Control of *Sclerotinia homoeocarpa* in Turfgrass Using Effective Microorganisms

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Abstract: *Different species of turfgrass are widely used worldwide for golf courses, athletic fields, and landscaping.* ***Sclerotinia homoeocarpa*** *[(Lib.) Korf & Dumonf], causal agent of "dollar spot," is considered the most prevalent turfgrass pathogen in North America Fungicides are a major input for controlling this disease. To evaluate effective alternative control approaches, research was conducted to (i) determine effects of Effective Microorganisms (EM) on growth and development of* ***S. homoeocarpa*** *in bioassays and (ii) effects of EM on disease development by* ***S. homoeocarpa*** *in turfgrass and turf quality.*

*A laboratory bioassay (*in vitro*) was conducted on* ***S. homoeocarpa*** *isolated from various turfgrass species using EM on potato dextrose agar (PDA). For the greenhouse study (*in vivo*), standard golf course soil substrate was amended with various rates of EM Bokashi made with local organic wastes. The fungus, cultures on autoclaved millet seeds, was inoculated into golf course substrates in pots four weeks after seeding with Penncross creeping bentgrass. Developing turfgrass was evaluated for disease infection and turf quality (disease rating). Laboratory results showed that EM amended in PDA at 1.0 and 4.0% significantly inhibited hyphal growth of* ***S. homoeocarpa****. In the greenhouse study, the EM Bokashi treatments had significantly less disease during the 3 week period than the standard golf green substrate. Increased turf quality was observed with increasing EM Bokashi concentration. Results suggest that EM cultures can potentially inhibit* in vivo ***S. homoeocarpa*** *growth. Further investigations into potential beneficial uses may lead to adaptation of biologically based technology such as EM to develop more sustainable environmental systems.*

Introduction Different species of turfgrass are widely used in the United States and world wide for golf courses, sports fields and landscaping. In 1989, turfgrass in the United States was estimated to cover between 10 and 12.5 million hectares, thus making the turfgrass industry one of the largest segments of US agriculture (Smiley et al., 1993). "Dollar spot" is a turfgrass fungal disease caused by *Sclerotinia homeoecarpa* classified as a summer disease that is widespread in North America, Central America, Australia, New Zealand and Europe. In 1992, the golf course industry in the United States spent around $56.5 million on fungicides trying to control the dollar spot fungus (Smiley et al, 1993*). Sclerotinia homoeocarpa* creates small circular, sunken patches of no more than 6 cm in diameter, which can coalesce destroying large areas of turf (Smiley et al., 1993). *Sclerotinia homoeocarpa* is a facultative saprophyte, living primarily as a parasite, but able to survive short periods as a saprophyte,

generally before host tissue becomes highly decomposed. Acquired pesticide resistance largely occurs in obligate parasites and facultative saprophytes because as pesticides eliminate sensitive strains, only resistant biotypes survive and become dominant. For this reason dollar spot management has become more difficult. *Sclerotinia homoeocarpa* biotypes with acquired resistance to contact fungicides such as cadmium, and to systemic demethylation inhibitor fungicides as well as the systemic benzimidazole fungicide benomyl have been reported (Vargas, 1994). Certain cultural practices can be used to prevent or reduce damage caused by dollar spot but none can be relied on for full control (Vargas, 1994). Cultural practices that promoted nitrogen availability can augment control of dollar spot but may simultaneously increase susceptibility to diseases such as pythium blight or brown patch. Currently, dollar spot is controlled by alternating different systemic and contact fungicides (Christians, 1998).

As an alternative to using fungicides for control of dollar spot and potentially developing more fungicide-resistant biotypes, considerable interest has developed in exploiting antagonistic microorganisms for biological control of plant diseases. One approach is to use Effective Microorganisms (EM) as a potential biological control agent based on previous reports of its ability to control plant pathogens through processes of competitive exclusion or pathogen inhibition. Suspensions of EM are mixed cultures of naturally-occurring beneficial microorganisms that can be applied as inocula to restore nutritionally and biologically degraded soils (Higa, 1994). As a soil inoculant, EM reportedly decreased the inocular density of several plant pathogens (Lin, 1991; Higa 1994). Myint et al. (1994) found that EM controlled bacterial leaf blight of rice caused by *Xanthomonas oryzae pv. Oryzae*. Jonglaekha et al. (1993) reported that EM controlled the pathogenic fungus *Rhizoctonia fragariae* (casual agent of root rot) in strawberry. When EM treatments for reducing incidence of *Xanthomonas campestris pv vesicatoria* in sweet pepper were applied, severe disease symptoms on leaves were reduced (Castro et al, 1996).

Research objectives of the present study were to (I) determine the effects of EM on growth and development of *S. homoeocarpa* in *in vitro* bioassays and (ii) determine the effects of EM on disease development by *S. homoeocarpa* in turfgrass and on turf quality.

Materials and Methods

Cultures of *S. homoeocarpa* were isolated from diseased tissue of creeping bentgrass (*Agrostis palustris* Huds.), Kentucky bluegrass (*Poa pratensis* L.), and perennial ryegrass (*Lolium perenne* L.) plants collected during 1998 from turfgrass plots at the University of Missouri Turfgrass Research Center, Columbia, Missouri or from golf greens at the Whitmoor Country Club in St. Charles County, Missouri following standard procedures (Dhingra and Sinclair, 1985). All isolates were maintained on potato dextrose agar (PDA) and stored at 8°C prior to use in experimental trials.

Effective Microorganisms (EM), a mixed culture of compatible and beneficial micorganisms selected from natural environments and not exotic or genetically engineered (Higa, 1994) was used in all experiments. EM consists of high populations of lactic acid bacteria, yeasts, phototrophic bacteria, and actinomycetes.

Laboratory Experiment

EM suspensions were mixed into autoclaved PDA cooled to ca. 40°C to yield final concentrations of 0.4, 1.0, and 4.0 percent EM (v/v). Additional treatments included a suspension of EM autoclaved at 121°C and 0.10 Mpa for 20 min before adding to molten PDA at 1.0 percent (v/v); PDA amended with 250 µg/ml fungicide (benomyl); and unamended PDA. All PDA treatments were allowed to solidify at room temperature. *Sclerotinia homoeocarpa* was cultured on PDA for 7 to 10 days at 27°C. Using a cork borer, uniform agar disks (5.0-mm diam) of fungal mycelia were obtained for bioassays on PDA amended with EM. A mycelial disc was inverted and placed into the center of each amended PDA plate at 0 and 48 h after preparation and incubated for 48 or 120 h. Growth was determined by measuring the diameter of fungal mycelia developed after incubation. The bioassays were conducted on duplicate plates for each treatment and were repeated once. Growth measurement data were subjected to analysis of variance and where F-values were significant at $P<0.05$, means were compared using the least significant difference (lsd) test.

Greenhouse Experiment

The experiment was conducted inside a greenhouse with a temperature such that 21°C was the target temperature, however, fluctuating sunlight tended to cause temperature variation (ca. $\pm$ 9°C).

EM Bokashi was prepared with 50 percent barnyard horse manure, 25 percent brewery waste, 25 percent wheat bran and charcoal (v/v) and anaerobically fermented for approximately 21 days. The EM Bokashi was used to prepare golf green substrates containing 20 percent EM + 80 percent sand and 40 percent EM + 60 percent sand (v/v). A standard golf green substrate containing 10 percent peat + 90 percent sand was used as control. Sand was autoclaved prior to mixing in EM Bokashi. Each treatment consisted of three replicate pots. 'Penncross' creeping bentgrass seed (70 mg) was spread over pots containing the greens mixtures arranged in a superficial area of $0.015m^2$, resulting in every pot receiving approximately 50 ml of a 1 to 1000 dilution of EM in water.

S. homoeocarpa culture growing on autoclaved millet seeds served as inoculum for infesting the greens mixtures. Pots were inoculated 4 wk after seeding using 10 inoculated millet seeds/pot. The millet seeds were randomly placed at the soil level. Prior to inoculation the pots were watered and after inoculation the pots were covered with plastic bags to improve the conditions for growth of the pathogen. At 3 wk after seeding, all treatments were fertilized weekly with a commercial fertilizer based on a golf course standard of 2.4 kg N/ha.

The variables analyzed were percentage area infected and visual disease rating. Percentage area of infection was collected by setting a plastic sheet over the turf and drawing the boundaries of the area infected. Each plastic sheet was then set over a grid and the area was measured. Visual disease rating (turf quality) consisted of observation of color and overall appearance of the grass. A scale of 1 to 6 was used, with 6 as no disease and 1 as worst overall appearance. Data was collected 6, 7 and 8 wk after seeding.

Results and Discussion

Laboratory Experiment

Compared to the nontreated control, EM amended in PDA at 1.0 and 4.0 percent significantly inhibited hyphal growth of *S. homoeocarpa* regardless of pre-incubation (Table 1) while the benomyl treatment completely suppressed fungal development. When EM was autoclaved and mixed with PDA at 1.0 percent, fungal growth was significantly inhibited. Organisms in EM may have been killed or suppressed in forming colonies on agar, thus results suggest that either extra cellular and heat-stable toxins produced during EM fermentation or intracellular metabolites lysed from heat-sensitive microbial cells on autoclaving were largely responsible for fungal growth inhibition. Results from the overall study suggest that both living organisms and substances produced or released from heat-labile EM organisms may inhibit growth but the mode of action is not evident. Future trials examining cell-free culture filtrates of EM will aid in assessing the contribution of extra-and intercellular metabolites to fungal growth inhibition. Indications of potential nonchemical management of *S. homoeocarpa* in turfgrass systems are supported by the current preliminary study.

Table 1.Effect of EM Treatments on *in vitro* Growth of *Sclerotinia homoeocarpa*

Treatment	Fungal Growth (mm diam)	
	Trial 1[a]	Trial 2[b]
Control	4.3	6.8
Benomyl	0	0
EM (0.4%)	4.4	7.2
EM(1.0%)	3.4	6.0
EM (4.0%)	3.8	4.6
EM (1.0%, autoclaved)	3.0	5.3
lsd (0.05)	0.5	0.6

[a] Treatments exposed to fungus immediately after PDA amendment; fungal growth measured at 48 h incubation

[b] Treatment incubated 48 h prior to exposure to fungus; fungal growth measured at 120 h incubation

Greenhouse Experiment

A decreasing percentage area infected was observed with an increasing percentage of EM Bokashi (Table 2). The 40 percent EM Bokashi + 60 percent sand treatment had significantly less disease during the 3 wk period than the 20 percent EM Bokashi + 80 percent sand treatment and standard golf green substrate. Similarly, increased turf quality, based on visual disease rating, was observed with increasing Bokashi concentration.

Based on the results, there is evidence suggesting that EM cultures have the potential to inhibit *in vivo* growth of *Sclerotinia homoeocarpa*.

Other effects observed from the use of EM Bokashi as part of the growing media for turf included increase in turfgrass seed germination, increased daily growth rate and decreased establishment time for turfgrass and an overall improved appearance and color of the turfgrass.

Table 2. Area Infected by *Sclerotinia homoeocarpa* and Turf Quality of Creeping Bentgrass as Affected by EM Amended Golf Green Substrate

Golf Green Substrate	Area infected (%)			Turf quality [a]		
	6 WAS [b]	7 WAS	8 WAS	6 WAS [b]	7 WAS	8 WAS
Standard	14.2	26.6	19.1	1.0	2.0	3.0
80%sand+20% EM	13.3	16.8	9.8	2.0	3.3	4.6
60% sand+40% EM	6.6	2.2	0.4	4.0	5.0	5.0
lsd (0.05)	4.8	15.2	12.3	1.3	3.0	0.8

[a] Turf quality rating based on visual disease rating with 1 = severely diseased and 6 = no disease symptoms

[b] WAS = Weeks after seeding

With the current and valid concerns of compromised environmental quality due to the use of agricultural chemicals, investigations of numerous natural product and microorganisms for biological control of pests become more important. Possible beneficial effects of preparations of EM as demonstrated in the present report have been evaluated to only a limited extent. Further intensive investigations into potential beneficial uses may lead to adaptation of biologically based technology such as EM to develop more sustainable environmental systems.

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