Effect of Fungicides on the Occurrence and Growth In Vitro of Basidiomycetes Associated with Superficial Fairy Rings in Creeping Bentgrass

K. E. KACKLEY, Graduate Research Assistant, Botany Department, P. H. DERNOEDEN, Associate Professor, Agronomy Department, and A. P. GRYBAUSKAS, Associate Professor, Botany Department, University of Maryland, College Park 20742

ABSTRACT

Kackley, K. E., Dernoeden, P. H., and Grybauskas, A. P. 1989. Effect of fungicides on the occurrence and growth in vitro of basidiomycetes associated with superficial fairy rings in creeping bentgrass. Plant Disease 73:127-130.

Superficial fairy rings (SFR) in turf are incited by several, mostly unidentified, basidiomycetes. The appearance of SFRs has previously been associated with the use of benomyl. Observations at three sites in Maryland and one site in Delaware revealed that SFRs developed in creeping bentgrass (*Agrostis palustris*) in the absence of fungicide use. Two isolates of basidiomycetes exhibiting similar colony characteristics and temperature optima for growth were obtained from SFRs where benomyl was either not used or was used extensively. Growth of the isolates was not stimulated by incubation at 25 C on PDA amended with 0–100 μg a.i./ml of benomyl, carbendazim, or iprodione. Observations of growth in vitro and in the field do not support the premise that benomyl predisposes turf to SFRs by stimulating mycelial growth.

Superficial fairy rings (SFR) in turf are incited by numerous thatch-inhabiting basidiomycetes. The term superficial fairy ring was first used in 1981 by Redhead and Smith (7) and Smith and Jackson (12) to identify this diverse group of diseases also described in the literature as white patch (10), superficial white patches or rings (10), or simply as circular patches (11). Some of the basidiomycetes that have been associated with SFRs include *Coprinus kubickae* Pilat & Svrek (7), *Trechispora coharens* (Schw.) Jülich & Stalpers (5,10), and *T. farinacea* (Pers. & Fr.) Lib. (10). However, the majority of the causal fungi remain unidentified. Superficial fairy rings occur most frequently in creeping bentgrass (*Agrostis palustris* Huds.) turf, but they have also been observed in Kentucky bluegrass (*Poa pratensis* L.) (9,10). Superficial fairy rings may appear as white rings or circular patches ranging from 15 cm to over 1.0 m in diameter in closely mown creeping bentgrass (11). Upon close inspection, white mycelial growth can be observed on the lower leaves of affected plants, permeating the thatch layer to a depth of 7 mm (5). The infested thatch possesses a strong "mushroom odor" and young roots forming at the nodes may be temporarily stunted (5). Superficial fairy rings differ from other fairy rings in that the causal fungi only inhabit the thatch layer and senescent tissue, while the other fairy ring fungi usually colonize both thatch and soil.

Smith and Jackson (12) described three types of SFRs: 1) those that produce abundant mycelia and have little effect on grass growth, 2) those that severely injure turf, and 3) those that markedly stimulate growth. Although SFR fungi are not highly pathogenic, they are important because they may mar the appearance of turf, they may degrade thatch and disrupt the "trueness" of a putting surface, and they may render thatch and soil hydrophobic (12).

In 1970, Smith et al (11) reported the occurrence of a basidiomycete-induced patch disease on bentgrass in Australia. The SFRs appeared only on turf previously treated with the fungicide benomyl. Results of fungicide tests in vitro, conducted on an isolate from the diseased patches, indicated that this particular basidiomycete was tolerant to benomyl at rates of up to 25 ppm; in fact, its growth was stimulated by 0.5 and 1.0 ppm of benomyl in the culture medium. These results coupled with earlier field observations led Smith et al (11) to conclude that applications of benomyl predispose turf to attack by SFR basidiomycetes. Reports (5,7,12) on SFRs both preceding and following that of Smith et al (11) do not include information on the history of fungicide use in relation to disease incidence. This study was undertaken to examine the relationship between SFRs and fungicide use.

Maryland Agricultural Experiment Station Contribution No. 7775, Scientific Article No. A-4771.

Accepted for publication 25 August 1988 (submitted for electronic processing).

© 1989 The American Phytopathological Society

MATERIALS AND METHODS

Fungal isolates. Two isolates of nonfruiting basidiomycetes associated with superficial fairy rings were collected. One isolate (DE) was obtained from a newly seeded creeping bentgrass cultivar Penncross putting green that was established in recently cleared woodland at Cripple Creek Golf and Country Club in Bethany Beach, DE. No fungicides had been applied to this green before the appearance of the SFRs or to isolation of the fungus. A second isolate (MD) was obtained from a creeping bentgrass cultivar Penncross nursery on the University of Maryland golf course in College Park. The bentgrass nursery had been treated for many years with a wide variety of chemicals, including the benzimidazole fungicides. Isolations were made from thatch tissues permeated by white mycelia. The thatch was surface-sterilized for 10 sec in 0.05% NaOCl solution, blotted dry on sterile tissue paper, and incubated at 25 C on Difco potato-dextrose agar (PDA).

Growth curve. *Temperature studies.* Both isolates were evaluated for growth at various temperatures on Difco PDA. A 5.0-mm-diameter disk was aseptically removed from the edge of an actively growing colony and placed in the center of a sterile petri dish (100 × 15 mm) containing 20 ml of PDA. Dishes were sealed with parafilm and incubated without light at 15, 20, 25, 30, and 35 ± 1 C. Growth was determined by measuring colony diameter (the mean of two perpendicular measurements) every 48 hr. The experimental design was completely randomized with four replicates per treatment.

Fungicide studies. Growth of the DE and MD fungal isolates was determined on PDA amended with either benomyl (methyl 1-[butylcarbamoyl]-2-benzimidazolecarbamate), carbendazim (2-[methoxycarbomylamino]-benzimidazole), or iprodione (3-[3,5-dichlorophenyl]-*N*-[1-methylethyl]2,4-dioxo-1-imidazolidinecarboxamide) at rates of 0, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, 50.0, and 100 μg a.i./ml. Fungicide-amended media were prepared by serial dilution of a 100 μg a.i./ml PDA stock solution with unamended PDA. Technical grade fungicides were dissolved in acetone and added after the PDA was autoclaved and cooled to 45 C. The control (0 μg/ml) plates contained unamended PDA without acetone. Fifteen milliliters of the appropriate media were aseptically dispensed into sterile petri dishes (100 ×15 mm) with a sterile repeating syringe. Five replicate plates of each rate of fungicide were seeded with a single 5.0-mm disk taken from the edge of an actively growing colony of each fungal isolate on PDA. Plates were sealed with parafilm and maintained in a dark incubator at 25 ± 1 C for 12 days. Growth was determined by measuring colony diameter every 48 hr, as previously described. The experiment was repeated and the data were combined for analysis. The data were analyzed as a completely randomized 2 × 3 × 9 factorial by SAS general linear models procedure, using type III sums of squares (8). Orthogonal polynomial contrasts were used to determine dose-response relationships. Significantly different means were separated using the FLSD multiple comparison prooedure.

Isolates produced very limited growth on media amended with 10–100 μg/ml of benomyl. Therefore, in order to determine if the benomyl treatments were fungicidal or fungistatic, a 5.0-mm disk or an equivalent volume of colonized medium of each isolate from each benomyl concentration was placed on four replicate plates of unamended PDA. In the instance where no growth was produced (100 μg/ml), the original disk was removed and placed on the unamended PDA. The isolates had been grown on benomyl-amended PDA for 17 days. Growth was determined by measuring colony diameter every 48 hr for 10 days. The data were analyzed as a completely randomized 2× 9 factorial by SAS general linear models procedure (8). Significantly different means were separated using the FLSD multiple comparison procedure.

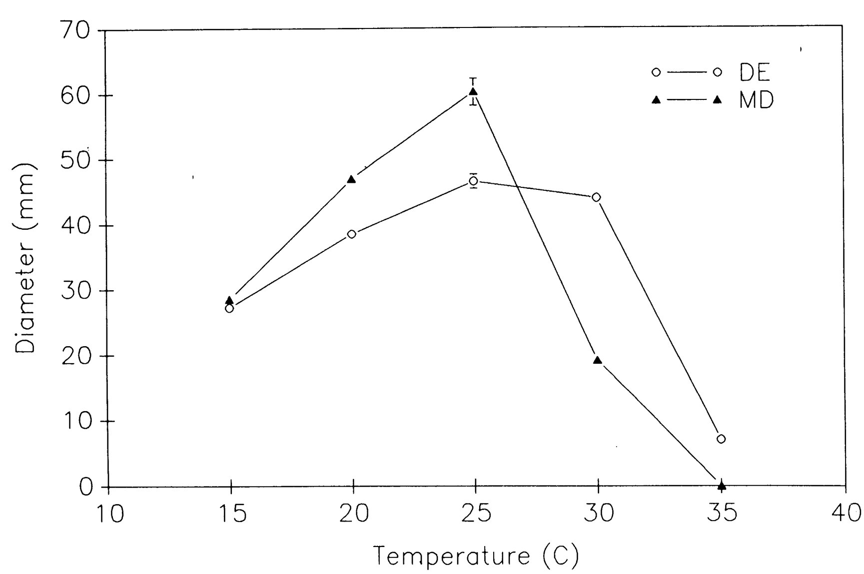

Fig. 1. Mean colony diameter of a Delaware isolate (DE) and a Maryland isolate (MD) of superficial fairy ring fungi grown on PDA at five temperatures after 8 days of incubation. Each point represents the mean of four replications and each bar represents the standard error of the mean.

RESULTS

Field observations. During the years 1981–1985, four creeping bentgrass turfs that were not treated with fungicide were found exhibiting superficial fairy rings in Maryland and Delaware. These areas were located as follows: at the O. M. Scott & Sons turfgrass research center in Port Republic, MD; a home lawn putting green in Baltimore, MD; two tees at Columbia Country Club in Chevy Chase, MD; and a putting green at Cripple Creek Golf and Country Club in Bethany Beach, DE. In Port Republic, MD, three separate areas were seeded to creeping bentgrass cultivars Seaside, Penncross, and Emerald in the fall of 1979. In previous years, the land was a part of a farm that grew soybeans (*Glycine max* (L.) Merr.) and tobacco (*Nicotiana tabacum* L.). According to Richard J. Schneider, Research Agronomist for the O. M. Scott & Sons Mid-Atlantic Research Station in Port Republic (*personal communication*), superficial fairy rings first appeared in 1981 and developed only in cultivar Seaside turf. The turf had not been treated with fungicides before the appearance of superficial fairy rings.

In Baltimore, MD, a golf course superintendent backfilled a swimming pool with unsterilized top soil and established a creeping bentgrass cultivar Penneagle putting green in May of 1981. The following spring and early summer, numerous superficial fairy rings appeared in the turf. Before the appearance of the rings, no fungicides had been applied. Subsequent to the appearance of rings, a wide range of fungicides were applied unsuccessfully to control the disease.

At Columbia Country Club in Chevy Chase, MD, several new tees were built and seeded to creeping bentgrass cultivar Penncross in the fall of 1980. During early summer of 1981, superficial fairy rings were observed on two tees. Chlorothalonil (tetrachloroisophthaloinitrile) may have been applied in the spring of 1981, but benzimidazole fungicides had not been applied before the appearance of rings.

A new practice putting green was seeded to creeping bentgrass cultivar

Penncross at Cripple Creek Golf and Country Club in Bethany Beach, DE, in the spring of 1985. This was a new golf course on land that was previously wooded. In the summer and fall of 1985, superficial fairy rings appeared on the practice green. No fungicides had been applied to this area before the appearance of the rings and subsequent fungicide treatments did not appear to influence the disease.

Growth curve. *Temperature studies.* The growth temperature optimum for both the DE and MD isolates at day 8 was 25 C (Fig. 1). Day 8 data were selected for analysis because at this time both isolates were exhibiting active, log-phase growth but had not yet reached the plate edges. The MD isolate grew faster than the DE isolate at the optimal (25 C) and suboptimal (15 and 20 C) temperatures, but was apparently more sensitive to supraoptimal (30 and 35 C) temperature growth suppression than the DE isolate.

Fungicide studies. There were no significant differences in fungal growth due to the type of fungicide or to the fungicide-by-isolate or the fungicide-by-rate treatment combinations. No stimulatory effect was detected with any fungicide at any rate tested, in either the DE or MD isolate (Table 1). There was no significant difference in the 0 (control) versus the 0.5 and 1.0 μg/ml rates. Fungicide rate and the fungicide rate-by-isolate interaction were highly significant ($P < 0.01$). As fungicide rate increased, fungal growth decreased quadratically. The equations for the quadratic response to fungicide of the fungal isolates are as follows: DE isolate, diameter = $38.70 - 1.35X + 0.01X^2$; MD isolate, diameter = $40.36 - 1.65X + 0.01X^2$; where diameter is the diameter of the mycelial growth at day 8, and X is the fungicide rate. The decrease in diameter with an increase in fungicide rate was greater for the MD than for the DE isolate.

Benomyl treatments were determined to be fungistatic by the transfer of isolates from benomyl-amended PDA to unamended PDA (Table 2). Data from day 5 are presented because all treatments were exhibiting active, log-phase growth at this time but had not yet reached the edges of the plates. Benomyl was not fungitoxic to either the DE or MD isolate at any rate tested. There were no significant differences between the isolates. There was very little difference in the growth of the isolates previously exposed to the 0–5.0 μg/ml rate. The reduced growth seen in the isolates previously exposed to the higher rates of benomyl (10.0–100.0 μg/ml) was due mainly to a delay in the onset of growth rather than a reduction in rate.

DISCUSSION

Superficial fairy rings have generally been considered to be a nontarget effect associated with the use of fungicides. This is due, in large part, to the reports of Smith et al (11) and Smiley (9). Smith et al (11) reported that a SFR in Australia appeared only in turf previously treated with benomyl. This report has been widely cited in both popular and scientific literature (1–3,6) as an example of a nontarget disease or one stimulated by the use of benomyl. Smiley (9) reported the occurrence of a disease in Cornell's Kentucky bluegrass cultivar trials that appeared identical to that described by Smith et al on bentgrass (11). This disease was greatest in areas treated with fungicides and less active or absent in the untreated areas (9). In Maryland and Delaware we have observed SFRs occurring on turf with no previous fungicide applications. These observations indicate that the occurrence of SFRs cannot always be regarded as a nontarget effect of fungicide use.

Differences among these studies can be attributed to working with unknown species that are possibly different biotypes of the same fungus, or even different species. Our fungal isolates (DE and MD) have similar morphological characteristics to those described by Smith et al (11) and occur in the field under similar weather conditions (i.e., those that favor the development of dollar spot incited by *Sclerotinia homoeocarpa* F. T. Bennett). Results of our growth curve temperature studies indicate that 25 C is the optimum for the DE and MD isolates. This is the same temperature that Smith et al (11) used to promote growth of their isolate in culture. Our isolates did, however, produce more mean growth at day 8 at 25 C (DE = 46.5 mm, MD = 60.3 mm) than the Smith et al (11) isolate (20.4 mm).

Benomyl was included in the in vitro study to examine its possible role in predisposition to SFRs and because the MD isolate was found in turf previously treated with benomyl. When benomyl is dissolved in water it breaks down to give carbendazim (the active moiety) and side chain products (4). Carbendazim was included to determine if the side chain products of benomyl were responsible for any possible observed effects. Iprodione was included because the MD isolate was found in plots previously treated with this fungicide.

We observed no growth stimulation of either isolate (DE or MD) by any fungicide at any rate. The rates of benomyl tested include the rates at which Smith et al (11) observed stimulation of growth of their isolate. As fungicide rate increased growth decreased in both isolates. The MD isolate, which has a history of fungicide exposure, was more sensitive to the high rates of fungicide than the DE isolate, which had never been exposed to fungicides. There is little evidence for selection for fungicide stimulation in the MD isolate or enhancement of growth of the DE isolate by fungicides. This is true for growth both during fungicide exposure and after removal from fungicide-amended media.

Smith et al (11) suggested that benomyl may predispose turf to SFRs by stimulating the growth of the causal fungus, by reducing the population of its competitors, or both. Our in vitro data do not support the theory of stimulation of fungal growth by benomyl or any

Table 1. Mean colony diameter of a Delaware isolate (DE) and a Maryland isolate (MD) of superficial fairy ring fungi incubated for 8 days on fungicide-amended PDA

	Mean colony diameter (mm)					
	Fungicide[a]					
Rate	Benomyl		Carbendazim		Iprodione	
(μg a.i./ml)	DE	MD	DE	MD	DE	MD
0	46.3	55.5	46.3	55.5	46.3	55.5
0.5	45.6	50.6	44.6	50.4	43.3	56.1
1.0	45.1	50.3	44.4	43.9	41.0	54.6
2.5	41.1	37.9	41.1	36.4	33.5	42.2
5.0	26.5	13.7	30.3	18.4	27.2	31.4
10.0	6.8	1.9	14.1	5.4	16.0	12.7
25.0	3.8	1.0	7.3	1.7	10.5	2.8
50.0	3.0	0.2	4.3	0.9	8.5	2.2
100.0	2.2	0.0	3.4	0.5	7.8	2.1

[a] $LSD_{0.05} = 1.32$ was used to separate individual rate means within a column.

Table 2. Mean colony diameter of a Delaware isolate (DE) and a Maryland isolate (MD) of superficial fairy ring fungi initially grown on benomyl-amended PDA for 17 days then transferred to unamended PDA and incubated for 5 days

Initial benomyl concentration	Colony diameter (mm)[a]	
(μg/ml)	DE	MD
0	23.0	31.4
0.5	24.8	29.1
1.0	24.0	30.3
2.5	24.5	30.8
5.0	25.6	23.3
10.0	22.8	12.9
25.0	19.4	11.5
50.0	21.4	18.1
100.0	18.0	13.6

[a] $LSD_{0.05} = 2.57$ was used to separate isolate means within a column.

other fungicide. Many of our observations of SFRs in the field have been in newly established areas with no history of fungicide use, and thus, do not support SFRs as nontarget effects of fungicide use. It is possible that any predisposition to disease is due to effects on the population of competitors. Smiley (10) notes that SFRs are associated with the use of fungicides that are more toxic to the ascomycetes and deuteromycetes than to basidiomycetes. Therefore, where fungicides are used, organisms that are competitors or antagonists of basidiomycetes that cause SFRs are suppressed. In newly established areas with no history of fungicide use, it is possible that sufficient populations of competitors or antagonists may not yet be present to result in suppression of SFRs. It is feasible that certain species or genera of SFR fungi may be stimulated by fungicides, but we did not find evidence for this with our isolates.

ACKNOWLEDGMENTS

We wish to thank Marla McIntosh and Thomas Harris for statistical advice and James Christy, Superintendent at Cripple Creek Golf and Country Club, for valuable assistance.

LITERATURE CITED

1. Brede, A. D. 1980. Side effects of fungicides. Golf Course Manage. 48(6):30-35.
2. Erwin, D. C. 1973. Systemic fungicides: Disease control, translocation, and mode of action. Annu. Rev. Phytopathol. 11:389-422.
3. Griffiths, E. 1981. Iatrogenic plant diseases. Annu. Rev. Phytopathol. 19:69-82.
4. Hammerschlag, R. S., and Sisler, H. D. 1973. Benomyl and methyl-2-benzimidazolecarbamate (MBC): Biochemical, cytological and chemical aspects of toxicity to *Ustilago maydis* and *Saccharomyces cerevesiae*. Pestic. Biochem. Physiol. 3:42-54.
5. Jackson, N. 1972. Superficial invasion of turf by two Basidiomycetes. J. Sports Turf Res. Inst. 48:20-24.
6. Munnecke, D. E. 1972. Factors affecting the efficacy of fungicides in soil. Annu. Rev. Phytopathol. 10:375-398.
7. Redhead, S. A., and Smith, J. D. 1981. A North American isolate of *Coprinus kubickae* associated with a superficial fairy ring. Can. J. Bot. 59:410-414.
8. SAS Institute, Inc. 1985. SAS User's Guide: Statistics. Version 5 ed. SAS Institute, Inc., Cary, NC. 956 pp.
9. Smiley, R. W. 1981. Nontarget effects of pesticides on turfgrasses. Plant Dis. 65:17-23.
10. Smiley, R. W. 1983. Compendium of Turfgrass Diseases. American Phytopathological Society, St. Paul, MN. 102 pp.
11. Smith, A. M., Stynes, B. A., and Moore, K. J. 1970. Benomyl stimulates growth of a Basidiomycete on turf. Plant Dis. Rep. 54:774-775.
12. Smith, J. D., and Jackson, N. 1981. Superficial fairy rings. Page 555 in: Proc. Int. Turf Res. Conf. 4th. R. W. Sheard, ed. Guelph, Ontario.