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Exserohilum Leaf Spot on Tiger Grass

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Abstract

Tiger grass [*Thysanolaena maxima* (Roxb.) Kuntze] is a popular ornamental grass grown throughout landscapes in South Florida. In the summer of 2006, a leaf spot was observed on tiger grass in the landscape and a commercial nursery in Homestead, FL. The causal agent of the leaf spot was isolated and characterized morphologically and molecularly as *Exserohilum rostratum* (Drechsler) Leonard & Suggs. At high inoculum densities, symptoms were apparent as early as 12 h after inoculation, and caused widespread necrosis. Germinating conidia of *E. rostratum* and appressoria were observed in direct association with lesions. This newly discovered disease could potentially have a dramatic effect on the aesthetic quality and salability of this landscape ornamental.

Introduction

Thysanolaena maxima (Roxb.) Kuntze [excluded] (tiger grass) is a commercial containerized and landscape ornamental grass from the family Poaceae (subfamily:Centothecoideae, tribe:Thysanolaeneae) and similar in appearance to bamboo (8). In the summer of 2006, leaf spot on Tiger grass was first noticed in a South Florida nursery, and subsequently in several nurseries and landscapes throughout Miami-Dade Co. and the Florida Keys in Monroe Co. In addition, the same leaf spot symptoms were observed on young transplants from a production greenhouse at Apopka, FL.

Symptoms

Fig. 1. Tiger grass showing tan-colored lesions that are typical of leaf spot symptoms on day of receipt from the supplier.

Symptoms start as minute tan colored flecks often turning chlorotic to necrotic. These initial lesions elongate elliptically between the leaf veins, sometimes with a yellow halo, eventually turning necrotic. Lesions on young, naturally infected leaves vary from pinpoint size up to 0.2 cm wide (between the veins) and 1.2 cm long. Individual lesions gradually coalesce into large necrotic elliptical spots to blotches, sometimes interspersed with chlorosis (Fig. 1). Infected leaf tips may turn light brown to brown, curl and turn yellow below the necrotic leaf tip. The purpose of this study was to identify and characterize the causal agent of the disease.

Pathogen Characterization

Tiger grass leaf pieces each containing part of a lesion were excised with a small amount of surrounding asymptomatic tissue, surface sterilized for 45 sec in 50 ml 10% commercial NaOCl solution (Clorox, Clorox Co., Oakland, CA) with a drop of detergent (Tween 20), and rinsed three times in sterile deionized water. The leaf pieces were picked up with sterile tweezers, surface water was removed by brief placement on filter paper (Whatman #1), and placed in the center of V8-juice agar plates. V8-juice agar was prepared by mixing 1 can (330 mL) V8-juice (Campbell Soup Co., Camden, NJ), 670 mL deionized water, 3 mg calcium carbonate, and 15-gram agar, and autoclaving (121°C, 16 psi, 15 min). Single-spore isolates were obtained by transferring individual germinating conidia to fresh plates of V8-juice agar media. Cultures were maintained in an incubator at 21.1 to 26.3°C under fluorescent lighting at 970 lux for 12 h light and 12 h dark.

A dematiaceous fungus was consistently isolated from naturally occurring lesions on tiger grass and identified as *Exserohilum rostratum* based on conidial morphology (Fig. 2 and 3) according to Sivanesan's key (9). For confirmation of species identity, polymerase chain reaction was performed with the MyCycler thermal cycler (Bio-Rad Laboratories, Hercules, CA) using Internal Transcribed Spacer region primers ITS1 and ITS4 (7), and genomic fungal DNA isolated with the Extract-N-Amp kit (Sigma-Aldrich, St. Louis, MO). PCR products were cleaned up with the QIAquick PCR purification kit (Qiagen, Germantown, MD), and sequenced by the ICBR sequencing core at the University of Florida. BLAST searches (1) were performed, and sequences were aligned with ClustalW (6).

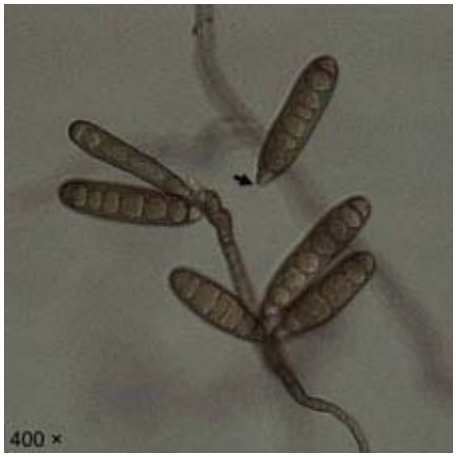

Fig. 2. Conidia of *Exserohilum rostratum* with the protruding hilum (arrow), which distinguishes the genus *Exserohilum* from *Bipolaris*.

Fig. 3. Bipolar conidial germination of *Exserohilum rostratum*.

The ITS1/4 sequences derived from several isolates were identical, and Blastn (1) results indicated 100% alignment with the *E. rostratum* sequence deposited in GenBank (gi: 76555872). This confirmed the identity of the fungal plant pathogen as *E. rostratum*. The ClustalW (6) sequence alignment with the closest blast hit (*E. rostratum*) is displayed in Fig. 4. One of the Blastn hits had a very high similarity (99% over 92% of the sequence) to gi: 55586034, *Alternaria* sp. This sequence was most likely mis-identified by the depositors, since there were no other sequences from the genus *Alternaria* that were similar in Blast hits. In addition, the conidial morphology of *E. rostratum* was sufficiently distinct from those of *Alternaria* to rule out the possibility that the isolate belonged to the genus *Alternaria*.

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0706019         ---CATTACACAACAAAAATATGAGGGTGTGGTTTGCTGGCAACAGCGTCCGCCCCAAGT 57
gi_76555872     GATCATTACACAACAAAAATATGAGGGTGTGGTTTGCTGGCAACAGCGTCCGCCCCAAGT 60
                   ************************************************************
0706019         ATTTTTCACCCATGTCTTTTGCGCACTTTTTGTTTCCTGGGCGAGTTCGCTCGCCACCAG 117
gi_76555872     ATTTTTCACCCATGTCTTTTGCGCACTTTTTGTTTCCTGGGCGAGTTCGCTCGCCACCAG 120
                ************************************************************
0706019         GACCCAACCATAAACCTTTTTTTATGCAGTTGCAATCAGCGTCAGTATAATAATTCAATT 177
gi_76555872     GACCCAACCATAAACCTTTTTTTATGCAGTTGCAATCAGCGTCAGTATAATAATTCAATT 180
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0706019         TATTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT 237
gi_76555872     TATTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT 240
                ************************************************************
0706019         GCGATACGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGC 297
gi_76555872     GCGATACGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGC 300
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gi_76555872     CGGCATCCAGCAAGCCTTTGTTTTCTATAACAAATCCACATTTTGACCTCGGATCAGGTA 540
                ************************************************************
0706019         GGGATACCCGCTGAACTTAAGCATATCAATA 568
gi_76555872     GGGATACCCGCTGAACTTAA----------- 560
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Fig. 4. ITS sequence alignment: ITS1/4 sequences derived from *Exserohilum rostratum* isolate 0706019 compared with the ITS sequence from GenBank gi: 76555872 *Exserohilum rostratum*.

Exserohilum rostratum grew on all media tested (water, potato dextrose, V8-juice, and lactose casein hydrolysate agars), but sporulated most profusely and consistently on V8-juice agar, which was used for subsequent experiments. Sporulation only occurred after the mycelium had reached the edge of the Petri dish, 6 to 7 days after transfer to the medium. The mycelium was initially white in culture, turning dark brown as the culture aged. Conidiophores were brown and geniculate. Conidiogenesis was blastic and sympodial. The conidia were 11-18 µm × 56-128 µm, brown, straight to slightly curved, pseudoseptate with the terminal septa dark and thick, and with a protruding hilum (Fig. 2). Germination was most often from both terminal cells of the conidium (Fig. 3). The inoculum density of *E. rostratum* applied to leaves affected the final disease severity on tiger grass (Fig. 5). Based on the results depicted in Fig. 5, all subsequent experiments were performed with 5×10^3 conidia/ml, harvested from 7 to 11-day-old cultures.

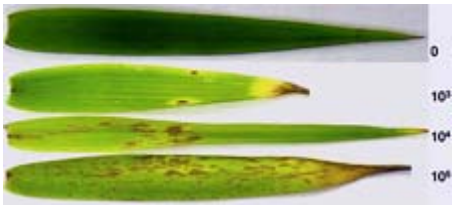

Fig. 5. The effect of inoculum density of *Exserohilum rostratum* on leaf spot development on tiger grass. Numbers to the right of each leaf define inoculum densities of 0, and 5×10^3, 10^4 or 10^5 conidia/ml applied to leaves during inoculation studies.

Tiger grass plants often arrived with leaf spot symptoms (Fig. 1) from the supplier, and all plants were pruned upon arrival to produce healthy leaves without leaf spot symptoms. This confirmed that the disease is a serious problem in the nursery, although during a discussion with the greenhouse manager of AgriStarts III, it became clear that it was not perceived as such (D. Hartman, *personal communication*). A targeted or regular fungicide spray schedule of either Dithane (Mancozeb, Dow Agrosciences, Indianapolis, IN), Medallion (Fludioxonil, Syngenta, Greensboro, NC), and Cleary 3336, and Chipco 26 GT (Iprodione, Bayer, Montvale, NJ) was maintained to adequately control disease symptoms in the nursery greenhouse.

To observe germination of conidia and the process of infection of tiger grass leaves, leaf sections with clearly visible lesions were cleared by heating (but not boiling) for 5 to 10 min in lactophenol blue, and rinsed briefly in lactophenol essentially as described by Dhingra and Sinclair (4). Excess lactophenol was then removed by placing the leaf sections on a paper towel for a few seconds. Slides were prepared with lactophenol, and lesions were photographed using an Olympus BX51 bifocal microscope with an Olympus DP12 digital camera. Conidial size was determined by measuring the width and length of 50 conidia derived from several isolates using the bifocal microscope at a 400-fold magnification. Fig. 6A shows a lesion on tiger grass associated with a germinated conidium of *E. rostratum* that produced a very pronounced bulbous appressorium on one of the germ tubes (Fig. 6B). This appressorium possessed two, distinct cell walls.

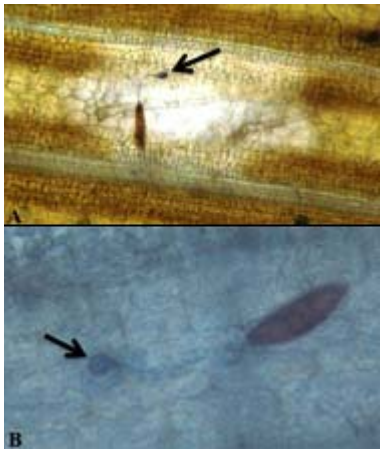

Fig. 6. Conidia of *Exserohilum rostratum* on tiger grass: A. Association of a germinated conidium of *E. rostratum* and germ tube with an appressorium (arrow) on tiger grass lesion (light-colored area of the leaf), B. Higher magnification of conidium of *E. rostratum* and appressorium (arrow) on a tiger grass leaf cleared and stained with lactophenol blue.

Pathogenicity Tests

Koch's postulates were performed by growing single-spore isolates on V8-juice agar medium, harvesting conidia from 7 to 10-day-old cultures, re-suspending the conidia in sterile water with Tween 20 to produce inoculum densities of 5×10^3 to 5×10^5 conidia/ml. Inoculum was sprayed onto symptomless tiger grass plants with a Crown #8211 Sprā-tool (Gardnerville, NV). Inoculated plants were placed in plastic bags for 24 h after inoculation to maintain high humidity and incubated in an air-conditioned greenhouse (temperatures ranged from 22 to 32°C).

Inoculations of tiger grass with conidia of *E. rostratum* resulted in symptoms appearing as early as 12 h after inoculation with 5×10^5 conidia/ml (Fig. 5). Reducing inoculum density to levels of 5×10^3 or 5×10^4 conidia/ml resulted in symptoms appearing at 2 to 5 days after inoculation. *Exserohilum rostratum* was re-isolated from the lesions resulting from the spray inoculation experiments.

Conclusions

Exserohilum rostratum (Drechsler) Leonard & Suggs was identified as the causal agent of leaf spot on tiger grass using morphological and molecular characteristics, and performing Koch's postulates. This fungal pathogen has not previously been reported on tiger grass, although it is known to be common on grasses, other plants and substrates, and in soil (9). Whitehead and Calvert (10) reported *E. rostratum* as the causal agent of ear rot of corn and leaf spot of 13 different grasses, while Kucharek (5) reportedly isolated it from rotted corn stalks in Florida in 1971 and confirmed it as the cause of the disease.

Even relatively low levels of inoculum (5×10^3 conidia/ml) resulted in substantial disease severity in artificial inoculation experiments. Inoculation of tiger grass at high inoculum densities (5×10^5 conidia/ml) caused such severe leaf spot symptoms that the coalescing lesions resulted in widespread necrosis and death of the leaf. This implied that the disease has the potential to be severe under favorable conditions for sporulation, dissemination, and germination of conidial inoculum. Indeed, tiger grass liners supplied by the grower were often heavily infected. *Exserohilum rostratum* is the anamorph of *Setosphaeria rostrata* Leonard. The species is heterothallic, producing a teleomorph *in-vitro* after pairing different mating types in culture at low temperature for 1 to 3 months (9). Since tiger grass is utilized as a landscape ornamental in Florida, any disease symptoms at the time of sale, compromises the aesthetic quality and will greatly reduce its salability.

Most conidia of *E. rostratum* germinated bipolarly. The fungus produced prominent appressoria that were associated with tiger grass lesions. Individual lesions expanded longitudinally, and were for the most part restricted to the area between the leaf veins. However, multiple lesions could coalesce to make larger lesions that might span the veins. Symptoms appeared at times very rapidly, implying that fungal toxins may be involved in the infection process. This is consistent with the report of Cuq et al. (3) on the presence of a non-specific toxin, monocerin, and the report of Bashan and Levy (2) on cultivar-specific phytotoxic water-soluble compounds in *E. turcicum*, [teleomorph *Setosphaeria turcica* (Luttr.) K. J. Leonard & Suggs 1974], the causal agent of Northern corn leaf blight.

Acknowledgments

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