Effects of Fungicides on Mycorrhizal Development of Creeping Bentgrass

L. H. RHODES, Assistant Professor, and P. O. LARSEN, Associate Professor, Department of Plant Pathology, The Ohio State University and Ohio Agricultural Research and Development Center, Columbus 43210

ABSTRACT

Rhodes, L. H., and Larsen, P. O. 1981. Effects of fungicides on mycorrhizal development of creeping bentgrass. Plant Disease 65:145-147.

Chlorothalonil, benomyl, and iprodione reduced mycorrhizal development of creeping bentgrass when applied in the spring to golf course greens or 4–8 wk after bentgrass was seeded and inoculated with *Glomus fasciculatus* in the greenhouse. Other fungicides significantly reducing mycorrhizal development in the greenhouse were pentachloronitrobenzene, triadimefon, anilazine, and chloroneb. Fungicides applied 16–20 wk after bentgrass was seeded did not affect mycorrhizal development in the greenhouse. Results indicate that spring application of several turf fungicides may cause reduced mycorrhizal development in bentgrass turfs.

Additional key words: nontarget organisms, vesicular-arbuscular

Vesicular-arbuscular (VA) mycorrhizae have been known to occur in grasses since 1888 (8). However, most field studies on noncereal grasses have dealt with the distribution of mycorrhizae in pasture lands or in natural vegetation systems. Little is known about effects of VA mycorrhizae in intensively managed turfgrasses or of the influence of pesticide applications on mycorrhizal associations in turf.

A recent survey in Ohio showed a single season total of more than 2,800 turf fungicide applications on 645 golf courses, with the five most frequently applied compounds accounting for more than 125 t a.i. (P. O. Larsen, D. P. Martin, and R. L. Miller, *unpublished*). With such high fungicide use, attention has understandably been directed recently toward potential toxicity of these compounds on nontarget soil microorganisms associated with turfgrasses. For example, Smiley and Craven (9) found that certain fungicides altered microbial populations in the thatch layer of a Kentucky bluegrass turf. Since VA mycorrhizal fungi are obligate symbionts, their populations would not have been quantified by the isolation procedure used in their study. Our observations indicate, however, that VA mycorrhizal fungi are major components of the soil microflora in intensively managed turf.

Because mycorrhizae improve growth of several grass species (1, 6), commonly used turfgrass fungicides should be evaluated for effects on mycorrhizal development and subsequent influence on turfgrass vigor. The purpose of this study, therefore, was to determine the effects of several turfgrass fungicides on the development of VA mycorrhizae in creeping bentgrass turfs.

This work was supported in part by grants from the Graduate School of The Ohio State University and the Ohio Turfgrass Foundation.

Approved for publication as Journal Article 44-80 of the Ohio Agricultural Research and Development Center, Wooster 44691.

0191-2917/81/02014503/$03.00/0

MATERIALS AND METHODS

In this study of creeping bentgrass (*Agrostis palustris* Huds.), the cultivar Toronto was used for field experiments and Penncross was used for greenhouse experiments.

The fungicides used were anilazine 50W, benomyl 50W, chloroneb 65W, chlorothalonil 500F, iprodione 50W, maneb 80W, pentachloronitrobenzene 75W, and triadimefon 50W.

Field study. Experiments were conducted on bentgrass putting greens on a northeastern Ohio golf course. Greens were established on a loamy sand, which had a pH of 7.1 and an available phosphorus level of approximately 40 kg/ha. Turf was maintained at a height of 5–7 mm by regular mowing. Mycorrhizal fungi identified from these sites included *Glomus microcarpus* Tul. and Tul., *Glomus macrocarpus* Tul. and Tul. var. *macrocarpus* Gerd. and Trappe, and *Glomus tenuis* (Greenall) Hall.

Fungicides were applied during April with a single nozzle CO_2-powered sprayer to 1.5-m^2 plots replicated four times in randomized complete block designs. On green 2, in some treatments, a fall application preceded the two spring applications.

Four soil and root sample cores 2.5 cm in diameter were removed from each plot 2 wk after the last fungicide application. Roots from these samples were cleared in 10% KOH and stained with trypan blue in a solution of 85% lactic acid-glycerine-water (2:2:1, v/v/v). Mycorrhizal development was based on an estimate of the percent root length colonized by

Table 1. Effect of spring[v] or fall/spring[w] fungicide applications on mycorrhizal development in Toronto creeping bentgrass on golf course greens[x]

Fungicide	Rate (g a.i./m^2)	Mycorrhizal development[y]
	Green 1	
Chloroneb	0.8	5.0 a[z]
Control	···	4.8 a
Maneb	1.0	3.8 ab
Chlorothalonil	1.2	2.3 b
Benomyl	0.3	1.3 c
	Green 2	
Control	···	5.0 a
Iprodione		
Spring	0.6	4.0 b
Fall/spring	0.3	3.9 b
Fall/spring	0.15	3.8 b
Spring	0.3	3.8 b
Spring	0.15	3.8 b
Fall/spring	0.6	3.6 b
Chlorothalonil		
Spring	1.2	3.0 c
Chlorothalonil		
Fall/spring	1.2	2.3 d

[v] Fungicides applied during second and fourth weeks of April.
[w] Fall application in October; spring applications during second and fourth weeks of April.
[x] On a northeastern Ohio golf course.
[y] Rated on a 1–10 scale: 1 = 0–10% and 10 = 91–100% colonized root length.
[z] Means for each location not followed by the same letter are significantly different (P = 0.05) according to Duncan's new multiple range test.

Table 2. Effect of early and late application of turf fungicides on mycorrhizal development in Penncross creeping bentgrass in the greenhouse

Fungicide	Rate (g a.i./m^2)	Mycorrhizal development[w]	
		Early application[x]	Late application[y]
Control	...	4.5 a[z]	4.0 a
Chloroneb	0.8	3.3 b	4.0 a
Benomyl	0.3	2.8 bc	3.5 a
Iprodione	0.3	2.8 bc	4.3 a
Anilazine	0.6	2.3 bcd	4.0 a
Triadimefon	0.3	1.8 cde	4.3 a
Chlorothalonil	0.6	1.5 de	4.5 a
Pentachloronitrobenzene	0.9	1.0 e	4.3 a

[w] Rated on a 1–10 scale: 1 = 0–10% and 10 = 91–100% colonized root length.
[x] Each fungicide applied 4, 6, and 8 wk after seeding; experiment terminated at 10 wk.
[y] Each fungicide applied 16, 18, and 20 wk after seeding; experiment terminated at 22 wk.
[z] Means in a column not followed by the same letter are significantly different ($P = 0.05$) according to Duncan's new multiple range test.

mycorrhizal fungi. This was ascertained microscopically at ×40–100. A 1–10 scale was used to express this estimate, with each integer approximating a range of 10% colonized root length. Therefore, 1 approximated 0–10% and 10 approximated 91–100% colonized root length.

Greenhouse study. An Ohio isolate of *Glomus fasciculatus* (Thaxter) Gerdemann and Trappe was incorporated into the upper 10 cm of a loam-sand (1:1, v/v) potting mixture in 9-cm-diameter, 500-ml plastic pots. Bentgrass was seeded at seven seeds per square centimeter. The potted turf was maintained at a height of 3–5 cm by clipping weekly. Each pot received 100 ml of a 20 mM solution of KNO_3 at 2 wk, and pots maintained for later fungicide applications received another 100 ml of solution again at 12 wk.

Each fungicide was applied with an artist's air brush at three 2-wk intervals, beginning 4 or 16 wk after seeding. The volume of spray applied to each pot was 1.2 ml. This was allowed to dry on grass blades overnight before regular overhead watering was resumed. All fungicides, except PCNB, were applied within the range of label use rates. PCNB, which can be phytotoxic on bentgrass, was also included because it has well-documented effects on mycorrhizal fungi in other crops. Each fungicide treatment was replicated four times, with one pot serving as the control unit.

Root samples were removed from each pot 2 wk after the last fungicide application. Roots were cleared, stained, and evaluated for mycorrhizal development.

RESULTS

Field study. Mycorrhizae were in all samples. The narrow angular hyphae and abundant small intercalary vesicles characteristic of *G. tenuis* (7) predominated in roots from green 2 but were also present in roots from green 1. Other mycorrhizal endophytes colonized roots from both greens as indicated by the presence of larger hyphae and vesicles.

Spring or fall/spring applications of benomyl, chlorothalonil, or iprodione reduced mycorrhizal development of bentgrass in the field in comparison to that in untreated controls (Table 1).

On green 2 only iprodione and chlorothalonil were used. Among iprodione treatments, variations in rates or inclusion of a fall application did not significantly affect mycorrhizal development (Table 1). However, with chlorothalonil, a fall/spring application caused a slight reduction in mycorrhizal development compared with two spring applications alone.

Greenhouse study. All fungicides reduced mycorrhizal development of creeping bentgrass when applied 4–8 wk after seeding and inoculation in the greenhouse (Table 2). Fungicides had no detectable effect on mycorrhizae when applied 16–20 wk after seeding. Phytotoxicity of PCNB, as indicated by some necrosis of grass blades, was evident in both early and late application experiments and was apparently not related to mycorrhizal development.

DISCUSSION

Mycorrhizae are normal components of intensively managed turfgrasses. Results of our study indicate that application of label use rates of several turf fungicides early in the growing season may result in reduced mycorrhizal development in creeping bentgrass. Maximum initiation and growth of turfgrass roots usually occurs in the spring (10), and fungicides applied then apparently prevented establishment of mycorrhizal fungi in newly produced root tissue. A similar situation was simulated in the greenhouse when fungicides were applied 4–8 wk after bentgrass was seeded.

When fungicides were applied to bentgrass after 16 wk in the greenhouse, no effects on mycorrhizal development were observed, indicating that mycorrhiza formation was not occurring to a great extent at this time. Data from a separate field study (Rhodes and Larsen, *unpublished*) also indicate that late season fungicide applications do not affect mycorrhizal development as measured by amount of fungal-colonized root length.

Because the histological techniques we used only allowed measurement of fungal colonization of roots, no information was obtained on the viability of the fungal components of the mycorrhizae, extent of external mycelium development, or function of the nutrient uptake and transfer process. Gray and Gerdemann (2) observed a 16-fold reduction of ^{32}P uptake in 12-wk-old mycorrhizal onions when PCNB was applied 48 hr before ^{32}P application. Likewise, Hirrel and Gerdemann (3) found that, in 10-wk-old onion plants, PCNB (applied 2 and 5 days before ^{14}C-glucose injection into soil) inhibited ^{14}C translocation through mycorrhizal hyphae. Studies to assess such fungicide effects on mycorrhizae of turfgrasses are in progress.

Two spring applications of chloroneb did not reduce mycorrhizal development in the field. Also, chloroneb resulted in the smallest numerical reduction of mycorrhizal development rating of any fungicide tested in the greenhouse. This agrees with information on ethazole (4) and metalaxyl (5), which also have activity against oomycetes but do not appear to be detrimental to mycorrhizal fungi. It may therefore be possible to selectively control pythiaceous fungi without substantially reducing mycorrhizal development. Studies involving a wider range of rates and application schedules are needed before the effects of fungicides on mycorrhizal fungi can be compared adequately.

Turfgrass disease control programs involve the use of specific fungicides applied during seasonal periods of pathogen activity. In Ohio, applications of turf fungicides often begin in the spring. Such fungicide programs appear likely to reduce mycorrhizal development in creeping bentgrass.

ACKNOWLEDGMENTS

We wish to thank Danette Cotton, Lee Wilson, Billie Harrison, and David Spring for technical assistance.

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