Flutolanil for Control of Necrotic Fairy Rings on Bermudagrass Putting Greens

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SUMMARY. **Type 1 (necrotic) fairy rings in turfgrass result in dead or badly damaged grass. This type of fairy ring is a severe problem on golf course greens as they interfere with the aesthetics and playability of the putting surface. In Florida, *Lycoperdon* spp., basidiomycetes that produce puffball mushrooms, have been implicated as a common cause of Type 1 fairy rings on hybrid bermudagrass (*Cynodon dactylon* × *C. transvaalensis*) putting greens. The fungicide flutolanil has basidiomycetes as the sole fungal target. It is also the only carboxin-related fungicide registered for use on turfgrass. Two experiments were conducted to examine the effect of flutolanil as a curative and preventive treatment for fairy ring caused by *Lycoperdon*. One experiment, established after the rings were present, determined that flutolanil significantly reduced mushroom production. The second experiment was conducted on a golf course that had experienced Type 1 fairy rings previously. One-half of each of nine putting greens was treated with flutolanil on a preventive basis. The other half of each green served as an untreated control. Type 1 fairy rings, due to *Lycoperdon*, developed only on the untreated control half of each green. These experiments confirm that flutolanil does have curative and preventive activity against *Lycoperdon* spp. that cause Type 1 fairy rings.**

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Fairy rings are normally divided into three types, based on the symptoms expressed by the affected grass (Smith et al., 1989). As first classified by Shantz and Piemeisel (1917), a Type 1 fairy ring results in grass that is killed or badly damaged. These Type 1 fairy rings often are observed on new or rebuilt hybrid bermudagrass putting greens in Florida and, because the turf is usually killed, are referred to as necrotic fairy rings. In most cases, the soil or root-zone mix underlying these necrotic rings repels water. Also, if the ring and surrounding area are left undisturbed, small [1-inch (2.5-cm) diameter] fungal fruiting structures usually form above ground at the soil surface. These structures are basidiocarps, often referred to as puffball mushrooms. The genus of basidiomycete fungi most often associated with these necrotic fairy rings in Florida is *Lycoperdon* (personal observations).

Lycoperdon spp. have been associated with fairy rings in other areas of the southern U.S., including *L. pyriforme* in bentgrass (*Agrostis palustris*) and bermudagrass putting greens in South Carolina (Martin et al., 1994), and *L. gemmatum* in bentgrass turf in Arkansas (Dale 1973). In the experiments reported herein, *L. marginatum* was the species causing the fairy rings (J.W. Kimbrough, personal communication). In the western U.S., the puffball mushroom associated with fairy rings is *Bovista plumbea* (Gelernter and Stowell, 1999). Other puffball mushroom genera associated with fairy rings include *Calvatia* and *Scleroderma* (Couch, 1995; Smiley et al., 1992). Basidiomycetes that do not produce puffball mushrooms but are also associated with fairy rings are numerous, with *Marasmius oreades* the most common one in temperate regions (Smith et al., 1989), and *Chlorophyllum molybdites* the most common one in tropical and subtropical regions (Kimbrough, 2000).

Basidiomycete fungi also have been implicated as a possible cause of localized dry spot, areas of turf that develop water-repellent soils but do not have a typical fair ring shape (Wilkinson and Miller, 1978; York and Baldwin, 1992). In both situations, hydrophobic Type I fairy rings and localized dry spot, this hydrophobicity is either due to production of extensive fungal mycelia, which alone may be hydrophobic, or due to production of an organic coating, perhaps fungal in origin, around the soil particles in the putting green (Bond and Harris, 1964; Djajakirana and Joeregensen, 1996; Doerr et al., 2000; Tucker et al., 1990; York and Canaway, 2000). A study in Australia on nonwetting sands demonstrated that hydrophobicity was primarily due to polar waxes of plant origin and not microbial origin; however, the waxes appeared to result from the microbial breakdown of plant constituents (Franco et al., 2000). With both necrotic fairy rings and localized dry spot, the drought conditions created by the hydrophobic soil result in stress that may kill the turfgrass. It has also been speculated that organic compounds being produced by fairy ring fungi could be directly toxic to the turfgrass, as was demonstrated for *Marasmius oreades* (Lebeau and Hawn, 1963).

Methods to effectively control fairy rings of all types are lacking. Early chemical controls ranged from ferrous sulfate to a homemade solution containing formaldehyde (Smith et al., 1989). Volatile fungicides are considered the most reliable fungicides for fairy ring control (Smith, 1978). While these compounds kill the fungus, they often also kill the turfgrass. While a few modern fungicides have appeared to be effective for fairy ring control, the products were not registered for use on turfgrass (Dale, 1973; Blenis et al., 1997). It has also been noted that due to the soil hydrophobicity often associated with fairy rings, the best control will require the drenching of fungicides, with or without surfactants, rather than the classic leaf/soil topical spray program (Smith et al., 1989; Blenis et al., 1997).

Since it is not possible to artificially induce necrotic fairy rings, a researcher must use a field site as it becomes available. This may preclude testing of large numbers of fungicides or other nonfungicide treatments. In 1994, when the experiments described herein were initiated, flutolanil (ProStar 50%WP, AgrEvo USA Co., Wilmington, Del.) was the only fungicide registered for use on turfgrass that had basidiomycete fungi as its sole fungal target. Therefore, this was the only fungicide evaluated for curative and preventive effects on Type 1 fairy rings in this study. Preliminary data have been presented previously (Fidanza et al., 2000).

Materials and methods

Experiment 1. The experimental area was a research putting green located at the Fort Lauderdale Research and Education Center, Florida. This green was built and planted with 'Tifdwarf' hybrid bermudagrass in late 1992. It was maintained by mowing six times each week at a height between 5/32 inch (4.0 mm) and 3/16 inch (4.8 mm). Nitrogen applications of 18 lb/1000 ft^2 (878 kg·ha^{-1}) per year were made using polymer-coated urea as the nitrogen source in a 12N–0P–10K fertilizer blend. No chemical pesticides were applied during the experimental period (August and September 1994). No fungicides had ever been used on the experimental site before initiation of this experiment. Also, mowing was suspended when necessary to allow for the development of mushrooms.

Necrotic fairy rings and associated mushrooms began to appear in mid-July 1994. The experiment was initiated in mid-August 1994, using a split-plot design. Main plot treatments consisted of two cultural treatments, core aerification or no core aerification. The main-plot treatment of core aerification was completed on 19 Aug. 1994, using a solid tine aerifier with 5/8-inch (15.9-mm) diameter tines to a depth of 2.5 inches (6.35 cm). Both core aerified and nonaerified main plots were then topdressed with a 1/8-inch (3.2-mm) layer of a root-zone mix consisting of 80% sand and 20% Canadian sphagnum peat. Main plots were 10 × 20 ft (3.0 × 6.1 m) in size. The subplots were 5 × 5 ft (1.5 m) in size. Each main × subtreatment combination was replicated three times, except the control which was replicated six times.

The subplot treatments included the fungicide flutolanil and the soil wetting agent AquaGro•L (47% polyoxyethylene esters of cyclic acids, 47% polyoxyethylene esters of alkylated phenols, and 6% silicon antifoam emulsion; Aquatrols, Cherry Hill, N.J.), and combinations of these two chemical compounds. Flutolanil was applied at a.i. 3 oz/1000 ft^2 (9.15 kg·ha^{-1}). AquaGro•L was applied at 8 fl oz/1000 ft^2 (25.5 L·ha^{-1}). Treatments were: 1) flutolanil applied once; 2) AquaGro•L applied once; 3) flutolanil mixed with AquaGro•L and applied once; 4) flutolanil mixed with AquaGro•L and applied once, followed by subsequent weekly applications of AquaGro•L alone for 3 consecutive weeks; 5) AquaGro•L applied once per week for 4 consecutive weeks; 6) flutolanil applied once, followed by subsequent weekly applications of AquaGro•L for 3 consecutive weeks; and 7) untreated control. Chemical treatments were initiated on 23 Aug. 1994. Materials were mixed with deionized water, and applied as a drench at the rate of 20 gal/1000 ft^2 (8149 L·ha^{-1}) in two different directions using a watering can. After all materials had been applied, the area was irrigated with 1/10 inch (2.5 mm) water.

Plots were evaluated by counting the number of mushrooms present on 30 Aug., 8 Sept. and 22 Sept. After mushrooms were counted on each date, the plots were immediately mowed to remove the mushrooms present.

Analysis of variance was performed using the GLM procedure (SAS Institute, Cary, NC). Means were compared using the Waller Duncan k ratio *t* test.

Experiment 2. For this experiment, nine putting greens on a golf course in Sebring, Fla. (Sun N Lake Golf and Country Club) were used. These nine greens were rebuilt and planted with 'Tifdwarf' hybrid bermudagrass in May 1996. On 11 Nov. 1996, the greens were overseeded with 'Gator' perennial ryegrass. Normal maintenance practices for a Florida golf course were used during the experiment. There were only two treatments in this experiment. One half of each green was treated with a tank mix of flutolanil [a.i. 1.5 oz/1000 ft^2 (4.58 kg·ha^{-1})] and Primer soil wetting agent [6 fl oz/1000 ft^2 (19.1 L·ha^{-1}); Aquatrols, Cherry Hill, N.J.). Primer is composed of polymeric polyoxyalkylenes and oxoalkenyl hydroxy polyoxyalkane diyl. The tank mix was applied with a commercial sprayer at a water delivery rate of 3 gal/1000 ft^2 (1222 L·ha^{-1}). The other half of the green was an untreated control. The chemical application began on 19 Sept. 1996 and was reapplied at 6-week intervals through Jan. 1997 for a total of four applications.

Necrotic rings began to appear on the untreated half of the greens in February 1997. On 13 Mar., the number of rings, their diameter and turf injury were evaluated on each green. Turf injury for each untreated green half was evaluated based on density and color of the turfgrass. Analysis of variance was performed using the ANOVA procedure (SAS Institute, Cary, N.C.).

Results and discussion

Experiment 1. This experiment was initiated to evaluate the effectiveness of flutolanil as a curative control for fairy rings. Before the chemical treatment applications, extensive formation of necrotic rings had occurred and a few mushrooms had developed at this site. These mushrooms previously had been identified as *Lycoperdon marginatum*. Although a few mushrooms had been observed in the area before chemical treatments, a substantial increase in mushroom production occurred after treatments were applied due to extensive rainfall [3.5 inches (8.89 cm) the last week of August and 13.0 inches (33.02 cm) during September]. Since the putting green was at the research center, mowing could be temporarily suspended to allow for undisturbed mushroom production. This rainfall, however, did eliminate the soil hydrophobicity and, therefore, the necrotic ring symptoms as well. This prevented evaluation of the treatments on that basis. Therefore, treatment evaluation was based solely on the direct effect on the fungus, as measured by mushroom production.

There were no significant differences between core aerification and no aerification on mushroom production (P = 0.27 for 30 Aug., P = 0.17 for 8 Sept., and P = 0.06 on 22 Sept.), and there were no significant interactions between aerification treatments and chemical treatments (P = 0.52 for 30 Aug., P = 0.83 for 8 Sept., and P = 0.26 for 22 Sept.). Therefore, data concerning mushroom numbers were analyzed across aerification treatments. Table 1 is a summary of the effect of the chemical treatments on *L. marginatum* mushroom production. Although the data obtained for 8 Sept. were not significant, the trends were similar to the other two dates. The use of AquaGro•L alone, either applied once or on a weekly basis, did not significantly ($P \leq 0.05$) decrease mushroom production compared to the untreated control. However, all treatments containing flutolanil did result in significantly fewer mushrooms than the control, whether applied alone, in a tank mix with AquaGro•L, or supplemented with weekly applications of AquaGro•L. Although not significantly different, it should be noted that the least number of mushrooms was associated with the flutolanil/AquaGro•L tank mix application supplemented with weekly applications of AquaGro•L. After the 22

Table 1. Effect of chemical treatments on mushroom production of *Lycoperdon marginatum* on a bermudagrass putting green.

Treatment	No. of applications[y]	No. of mushrooms[z]			
		30 Aug.	8 Sept.	22 Sept.	Total
Flutolanil	1	3.3 bc	3.2	2.0 c	8.5 bc
Flutolanil	1				
plus AquaGro•L	4	1.7 c	7.2	4.3 bc	13.2 bc
AquaGro•L	1	5.3 ab	23.7	17.7 abc	46.7 ab
AquaGro•L	4	7.3 a	20.3	25.0 ab	52.7 a
Flutolanil/AquaGro•L tank mix	1	1.0 c	0.7	4.3 bc	6.0 c
Flutolanil/AquaGro•L tank mix	1				
plus AquaGro•L	4	0.2 c	0.8	0.0 c	1.0 c
Control	---	8.2 a	11.2	28.2 a	47.5 a
$P > F$	---	0.0001	0.0793	0.0118	0.0061

[z]Values are means of six replicate plots, except for the control treatment, which are means of twelve replicate plots. Means in the same column followed by the same letter are not significantly different ($P \leq 0.05$), according to Waller-Duncan k ratio *t* test.
[y]Chemical treatments were initiated on 23 Aug. 1994. AquaGro•L soil wetting agent (Aquatrols, Cherry Hill, N.J.) was reapplied on 31 Aug., 7 Sept., and 14 Sept. Flutolanil fungicide was applied at a.i. 3 oz/1000 ft^2 (9.15 kg·ha^{-1}) as ProStar 50WP (AgrEvo USA Comp., Wilmington, Del.). AquaGro•L was applied at 8 fl oz/1000 ft^2 (25.5 L·ha^{-1}).

Sept. flush of mushroom growth, very few mushrooms were produced. No necrotic rings or mushrooms were produced the following summer in this experimental area; therefore, the experiment could not be repeated at this site.

EXPERIMENT 2. This experiment was conducted on a golf course. Severe necrotic fairy rings had developing during previous renovation projects at this course. Therefore, the membership was willing to allow the nine newly renovated putting greens of 1996 to be used for experimental purposes to determine the efficacy of flutolanil in the prevention of necrotic fairy rings. On all nine putting greens, the half that received the flutolanil fungicide treatment did not develop any necrotic fairy rings. The untreated half of all nine greens did develop necrotic fairy rings, beginning in February 1997. The number of rings per untreated green half averaged 23 and ranged from 5 to 38 on 13 Mar. (Table 2). Compared to the treated green halves, this was highly significant ($F = 39.39$; $P = 0.0001$). No additional data were collected because, due to these results, the untreated halves of each green were treated with flutolanil by the superintendent. By May 1997, rings and mushrooms on the previously untreated halves had disappeared, and the grass had fully recovered.

At the time these experiments were initiated, flutolanil was the only fungicide registered for use on turfgrass that had basidiomycetes as the specific fungal target. Flutolanil is a member of the benzanilide group of fungicides, which are based on the carboxin fungicide structure (Kulka and von Schmeling, 1995). These fungicides target Complex II of the succinate oxidase system within the fungal mitochondria (Schewe and Lyr, 1995).

In previous in vitro studies, the most effective fungicides for inhibiting *Lycoperdon* were carboxin and the carboxin-related fungicide, furcarbanil (BAS 3191 F; BASF Corp., Research Triangle Park, N.C.) (Dale, 1973). When these materials were evaluated as drenches on bentgrass, carboxin resulted in phytotoxicity, even at low levels [400 mg·mL^{-1} (ppm)], whereas furcarbanil was not phytotoxic to bentgrass, even at the highest application rate (2000 mg·mL^{-1}) used in the study (Dale, 1973). The other systemic fungicides registered for use on turfgrass in 1994 that had basidiomycete activity were the demethylation-inhibiting and benzimidazole fungicides. However, previous studies had indicated the demethylation-inhibiting fungicides were not a good choice due to their potential phytotoxicity effects on close-cut bermudagrass (Elliott, 1995; Elliott, 1999). In vitro studies had demonstrated little inhibition of *Lycoperdon* by benzimidazole fungicides, even at 6000 mg·mL^{-1}, and similar results were obtained with com-

Table 2. Fairy ring counts and sizes of rings on untreated halves of nine putting greens at the Sun N Lake Golf and Country Club, Sebring, Fla., on 13 Mar. 1997. The green halves treated with flutolanil had no fairy rings.

Green	Total no. of rings	Total no. of rings with diam of (in ft)				Turf injury[y]
		0–1[z]	1–2	2–3	>3	
1	14	7	6	1	0	Severe
2	5	2	2	1	0	Light
3	30	7	14	6	3	Severe
4	30	14	10	5	1	Severe
5	22	4	12	6	0	Moderate
6	38	12	8	13	5	Severe
7	23	6	12	4	1	Severe
8	11	7	1	2	1	Severe
9	22	13	4	3	2	Severe

[z]1 ft = 0.3 m.
[y]Turf injury for each green half was based on density and color of the turfgrass. Light = turfgrass had a noticeable color difference in the ring pattern; moderate = turf was thin in ring pattern and a noticeably different color than surrounding turfgrass; severe = turfgrass in the ring was dead.

mon contact fungicides used in the golf course industry, such as maneb, mancozeb, and chlorothalonil (Dale, 1973).

In Expt. 1, the effect observed was a direct one on the fungus, since mushroom production was reduced. The use of the soil wetting agent AquaGro•L alone had no effect on mushroom production. This is in contrast to the results obtained with *M. oreades* in a Canadian study (Blenis et al., 1997). However, different soil wetting agents [Sylgard (Setre Chemical Comp., Memphis, Tenn.) and Silwet L-77 (Dow Corning Corp., Midland, Mich.)] were used in the Canadian study, and the fungicide evaluated was chlorothalonil, a product not normally associated with fairy ring control. The reason for using soil wetting agents with the fungicides is the hydrophobic soil layer that develops with fairy rings and that resists penetration by fungicide solutions (Nadeau et al., 1993).

In Expt. 2, it was not possible to definitely determine if the fungicide alone, the soil wetting agent alone, or the combination of the two products were preventing development of necrotic fairy rings, since there was no treatment using the flutolanil alone or the soil wetting agent alone. It only was possible to have two treatments on the golf course. Since the flutolanil label does recommend the use of a surfactant (wetting agent) when applications are made for fairy ring control, such a mixture was used. However, since the necrotic fairy rings that developed on the untreated half of the golf greens were due to *Lycoperdon* spp., it seems more likely that the fungicide was the active compound in the treatment. The conclusion from an in vitro study with the surfactant Silwett L-77 was that it was not likely that the surfactant would be able to directly kill the fungus if it was applied to the soil (Nadeau et al., 1993). Furthermore, studies in California also have demonstrated that while the fungicides flutolanil and azoxystrobin (Heritage; Syngenta Crop Protection, Inc., Greensboro, N.C.) alleviated both fairy ring and localized dry spot symptoms, the Primer soil wetting agent alleviated only localized dry spot and not fairy ring symptoms (Gelernter and Stowell, 1999).

A recent study in Georgia demonstrated that after hydrophobic soils have developed, flutolanil alone did not decrease soil water repellency, whereas the use of Primer soil wetting agent alone did (Karnock and Tucker, 2001). However, since there were no fairy rings observed in the Georgia experiment, it was not known if the hydrophobicity was due to fungi. It also was noted in the Georgia experiment that core aerification did not have a significant effect on eliminating water repellency.

In summary, the use of flutolanil fungicide was effective for curative and preventive control of the fairy ring fungus *Lycoperdon*. However, as the study in Georgia has demonstrated, the fungicide should not be relied on to eliminate hydrophobicity of putting green soils. Therefore, if the necrotic rings are present and soil hydrophobicity has developed already, soil wetting agents should be incorporated into the recovery program.

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