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Fungi and Diseases Associated with Cultivated Switchgrass in Iowa

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Switchgrass (*Panicum virgatum* L.) is a native perennial prairie grass that is now cultivated as a forage crop and a biomass crop for renewable energy. Biomass yields of switchgrass in southern Iowa have recently dropped significantly in some fields and the reduction has been attributed to disease. A disease survey was conducted in 1999 to assess the ptevalence of major diseases in Chariton Valley switchgrass production. There were disease symptoms present on switchgrass plants in each field and thirteen fungal species were identified from leaf, stem, and root samples. Two pathogenic fungi, *Tilletia maclaganii* and *Colletotrichum graminicola,* were present in 88% and 100% of fields, respectively. Severity (% diseased leaf area) of *C. graminicola* was low in each field. However, *Tilletia maclaganii* was at high incidence (>70%) in some fields and apparently is causing significant reductions in biomass and seed production. Nine of the other fungi identified in the survey have not been reported previously from switchgrass in Iowa.

INDEX DESCRIPTORS: biomass, fungi, *Panicum virgatum,* switchgrass, *Tilletia maclaganii.*

Switchgrass (*Panicum virgatum* L.) is a native perennial prairie grass in which cultivars have been developed for use as forage (Hughes et al. 1984) and biomass crops. In southern Iowa, a consortium of private groups and government agencies has established an infrastructure for using switchgrass as a cash crop for renewable energy, by combining biomass from switchgrass with coal for combustion. This project also was designed to provide erosion control for the Lake Rathbun watershed and provide wildlife refuge land (Chariton Valley Resource Conservation and Development 2000).

The four-county area, known as the Chariton Valley, is composed of Lucas, Monroe, Wayne, and Appanoose Counties in south-central Iowa. Landowners have committed 1,600 hectares in the area to the production of switchgrass for biomass (M. Braster, pers. comm.). Biomass harvested from switchgrass fields in the Chariton Valley is co-fired with coal at the Alliant Energy Ottumwa Generating Station in Chillicothe, Iowa.

In 1998 the Chariton Valley project coordinators became aware that biomass and seed yields were beginning to decline. Expected biomass yields are approximately 9.0 Mg/ha, but in some fields there had been a decline of approximately 5.0 Mg/ha in the past two years (M. Braster, pers. comm.). Diseases were suspected of contributing to the declining yields, but it was unclear which diseases might be involved or to what extent they were affecting biomass production.

At least 42 fungal species have been reported to occur on switchgrass in the U.S., but only 10 have been reported previously in Iowa (Table 1). Pathogenicity of many of these fungi is unknown. Because switchgrass has not been an economically important plant in the state, there has not been extensive research on its diseases, and there is a strong likelihood that many of the other 32 fungi occur in Iowa but have not been reported.

Our objectives were to: investigate fields with declining yields for possible disease-related causes, and to assess the occurrence of diseases of switchgrass in biomass production areas.

METHODS

Preliminary sampling was conducted on 27 May and 24 June 1999, in fields previously identified as low-yielding, and at a cultivar trial at the Iowa State University McNay Research Farm near Chariton, IA, in Lucas Co. Stem, leaf, and root samples with disease symptoms were collected from cooperator fields near the towns of Derby, Lucas (Lucas Co.), Iconium (Appanoose Co.), and Millerton (Wayne Co.). Samples were collected from several cultivars. During the preliminary sampling, a seed smut was observed in several fields.

A more intensive survey, focused primarily on the smut disease, was conducted in late August. We used a weighted random sampling procedure to select 20 switchgrass fields from approximately 60 switchgrass fields involved in biomass production. Neither yield nor suspected disease status were considered in the field selection. The sampling procedure was designed so that the probability of each field being chosen was proportional to its area. This resulted in samples being taken from 16 fields representing approximately 50% of the total area of the 60 fields. Four of the 20 selected fields were not suitable for sampling due to low switchgrass densities. In addition to the randomly selected fields, one additional field (field 2) was sampled in Appanoose Co. This field was chosen because of its high incidence of smut. All 17 fields had been planted to the predominant cultivar, Cave-in-Rock. In each field, five samples were taken from arbitrary locations. Each sample consisted of one square meter in which all vegetation was clipped approximately 15 cm above the soil and brought to the laboratory. Samples were stored at 4°C until they were assessed for disease. The total number of tillers and the number of tillers with smut were counted. The incidence of smut (% of tillers with smut) was calculated for each sample. The mean incidence of smut for the sampled area was calculated as a weighted average of the 16 randomly selected fields. Linear correlation analysis (SigmaStat, Jandel Scientific, San Diego, CA) was conducted to assess the relationship between tiller density (tillers/m^2) and smut incidence. Other disease symptoms were recorded and sub-samples were retained for disease identification.

In order to identify diseases and fungi other than smut, leaf and stem samples from the surveyed fields were placed into moist chambers (sterile petri dish with moistened filter paper) and allowed to develop for four to seven days. Fungi developing on symptomatic tissue were microscopically examined and identified. Fungi that

Table 1. Fungi and Oomycetes reported to infect switchgrass in Iowa and other areas of the United States.

Class or Phylum	Species	Reference	Iowa Reports
Oomycetes	*Pythium arrhenomanes* Drechs.	Farr et al. 1995	
	Pythium deharyanum R. Hesse	Farr et al. 1995	
	Pythium graminicola Subramanian	Farr et al. 1995	
	Sclerophthora macrospora (Sacc.) Thirumalachar, C.G. Shaw & Narasimhan	Farr et al. 1995	
Ascomycota	*Balansia epichloe* (Weese) Diehl	Farr et al. 1995	
	Balansia henningsiana (A. Möller) Diehl	Farr et al. 1995	
	Claviceps Tul.sp.	Farr et al. 1995	
	Elsinoë panici L.H. Tiffany & Mathre	Tiffany and Mathre 1961	Tiffany and Mathre 1961
	Exarmidium fusariisporum (Ellis & Everh.) Theiss. & Syd.	Farr et al. 1995	
	Graphyllium hysterioides (Ellis & Everh.) Barr	Farr et al. 1995	
	Leptosphaeria orthogramma (Berk. & M.A. Curtis) Sacc.	Tiffany and Knaphus 1985	Tiffany and Knaphus 1985
	Metasphaeria subseriata Ellis & Everh.	Farr et al. 1995	
	Phyllachora graminis (Pers.:Fr.) Nitschke	Gabel and Tiffany 1999	Gabel and Tiffany 1999
Basidiomycota	*Puccinia emaculata* Schwein.	Farr et al. 1995	Gilman and Archer 1929
	Puccinia graminis Pers.:Pers.	Farr et al. 1995	
	Puccinia virgata Ellis & Everh.	Cummins 1971	
	Uromyces graminiciola Burrill	Farr et al. 1995	Gilman and Archer 1929
	Sporisorium cenchri (Lagerh.) K. Vánky	Farr et al. 1995	
	Tilletia barclayana (Bref.) Sacc. & Syd.	Fischer 1953	Farr et al. 1995
	Tilletia maclaganii (Berk.) G.P. Clinton	Fischer 1953	Gilman and Archer 1929
	Ustilago heterogena Henn.	Fischer 1953	Farr et al. 1995
	Ustilago trebouxii Syd. & P. Syd.	Fischer 1953	
	Thanatephorus cucumeris (A.B. Frank) Donk	Farr et al. 1995	
	Rhizoctonia solani Kühn	Farr et al. 1995	
Hyphomycetes	*Alternaria* Nees sp.	Farr et al. 1995	
	Beniowskia sphaeroidea (Kalchbr. & Cooke) E. Mason	Farr et al. 1995	
	Bipolaris sorokiniana (Sacc.) Shoemaker	Farr et al. 1995	
	Cerebella andropogonis Ces.	Farr et al. 1995	
	Curvularia geniculata (Tracy & Earle) Beodijn	Farr et al. 1995	
	Fusarium acuminatum Ellis & Everh.	Farr et al. 1995	
	Fusarium equiseti (Corda) Sacc.	Farr et al. 1995	
	Micordochium bolleyi (R. Sprague) DeHoog & Hermanides-Nijhof	Farr et al. 1995	
	Phaeoramularia fusimaculans (Atk.) X. Liu & Guo	Farr et al. 1995	
Coelomycetes	*Ascochyta missouriensis* R. Sprague & A.G. Johnson	Farr et al. 1995	
	Ascochyta Lib. sp.	Farr et al. 1995	Tiffany et al. 1990
	Colletotrichum graminicola (Ces.) G.W. Wils.	Farr et al. 1995	
	Hendersonia panicicola Petr.	Farr et al. 1995	
	Phoma sorghina (Sacc.) Boerema, Dorenbosch, & Van Kesteren	Farr et al. 1995	
	Phoma terrestis E.M. Hans.	Farr et al. 1995	
	Phyllosticta panici E. Young	Sprague 1950	
	Pseudoseptoria donacis (Pass.) Sutton	Farr et al. 1995	
	Septoria sigmoidea Ellis & Everh.	Farr et al. 1995	Farr et al. 1995
	Wojnowicia hirta Sacc.	Farr et al. 1995	

could not be identified from moist chambers were aseptically transferred to potato dextrose agar (PDA, Difco Brand, Becton Dickinson and Co., Sparks, MD) amended with 50 mg/L chlortetracycline hydrochloride, 120 mg/L neomycin sulfate, and 200 mg/L streptomycin sulfate, or carnation leaf agar (CLA) (Nelson et al. 1983). Root samples from the field were cut into three to four 1-cm sections, including necrotic areas, surface disinfested in 10% bleach for one minute, rinsed with sterile water, and placed onto PDA. The samples were incubated for 7 days in the dark. Fungal colonies then were examined microscopically for identification according to morphological characters. Colonies that could not be identified from the PDA cultures were transferred to CLA, incubated for 7 additional days,

and then examined microscopically. Presence of each fungus recovered from leaves, stems, or roots was recorded for each sample and prevalence (% of fields from which the fungus was recorded) was calculated for the more common fungi.

Seeds of the cultivar Cave-in-Rock, contributed by a commercial seed producer in Lucas County, were cultured for seedborne diseases. Approximately 75 seeds were surface sterilized for 3 min in 0.5% NaOCl, rinsed with sterile distilled water, and placed in moist chambers for 24 h to initiate germination. The moist chambers were then placed in a freezer (−20°C) for 48 h; seeds were removed and cultured on PDA for 7 days. Fungal colonies growing on PDA were transferred to CLA for identification. Additional seeds of cultivars Forestburg and Sunburst, contributed by Dr. Charles Brummer (Iowa State University Dept. of Agronomy), were planted in greenhouse soil mix. After 3 weeks, seedlings were removed and rinsed with tap water to remove soil. Seedlings were dissected into root, stem, and leaf pieces, which were surface sterilized in 0.5% NaOCl for 3 min and rinsed with sterile distilled water. Plant tissue pieces were cultured on PDA for 7 days. Fungal colonies growing on PDA were transferred to CLA for identification.

RESULTS

Thirteen fungal species were identified during the preliminary sampling or survey sampling. Additional fungi were collected but not identified due to a lack of sufficient taxonomically meaningful morphological characters.

Alternaria alternata (Fr.) Keissler (Ellis 1971) was found on fresh leaves from the field, seeds, and from the base of seedling leaves in the greenhouse. Clavate or pyriform, olive-pigmented conidia formed in chains from conidiophores on leaf tissue and in culture. Conidia had transverse and longitudinal septa, short beaks, and a verrucose surface. This fungus can be parasitic or saprophytic on plant material (Agrios 1997) and it is unclear from our examination whether the fungus was present as a leaf pathogen or saprophyte.

Bipolaris sorokiniana (Sacc.) Shoemaker was found on lesions on leaves collected in the field, on the bases of seedling leaves from the greenhouse, and also was isolated from seed. Isolates from each source were morphologically identical. The leaf lesions were elliptical, approximately 1 to 3 mm long, and had yellowish halos. The halo was surrounded by tissue that had a reddish-purple tint. Conidia formed sympodially on dark-pigmented, erect conidiophores arising from the lesions or in culture. Conidia were brown, 3 to 10 pseudoseptate, elliptical, and slightly curved, as described by Ellis (1971), as *Drechslera sorokiniana. Bipolaris sorokiniana* (Sacc.) Shoemaker (*Helminthosporium sativum*) has been reported to cause a seedling blight (Farr et al. 1995) and leaf disease (Zeiders 1984) on switchgrass.

Colletotrichum graminicola (Ces.) G.W. Wils. (Sprague 1950) was found on lesions on leaves from the field. Lesions were 3 to 5 mm long, elliptical, and tan with a brown border and a yellow halo. Within the lesions were numerous acervuli with dark setae. Abundant conidia formed in mucilage in the acervuli; conidia were single-celled, falcate, hyaline, and slightly pink in mass. This fungus has been reported from switchgrass in Iowa (Tiffany et al. 1990).

Elsinoë panici L.H. Tiffany & Mathre (Tiffany and Mathre 1961) was found on leaves in the field, causing elongated white to cream-colored lesions, 10 to 20 mm long, or with a black fungal stroma on the leaf surface. Conidia were hyaline, single-celled and ovoid. Globose, eight-spored asci were present in some samples, with ascospores that were three- to four-celled, ellipsoid, and hyaline.

Species of *Fusarium* Link were isolated from fresh leaf tissue, root tissue, and seeds. *F. acuminatum* Ellis & Everh. was isolated from leaf tissue and *F. oxysporum* Schlechtend.:Fr. and *F. solani* (Mart.) Sacc. were isolated from root tissue and seeds. The necrotic lesions on the roots were brown to dark brown in color and covered 1 to 3 mm of tissue. *Fusarium* isolates were identified to species by morphological characters on CLA as described by Nelson et al. (1983).

Isolates of *Penicillium* Link:Fr. were isolated from root tissue and seed. Root lesions covered 2 to 5 mm on the roots and were brown to dark brown. In culture on CLA, the fungus produced long branched conidiophores tipped with clusters of phialides. Conidia were small, globose, hyaline (but blue-green in mass), and produced in chains, as described by Barnett and Hunter (1998).

A species of *Phyllosticta* Pers. was found on leaf lesions from the field. Lesions were elliptical, 3 to 5 mm long, and yellow to tan with dark spots (pycnidia) in the middle of the necrotic tissue. Pycnidia were globose with a short beak. Conidiophores were indistinguishable. Conidia were small, single-celled, ovoid, and hyaline, as described by Barnett and Hunter (1998). *Phyllosticta panici* E. Young has been reported previously from switchgrass (Sprague 1950).

Pseudoseptoria donacis (Pass.) Sutton was found on leaf lesions from the field. Lesions were elliptical, 2 to 5 mm long, and tan with black spots (pycnidia) in the middle of the lesion. Pycnidia were globose and about 100 μm in diameter. Conidia were falcate, nonseptate, and hyaline, as described for *Pseudoseptoria donacis* (Sprague and Johnson 1950) (as *Selenophoma donacis*). This fungus has been reported previously on switchgrass (Farr et al. 1995).

Puccinia emaculata Schwein. was found on fresh leaves and year-old dead stems. Pustules were 1 to 2 mm long, black, linear, and contained between veins on the stems. Urediniospores and teliospores were found on leaf samples, but stem pustules contained only teliospores. Urediniospores were almost globose and approximately 24 μm long; teliospores were two-celled, ellipsoidal, and 32–40 μm × 15–20 μm, consistent with *P. emaculata* as described by Cummins (1971). *Puccinia emaculata* Schwein., *P. virgata* Ellis & Everh., and *P. graminis* Pers:Pers. have been reported previously on switchgrass (Gilman and Archer 1929, Cummins 1971, Tiffany and Knaphus 1985, Farr et al. 1995).

Tilletia maclaganii (Berk.) G.P. Clinton was found on inflorescences in the field. This smut disease was characterized by a purpling of the panicles, early heading, stunted plants, and seeds replaced by orange-brown teliospore masses. Sori remained covered by the glumes even after the plants matured, with small inconspicuous spore masses exuding from the tips of the florets. Teliospores were reddish-orange when immature, then becoming dark brown as they matured. They were globose to slightly irregular, approximately 18–25 μm in diameter, finely verrucose, and with a thick exospore. True sterile cells also were present, as described by Fischer (1953).

A species of *Trichoderma* Pers:Fr. was isolated from root lesions that were 2 to 5 mm long and brown to black in color. In culture on CLA, the fungus produced hyaline, branched conidiophores with hyaline to pale green single-celled, ovoid conidia in terminal clusters, as described by Barnett and Hunter (1998). Conidia were bright green in mass.

Among the fungi we identified, *T. maclaganii* and *C. graminicola* were the most commonly encountered (Tables 2 and 3). Although *C. graminicola* was found on several cultivars, *T. maclaganii* was found only on Cave-in-Rock, which is the most commonly planted cultivar. Other cultivars were observed primarily at the cultivar trial at the ISU McNay Research Farm, and the smut disease did not occur there.

Smut caused by *T. maclaganii* was widespread in the Chariton Valley switchgrass plantings. It was found in each of the four counties, in 15 of the 17 fields surveyed (Table 3) at an incidence of 0.2 to 70.5% of tillers. Mean incidence was 10.1%. Field 2 was not included in this calculation since it was not one of the randomly selected fields. Based on the occurrence of the disease in individual subsamples, we estimate that 50–82% of the sampled land area was infested with smut in 1999. A more precise estimare is not possible

Table 2. Fungi isolated from switchgrass in the Chariton Valley in 1999; cultivars, infected tissue, and prevalence in biomass production fields.

Fungi	Cultivars	Plant Tissue	Prevalence (%)[a]
Alternaria alternata	Blackwell Cave-in-Rock Sunburst	Leaf	82
Bipolaris sorokiniana	Blackwell Cave-in-Rock Sunburst	Leaf, seed	24
Colletotrichum graminicola	Blackwell Carthage/Shawnee Cave-in-Rock Forestburg IALM NU94-2CH Sunburst	Leaf	100
Elsinoë panici	Cave-in-Rock	Leaf	29
Fusarium acuminatum	Cave-in-Rock	Leaf, root, seed	NR
F. oxysporum	Cave-in-Rock	Root	NR
F. solani	Cave-in-Rock	Root	NR
Penicillium sp.	Cave-in-Rock	Root	NR
Phyllosticta sp.	Cave-in-Rock	Leaf	47
Pseudoseptoria donacis	Blackwell	Leaf	NR
Puccinia emaculata	Cave-in-Rock	Leaf, stem	47
Tilletia maclaganii	Cave-in-Rock	Head	88
Trichoderma	Blackwell	Root	NR

[a]Percentage of fields (out of 17) in which the fungus was found or isolated. NR = not recorded

Table 3. Incidence of seed smut caused by *Tilletia maclaganii* in cultivated switchgrass stands sampled in the Chariton Valley in southern Iowa.

Field	County	Hectares	Tillers /m^2	Incidence (%)[a]
1	Appanoose	41.3	81	46.5
2	Appanoose	53.4	252	70.5
3	Lucas	5.7	128	9.4
4	Lucas	13.0	61	0.0
4-1	Lucas	13.8	86	1.2
5	Lucas	2.8	97	1.4
7	Lucas	57.5	95	3.2
8	Lucas	16.2	122	15.0
9	Lucas	48.2	121	6.6
12	Lucas	80.9	127	11.8
13	Monroe	6.9	152	1.7
14	Wayne	8.1	176	0.8
15	Wayne	40.9	96	0.2
16	Wayne	68.9	112	0.0
18	Wayne	30.8	171	21.4
19	Wayne	6.5	147	13.2
20	Wayne	12.1	126	8.8
Total		507		
Weighted mean[b]			114	10.1

[a]% of tillers
[b]Does not include field 2

without a more intensive sampling procedure. There was a positive linear correlation (correlation coefficient, $R = 0.57$, $P = 0.017$) between the number of tillers/m^2 and smut incidence, but only when Field 2 was included in the analysis. A preliminary report on the occurrence of *T. maclaganii* was published (Gravert et al. 2000).

DISCUSSION

Of the 13 fungi identified (Table 2), only four (*C. graminicola, E. panici, Puccinia emaculata,* and *T. maclaganii*) have been reported previously from Iowa switchgrass. Five of the fungi identified have been reported previously from switchgrass in areas other than Iowa. This represents the first reports for *Alternaria alternata, Bipolaris sorokiniana, Fusarium acuminatum, Phyllosticta* sp., and *Pseudoseptoria donacis* from switchgrass in Iowa. Four species reported here (*Fusarium oxysporum, Fusarium solani, Trichoderma* sp., and *Penicillium* sp.) have not been reported previously from switchgrass. However, it is not clear whether any of these four fungi are pathogenic to this host.

Rusts are recognized as a potential threat to cultivated switchgrass and some cultivars have been selected for resistance to rust (Hughes et al. 1984). At least three *Puccinia* species and one species of *Uromyces* have been reported on switchgrass, but only *P. emaculata* was found in our survey. Mycologists surveying native prairie grasses in Iowa in the 1920s and 1980s also did not report other *Puccinia* species on switchgrass (Tiffany and Knaphus 1985, Tiffany et al. 1990). In 1999, rust was not present at a high incidence. In 2000, a systematic survey was not conducted, but rust was observed in several fields in the area and appeared to be more prevalent than in 1999.

The results indicate that there is a wide distribution of two pathogenic fungi on switchgrass cultivated in southern Iowa: *Tilletia maclaganii* and *Colletotrichum graminicola.* Both are well-known pathogens; *Tilletia maclaganii* is the causal agent of seed smut and *Colle-*

totrichum graminicola causes anthracnose. Both fungi were found in the majority of samples, but only *T. maclaganii* appeared to have a significant impact on plant growth. Plants infected by *T. maclaganii* were 30 to 50 cm tall, whereas healthy plants grew to a height of 2 m or more. Conversely, *C. graminicola* lesions affected only a small amount of leaf area on infected plants and were not associated with stunting of the plants.

Tilletia maclaganii greatly reduces biomass yields because infected plants flower prematurely, when the plant is less than 1 m in height. The disease cycle for this pathogen is unknown. The source of inoculum for cultivated switchgrass fields is a high priority for current research. No other hosts are reported for this fungus (Farr et al. 1995). Some species of *Tilletia* are primarily seedborne while others overwinter as teliospores in the soil and infect plants through the roots. Covered smuts of wheat, *Tilletia caries* and *T. foetida,* have been found to survive up to three years in the soil in the teliospore stage (Agrios 1997). Additional research is needed on overwintering and seed-related aspects of *T. maclaganii* in Iowa.

Tilletia maclaganii occurs on native switchgrass in Iowa, but is not common (L. H. Tiffany, personal communication). Previous surveys of fungi found on switchgrass have been conducted primarily in prairie remnants (Tiffany and Knaphus 1985, Tiffany et al. 1990, Gabel and Tiffany 1999). The limited genetic diversity and higher density of switchgrass in cultivated fields may lead to predominance of specific fungi that are not common in prairies. A positive relationship between tiller density and smut incidence could indicate a greater tendency for disease spread when switchgrass density is high, but the evidence for this relationship is not clear-cut.

The high prevalence and incidence of the disease in cultivated switchgrass may be a result of the widespread planting of a single cultivar, Cave-in-Rock, which is obviously highly susceptible to the pathogen. Effective control practices are difficult to determine, but alternative cultivars must be investigated, and planting a greater diversity of cultivars would reduce the risk of widespread disease losses.

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