to this was the stimulation of growth of *R. solani* at 35 C by two rates of NC8438 and one rate of bensulide; stimulation of growth of *R. solani* by herbicides also has been reported previously (1). None of the growth effects of the herbicides observed in our studies appeared to be irreversible. When any of the fungi on herbicide-treated medium was transferred to fresh, untreated medium, growth was similar in rate and appearance to that of a corresponding transfer from a control culture (author's, *unpublished*).

Dinitroanilines, of which benefin is a member, disrupt mitotic processes in cells of higher plants, and there is some indication that the mechanism of action is at the site of nucleic acid synthesis. Also, the dinitroanilines are uncouplers of oxidative phosphorylation in higher plants (2). Bensulide also disrupts the mitotic process in cells of higher plants, but the exact mechanism of action is not yet known (15). NC8438 is a relatively new herbicide and little is known about its action; however, it is a growth regulator type of herbicide (20). Mechanism(s) for the inhibitory effects of the herbicides on fungi may be similar to those operative in higher plants. The bases for stimulation of growth of *R. solani* at 35 C by some rates of bensulide and NC8438 also are not known. Possibly, the herbicides counteracted the effects of high temperature on metabolism of the fungus (8) or altered the water potential of the agar medium (9) such that growth was enhanced at the high temperature.

Effects of the herbicides on disease did not always parallel apparent effects on the pathogens in vitro. NC8438 reduced disease caused by *P. aphanidermatum* and *S. homoeocarpa*, and it also inhibited their growth in culture. This herbicide also reduced brown patch symptoms but stimulated growth of the pathogen, *R. solani*. Generally, benefin was inhibitory to growth of all the fungi but it increased the amount of disease caused by *S. homoeocarpa* and *R. solani*. One possible explanation for the discrepancy between results of the growth and disease studies is that direct

TABLE 1. Development of disease in turfgrasses treated with herbicide prior to inoculation with *Rhizoctonia solani* (brown patch), *Sclerotinia homoeocarpa* (dollar spot), or *Pythium aphanidermatum* (Pythium blight)

Herbicide		Disease rating[w]			
Name	Concentration (μg/ml)	Brown patch[x]	Dollar spot[y]	Pythium blight[z]	
Benefin	174	2.44 c	2.79 d	1.83 ab	3.17 a
Benefin	522	1.86 ab	2.25 bc	1.79 ab	2.08 a
Bensulide	928	1.88 ab	2.62 cd	2.16 b	4.00 b
Bensulide	2,784	2.13 bc	2.31 bc	2.00 ab	4.00 b
NC8438	186	1.61 a	2.18 b	1.75 ab	3.67 b
NC8438	558	1.61 a	1.77 a	1.50 a	2.33 a
Control	0	1.97 b	2.31 bc	2.17 b	3.17 ab

[w] Means within a column and followed by the same letter are not significantly different ($P = 0.05$).

[x] Each value is the average for ratings of 18 inoculation sites in six pots of bermudagrass. Rating scale, 1–5: 1 = no signs or symptoms apparent beyond point of inoculation, 2 = area of diseased grass to 1.5 cm diameter, 3 = 2.5 cm, 4 = 4.0 cm, and 5 = greater than 4.0 cm.

[y] Each value is the average for ratings of 24 inoculation sites in six pots of bermudagrass; rating scale 1–5 as above.

[z] Each value is the average for ratings of 12 inoculation sites in six pots of bermudagrass (left column) or six pots of annual ryegrass (right column); rating scale 1–5 as above.

contact between pathogen and a herbicide concentration that was effective in vitro would not have been as likely to occur in the complex interactions among herbicide, plant, pathogen, and environment in disease. Besides affecting growth of the pathogens, other possible herbicide effects leading to increase or decrease in the turfgrass diseases could have included effects on antagonists to the pathogens and alterations in host biochemical and structural defenses and exudation (1).

The increase in dollar spot and brown patch associated with benefin treatment differs from the results of Grinstein et al (12) who reported that the herbicide had no effect on resistance of corn, oats, and several nongrass plants to *R. solani*, and also those of Buczacki (5) who observed less club root of cabbage in soil treated with benfluralin (benefin) before planting. However, there are several reports in which increases in diseases caused by *R. solani* and other pathogens have been associated with herbicide treatments (1,12–14,19).

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