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Biomass Reducing Potential and Prospective Fungicide Control of a New Leaf Blight of *Miscanthus×giganteus* Caused by *Leptosphaerulina chartarum*

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Abstract During a multistate survey (2009) of diseases of perennial grasses under consideration as biofuel feedstocks, a new leaf blight of *Miscanthus×giganteus* caused by *Leptosphaerulina chartarum* was observed on 100 % of the plants evaluated in research plots near Lexington, Kentucky. A greenhouse study was conducted to evaluate the effect of *L. chartarum* on *M.×giganteus* biomass and to identify effective foliar fungicides against the disease. Eleven broad-spectrum fungicides were tested with rates typically used in agronomic crops. Fungicides tested included active ingredients from five different chemical groups, demethylation inhibitors (prothioconazole, tebuconazole, cyproconazole, propiconazole, tetraconazole, flutriafol, and metconazole), quinone outside inhibitors (pyraclostrobin), succinate dehydrogenase inhibitors (boscalid), methyl benzimidazole carbamates (thiophanate-methyl), and chloronitriles (chlorothalonil). Infection by *L. chartarum* significantly lowered aboveground biomass by an average of 33 %. Application of cyproconazole, flutriafol, tebuconazole, or prothioconazole significantly reduced disease severity, with cyproconazole and flutriafol ranking best at an average of 29 % disease control. However, no fungicide treatment resulted in biomass loss abatement. Greater rates or a combination of fungicides might be needed to achieve adequate control.

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Introduction

Miscanthus×giganteus is a high-yielding, rhizomatous C4-perennial grass considered to be a highly promising biomass feedstock for renewable biofuel production [4, 17]. At differing times, *M.×giganteus* has been called *Miscanthus sinensis* 'Giganteus', *Miscanthus giganteus*, *Miscanthus ogiformis* Honda, *Miscanthus sacchariflorus* var *brevibarbis* (Honda) Adati, and *M.×giganteus* (Greef & Deuter ex Hodkinson & Renvoize) [4, 18]. *M.×giganteus* is a sterile triploid interspecific natural hybrid of *M. sinensis* Anderss and *M. sacchariflorus* (Maxim.) Hack and is native to tropical and subtropical regions of Africa and southern Asia and temperate Eastern Asia [18]. Its high biomass accumulation potential is due to its C4 photosynthesis (which means greater rates of carbon fixation) and greater water and nutrient-use efficiency as is typical of C4- grasses [4, 16, 17]. Naturally established hybrid vigor in *M.×giganteus* also could be an important factor that set it apart from many other similar grass species (even its natural parents, *M. sinensis* and *M. sacchariflorus*) in its ability to withstand stress, adapt to a wide range of environments, and more efficiently convert solar energy into biomass energy, therefore providing a high biomass/input ratio per cultivated land area [4, 16, 17]. *M.×giganteus* has been thought to be mostly free of damage from diseases and pests, an important trait that probably contributes to its high biomass yields even under a system using fewer inputs. Pest resistance and a perennial habit are two traits that would contribute to an ideal biofuel feedstock candidate [4, 17].

The limited number of reported pest infestations and diseases in *M.×giganteus* may partially be due to the limited scope of research conducted in this area, as well as the relatively small portion of agricultural land worldwide devoted to *M.×giganteus* production. As more and more plots of *M.×giganteus* and other *Miscanthus* spp are established for research in different agroecological zones of the world due to the escalating interests in second-generation biofuels, diseases of *Miscanthus* spp (including *M.× giganteus*) are beginning to emerge [2, 3, 5, 6, 14, 23, 24]. As this new crop goes into more intensive monoculture systems, it will begin to succumb to the pressure of pathogens that also are continually evolving to find new hosts. The risk of development of disease epidemics that could severely limit biomass production is particularly high for the *M.×giganteus* clone which is propagated from rhizome cuttings, and lacks genetic diversity. During our multistate survey (2009) of diseases in perennial grasses of potential use as feedstocks for biofuels, a severe occurrence of a new leaf blight of *M.×giganteus* (Fig. 1) was found in research plots near Lexington, Kentucky. The disease was determined to be caused by the fungus, *Leptosphaerulina chartarum* (anamorph: *Pithomyces chartarum*) [2]. Incidence of the disease was 100 % in both 2-year-old and first-year *M.×giganteus* plots. Damage was particularly severe on the first-year crop, where some tillers were killed [2].

L. chartarum has been known to be an important ascomycetous fungus, not because it causes plant diseases, but because of the worldwide mycotoxicosis of ruminant animals from its potent toxic metabolite sporidesmin produced in its spores and ingested through grazing on infected pastures [1, 8, 12, 15, 19, 21, 29, 33]. Sporidesmin, a hepatotoxin produced by the fungus is associated with 'pithomycotoxicosis' (or pithomyces poison-induced liver damage), a potentially fatal photosensitization disease of sheep and other grazing ruminants, commonly referred to as 'facial eczema' because of its post-liver-damage symptoms [8, 9]. In addition to a recent report of the *Pithomyces* blight of *M.×giganteus* [2], there have been sporadic reports of diseases that *L. chartarum* causes on plants [10, 32, 34]. Wilkinson [36] found masses of dark spores in July and August during 1985 and 1986 as bluegrass sod (*Poa pratensis* L.) was being mowed at several farms in Illinois, Indiana, and Wisconsin. The fungus was isolated and identified as *L. chartarum*. It was the first report of the fungus in the north central USA and the first report of the fungus in bluegrass sod where it appeared to be saprophytic [36].

Pithomyces blight in *M.×giganteus* plots (Kentucky, 2009) [2] severely affected growth and plant establishment. However, the loss could not be estimated because the disease incidence was 100 %. The need for an effective control of *L. chartarum* has long been recognized, particularly in grass pastures due to its economic importance as a mycotoxigenic fungus. Some efforts directed at biological control using non-toxigenic *Leptosphaerulina* strains based on the competitive exclusion principle [7, 11] and use of chemical fungicides [25, 26, 28, 30, 37] have been made in the past but without any significant success. The objectives of our current study were to: (1) evaluate the potential effect of *Pithomyces* blight on *M.×giganteus* biomass and (2) determine the efficacy of selected fungicides for control of *Pithomyces* blight on *M.×giganteus*.

Fig. 1 *Pithomyces* blight on **a** first-year crop of *M.×giganteus* and **b** second-year crop of *M.×giganteus* in research plots new Lexington, Kentucky, in 2009

Materials and Methods

Three experiments ("1", "2a," and "2b") were conducted to determine the effect of *Pithomyces* blight on *M.×giganteus* biomass and screen fungicides for their efficacies to control the disease.

Fungicides Tested

In experiment 1, five broad-spectrum fungicide active ingredients (chlorothalonil, boscalid, pyracclostrobin, proclostrobin, prothioconazole, and thiophanate-methyl) from five different chemical groups (Table 1) were evaluated. In experiments 2a and 2b, six additional fungicide active ingredients from the demethylation inhibitor (DMI) fungicide group were added to the screening experiment (Table 2). Thus, 11 broad-spectrum fungicide active ingredients from five different fungicide groups (Table 1) were tested in experiments 2a and 2b. They included seven fungicides in the DMI group (prothioconazole, tebuconazole, cyproconazole, propiconazole, tetraconazole, flutriafol, and metconazole) and one fungicide within each of the other four fungicide groups: thiophanate-methyl (methyl benzimidazole carbamate group (MBC)), boscalid (succinate dehydrogenase inhibitor group (SDHI)), pyraclostrobin (quinone outside inhibitor group (QoI)), and chlorothalonil (chloronitrile group).

The Test Plant *M.×giganteus*

M.×giganteus 'Illinois' plants used for this study were established from 'plugs' (live plants generated in the greenhouse) supplied by Speedling, Inc. (Speedling Inc. Sun City, FL, USA). *M.×giganteus* plugs received from Speedling, Inc., were immediately removed from the shipment boxes and planted in 12×4 channel plastic inserts placed in plastic flats to re-establish growth in the greenhouse with a 15-h photoperiod, day temperature of 24–27 °C, and night temperature of 21–24 °C. Metro-Mix 510 growing mix (Sungro Horticulture Canada LTD., Bellevue, WA) (35–45 % Canadian Sphagnum peat moss horticulture grade vermiculite, composted pine bark, bark ash, and dolomitic limestone) was used as the growing medium.

Approximately 2 weeks later, after new leaves developed and the plants were established, plants were transplanted from the inserts into individual 12.7-cm plastic pots (0.6 L) containing Metro-Mix 510 growing mix. The potted plants were grown in the greenhouse under the same conditions previously described for an additional 2 to 3 weeks. Then, uniformly sized plants (4–5 weeks old, five to six leaves, and average height of 25 cm) were arbitrarily selected for the fungicide evaluation experiment. The growing mix within each pot was surface-dressed with slow release fertilizer, osmocote 15:9:12 (The Scotts Company LLC, Marysville, OH), at the time of transplanting at the rate of 2 g fertilizer per pot.

Production of *L. chartarum* Inoculum

Single-spore *L. chartarum* isolate Mxg-KY09-s4 (GU195649.1), isolated from symptomatic *M.×giganteus* plants near Lexington, Kentucky, USA, in 2009 [2], was used in this study. The fungal isolate was grown on half-strength potato dextrose agar (Oxoid Ltd, Basingstoke, Hampshire, England) amended with 25 mg/l of rifamycin sulfate. The 100×15 mm agar plates were initially incubated at 22 °C for 3 days in the dark, then under continuous white light for 2 weeks.

To prepare spore inoculum, Petri dish agar cultures were flooded with 20 ml/plate of sterilized water containing 0.05 % (*v/v*) Tween-20 as a wetting agent, and then cultures were scraped with microscope glass slides to loosen the mycelia and release conidia into the water. A collection of the conidia and mycelia suspension from all the culture plates was blended using a hand blender (Cuisinart Smart Stick Hand Blender Model CSB-76, Cuisinart, East Windsor, NJ). The blended spore suspension was then filtered through double layers of cheese cloth. The inoculum was adjusted to an approximate concentration of 2×10^6 conidia/ml.

Fungicide Application

A single dose of fungicide was applied to the *M.×giganteus* plants 24 h prior to plant inoculation with pathogen inoculum at rates (Table 1) typically used in agronomic crops. In preparation to spray the plants, each of the 11 fungicides was diluted to final application concentration (Table 1) with sterile water. The designated fungicide was applied as uniform fine sprays onto the *M.×giganteus* leaves using an automated pesticide spray containment chamber with a 80015EVS spray nozzle (Spraying Systems Co., Wheaton, IL) calibrated to deliver 187 L/ha. *M.×giganteus* plants designated as no fungicide (control) were sprayed with sterile water. The plants were allowed to air-dry outside the spray chamber for 1 to 2 h and then moved to the growth chamber room where they would be inoculated and incubated.

Plant Inoculation and Incubation for Infection

M.×giganteus plants were hand-spray-inoculated with an aqueous conidial suspension (approx. 2×10^6 conidia/ml) using a Preval spray gun (Preval, 1300 E North Street Coal City, IL) 24 h after fungicide application. They were

Table 1 Fungicides and application rates evaluated on *M.×giganteus* plants

Fungicide trade name	Fungicide active ingredient	Company (city, state)	Fungicide group name	FRAC group code[b]	Formulation	Concentration applied (ml or g product /L)	Rate applied (kg a.i./ha)
Proline	Prothioconazole[a]	Bayer CropScience (Research Triangle Park, NC)	Demethylation inhibitors (DMI)	3	SC	2.23	0.20
Topguard	Flutriafol	Cheminova (Research Triangle Park, NC)	DMI	3	SC	5.47	0.13
Alto	Cyproconazole	Syngenta Crop Protection (Greensboro, NC)	DMI	3	SL	2.15	0.04
Folicur	Tebuconazole	Bayer CropScience	DMI	3	F	1.57	0.13
Tilt	Propiconazole	Syngenta Crop Protection	DMI	3	EC	1.57	0.13
Domark	Tetraconazole	Valent USA Corp. (Walnut Creek, CA)	DMI	3	ME	1.96	0.08
Caramba	Metconazole	BASF Corp. (Research Triangle Park, NC)	DMI	3	SL	5.47	0.09
Headline	Pyraclostrobin[a]	BASF Corp.	Quinone outside inhibitors (QoI)	11	EC	3.52	0.17
Endura	Boscalid[a]	BASF Corp.	Succinate dehydrogenase inhibitors (SDHI)	7	WG	4.20	0.54
Topsin	Thiophanate-methyl[a]	United Phosphorus, Inc. (King of Prussia, PA)	Methyl benzimidazole carbamates (MBC)	1	L	7.82	0.79
Bravo Weather Stik	Chlorothalonil[a]	Syngenta Crop Protection	Chloronitriles	M5	SC	12.51	1.68

EC emulsifiable concentrate, *SC* suspension concentrate, *SL* soluble concentrate, *F* flowable, *ME* micro-emulsion, *WG* water-dispersible granules, *L* liquid

[a] Fungicide active ingredient included in experiment 1

[b] Fungicide Resistance Action Committee

Table 2 Effect of fungicide spray on the severity of *Pithomyces* blight on *M.×giganteus* under greenhouse conditions in experiment 1

Fungicide treatment (fungicide active ingredient)	Percent disease severity			
	15 days after inoculation		23 days after inoculation	
	Mean	Disease reduction by fungicide (%)[a]	Mean	Disease reduction by fungicide (%)[a]
No fungicide	47.1		49.5	
Chlorothalonil	30.9	34.3 NS	39.7	19.7 NS
Boscalid	40.6	13.8 NS	45.7	7.6 NS
Pyraclostrobin	37.0	21.4 NS	47.1	4.8 NS
Prothioconazole	18.4	61.0**	25.2	49.0*
Thiophanate-methyl	32.8	30.3 NS	42.9	13.3 NS
SE(±)	4.94		5.73	
$P>F$	0.0097		0.0734	

NS not significant (at $P\leq0.05$)

[a]Compared with no-fungicide-inoculated

*$P\leq0.05$, **$P\leq0.01$ levels of significance

then incubated in growth chambers (Conviron CMP6050 model # MTR26, Controlled Environment Limited). The plants were maintained in the dark at relative humidity of >85 %, and at 26 °C for 48 h initially following inoculation, and thereafter at alternating 15 h of 280 μmol light at 25 °C and 9 h of darkness at 23 °C until 2 week after inoculation. Thereafter, the plants were moved back to the greenhouse.

Disease Development in the Greenhouse and Data Collection

Following initial 1 week incubation in growth chamber, plants in all three experiments were maintained in the greenhouse with a 15-h photoperiod and day and night temperatures of 24–27 °C and 21–24 °C. In experiment 1, the plants were left in the greenhouse only until the last disease assessment (at day 23 after inoculation). In experiment 2a and 2b, the plants were maintained for 15 and 11 additional weeks in the greenhouse, respectively. During this period, the plants were lightly watered twice a day to meet the water need of the plants and to maintain adequate relative humidity for infection and disease development. To limit assessment to only the treated tillers, new and emerging tillers were removed regularly.

Disease severity was assessed as 'percent disease severity' per plant at 15 and 23 days after inoculation in experiment 1 and at 4 weeks after inoculation in experiment 2a and 2b. The percentage of leaf surface area covered by disease symptoms (compared with green tissues) on each of the three most infected leaves on a plant was estimated visually. Then, these estimates were averaged to get the percent disease severity per plant. Plant biomass accumulation was measured at 16 (for experiment 2a) or 12 weeks (for experiment 2b) after inoculation as 'aboveground dry-weight.' The aboveground part (shoot and leaves) of each plant was harvested, air-dried for 2 weeks in labeled brown bags on a greenhouse bench at 27 °C-30 °C, and then weighed to obtain the aboveground dry-weight per plant.

Experimental Design and Statistical Analysis

In the preliminary experiment (experiment 1), there were six fungicide treatments which included five 'fungicide-inoculated' treatments and one control which was 'no fungicide (sterile water applied)-inoculated.' In experiment 2a or 2b (experiment 2b is a repeat of 2a), there were 13 treatments, including 11 'fungicide-inoculated' treatments and two controls which were 'no fungicide-inoculated' and 'no fungicide-non-inoculated.'

Each experiment was designed as a randomized complete block design with eight blocks. Each potted plant (0.6-L pot) served as both an experimental and observational unit. For experiments 2a and 2b, with 13 treatments, each block was contained within a space of 0.55×0.55 m, with the eight blocks allocated to three greenhouse benches.

To provide a better understanding of the treatment responses in the repeated experiments, the percent disease severity or the aboveground *M.×giganteus* dry-weight per pot data were analyzed by experiment and as a pooled dataset. Data were subjected to analysis of variance (ANOVA) in SAS (SAS version 9.3, SAS Institute Inc., Cary, NC, USA) using the MIXED procedure [22]. Replication and experiment (for pooled data) were treated as random effects and treatment as a fixed effect in the model statement. To normalize percent disease severity data, they were arcsine-transformed. Also, since no disease was observed in the 'No inoculation' control, it was expunged from the ANOVA of the percent disease severity. Single-degree-of-freedom contrasts were made to test differences between specific treatment means with the mean of a control.

Results

Disease Severity and the Effect of Fungicide Application

Pithomyces blight of *Miscanthus* was observed in all inoculated *M.×giganteus* plants in all three experiments, but the average percent disease severity per plant varied according to fungicide treatment. In experiment 1, percent disease severity on *M.×giganteus* plants to which the DMI prothioconazole had been applied was significantly lower by 61 % ($P \leq 0.01$) and 49 % ($P \leq 0.05$) at 15 and 23 days after inoculation, respectively, compared with plants to which no fungicide was applied (Table 2).

There was reduced disease severity with each of the 11 fungicide treatments tested in experiment 2a and 2b compared with the no fungicide control, but only in a few fungicide treatments were reductions significant at the 5 % probability level (Table 3). Only applications of cyproconazole, flutriafol, prothioconazole, or tebuconazole, all of which belong to the DMI fungicide group, significantly ($P \leq 0.05$) reduced disease severity compared with the control (Table 3). Cyproconazole and flutriafol were the most effective across trials, with an average disease reduction of 29 % (Table 3). These were followed by tebuconazole (27 % disease reduction) and prothioconazole (25 % disease control) (Table 3).

Another DMI fungicide, tetraconazole, significantly lowered disease levels in experiment 2b but not in experiment 2a. Similarly, inconsistent results were obtained for pyraclostrobin (quinone outside inhibitor) and boscalid (succinate dehydrogenase inhibitor) applications in the three experiments (Tables 2 and 3).

Biomass Reduction Potentials of *Pithomyces* Blight of *Miscanthus* and the Effect of Fungicide Application

M.×giganteus plants infected with *Pithomyces* blight following inoculation with *L. chartarum* consistently resulted in significantly ($P \leq 0.05$) lower harvestable above-ground plant biomass compared with non-inoculated plants (Table 4). There was an average biomass reduction of 33 % ($P \leq 0.01$) when plants were inoculated with *L. chartarum* and not treated with a fungicide compared with disease-free plants that also were not treated with a fungicide (Table 4). None of the tested fungicide active ingredients (including the four DMI fungicides that significantly reduced disease severity (Table 3)) resulted in biomass loss abatement at the application rate used (Table 4). *M.×giganteus* plants that were sprayed with fungicides and inoculated with *L. chartarum* had lower above-ground biomass compared with the non-inoculated plants ($P > 0.05$) (Table 4).

Discussion

Results of our experiments (Tables 3 and 4) have demonstrated that infection of *M.×giganteus* plants by the pathogen *L. chartarum* could result in biomass loss of *M×giganteus*. The effect of the severe incidence of *Pithomyces* blight of *M.×giganteus* in research plots near

Table 3 Effects of fungicide spray on the severity of *Pithomyces* blight of *Miscanthus* on *M.×giganteus* under greenhouse conditions 4 weeks after inoculation

Fungicide treatment (fungicide active ingredient)	Percent disease severity					
	Experiment 2a		Experiment 2b		Experiment 2a and 2b combined	
	Mean	Disease reduction (%)[a]	Mean	Disease reduction (%)[a]	Mean	Disease reduction (%)[a]
No fungicide	40.2		61.8		51.0	
Cyproconazole	27.9	30.8*	44.2	28.4***	36.1	29.3**
Chlorothalonil	32.9	18.3 NS	56.8	8.1 NS	44.8	12.1 NS
Metconazole	34.7	13.8 NS	53.8	12.9 NS	44.3	13.2 NS
Tetraconazole	32.6	19.0 NS	49.4	20.0*	40.1	19.6 NS
Boscalid	32.5	19.3 NS	49.4	20.0*	41.0	19.7 NS
Tebuconazole	30.1	25.2 NS	44.6	27.7***	37.4	26.7*
Pyraclostrobin	26.5	34.1**	52.0	15.9 NS	39.3	23.1 NS
Prothioconazole	27.9	30.7*	48.6	21.3**	38.5	24.6*
Propiconazole	32.0	20.5 NS	53.5	13.4 NS	42.7	16.2 NS
Flutriafol	28.0	30.4*	44.2	28.4***	36.1	29.2**
Thiophanate-methyl	29.4	26.9 NS	50.2	18.8 NS	39.8	22.0 NS
SE(±)	2.93		2.95		3.25	
$P>F$	0.0905		0.0011		0.0674	

NS not significant (at $P \leq 0.05$)

[a]Compared with no-fungicide-inoculated

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$ levels of significance

Table 4 Effects of *Pithomyces* blight of *Miscanthus* on *M.×giganteus* biomass and the ability of fungicide spray to mitigate biomass loss

Fungicide treatment (fungicide active ingredient)	Aboveground *M.×giganteus* biomass (g dry-weight per pot)					
	Experiment 2a		Experiment 2b		Experiment 2a and 2b combined	
	Mean	Biomass reduction (%)[a]	Mean	Biomass reduction (%)[a]	Mean	Biomass reduction (%)[a]
No fungicide–non-inoculated	37.9		17.5		27.7	
No fungicide–inoculated	24.3	34.9***	12.6	28.1*	18.6	32.8**
Cyproconazole	16.6	56.3***	14.4	17.5 NS	15.5	44.1***
Chlorothalonil	24.5	35.2***	12.3	29.6*	18.4	33.5**
Metconazole	24.3	36.0***	14.9	14.8 NS	19.6	29.3*
Tetraconazole	18.8	50.3***	12.1	30.5*	15.5	44.1***
Boscalid	19.6	48.3***	12.2	30.1*	15.9	42.6***
Tebuconazole	25.0	34.0***	09.2	47.2***	17.1	38.2***
Pyraclostrobin	21.2	42.7***	10.8	38.2**	16.2	41.3***
Prothioconazole	21.6	43.0***	12.2	30.1*	16.9	38.9***
Propiconazole	20.5	46.0***	14.1	19.5 NS	17.3	37.6***
Flutriafol	24.3	36.0***	12.2	30.4*	18.2	34.2**
Thiophanate-methyl	20.6	45.7***	12.1	30.9*	16.3	41.0***
SE (±)	2.05		1.23		1.84	
P>F	<0.0001		0.0032		0.0007	

NS not significant (at $P \leq 0.05$

[a]Compared with no fungicide–non-inoculated

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$ levels of significance

Lexington, Kentucky, in 2009 [2] could not be estimated empirically in the field, because of the 100 % incidence. Notwithstanding, severe damage was obvious particularly on the first-year crop, where some tillers were killed. In addition, it was apparent that the leaf blades and sheaths that were covered with the brown, mosaic-like, coalesced necrotic lesions [2] could have had reduced chlorophyll-rich surface area and therefore a significant reduction in the overall photosynthetic efficiency [20], potentially culminating in a reduction in biomass accumulation.

To our knowledge, this is the first study that deliberately estimates the effects of a foliar disease on biomass yield of *M.×giganteus*. Prior investigations of the pathogenicity of soil-borne fungi, particularly *Fusarium* species, on *M.×giganteus* establishment have been reported in Europe [5, 13]. In 2010, *Fusarium avenacerum* was implicated as the major (65 %) soil-borne fungus responsible for rhizome rot of *Miscanthus* that led to an establishment failure of an estimated 90 % of transplanted *M.×giganteus* rhizomes [5]. About a decade earlier, a study on the role that *Fusarium* spp. play in field establishment challenges of *Miscanthus* in Europe (particularly with field establishment of in vitro propagated miscanthus plants) was published by Thinggaard [31]. Of the three *Fusarium* spp.: *Fusarium culmorum*, *Fusarium avenaceum*, and *Fusarium moniliforme* (tested isolates of which all were previously isolated from *Miscanthus* roots, rhizomes, and stems in the field), only *F. culmorum* isolates caused severe root rot and reduced root density of *M. sinensis* 'Goliath' and *M. sinensis* 'Giganteus' (*M.×giganteus*). None of the tested *Fusarium* isolates significantly reduced the height or dry weight of the two *Miscanthus* varieties 10 weeks after inoculation under greenhouse conditions [31].

In our study, a direct comparison of inoculated and non-inoculated *M.×giganteus* plants (Table 4) indicated that a severe infection by *L. chartarum* (Table 3) reduced ($P \leq 0.05$) biomass yield by 35 % and 28 % in two separate experiments under greenhouse conditions. The disease severity and impact on *M.×giganteus* growth and biomass accumulation in the greenhouse inoculation experiments may be comparable to the severe incidence observed on first year *M.×giganteus* research plots near Lexington, Kentucky, in 2009 and the observed, but not measured effect on the establishment of *M.×giganteus* [2]. The uniform (100 % incidence) infection of *M.×giganteus* in the field [2] and in the greenhouse study underscores the potential challenge of using a *Miscanthus* clone with no genetic variability as a biomass monocrop. The susceptibility of a *M.×giganteus* monocrop (particularly a first-year crop) to an aggressive pathogen strain under favorable environmental conditions could mean significant reduced growth, field establishment problems, and loss of biomass harvest and income to a farmer.

This study also represents the first time any management tactics have been attempted for a fungal pathogen of *M.×giganteus*. However, investigations conducted to identify effective chemical fungicide treatments for the control of *L. chartarum* on pasture grasses have been performed

[25, 26, 28, 30, 37] due to the veterinary importance of this fungus [1, 8, 12, 15, 19, 21, 29, 33]. In our experiments, cyproconazole, flutriafol, prothioconazole, and tebuconazole treatments resulted in consistent and significantly lower disease severity and belonged to the DMI group (Table 3). Early research on the chemical control of the fungus *L. chartarum*, the causative agent of facial eczema, determined that a number of compounds in the MBC group, which includes thiabendazole, benomyl, carbendazim, and thiophanate-methyl, reduced spore production in pastures [26–28, 30, 35]. Thiabendazole and benomyl reduced spore numbers during periods of accelerated production by 40 % to 90 % for 6 weeks [26]. Wallace [35] evaluated the effectiveness of three fungicides carbendazim, benomyl, and thiophanate methyl in the field and found that a carbendazim pre-danger spore level application (at 0.15 kg a.i./ha) was effective in significantly reducing *L. chartarum* spore counts in pasture to a below danger level on treated pasture (700×10^3 spores per m^3 air). Benomyl and thiophanate methyl also were effective at 0.3 kga.i./ha. Only carbendazim at a rate of 0.15 kga.i./ha kept the spore count at or below 700×10^3 spores per m^3 air [35]. Although spore count reduction cannot be directly translated to plant disease reduction, results from our testing of thiophanate-methyl indicated a consistent but non-significant reduction in *Pithomyces* blight of *M.×giganteus* (Tables 2 and 3). Although there was a statistically significant reduction in disease severity when cyproconazole, flutriafol, tebuconazole, or prothioconazole were applied (Table 3), the reduction was not sufficient to mitigate *M.×giganteus* biomass loss resulting from the infection by *L. chartarum* (Table 4).

It is reasonable to expect that more than one application or a higher application dosage of these fungicides, particularly cyproconazole, flutriafol, tebuconazole, and prothioconazole, may be needed to achieve adequate disease control that could result in biomass loss abatement. However, more challenges are expected under field conditions due to rain and other environmental factors that can render the fungicides less effective than under more controlled conditions in growth chambers and the greenhouse. Since the rates typically used for other diseases of agronomic crops in the field were tested in this study, it was expected that any fungicide that can be profitably and sustainably (in environmental sense) used in the field should give a near-complete control under controlled conditions of growth chambers and the greenhouse. The average percent disease severity on *M.×giganteus* treated with fungicide ranged from 36 % to 39 % for the four best fungicides compared with 51 % for the no fungicide-inoculated control (Table 3). This level of control achieved under greenhouse conditions, though statistically significant, does not seem to be practically promising under field conditions. An alternative approach to using unacceptably high application rates might be to test a combination of different chemical fungicides with different modes of action. For field setting, multiple applications of the fungicides may be necessary. Furthermore, recognizing the importance of alternative disease management, particularly for this new disease, we are currently screening different *Miscanthus* populations for sources of disease resistance.

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