Evaluation of Fungicides for Control of Pythium Blight in Overseeded Turfgrasses Using a Simple Field Inoculation Technique

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Abstract

A simple inoculation technique, which uses small plastic boxes as field inoculation chambers, was developed to enhance disease development in turfgrass inoculated with Pythium aphanidermatum. When temperatures were favorable and relative humidity was high under the plastic boxes, disease severity attained 70 to 90% in untreated controls. This technique was used to screen several fungicides for efficacy in controlling Pythium blight over four years. Preventative treatments of azoxystrobin, mefenoxam, and fosetyl-Al were highly effective in controlling Pythium blight even under very high disease pressure. Curatively, mefenoxam and azoxystrobin also controlled Pythium blight, if applied as soon as symptoms (≤5% disease severity) were detected.

Introduction

Pythium blight and damping-off affect most cool-season turfgrass species and are often severe on highly managed golf course and athletic field turfgrasses. Nutter et al. (7) found that favorable conditions for Pythium blight include high relative humidity (at least 14 h at >90%), minimum temperatures above 20°C, and a maximum daily temperature exceeding 30°C. In the temperate growing areas of the U.S., these conditions occur during the summer months, while in Florida and the southern U.S., these conditions may occur year-round. In Florida, Pythium damping-off and Pythium blight are primarily a problem in the fall, winter, and spring, when cool-season turfgrass species (6) are planted as overseed on bermudagrass (Cynodon dactylon × C. transvaalensis Burtt-Davy) golf courses or athletic fields.

Cultural practices to prevent damage from Pythium blight include maintaining soil moisture at appropriate levels and using balanced applications of nitrogen. Mowing or other mechanical practices may spread the pathogen during an active outbreak. In highly managed turfgrasses, even with appropriate cultural management, the risk of severe damage may still be high when environmental conditions are favorable and disease pressure is high, including rapid and complete loss of the overseeded playing surface. Therefore, turfgrass managers will often treat preventatively with fungicides in areas with a history of Pythium-incited diseases.

Several species of Pythium can incite damping-off and Pythium blight, including P. aphanidermatum (Edson) Fitzpatrick, P. torulosum Coker & F. Patterson, P. graminicola Subramanian, P. myriotylum Drechs. and P. ultimum Trow (8). In Florida, P. aphanidermatum has been most frequently used in studies of Pythium blight (6).

Cultivars of perennial ryegrass (Lolium perenne L.) and rough bluegrass (Poa trivialis L.), either as single cultivars or as mixtures, are commonly used to overseed bermudagrass in winter in the southeastern U.S. Since both of these species are susceptible to Pythium diseases, evaluation of fungicides, biological
control products, and cultural management techniques for effective control are crucial for successful management of overseeded turfgrasses.

Creating the conditions for consistent disease development in the field is critical to successful studies. Feng et al. (5) investigated two plastic covers and a thermal blanket for effectiveness of increasing humidity and temperature and subsequent disease development in plots inoculated with \( P. \ aphanidermatum \). They found that a black plastic cover produced higher blight severity ratings (mean of 26% of plot area with visual blight symptoms) than a clear plastic cover (11%) or a geothermal blanket (13%). These coverings were cut to fit the experimental site, secured to the area with metal sod staples, and removed during the day for irrigation; plots were covered an additional night if necessary to further enhance disease development.

A relatively simple inoculation technique was developed to determine the efficacy of a number of fungicides against Pythium blight when applied preventatively and curatively. In these experiments, plastic boxes that are readily available and easily manipulated in field studies were used to create conditions highly favorable to Pythium blight. The objective of this report is to describe this technique and to summarize the results of fungicide evaluation studies conducted over four years using this method to consistently enhance disease development.

**Preparation of \( P. \ aphanidermatum \)-Corn Meal/Sand Inoculum**

Magenta boxes (6.5 cm square \( \times \) 10 cm in height; Sigma, St. Louis, MO) were filled with a corn meal:sand mixture (30/70; v/v) to approximately \( \frac{3}{4} \) full; 50 to 100 ml water was added to each box before autoclaving. The boxes containing the corn meal/sand medium were autoclaved twice for 60 min each cycle. An isolate of \( P. \ aphanidermatum \) (designated LD-1), originally obtained from infected perennial ryegrass, was used in all field experiments. The isolate was grown on potato dextrose agar (Difco, Becton-Dickinson and Co., Sparks, MD) acidified with 3 ml of 50% lactic acid per liter (APDA) for 4 days. One-fourth of an APDA plate was cut into pieces and added to the autoclaved corn meal/sand medium in each box and shaken by hand to mix thoroughly. The Magenta boxes were then placed in the incubator at 25°C in the dark for 10 to 14 days. About 25 to 50 grams of the infested corn meal/sand medium was placed into sterile plastic containers before taking to the field.

**Establishment of Preventative and Curative Field Fungicide Efficacy Trials**

All field trials were conducted between November and March in 2001 through 2004. All field trials were conducted on a bermudagrass research putting green (cultivar Tifeagle) at the Ft. Lauderdale Research and Education Center, University of Florida, IFAS. \( Poa \ trivialis \) (cultivar Dark Horse) was overseeded into the putting green at a rate of 5.4 to 6.8 kg / 90 m\(^2\). The putting green was maintained at a mowing height of 0.34 cm with fertility applications as needed (about 8 kg of N per 90 m\(^2\) per year). After overseeding, irrigation was applied three times per day, for 10 min each time, to ensure germination and emergence of seedlings. Irrigation was turned off for 4 h after fungicide application and while inoculating plots, and then turned back on after plots were covered again. Preventative fungicide applications were typically made 14 days after overseeding or as soon as a newly established turfgrass canopy had developed that was capable of sustaining Pythium blight development (defined as "emergence" in these studies). Each fungicide (Tables 1 and 2) was applied using a \( CO_2 \), 2.07×-10-Pa\(^5\) (30-psi) backpack sprayer equipped with two flat fan nozzles on a hand-held boom. Experimental units treated were 1.0 m\(^2\). Treatments were delivered in a volume of 11.4 liters of water per 90 m\(^2\). Between 4 and 24 h after fungicide applications at emergence, each experimental unit received approximately 25 to 50 g \( Pythium \)-infested medium. A plastic box, 0.28 \( \times \) 0.55 m, was inverted in the middle of each experimental unit to mark the turfgrass area to be inoculated. After the inoculum was dispersed evenly by hand in the marked area, the plastic boxes were returned to cover the inoculated areas.
to increase the temperature and the relative humidity, and thus to enhance the potential for infection. For curative fungicide trials (Table 2), fungicides were applied after inoculated plots exhibited 0.5 to 5% disease severity, usually within 24 to 48 h after inoculation.

Table 1. Effects of preventative fungicide treatments on Pythium blight development (2001-2004)

<table>
<thead>
<tr>
<th>Treatment and rate (a.i., per 90 m²)</th>
<th>Final disease severity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>78.5 a*</td>
</tr>
<tr>
<td>mefenoxam 6.3 ml</td>
<td>16.1 b</td>
</tr>
<tr>
<td>azoxystrobin 5.6 g</td>
<td>21.5 b</td>
</tr>
<tr>
<td>fosetyl-Al 90.7 g</td>
<td>18.8 b</td>
</tr>
<tr>
<td>fosetyl-Al 181.4 g</td>
<td>3.2 b</td>
</tr>
</tbody>
</table>

* Means followed by the same letter are not significantly different based on Fisher’s Protected LSD ($P < 0.05$).

Table 2. Effects of curative fungicide treatments on Pythium blight development (2000 and 2003). Fungicides were applied after 0.5 to 5% disease severity was present.

<table>
<thead>
<tr>
<th>Treatment and rate (a.i., per 90 m²)</th>
<th>Final disease severity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>84.5 a*</td>
</tr>
<tr>
<td>azoxystrobin 5.6 g</td>
<td>19.2 b</td>
</tr>
<tr>
<td>mefenoxam 6.3 ml</td>
<td>11.1 b</td>
</tr>
</tbody>
</table>

* Means followed by the same letter are not significantly different based on Fisher’s Protected LSD ($P < 0.05$).

Assessment of Pythium Blight Severity, Experimental Design and Statistical Analyses

The inoculated areas were rated for *Pythium* blight development daily or every other day. Percent disease severity of Pythium blight was based on the total infected turfgrass area beneath the inverted boxes. Each experiment was a randomized complete block design with four replicates. All data collected were analyzed by analysis of variance (ANOVA) and means were compared using Fisher’s protected least significant difference test (FLSD). Data from four years of experiments was analyzed as a split-plot design; since there were no significant experiment × treatment interactions, data were combined. All analyses were conducted using SAS version 6.12 (SAS Institute, Cary, NC).

Efficacy of Fungicides and Management Implications

Azoxystrobin, mefenoxam, and fosetyl-Al were consistently highly effective (Table 1) when applied preventatively, even under very high disease pressure. When azoxystrobin and mefenoxam were applied curatively, after the onset of visible disease symptoms (0.5 to 5% disease severity), final disease severities were significantly lower than the untreated control (Table 2). Other rates of azoxystrobin also significantly reduced disease, but the final disease severities were higher (1,3). Even though the fungicides tested significantly reduced disease severity after curative application, in practice, golf course superintendents and the clientele of most golf courses will not tolerate turfgrass stands damaged to higher levels, and the superintendents would likely need to re-seed the affected areas. In these experiments, curative applications were applied at a very low level (0.5 to 5% disease) reiterating the need for the
turfgrass manager to carefully monitor for disease or to treat preventatively, especially in areas with a history of Pythium blight.

**Disease Enhancement Using Plastic Boxes**

Placement of plastic boxes over inoculated plots consistently enhanced disease development caused by \textit{P. aphanidermatum}. When temperature and humidity were favorable, disease severity increased to 85% in inoculated but untreated control plots. Condensation was abundant and relative humidity was very high under plastic boxes (Fig. 1) because the recently seeded plots were irrigated three times per day, for 10 min each time, to ensure seed germination and seedling emergence.

Disease symptoms were typical of Pythium blight under natural conditions (Fig. 2). Individual plants first became water-soaked and eventually the whole plant became necrotic. Within 48 h, patches of necrotic tissue had developed under the plastic boxes in untreated control plots (Fig. 3A) while turfgrass plots treated with an effective fungicide remained healthy (Fig. 3B). Abundant hyphae were present in the affected areas of each plot. \textit{Pythium aphanidermatum} was consistently re-isolated from infected tissue during the trials and identified microscopically.

The plastic boxes were easily obtained and easily handled by one person. Because the boxes are small [relative to covers used by Feng et al. (5)] and translucent, irrigation water and light can reach the emerging turfgrass plants,
and can be left in place for the duration of the experiment, particularly during the cooler winter months when these experiments are completed in Florida. In addition, because environment is enhanced in each experimental unit by a separate box, treatments such as timing of fungicide applications relative to time of inoculation can be varied within the same trial. This technique also was successfully used for two field experiments with Pythium blight in Lolium perenne L. (2), as well as one field experiment with brown patch of St. Augustinegrass [Stenotaphrum secundatum (Walt.) Kuntze], caused by Rhizoctonia solani Kuhn (4).

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Literature Cited