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9. ABSTRACT

This paper reports the results of a qualitative comparison of various genera of fungi isolated from creeping bentgrass turf plots treated with six fungicides for four and five years in comparison with a non-sprayed control plot. The fungicides used were Fore, Actidone-Thiram, Dyrene, PMA plus Thiram, Kromad plus Calaclor, and Tersan OM. There were no great differences found in the distribution of various fungal genera isolated from the nonsprayed and sprayed bentgrass turf. The high organic matter content in the soil and thatch layer associated with turf grass may be responsible for rapid chemical and/or microbial degradation of these fungicides, and may account for the lack of any qualitative effects on the fungal ecology.

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# FUNGICIDE EFFECTS ON FUNGAL ECOLOGY IN CREEPING BENTGRASS TURF *)

by

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ABSTRACT

A qualitative comparison was made of the genera of fungi associated with creeping bentgrass turf (*Agrostris palustris*) plots either nonsprayed or sprayed with six fungicides for 4 or 5 successive years. The fungicides used were:

Fore (manganese ethylene bisdithiocarbamate plus zinc ion) Actidone-Thiram (3[2—(3, 5-dimethyl-2-oxycyclohexyl)-2-hydroxy-ethyl]) glutarimide plus tetramethyl thiuram disulfide); Dyrene (2, 4-dichloro-6-0-chloroanillino-s-triazine; Kromad plus Calaclor (5% cadmium sebacate, 5% potassium chromate, 1% malachite green, 0.5% auramine, 16c tetramethyl thiuram disulfide plus 60% mercurous chloride, 30% mercuric chloride); PMA plus Thiram (phenylmercuric acetate plus tetramethyl thiuram disulfide); and Tersan OM (hydroxymercuric chlorophenol plus tetramethyl thiuram disulfide). None of the fungicides had any significant effect on the distribution of fungal genera studied.

## INTRODUCTION

Various fungicides are recommended for control of fungal diseases of creeping bentgrass (*Agrostris palustris* HUDS.) in Illinois (12). Little is known about the effects of the commonly used fungicides on the genera of fungi commonly associated with the soil and thatch layers of turfgrasses. MASSIE et al. (5) reported that the effectiveness of eight fungicides on five isolates of the phytopathogen *Sclerotinia homeocarpa* on creeping bentgrass depended upon the virulence of the isolate used as well as its tolerance to the fungicide being tested.

The purpose of this investigation was to make a qualitative comparison of various genera of fungi isolated from creeping bentgrass turf plots treated with six fungicides for 4 and 5 years in comparison with a non-sprayed control plot. GARRETT (3) states that quantitative studies using dilution techniques are almost meaningless since heavily sporulating fungi are represented out of proportion to their volume of active mycelium.

## MATERIALS AND METHODS

Six, 10′ × 20′ bentgrass turf (var. Seaside) plots received a weekly fungicide treatment for 16 weeks in each of five summers beginning in 1965 and continuing through 1969. A nontreated

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plot served as a control. Plots consisted of a 1:1 mixture of Drummer loam soil and sand, located near the Urbana-Champaign campus of the University of Illinois. The plots received a biannual topdressing consisting of nonsterile 1:1 mixture of soil and sand.

The fungicides, applied with rate in oz of active ingredient/1000 sq. ft., were: Fore (manganese ethylene bisdithiocarbamate plus zinc ion) at 4.8 oz; Actidione-Thiram (3[2-(3, 5-dimethyl-2-oxycyclohexyl)-2-hydroxy-ethyl] glutarimide plus tetramethyl thiuram disulfide) at 1.52 oz; Dyrene (2, 4-dichloro-6-0-chloroanilino-s-triazine) at 3 oz; Kromad plus Calaclor (5% cadmium sebacate, 5% potassium chromate, 1% malachite green, 0.5% auramine, 16% tetramethyl thiuram disulfide plus 60% mercurous chloride, 30% mercuric chloride) at 0.825 oz and 1.80 oz; PMA plus Thiram (phenylmercuric acetate plus tetramethyl thiuram disulfide) at 0.2 oz and 1.50 oz; and Tersan OM (hydroxymercuric chlorophenol plus tetramethyl thiuram disulfide) at 2.2 oz. All were applied as water suspensions using a hand sprayer with a constant pressure of 30 p.s.i.

There were three sampling dates: 23 September 1968 (after 4 years of fungicide treatment), and 4 and 14 June 1969 (during the fifth year). One additional sample was taken on 17 June 1969 because numerous *Trichoderma* sp colonies (40—50/plate) were recovered from the 14 June samples taken at 1 cm. Each sample consisted of two, 2 × 3 inch sod-soil cores. Samples were taken from each plot at each sampling date and placed separately in plastic bags. The coring tool was washed with a 0.52% sodium hypochloride solution before each sample was taken. All core samples were processed on the day collected, except those taken on 23 September, which were refrigerated until processed.

A modification of the technique described by RODRIGUEZ-KABANA (9) was used in isolating the fungi from the cores. Three separate soil samples were taken at a 1 cm depth from the edge of one core, and at a 3—4 cm depth from the edge of the other core. It was found by trial and error that 1 g of soil in 100 ml of sterile water, sampled with a 0.1 ml pipette, produced 30—50 colonies/plate with samples taken at 3—4 cm. One-half g of thatch in 275 ml of sterile water was used to obtain approximately 30—50 colonies/plate from the 1 cm thatch layer. Since the 1 cm soil samples contained a high percentage of decaying plant material, it was necessary to pulverize them in a soil-water solution using a Waring blendor before sampling. The 0.1 ml samples were drawn from the magnetically stirred soil-water solutions and mixed with 10 ml of molten dextrose-peptone-yeast agar in each of two plates. A separate, sterile pipette was used for each sample. The dextrose-peptone-yeast isolation medium (7) was modified to contain 100 ppm of the surfactant NPX (nonphenyl polyethylene glycol ether containing ethylene oxide concentration of 7 moles) and 30 ppm tetracycline in place of chlorotetracycline. The surfactant was used because of

its ability to suppress the growth of fast growing fungi (10). All ingredients were added before autoclaving except the antibiotics.

The plates were divided into groups of six representing the three samples from the same core and depth and incubated at room temperature (22—24°C). The groups were examined daily with a binocular microscope and the plate containing the largest number of genera was selected after 4 days. All fungi on the selected plates were identified to genus and recorded. Genera found on the other five plates that were not on the selected plate also were recorded.

## Results

There were no great differences in the types of fungi isolated from creeping bentgrass turf whether from nontreated or treated plots. Species of *Aspergillus*, *Fusarium*, *Gliocladium*, *Penicillium*, and *Trichoderma* were ubiquitous in the isolations made from all of the samples at both depths on the three sampling dates (Table I, II,

**Table I** Genera of fungi isolated at two depths measured in cm from sod-soil cores of bentgrass turf subjected to six different fungicide treatments after 4 successive years of spraying.

| | FUNGICIDE TREATMENT | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Check | | Acti-dione-Thiram | | Dyrene | | Fore | | Kromad Caloclor | | PMA Thiram | | Tersan OM | |
| | cm | | cm | | cm | | cm | | cm | | cm | | cm | |
| GENERA | 1 | 3-4 | 1 | 3-4 | 1 | 3-4 | 1 | 3-4 | 1 | 3-4 | 1 | 3-4 | 1 | 3-4 |
| Absidia | | | | X | | | | | | | | | | |
| Alternaria | | | | | | | | X | | | | | | |
| Aspergillus | | X | | X | | X | X | | X | X | X | X | | X |
| Chaetomium | | X | | X | | X | | X | | X | | X | | X |
| Curvularia | X | | X | | | | | | X | | | | X | |
| Doratomyces | | | | | | | | | | | | | | X |
| Epicoccum | | | | | | | | X | | | | | | |
| Fusarium | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Gliocladium | X | X | X | X | | X | X | X | X | X | X | X | X | X |
| Helminthosporium | | | | | | X | | | X | | X | X | | |
| Humicola | | | | | | X | | | | | | | | |
| Mucor | X | | | | | X | X | | X | | | | | |
| Penicillium | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Rhizoctonia | | | | | | | | | | | | X | | |
| Rhizopus | | | | | | | | | | | | X | | |
| Scolecobasidium | X | X | | X | X | X | | | X | | X | X | X | X |
| Stachybotrys | | X | | | | | | | | | | | | |
| Thamnidium | | | | | | | | | | | | | | X |
| Thielavia | | X | | | | | | | | | | X | | |
| Torula | | X | | | | X | | | | X | | | | |
| Trichocladium | | | | X | | | | | | | | | | |
| Trichoderma | X | X | X | X | X | X | | X | X | X | | X | X | X |
| Verticillium | | | | | | | X | X | | | | | | |

and III). A species of *Scolecobasidium* was consistently present throughout all plots except for the plot treated with Fore.

The genus *Curvularia* was isolated at the 1 cm depth more often in June than in September, and was isolated only once throughout the study at the 3—4 cm depth. In the June samples, *Curvularia* spp. were isolated from the 1 cm depth in all plots except the Dyrene plot.

*Pyrenochaeta* sp. was isolated only from the June samples and was found in all but the Dyrene plot on the 14 and 17 June sampling dates.

*Cladosporium* and *Verticillium* spp. were isolated from all plots in the 14 and 17 June samples, but only a few times on the other two dates.

Species of the genus *Chaetomium* were isolated only at the 3—4 cm depth from all plots on all sampling dates.

Other genera observed plots were inconsistent in their appearance on the isolation plates, such as: *Absidia*, *Mucor*, and *Alternaria* spp

**Table II** Genera of fungi isolated at two depths measured in cm from sod-soil cores of bentgrass turf subjected to six different fungicide treatments at the first sample during the fifth year of spraying

| | FUNGICIDE TREATMENT | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Check | | Acti-dione-Thiram | | Dyrene | | Fore | | Kromad calocior | | PMA Thiram | | Terran OM | |
| | cm | | cm | | cm | | cm | | cm | | cm | | cm | |
| GENERA | 1 | 3-4 | 1 | 3-4 | 1 | 3-4 | 1 | 3-4 | 1 | 3-4 | 1 | 3-4 | 1 | 3-4 |
| Absidia | | | | | | | | X | | | | | | |
| Alternaria | | | | | X | | | | | | | | | |
| Aspergillus | X | X | X | X | X | X | | X | X | X | X | X | X | X |
| Chaetomium | | X | | X | | X | | X | | X | | X | | X |
| Cladosporium | X | | | | | | | | | | X | | | |
| Curvularia | X | | X | | | | X | | X | | X | | X | X |
| Doratomyces | | X | | | | | | | | | | | | |
| Fusarium | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Gliocladium | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Humicola | X | | | | | | | X | | | | | | |
| Monila | | | | | | | | | | | | | | X |
| Mucor | | X | | | | | | | X | | | X | X | |
| Paecilomyces | | | | | | | | | | | | X | | |
| Penicillium | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Phoma | | X | | | | | | | | X | | | | |
| Pyrenochaeta | X | | | X | X | | X | X | | | X | | X | X |
| Sclerotinia | | | | | | | | X | | | | | | |
| Scolecobasidium | X | X | X | X | X | X | | | X | X | X | X | X | X |
| Trichocladium | | | | | X | | | | | | | | | |
| Trichoderma | X | X | X | X | X | X | | X | X | X | | X | X | X |
| Verticillium | | | | | | | | X | X | X | | | | |

Table III Genera of fungi isolated at two depths measured in cm from sod-soil cores of bentgrass turf subjected to six different fungicide treatments at the second and third sampling during the fifth year.

| | FUNGICIDE TREATMENT | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Check | | Acti-dione-Thiram | | Dyrene | | Fore | | Kromad Calclor | | PMA Thiram | | Tersan OM | |
| | cm | | cm | | cm | | cm | | cm | | cm | | cm | |
| GENERA | 1 | 3-4 | 1 | 3-4 | 1 | 3-4 | 1 | 3-4 | 1 | 3-4 | 1 | 3-4 | 1 | 3-4 |
| Absidia | | X | X | | | | X | | | | | X | | |
| Alternaria | | | | | X | | X | | | | | | | |
| Aspergillus | | X | X | X | | X | X | X | X | | X | X | X | X |
| Chaetomium | | X | | X | | X | | X | | X | | X | | X |
| Cladosporium | X | | X | | X | X | X | | X | X | X | | X | |
| Curvularia | X | | X | | | | X | | X | | X | | X | |
| Doratomyces | | | | | | | | | | X | | | | X |
| Fusarium | X | X | X | X | X | X | | X | X | X | X | X | X | X |
| Gliocladium | X | X | X | X | X | X | X | X | X | X | X | X | | X |
| Gliomastix | | | | | | X | | | | | | | | |
| Helminthosporium | | | | | | | | | X | | | | X | |
| Humicola | | | | | | | X | | | | X | | | |
| Monilia | | | | | | | | | | X | | | X | |
| Mucor | X | | X | | | | | | | X | X | | | |
| Penicillium | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Phoma | | | | | X | | | | | | | | | X |
| Pyrenochaeta | X | X | X | X | | | X | X | X | X | X | X | X | X |
| Rhizoctonia | | | | X | | | | | | | | | | X |
| Rhizopus | | X | | | | | | | | | | | | |
| Scolecobasidium | X | X | X | X | X | | X | X | X | X | X | X | X | X |
| Stachybotrys | | | | | | | | | | | | X | | |
| Thamnidium | | | | | | | | | | | | | | X |
| Trichoderma | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Verticillium | X | | X | | X | | X | X | | | | | | X |

## Discussion

The effect of fungicides on the distribution of the genera of fungi isolated in this study was not evident. The total number of genera and specific genera isolated from the check plot were very similar to those isolated from the individual plots which had received fungicide treatments for 4 and 5 years. It is possible that other genera, not isolated with the techniques used in this study, were affected by the fungicidal treatments.

The genera of fungi isolated in this study were similar to some isolated by Healy (4) and Endo (2) from plants of creeping bentgrass. Many of the genera isolated were synonymous with those isolated by Tjitrosomo (11) from forest and prairie soil in East-Central Illinois.

Both *Pyrenochaeta* and *Scolecobasidium* spp., relatively slow growing fungi, were isolated often in this study, probably because the fast growing fungi, which usually mask their growth on culture

plates, were restricted by NPX. *Pyrenochaeta terrestris*, isolated during these studies, was shown to be a pathogen on creeping bentgrass (6).

The fact that *Chaetomium* sp. was isolated only at the 3—4 cm depth in all plots may be due to the high number of competitive organisms present at the 1 cm depth.

The great increase in *Trichoderma* sp. isolated on 14 June could have resulted from an application to all plots of 1 lb actual nitrogen per 1000 $ft^2$ 10 days before sampling.

RICHARDSON (8) has reported that 90% of the amount of Thiram added to sandy soils at a rate of 50 ppm disappeared within 5 days as indicated by a bioassay with *Glomerella cingulata*. BURCHFIELD (1) has stated that it is likely that chemical and microbial degradation of fungicides occurs more rapidly in soils with high organic matter. Since the soil and thatch layers of creeping bentgrass are very high in organic matter, this may be a possible explanation for the small effect of the 4 and 5 year fungicide treatments on the distribution of various genera of fungi in these layers.

## Summary

There were no great differences found in the distribution of various fungal genera isolated from creeping bentgrass turf either nonsprayed or sprayed with six fungicides for 4 and 5 consecutive years. Species of *Aspergillus*, *Fusarium*, *Gliocladium*, *Penicillium*, *Scolecobasidium*, and *Trichoderma* were most frequently isolated at 1.0 and 3.0—4.0 cm sampling depths. *Curvularia* spp. were isolated primarily from the 1.0 cm samples, while *Chaetomium* spp. were isolated only from the 3.0—4.0 cm depth. *Pyrenochaeta* spp., shown to be pathogenic to creeping bentgrass, was isolated only from samples taken in midsummer. The high organic matter content in the soil and thatch layer associated with turf grass may be responsible for rapid chemical and/or microbial degradation of these fungicides and may account for the lack of any qualitative effects on the fungal ecology.

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