Fungicide Effects on *Acremonium* Endophyte, Plant-Parasitic Nematodes, and Thatch in Kentucky Bluegrass and Perennial Ryegrass

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ABSTRACT

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Foliar applications of benomyl, chlorothalonil, iprodione, thiram, and triadimefon were assessed for possible nontarget effects on *Acremonium loliae*, plant-parasitic nematodes, thatch accumulation, and soil pH. Fungicides were applied monthly from April to September during 1983–1987 to field plots of Regal and Fiesta perennial ryegrass (*Lolium perenne*) and South Dakota, Merion, Sydsport, and Vantage Kentucky bluegrass (*Poa pratensis*). None of the fungicides significantly affected soil pH and only thiram enhanced thatch significantly when compared with untreated plots. Fungicides did not significantly affect population densities of plant-parasitic nematodes. Nematode species showed significant cultivar preferences: *Xiphinema americanum* and *Hoplolaimus galeatus* for Fiesta and Regal, *Pratylenchus penetrans* for Sydsport, *Helicotylenchus pseudorobustus* for South Dakota and Merion, and *Tylenchorhynchus* spp. for South Dakota and Vantage. Fungicides did not significantly reduce the percent of perennial ryegrass plants infected with *A. loliae*.

Additional keyword: turfgrass

Among the deleterious, nontarget effects of fungicides applied to turfgrasses is enhanced accumulation of thatch (7,8). Smiley and Craven (7) studied the nature of thatch accumulation in Kentucky bluegrass (*Poa pratensis* L.) as influenced by long-term use of fungicides. They (7) reported that benomyl and high sulfur-containing fungicides such as mancozeb, maneb, and thiram acidified soil. Soil acidification presumably suppressed the decomposition capabilities of microorganisms, and thatch accumulated. Cadmium succinate and iprodione also caused thatch to accumulate, which Smiley and Craven (7) attributed to direct toxicity to microorganisms. After further study, Smiley et al (8) determined that the mechanism of thatch buildup induced by fungicides was an increase in the rate of root and rhizome production rather than a fungicide-induced reduction in the rate of thatch decomposition. Halisky et al (3) also reported that applications of benomyl and iprodione resulted in increased thatch in Kentucky bluegrass.

The influence of fungicides on plant-parasitic nematodes and *Acremonium* endophyte in turfgrasses has received little study. Laughlin and Vargas (5) and Halisky et al (3) reported that benomyl drenches reduced stunt nematode (*Tylenchorhychus* sp.) populations in turf. Latch and Christensen (4) observed that propiconazole and prochloraz seed treatment and benomyl drenches to potted plants eliminated an unidentified endophyte from perennial ryegrass. Although *Acremonium* endophyte causes maladies when ingested by animals (6), it provides improved insect resistance, persistence, and performance in some turfgrass species (2). Hence, control of plant-parasitic nematodes in turf by fungicides may be considered desirable, whereas eradication of *Acremonium* endophyte or enhancement of thatch would be deleterious.

The objectives of this study were: 1) to determine if multiple foliar fungicide applications would affect plant-parasitic nematode populations in soil underlying Kentucky bluegrass and perennial ryegrass or the survival of *Acremonium* endophyte in perennial ryegrass and 2) to gather more information on thatch accumulation and soil pH as influenced by fungicides.

MATERIALS AND METHODS

The study area was established at the University of Maryland Turfgrass Research and Education Facility in September 1981. Soil was a Chillum silt loam (fine silty, mixed mesic Typic Hapludult) with a pH of 6.8 (1:1 soil:distilled H_2O) and 19 mg of organic matter per gram of soil. Soil was not fumigated at any time before turf was established.

Four Kentucky bluegrass (*Poa pratensis* L.) cultivars—South Dakota, Merion, Sydsport, and Vantage—were seeded at the rate of 98 kg/ha of seed. Two perennial ryegrass (*Lolium perenne* L.) cultivars—Fiesta and Regal—were seeded at 293 kg/ha of seed. The seedbed was rototilled to a depth of 20 cm, cultipacked, dragged with a steel mat, and fine-graded by hand-raking. Before seeding, a 10-3-3 (ammonium-based N source) fertilizer was applied to supply 75 kg/ha of N. Individual plots were hand-seeded and raked, and the seedbed was firmed with a roller. The study area was maintained at a height of 6–8 cm, and clippings were not removed. Turf was irrigated as required to prevent severe drought stress and was fertilized two or three times annually in the fall with a total of 100–150 kg/ha of N from either urea or the aforementioned 10-3-3 fertilizer. The site was treated once a year in fall with a mix of 2,4-D + MCPP + dicamba to control broadleaf weeds. In April of 1986 and 1987, oxadiazon was applied and immediately watered in to control annual grass weeds.

Fungicides were applied to the same plots midmonth ±2 days from April to September during 1983–1987 for a total of six applications per year for 5 yr. When fungicide treatments were initiated, thatch levels were negligible (<0.5 cm). The fungicides and rates evaluated were: benomyl, 3.1 kg a.i./ha; chlorothalonil, 9.2 kg a.i./ha; iprodione, 3.1 kg a.i./ha; thiram, 13.8 kg a.i./ha; and triadimefon, 1.5 kg a.i./ha. Fungicides were applied in 1,020 L/ha of water at 260 kPa, and generally no rain or irrigation followed within 24 hr of application.

Plots were arranged in a randomized complete split-plot design with four replications. The cultivar main plots were 3 × 6 m and the fungicide subplots were 1 × 3 m. Thatch depth and soil pH were assessed in September 1987. Uncompressed thatch depths were measured with a ruler from three 5 cm wide × 8 cm deep plugs randomly removed from each plot. The three thatch measurements per plot were averaged for each replicate for the statistical analysis. Three 2 cm wide × 6–8 cm deep plugs were taken randomly from each plot, and

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thatch was removed for pH determination. The soil from each plot was mixed, air-dried, and ground. A 5-g composite sample from each plot was placed in 10 ml of 0.01 M $CaCl_2$, stirred, and allowed to equilibrate 1 hr before pH measurement.

Soil was sampled for plant-parasitic nematodes in September 1986 and 1987. Twelve 2 cm wide × 12 cm deep plugs were taken from each plot, thatch was removed, and soil was mixed. Nematodes were extracted from a 250-cm^3 composite soil sample from each plot by means of a roiling-sieving-Baermann funnel technique. Nematode adults plus juveniles were counted and identified at ×40 magnification. Species identifications were made from at least 10 randomly selected adult species of each genus with a compound light microscope. In June 1988, perennial ryegrass plants were assessed for percent of plants infected with *A. loliae* Latch, Christensen & Samuels. Six 5 cm wide × 8 cm deep plugs were selected randomly from each plot. Two plants were selected from each of six plugs, for a total of 48 plants per fungicide treatment. Dead leaf and sheath as well as root tissues were removed from each plant. A 1-cm-long section of sheath tissue was taken from the base of the second oldest living leaf of each plant. Sheath tissue was gently heated on a glass microscope slide for 30 sec in 0.05% trypan blue in lactophenol stain. Each sheath was examined for mycelium of *A. loliae* at ×400 magnification, and the stained mycelium observed was identical to that illustrated by Siegel et al (6). Infection percentages were not determined at the beginning of the experiment, and we assumed that seed infection by *A. loliae* was uniform at planting. Infection percentage was calculated by dividing the number of infected plants per plot by the total number of plants analyzed from each plot. All data were subjected to analysis of variance, and significantly different means were separated using the least significant difference multiple comparison test at $P = 0.05$.

RESULTS

None of the fungicides significantly reduced the percentage of perennial ryegrass plants infected with the *Acremonium* endophyte (Table 1). Regal, however, had significantly higher infection than Fiesta. The potential *A. loliae* infection percentages of Regal seed generally exceed 70%, whereas those for Fiesta seed are normally less than 15% (P. M. Halisky, *personal communication*). With Regal, infection percentages were significantly higher in benomyl- and thiram-treated plants than in chlorothalonil-treated plants. Percentage infection of Fiesta plants was very low, particularly in triadimefon-treated plants. There were, however, no significant infection percentage differences among Fiesta plants treated with fungicides. As previously noted, Fiesta is naturally low in endophyte, so the low infection percentages observed were not due to any of the treatments imposed.

Population densities of plant-parasitic nematodes in soil were not significantly affected by application of any fungicide, so the data were combined over fungicides to show host-nematode preferences (Table 2). Five plant-parasitic nematode species were detected in appreciable numbers among the six turf cultivars in both sampling years: dagger (*Xiphinema americanum* Cobb), lance (*Hoplolaimus galeatus* Cobb), lesion (*Pratylenchus penetrans* (Cobb) Chitwood & Oteifa), spiral (*Helicotylenchus pseudorobustus* (Steiner) Golden), and stunt (*Tylenchorhynchus claytoni* Steiner and *T. maximus* Allen); stunt nematode species counts were combined for data analyses (Table 2).

Several host species and cultivar preferences were detected among nematode species (Table 2). Lesion nematode populations tended to be highest from soil underlying Sydsport, Vantage, and Merion and lowest from soil under South Dakota, Fiesta, and Regal. Lesion nematode numbers were significantly higher in Sydsport than in South Dakota, Regal, or Fiesta during both 1986 and 1987. Spiral nematode counts were highest in South Dakota and Merion and lowest in Regal. Spiral nematode counts in Sydsport and Vantage were intermediate, although differences generally were not significant among Kentucky bluegrass cultivars. Stunt nematode counts were significantly higher in Vantage and South Dakota than in Sydsport in both years and Merion in 1987. Stunt nematode levels were extremely low in soil underlying Sydsport in both years and were highest both years in Vantage plots. Stunt nematode population densities were intermediate in the ryegrasses. Levels of lance nematodes were similar in soil underlying Regal, Fiesta, South Dakota, and Vantage. Lance nematode counts were lowest in Sydsport and Merion but were not significantly different from those in South Dakota and Vantage. Dagger nematodes showed the most obvious preferences; population densities were very low among all blue-

Table 1. Soil pH, thatch depth, and infection by *Acremonium loliae* as influenced by foliar fungicide applications to perennial ryegrass

Fungicides[v]	Soil (pH)	Thatch depth[w] (mm)	Percent infected[x] Regal	Percent infected[x] Fiesta
Benomyl	6.3 ab[y]	17 ab[y]	79 a[z]	10 c[z]
Triadimefon	6.3 ab	17 ab	71 ab	2 c
Iprodione	6.4 a	16 bc	73 ab	21 c
Chlorothalonil	6.3 ab	15 c	56 b	10 c
Thiram	6.2 b	18 a	83 a	10 c
Untreated	6.3 ab	16 bc	71 ab	10 c

[v] Fungicides were applied monthly between April and September during 1983 through 1987.
[w] Measurements were determined from samples collected in September 1987.
[x] Plants were sampled for endophyte in July 1988.
[y] Means in a column followed by the same letter are not significantly different at $P = 0.05$ according to the Bayes LSD.
[z] Means in columns and rows followed by the same letter are not significantly different at $P = 0.05$ according to the Bayes LSD.

Table 2. Plant-parasitic nematode[x] population densities from soil underlying two cultivars of perennial ryegrass and four cultivars of Kentucky bluegrass per 250 cm^3 of soil[y]

Cultivar/species	Lesion 1986	Lesion 1987	Spiral 1986	Spiral 1987	Stunt 1986	Stunt 1987	Lance 1986	Lance 1987	Dagger 1986	Dagger 1987
Regal/perennial ryegrass	16 bc[z]	15 c	29 bc	105 c	20 bc	99 ab	33 a	68 ab	57 b	133 b
Fiesta/perennial ryegrass	15 bc	14 bc	16 c	225 bc	21 bc	36 b	52 a	71 a	229 a	193 a
South Dakota/Kentucky bluegrass	14 c	6 c	76 a	652 a	42 ab	113 a	31 ab	22 abc	8 c	14 c
Merion/Kentucky bluegrass	36 ab	25 b	76 a	411 ab	19 bc	33 b	8 b	12 bc	2 cd	10 cd
Sydsport/Kentucky bluegrass	68 a	72 a	11 c	197 bc	5 c	3 c	4 b	11 c	3 cd	7 cd
Vantage/Kentucky bluegrass	44 a	31 b	40 ab	194 bc	76 a	173 a	18 ab	32 abc	1 d	3 d

[x] Lesion (*Pratylenchus penetrans*), spiral (*Helicotylenchus pseudorobustus*), stunt (*Tylenchorhynchus claytoni* and *T. maximus*), lance (*Hoplolaimus galeatus*), and dagger (*Xiphinema americanum*).
[y] Soil was collected down to 10–12 cm in September 1986 and 1987.
[z] Means in a column followed by the same letter are not significantly different at $P = 0.05$ according to the Bayes LSD.

grass cultivars and high in the ryegrasses during both 1986 and 1987. Dagger nematode populations were significantly higher in soil from Fiesta plots than in soil from Regal plots during both years.

No cultivar × fungicide interactions for soil pH or thatch depth were observed, so soil pH and thatch depth data were combined over fungicides and cultivars. Thatch accumulation was greatest among the bluegrass cultivars, and significant differences among the cultivars were: Sydsport (20 mm) = Vantage (19 mm) >Merion (18 mm) = South Dakota (17 mm) >Regal (13 mm) >Fiesta (11 mm). When data were combined over fungicides, thiram-treated turf had significantly deeper thatch than untreated plots (Table 1). No other fungicide had significantly promoted thatch accumulation when compared with untreated turf.

No significant differences in soil pH were detected among fungicide treatments when compared with untreated turf (Table 1). Soil from thiram-treated plots was more acidic than soil from iprodione-treated plots. When combined over fungicides, the data revealed that the pH of soil underlying the perennial ryegrasses (6.1–6.2) was significantly more acidic than soil underlying South Dakota, Sydsport, or Vantage (pH = 6.4) but not Merion (pH = 6.3) (*data not shown*).

DISCUSSION

Smiley and Craven (7) reported that benomyl, cadmium succinate, iprodione, mancozeb, and thiram caused thatch to accumulate significantly in Kentucky bluegrass. These researchers (7) initially attributed fungicide-induced thatch accumulation to inhibition of microbial degradation either by acidifying soil or by direct fungicide toxicity to microorganisms. In our investigation, lowest soil pH and deepest thatch were detected in thiram-treated plots. Smiley and Craven (7) observed that thiram acidified soil and the lower pH may have inhibited microbial activity, allowing thatch to accumulate. There were, however, few statistically significant or agronomically important differences in thatch depth (17–20 mm for Kentucky bluegrass and 11–13 mm for perennial ryegrass) or soil pH (6.1–6.4) among treatments at the end of our 5-yr study. Later, Smiley et al (8) reported that fungicides caused thatch to accumulate by eliciting an increase in root and rhizome growth, rather than by retarding thatch decomposition. The differences observed in thatch accumulation between our study and those in New York (7,8) could be attributed to the following: 1) more frequent fungicide applications in New York (7–9 vs. 6 per year), 2) generally higher N fertility in New York (200 kg/ha vs. 100–150 kg/ha per year), 3) more thatch in the Kentucky bluegrass in the New York study when fungicide applications were initiated (2.0 cm vs. <0.5 cm), and 4) generally better environmental conditions for growth of Kentucky bluegrass and thatch development as well as lack of severe disease injury to turf in the New York study. In our study, stripe smut (*Ustilago striiformis* (Westend.) Niessl) and summer patch (*Magnaporthe poae* Landschoot & Jackson) chronically injured Merion. Sydsport was injured significantly by summer patch, and South Dakota was damaged annually by leaf spot (*Drechslera poae* (Baudys) Shoemaker). Both perennial ryegrass cultivars were blighted by low levels of brown patch (*Rhizoctonia solani* Kühn). Frequent and severe damage to Merion and South Dakota by the aforementioned diseases may have influenced thatch accumulation in our study.

Halisky et al (3) also reported that benomyl and iprodione caused a significant accumulation of thatch in Kentucky bluegrass. In that study (3), however, fungicides were applied more frequently, at higher dosages, and in more water than in either the New York (7,8) or our studies.

Foliar applications of fungicides did not significantly reduce *Acremonium* endophyte infection in either Regal or Fiesta. Latch and Christensen (4) reported that the triazole fungicides propiconazole and etaconazole, as well as prochloraz seed treatments, eradicated an undescribed endophyte in perennial ryegrass. Benomyl soil drenches also controlled a perennial ryegrass endophyte in greenhouse pot studies (4). The fungicides used in our study represent five classes: benzimidazole (benomyl), ethylene bisdithiocarbamate (thiram), dicarboximide (iprodione), organic (chlorothalonil), and triazole (triadimefon). It cannot be definitively concluded that all fungicides representing these classes would have no impact on *Acremonium* endophyte in field-grown, mature perennial ryegrass plants. However, in view of the data and the frequency of fungicide application in our study (i.e., 30 applications over a 5-yr period to the same plots), we conclude that these fungicides (applied in 1,020 L/ha of water) do not significantly affect *Acremonium* endophyte in field-grown perennial ryegrass. Because of the beneficial attributes of endophytic fungi in turfgrasses and the extensive use of these fungicides on intensively managed turfs, these findings are important to turfgrass managers.

Vargas (9) has listed nematode species associated with turfgrasses and states that their pathogenicity is influenced by complex biotic and abiotic factors. Very little is known, however, about turfgrass cultivar preferences of plant-parasitic nematodes. Cole et al (1) found lesion, spiral, pin (*Paratylenchus* sp.), and ring (*Criconemoides* sp.) nematodes in at least 25% of Kentucky bluegrass sod fields surveyed in Pennsylvania. Similarly, Halisky et al (3) observed significant levels of stunt, lance, ring, and pin nematodes in Merion Kentucky bluegrass in New Jersey. Vittum et al (10) surveyed *Agrostis* spp. in New England and found that stunt, lance, spiral, lesion, ring (*Criconemella* sp.), and needle (*Longidorus* sp.) nematodes to be commonplace. The nematodes reported here were generally the same as those species associated with cool-season grasses in other parts of the northeastern United States. Significant host preferences determined here (Table 2) among nematodes were: dagger and lance in Fiesta and Regal, lesion in Sydsport, spiral in Merion and South Dakota, and stunt in South Dakota and Vantage.

None of the fungicides evaluated showed significant nematicidal activity. Laughlin and Vargas (5) and Halisky et al (3) reported that benomyl reduced the soil population densities of stunt nematodes in field studies. In those studies, benomyl was applied at higher rates than we used and in 12,000-16,000 L/ha of water. It is likely that those higher benomyl dosages were more effectively moved into the root zone by drenching than by the 1,020 L/ha we used. Despite the greater frequency of fungicide applications in our study, the data indicate that foliar applications of the fungicides evaluated are not likely to provide any significant control of the nematode species studied in this investigation.

LITERATURE CITED

1. Cole, H., Forer, L. B., Nelson, P. E., Bloom, J. R., and Jodon, M. H. 1973. Stylet nematode genera and *Fusarium* species isolated from Pennsylvania turfgrass sod production fields. Plant Dis. Rep. 57:891-895.
2. Funk, C. R., Halisky, P. M., Ahmad, S., and Hurley, R. H. 1985. How endophytes modify turfgrass performance and response to insect pests in turfgrass breeding and evaluation trials. Pages 137-145 in: Proc. Int. Turfgrass Res. Conf. 5th. F. Lemarie, ed.
3. Halisky, P. M., Myers, R. F., and Wagner, R. E. 1981. Relationship of thatch to nematodes, dollar spot, and fungicides in Kentucky bluegrass turf. Pages 415-420 in: Proc. Int. Turfgrass Res. Conf. 4th. R. W. Sheard, ed.
4. Latch, G. C. M., and Christensen, M. J. 1982. Ryegrass endophyte, incidence, and control. N.Z. J. Agric. Res. 25:443-448.
5. Laughlin, C. W., and Vargas, J. M. 1972. Influence of benomyl on the control of *Tylenchorhynchus dubius* with selected nonfumigant nematicides. Plant Dis. Rep. 56:546-548.
6. Siegel, M. R., Latch, G. C. M., and Johnson, M. C. 1985. *Acremonium* fungal endophytes of tall fescue and perennial ryegrass: Significance and control. Plant Dis. 69:179-183.
7. Smiley, R. W., and Craven, M. M. 1978. Fungicides in Kentucky bluegrass turf: Effects on thatch and pH. Agron. J. 70:1013-1019.
8. Smiley, R. W., Craven Fowler, M., Kane, R. T., Petrovic, A. M., and White, R. A. 1985. Fungicide effects on thatch depth, thatch decomposition rate, and growth of Kentucky bluegrass. Agron. J. 77:597-602.
9. Vargas, J. M., Jr. 1981. Management of Turfgrass Diseases. Burgess Publishing Co., Minneapolis, MN. 204 pp.
10. Vittum, P. J., Wick, R. L., and Swier, S. R. 1987. Nematodes on New England greens. Golf Course Manage. 55(8):58, 60, 62.