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Field Disease Incidence, Fungal Collection, and Evaluation of Koch's Postulates with Isolated Fungi from Giant Miscanthus (Miscanthus X Giganteus) in Mississippi

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Field disease incidence, fungal collection, and evaluation of Koch's postulates with isolated fungi from giant miscanthus (*Miscanthus x giganteus*) in Mississippi

By

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The establishment of perennial grasses as biomass crops has increased the production acreage of giant miscanthus (*Miscanthus x gigantues* Greef et Deu, MXG). Yield loss and establishment failure could be detrimental to the sustainable production of this crop, and therefore, exploitation of differentiation in cultivar response to fungal diseases could be a key management strategy. A study was initiated in 2010 to evaluate MXG cultivars for foliar disease incidence (FDI) and compare to switchgrass (*Panicum virgatum* L., SG) cultivars, isolate and identify fungi from symptomatic leaf material, and demonstrate through Koch's postulates the ability of these fungi to incite symptoms observed in the field.

Giant miscanthus FDI ratings were similar between MXG cultivars, but significantly lower when compared to SG cultivars. Thirty genera of fungi were identified from fungal collections, and 16 pathogenic genera were isolated. Twelve isolates were selected and four were demonstrated to be pathogenic on Mxg.

DEDICATION

This research is dedicated to my parents, Dan and Anne Gilley. Their unending love, support, and encouragement carried me throughout this process, and without their instillation of persistence and determination this thesis and degree would not have been possible. Also, to my brother, Trevor, who is always a positive role model and a glimpse of inspiration.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Giant miscanthus, *Miscantus x giganteus* (Greef et Deu ex. Hodkinson et Renvoize) is a tall, C_4 perennial grass in the Poaceae family (Greef and Deuter 1993). The genus *Miscanthus* includes species with great phenotypic and genotypic variation. The vast physical and physiological differences allows for the application of this crop in a variety of ways. Historically used as a forage grass (Stewart et al. 2009), improved miscanthus species are used in landscape settings as ornamental grasses, cultivated and bred to develop into specimen plantings with large biomass and large, flowering plumes.

Recent legislation passed by the United States government in 2006 outlined support for research on cellulosic ethanol to be competitive with corn based ethanol has increased production of certain non-traditional crops as energy crops. Similar goals have been set forth in the European Union. Perennial rhizomatous grasses such as giant miscanthus, switchgrass (*Panicum virgatum L.*), and others have advantages for use as alternative bioenergy sources. In general, perennial grasses produce large amounts of biomass annually on relatively small acreages. High production yields are obtainable with minimal cultural inputs. The biomass of perennial grasses contains high levels of cellulose and relatively low lignin compared to wood, important for low ash when converting into ethanol as well as enabling the crop to stand upright through dry-down until harvest. A far greater amount of energy can be produced from the conversion of

these grasses to ethanol than what is required to produce them. High yields combined with minimal production inputs allow for an overall positive net energy gain when using perennial rhizomatous grasses for bioenergy feedstock (Lewandowski et al. 2003).

Giant miscanthus is one of the perennial grasses currently being produced for energy conversion. With a maximum height averaging 3 m, biomass yields of up to 30 T dry mass per hectare are attainable on suitable lands (Lewandowski et al. 2003). High yields can be achieved with minimal fertility and irrigation inputs thus reducing the financial and energy cost of production inputs. At initial planting, tilling and weed control is required; however, no continual annual cultivation practices are needed. The minimal tillage involved helps reduce costs as well as reducing soil erosion. Members of the genus miscanthus have a large range of native habitat in East Asian countries allowing it to be suitable for production across a wide variety of environments and growing conditions (Stewart et al. 2009).

Giant miscanthus is a triploid hybrid, consequently the plant is sterile. This trait is advantageous for preventing the dissemination of giant miscanthus resulting in invasive weed issues, but it also creates strict requirements for propagation and establishment. Rhizomes are the primary source of propagation material. Field establishment is accomplished by planting rhizomes either by hand or mechanically. Due to the need for vegetative propagation, many times a single genotype is used for widespread dispersal and establishment. This creates a situation where little to no genetic diversity exists. Monocropping systems such as those used for giant miscanthus production can be particularly vulnerable to disease epidemics. Once natural host susceptibility is established, any pathogen could readily spread between plants,

eventually colonizing and infecting entire fields. Determining the breadth of pathogenic organisms with the ability to infect, colonize and reduce yield is an important first step for giant miscanthus production to remain a viable biofuel alternative.

Giant miscanthus is produced in a monocropping system and information regarding pathogenic relationships in the production of the grass as a biofuel is extremely limited. Research programs to monitor and evaluate this crop for potential pathogens before the mass production stages can be initiated are an important first step. Research and the development of understanding in regards to the pathology of giant miscanthus allows for the addressing of possible issues before production stages. It would also help to create future control programs that are specific to the crop and area being produced.

The goal of this research is to identify plant pathogen relationships that exist within giant miscanthus genotypes produced as biofuel crops. Achievement of this goal will be determined upon the ability to; (i) assess field disease incidence of giant miscanthus cultivars and compare to switchgrass for the growing seasons of 2011 and 2012, (ii) isolate and identify fungi associated with foliar symptoms of giant miscanthus and switchgrass cultivars, and (iii) evaluate pathogenicity of the isolated fungi on giant miscanthus cultivars.

CHAPTER II

LITERATURE REVIEW

Miscanthus (Andersson) is a diverse genera of perennial grasses with origins in the East Asian countries of China, Japan, Korea, and Taiwan (Greef and Deuter 1993). It occurs naturally in regions ranging from the tropics to the subartic (Clifton-Brown and Lewandowski 2002; Stewart et al. 2009) and is one of only a few C_4 grasses able to withstand the environmental fluctuations associated with the temperate zone (Lewandowski et al. 2000). Miscanthus was first introduced to Europe in the 19th Century (Deuter 2000). In 1935, the Dutchmen, Aksel Olsen, traveled to Yokohama, Japan and observed a miscanthus species with exceptionally vigorous growth and brought it back to Denmark to be distributed in many European gardens as "Clone Aksel Olsen" (Deuter 2000; Lewandowski 2000). The native grassland setting of miscanthus in Asia gave rise to many natural hybrid crosses due to various species being located in close proximity. The clone returned back by Olsen is thought to be a cross of *Miscanthus sacchariflorus* and *Miscanthus sinensis* (Greef and Deuter 1993; Greef et al. 1997; Lewandowski et al. 2000). This particular species, noted for its outstanding growth when compared to other miscanthus species, was later categorized by Greef and Deuter (1993) to be *Miscanthus x giganteus* Greef et Deu ex. Hodkinson et Renvoize (Hodkinson and Renvoize 2001); however, the taxonomy is easily confused within the genus (Hodkinson et al. 2002).

The management of miscanthus dates back as far as 1,000 years based on ancient Japanese historical books describing vast grasslands making the sight of nearby villages impossible (Stewart et al. 2009). People indigenous to the area where miscanthus grows naturally have utilized its long stems as thatching material for buildings and houses as well as using the leaves to weave bags for charcoal storage (Stewart et al. 2009). Due to the availability and dispersion of this grass across its native area, it has been widely used as a feed for livestock. Many *M. sinensis* cultivars have been selected for the sole purpose of livestock feed, focusing on traits such as shorter heights and broader leaves. Miscanthus cultivars are used today as ornamental grasses in landscape settings as well as land stabilization plantings on hills along highways (Deuter 2000). Modern day applications of miscanthus use include insulation material, lumber for floor construction, as well as additives in potting mixes. The most recent work being done with miscanthus species involves breeding programs focusing on exploiting the wide array of genetic variances among the species in order to generate new superior genotypes better suited to the production processes and the environment in which it is produced (Clifton-Brown and Lewandowski 2002, Greef et al. 1997).

The genus Miscanthus was first described by Andersson in 1855 and includes 17 species to date (Hodkinson and Renvoize 2001). The species *Miscanthus* × *giganteus* is an interspecific hybrid resulting from a natural cross between the tetraploid, *M. sacchariflorus* and the diploid *M. sinensis*, producing a allotriploid (2n=3x=57) hybrid incapable of producing fertile seed (Hodkinson and Renvoize 2001). The genus is considered to have high genetic diversity among the species, explaining the vast

differences and confusion when trying to identify at this level (Hodkinson and Renvoize 2001).

Giant miscanthus (Mxg), *Miscanthus* × *giganteus*, is a highly persistent rhizomatous, C_4 perennial grass located within the Poaceae family. It occurs naturally in the form of grasslands, however younger stands will develop circular masses of tillers reaching 1 to 2 meters in diameter. New growth measuring 2 m high with erect culms, 5 to 10 mm diameter, is initiated each spring from the clump of rhizomatous roots (Hodkinson and Renvoize 2001). Leaf blades are linear, flat, 50 to 66 cm long × 2.2 to 2.5 cm wide with serrated edges (Hodkinson and Renvoize 2001). Leaf arrangement is alternate on the stem and height can range from 1 to 3 m depending on the species and limiting growth conditions such as water and light (Deuter 2000). Christian et al. (2008) reported a maximum stem height of 3.6 meters in a ten-year-old stand of Mxg cultivated in England. It was reported by Liou in 1989 that some tropical and subtropical species of miscanthus can reach heights in excess of 5 m (Deuter 2000). These genotypes are typically used for pulp and paper-making raw material. As the plant flowers mid-summer, large panicles with racemes form. The sterile inflorescence is 30 cm long, covered with small hairs with 24 racemes which can be 10 to 20 cm long (Hodkinson and Renvoize 2001).

Field trial yields of up to 30 t dry matter (DM) per hectare (ha) have been reported in areas with long growing seasons, high average temperatures and supplemental irrigation. The soil type greatly influences the crop. A sandy soil has faster establishment rates, while greater yields can be obtained on clay soils in older stands (Venendaal et al. 1997). Yields of 10 to 25 t DM ha^{-1} are reported in non-irrigated fields

with shorter growing seasons and lower temperatures (Lewandowski 2003). Giant miscanthus can be grown on a wide range of soils; however, sites with stagnant water are unsuitable. A soil profile with good drainage while having enough water holding capacity to supply the plant with adequate water for optimum growth will produce the largest amount of biomass. The greatest yields have been observed on well-drained loamy soils (Lewandowski 2003). Giant miscanthus can withstand soil pH levels ranging from 3.5 to 7.5, with a range of pH 4 to 6 being ideal. A miscanthus productivity model (MISCANMOD) was developed by John C. Clifton-Brown and has been used to predict potential yields in the United States. Yields of 33 t of DM ha^{-1} were projected using this model for research sites in Illinois (Heaton et al. 2003).

Giant miscanthus has a characteristically high rhizomatous growth habit which makes it a desirable host for biofuel production. Giant miscanthus, like most perennial grasses, has the ability to translocate phytonutrients such as N, P, and K to the rhizomes at the end of each growing season. Storage of these nutrients allow for less fertilizer inputs. Also, the reduced nutrient concentration in harvested plant material is desirable to help minimize the problems of corrosion, slagging, fouling and emissions that can occur during combustion (Beale and Long 1997). Beale and Long (1997) did extensive studies in northern Europe on the translocation and location of these vital nutrients at different times throughout the growing season. The greatest concentrations of N, P, and K were detected in the start of the growing season in June, and thereafter nutrient concentrations became diluted with increased amounts of above ground dry matter. The proportion of total nutrients was determined to increase in shoot material in mid-summer, where a steady decline in total nutrient allocation occurred. The study reported that in general,

Mxg fertilizer requirements are lower than most agronomic crops such as corn (*Zea maize* L.), sorghum (*Sorghum bicolor* L.) and wheat (*Triticum* spp. L.) (Beale and Long 1997). Giant miscanthus was also determined to have more efficient nutrient use than the abovementioned crops.

Establishment is a key step in the production of any bioenergy crop, limiting the costs associated with this factor helps increase the net energy gain. Giant miscanthus is a sterile hybrid requiring means other than seed to be used in establishing a crop. Currently this is done either by cuttings or through tissue culture, both of which can be an expensive input cost at around $4,000 per hectare (Lewandowski et al. 2000). Mechanical methods are being developed to relieve some of these establishment costs, that when depreciated over the crop lifetime can constitute 50 to 60% of the annual variable costs (Venendaal et al. 1997). Methods such as disc cultivating to separate rhizomes, or a process using modified potato pickers and planters can drastically reduce propagation costs to an estimated $400 per hectare (Lewandowski et al. 2000).

Fertilization requirements are low due to the ability of Mxg to redirect stored nitrogen to the roots for energy reserves. A study conducted on Mxg productivity levels concluded that no additional yields were noticed with increased nitrogen levels, and that if grown on soils with adequate nitrogen levels average yields may be achieved (Beale and Long 1997, Christian et al. 2008). The study also noted that nitrogen applications may increase the amount of weeds present during establishment. Weed control is necessary only during the establishment period, but after 1 to 3 years Mxg is capable of controlling weeds through competition. Chemical control with herbicides labeled for corn has been successful for weed control in Mxg (Lewandowski et al. 2000). Venendaal

(1997) reported in Denmark glyphosate can be used in the spring on dormant material. However, it was noted that caution should be used in warmer climates where material can be taken up more readily. Mechanical weed cultivation is possible through long tine harrow and row cultivation.

Harvest of foliar tissue occurs after senescence, usually in the fall when temperatures start to cool. In Denmark, lethal frost damage can occur at temperatures of -2.8°C to -5°C (Jorgensen 1996). Fields are allowed to stand and dry and are harvested when a moisture level of 30.0% is achieved. A prolonged harvest was reported in Denmark, Netherlands, Germany, Austria, and Switzerland which reduces the moisture and mineral content of Mxg which is undesirable (Lewandowski et al. 2000). In Germany, a March harvest was recommended to take advantage of optimum dry down times; however, harvesting can also be done in the fall of the year to avoid drastic winter yield losses, which can be as much as 30.0% (Venendaal et al. 1997). Harvest methods are similar to those of forages used for hay production, and Mxg material is stored in round bales (Lewandowski et al. 2000).

Characteristics of Mxg including high dry matter yields as well as its adaptability across a wide soil range has led to research in exploring the use of Mxg as a source of biomass feedstock for bioenergy production. Government legislation in the United States as well as the European Union (EU) set specific goals for the contribution of renewable resources. The Advanced Energy Initiative (AEI) established in 2006 by the U.S. government set a goal in which 30.0% of petroleum dependency be offset by renewable biomass, and going further, called for the production of cellulosic ethanol to be competitive with its gasoline counterparts by 2012 (Heaton et al. 2008, Perlack et al.

2005). Similar goals have been set forth in the EU with a target goal of 12.0% of total energy consumption displaced by renewable sources (Clifton-Brown et al. 2003). A study funded by the U.S. Department of Energy (DOE) and U.S. Department of Agriculture (USDA) was conducted to determine whether land resources in the U.S. can produce enough biomass to sustainably meet the goal of displacing 30.0% or more of the country's current petroleum usage. Estimates figured in the study stated that to displace 30%, approximately a billion tons of dry matter would need to be produced. This amount is unachievable without disturbing current food production systems (Perlack et al. 2005). If all U.S. land used for corn and soybeans (*Glycine max* (L.) Merr) were converted to biofuel production, only 12.0% of total petroleum usage would be replaced (Hill et al. 2006). However, the study conducted by Perlack et al. (2005) reported that with the development of science and technology to streamline production, amounts of approximately 1.4 billion tons of dry matter could be available by 2030 without creating impacts on the food production industry (Perlack et al. 2005).

Many qualities must be present in order for a crop to be considered an ideal energy crop. These include, but are not limited to: 1) having a high concentration of lignin and cellulose content; 2) ability to be produced economically on marginal land; 3) providing a positive net energy gain; and 4) ability to be produced in large quantities without detrimental consequences to the food chain and environment (Beale and Long 1997; Hill et al. 2006; Lewandowski 2003). Perennial grasses have high lignin and cellulose contents which is especially important for high combustion rates. This characteristic also allows for the vegetation to stand upright for ease of harvest as well as enhancing the drying process. High importance is placed on the ability of the grass to be

produced on marginal land. This enables soils once unsuitable for agricultural production to be utilized without reducing current allocations for food production. The ability of biofuel to be produced with a net energy gain is based on the principal of increased energy production over energy spent. Therefore, the crop must be economically capable of producing large amounts of biomass with limited inputs.

Many studies have been conducted to analyze various perennial grasses to be used as bioenergy feedstocks. In 1984 the Herbaceous Energy Crops Research Program (HECP) was initiated under the U.S. Department of Energy (DOE) to evaluate 35 various herbaceous crops for further research as energy crops, 18 of which were perennial grasses. Results of this study showed switchgrass (*Panicum virgatum* L.) to have greatest potential. In 1991 HECP evolved into the DOE's Bioenergy Feedstock Development Program (BFDP), and switchgrass was chosen as a model crop to focus research efforts in hopes to expedite information concerning the use of perennial grasses as energy crops (Lewandowski 2003). Switchgrass was chosen due to its ability of high yields on marginal land, desirable attributes for biofuel, and lack of production limitations. Europe was the first to initiate trials investigating the potential use of the *Miscanthus* species as a bioenergy feedstock in Denmark in the late 1960's (Heaton et al. 2008; Lewandowski et al. 2003; Venendaal et al. 1997). Breeding trials were set up by the European AIR program in 1993 to identify cultivars within *Miscanthus* spp. having genetics applicable to the bioenergy field. It was found that although *M. sinsensis* showed improved combustion quality of biomass; higher yields were obtained using the hybrid, Mxg.

While much research has been focused on the viability of Mxg as a biofuel feedstock in terms of production and energy harnessing power, limited importance has

been placed on the interaction of plant pathogens and its potentially limiting role in biomass yield production. The possible presence of a pathogen and characteristics of miscanthus production warrants investigation.

Some disadvantages with respect to plant parasitic relationships exist in the cultivation of Mxg for biofuel. Given the perennial nature of Mxg, and harvesting techniques, large quantities of plant debris remain in the field providing a source of substrate for possible pathogens. Inoculum may accumulate over time to a sufficient level causing an epidemic in Mxg fields. Giant miscanthus is vegetatively propagated; therefore genetic diversity is limited, which creates entire fields of genetic clones that may be susceptible to a particular disease. The monocrop nature of Mxg predisposes the crop to rapid destruction with little or no means for control.

Accounts of vast crop destruction due to monocropping have been witnessed throughout history. Dating back to 1840 in Ireland where nearly 1 million citizens died of starvation due to entire losses of the potato crop (Donnelly 2001). The Irish were dependent on one variety of potato which was vegetatively propagated. An outbreak of potato blight caused by the fungus *Phytophthora infestans* (Mont.) de Bary spread quickly and wiped out entire fields (Ristaino 2002). Other accounts of monocropping disasters have been witnessed throughout time. An epidemic within the last 30 years was the approximate loss of over 1 billion dollars or 710 million bushels of corn due to southern corn leaf blight, causal organism *Bipolaris maydis* (Y. Nisik. & C. Miyake), in the southeastern United States (Tatum 1971). The majority of hybrid corn planted throughout the Southeast and Midwest at that time contained the Texas male-sterile (TMS) cytoplasm. Vast acres of hybrid TMS corn production were susceptible to a new

race of *B. maydis* identified as Race T (Tatum 1971). Past historical epidemic occurrences in monocropping production systems raises awareness to potential losses and vulnerabilities associated with Mxg.

Current research is limited concerning pathogens associated with bioenergy crops due to their recent emergence into the production market. However, as production yields across different species become more important, so does the relationship of pathogens that may potentially limit yield.

Switchgrass diseases and associated pathogens could be potential pathogens of Mxg due to the close proximity associated when these grasses are grown side by side for research. Currently identified pathogenic relationships of switchgrass include rust caused by *Puccinia emaculata* Schwein (Frazier et al. 2013; Hirsch et al. 2010; Zale et al. 2008), as well as *Colletotrichum navitas* Crouch causing anthracnose (Crouch et al. 2009), leaf spot caused by *Bipolaris oryzae* Breda de Haan (Krupinsky et al. 2005; Tomaso-Peterson and Balbalian 2010; Waxman and Bergrstom 2011) and leaf smut disease caused by *Tilletia maclaganii* (Berk.) (Thomsen et al. 2008). Switchgrass smut can have a deleterious effect on biomass and tiller production (Thomsen et al. 2008). Yield reductions of 17.0% and 6.6% caused by switchgrass smut in 2002 have been documented (Thomsen et al. 2008).

Limited information is available concerning diseases of Mxg. Current research proposed by M. Gray, Ph.D, University of Illinois Energy Biosciences Institute, focuses on potential pest relationships among certain insects, plant diseases, and nematodes in Mxg and switchgrass (Gray et al. 2009). A first report of *Pithomyces chartarum* (Berk. and Curt.) causing a leaf blight of Mxg in Kentucky was published in 2010 (Ahonsi et al.

2010). Leaf blight, caused by *Leptosphaeria* sp. and it's anamorph *Stagonospora* sp., has been described occurring on *Miscanthus* spp. (O'Neill and Farr 1996) as well as more recently described occurring on Mxg (Pusz and Plaskowska 2010). Symptoms of very small brown spots which further progressed into complete blighting and necrosis were reported in both instances.

CHAPTER III

MATERIALS AND METHODS

Field Disease Ratings

Field establishment

Field research sites were selected to assess foliar disease incidence (FDI) from three Starkville, MS locations with two sites located at the Leveck Animal Research Center (South Farm) and a third located at the Bearden Dairy Research Center. All research sites were previously established as part of a broader alternative crop breeding research program directed by Dr. Brian S. Baldwin, Mississippi State University. The "Dairy" site, established in 2010 at the Bearden Research Center, consisted of 'Freedom', 'Illinois', and 'Nagara' giant miscanthus (Mxg). Each cultivar was replicated four times in a randomized complete block with plot dimensions of 1.5 m × 3.0 m. The "Forage" site, established in 2009 at the Leveck Research Center, consisted of Freedom and Illinois Mxg and 'EG1101' switchgrass (SG). Each cultivar was replicated four times in a completely random design with plot dimensions of 9.1 m^2. The final site, "Variety", established in 2002 and also at the Leveck Research Center, consisted of Freedom Mxg and 'Alamo' SG. Both cultivars were replicated eight times in a completely random design.

Disease Assessment and Statistical Analysis

All cultivars were assessed for FDI on a monthly basis from Jun to Oct, 2011 and Apr to Oct for 2012. FDI was based on the percentage of foliar disease symptoms observed within each cultivar plot on a monthly basis throughout a growing season. Disease incidence was compared by repeated measures covariate analysis using PROC MIXED program in SAS (SAS 9.2; SAS institute, Cary, NC). Each location was analyzed separately due to unique differences in cultivar and conditions. Disease incidence (back transformed) is reported as percent plot displaying foliar disease symptoms following arcsine transformation. Fisher's protected least significant difference at ($P \leq 0.05$) was used to compare least square means to determine statistical significance among cultivars. Best fit models were developed when significant cultivar variation occurred.

Fungal Collections

Disease sampling and fungal isolation

Samples of leaf material displaying symptoms of fungal infection (Fig. 4.1, 4.2, 4.3) were harvested from Freedom, Illinois, and Nagara Mxg as well as Alamo and EG1101 SG at the Dairy, Forage, and Variety locations. Cultivars were sampled monthly, Jun to Sep, 2011 and Apr to Sep, 2012. Leaves displaying symptoms were removed from the plant, sealed in a labeled plastic bag, and held under refrigeration at approximately 4°C until being processed within 48 h of collection. The diseased material at a location was pooled across replicates to ensure an adequate number of tissue samples (n = 50).

Surface disinfestation was performed using a modified protocol to remove contaminants from the leaf surface (Plant Pathologists's Handbook, 1968). Briefly, foliar lesions were excised with scissors and sectioned to create small segments not exceeding 4 mm^2. Modified 50 ml Falcon conical centrifuge tubes (BD Biosciences San Jose, CA) with holes in the caps facilitated efficient rinsing of leaf lesions during the disinfestation process. Leaf segments were placed in the cleaning tubes, vigorously agitated for 120 s in 70% ethanol (ETOH), and 120 s in 0.6 % sodium hypochlorite, then rinsed three times in sterile distilled water (sdH_2O) for 120 s. Leaf segments were then placed onto sterile filter paper under a laminar flow hood to dry. Five random leaf segments were plated onto a 100 × 15 mm petri plate containing 1.5% water agar (WA). Fifty randomly selected leaf segments were plated for each cultivar at each location. Leaf segments were incubated on a laboratory bench top at 23°C under continuous cool white fluorescent lighting for a minimum period of 2 to 4 weeks. Identification was initially to be conducted after 2 to 4 weeks incubation; however, this proved to be an insufficient amount of time for mature development of fungal structures and a minimal incubation period of 4 weeks was used.

Fungal Identification and Preservation

Identification was based on vegetative (chlamydospores, appressoria) and reproductive (asexual fruiting bodies and ascocarps) feature morphology following incubation of leaf segments. Stereo (Meiji Techno RZ, Meiji Techno, Saitama, Japan) and compound (Nikon Labophot, Tokyo, Japan) microscopy along with taxonomic keys and reference guides were used to facilitate morpho-taxonomic identification to the genera level, and to the species level when morphological features allowed (Barnett and

Hunter 1998; Domsch et al. 2007; Ellis 1971; Ellis 1976; Guarro et al. 2012; Hanlin 1990; Murray et al. 2009; Seifert et al. 2011; Sutton 1980; Sivanesan 1987; Ulloa and Hanlin 2000; Watanabe 2002).

Pure fungal cultures were generated for fungi known to be pathogenic to gramineaceous hosts (Farr et al. 1989) and from these cultures, isolates were selected for pathogenicity evaluations. Isolates selected for pathogenicity evaluations were identified to species level using molecular sequencing, and taxonomic morphology when applicable. Numerous isolation techniques were used including; hyphal tip transfer, single conidia or ascospore transfer, as well as the transfer of entire asexual or sexual fruiting bodies (Plant Pathologists Pocketbook 1968). Axenic cultures were established on WA and 3.9% potato dextrose agar (PDA). These cultures were maintained for further research purposes including molecular sequencing, long term storage and pathogenicity testing.

Fungal mycelium from one week old cultures grown on PDA was used as material for genomic DNA extraction. Fresh mycelium was lyophilized with liquid nitrogen and stored in 1.5 ml microcentrifuge tubes. DNA was extracted from lyophilized material using DNeasy Plant Mini Kit (Qiagen, Valencia, CA) according to manufacturer's instructions.

Polymerase chain reaction (PCR) was used to amplify the internal transcribed spacer (ITS) region. This portion of the fungal genome is highly variable and widely used to identify fungi (White et al. 1990). The ITS region was used to allow comparative analysis and identification with other known sequenced fungi. Fungal specific primers ITS1 and ITS4 were used to amplify ITS1 and ITS2 nucleic acid sequences that represent

the 18s rRNA partial sequence; ITS1, 5.8S, and ITS2 complete sequences, and 28S rRNA partial sequence (Martin and Rygiewicz 2005). The master mix used for each aliquot of DNA included 28 µl millipore water, 10 µl of 5x GoTaq buffer, 4 µl of 25 mM MgCl2, 0.8 µl dNTP's, 3.0 µl ITS1 (5mM), 3.0 µl ITS4 (5 mM), and 0.2 µl TAQ. The thermal cycling protocol used to amplify DNA was as follows: 95°C cycle for 120 s to denature material, followed by 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 60 s, followed by a final extension stage at 72°C for 10 min. A 1.5% agarose electrophoresis gel was used to confirm amplification of genomic material. Amplified ITS1 and ITS4 was purified using ExoSap-IT (USB Products, Cleveland, OH) prior to submission for nucleotide sequencing at Eurofins MWG Operon (Huntsville, Al). Resultant sequences were inspected and manually edited with DNASTAR Lasergene Software (DNASTAR Inc., Madison, WI) and subjected to BLAST searches in the National Center for Biotechnology Information (NCBI) GenBank database.

Fungal isolates were maintained for long term storage on glass fiber filter paper (GFFP) by placing sterilized GFFP onto PDA plates (Young et al. 2010). The subcultured fungi were incubated on GFFP under temperature and light conditions previously described. The GFFP was removed when completely colonized and dried in sterile glass petri dishes in a laminar flow hood, cut into 3 mm^2 pieces then stored in parafilm (American National Can, Greenwich, CT) wrapped 60 mm petri dishes. Fungal isolates were maintained at -20°C. Long term storage maintains the viability and virulence of fungal isolates indefinitely as well as allowing fresh cultures to be available when needed.

A frequency of fungal occurrence was generated for all fungi identified from symptomatic tissue for each cultivar at each location. Tissue samples were pooled across replicates for each cultivar per location to generate the requisite 50 samples per month.

Koch's Postulates

Koch's postulates were conducted in a greenhouse at the Rodney R. Foil Research Center (North Farm) in Starkville, MS. Stock plants of Freedom and Illinois Mxg were maintained in the greenhouse for use in pathogenicity evaluations. Stock plants were established from field harvested rhizomes of Freedom and Illinois Mxg in early spring of 2011 from the Forage location. Each 15 cm pot contained three washed rhizomes, 2.5 to 7.5 cm segments, planted in a 3:1 (v:v) Miracle Grow (Scotts, Vernon Hills, IL): sand mix. Rhizomes were placed in a 26 to 35°C greenhouse with supplemental 117,000 lumen watt high pressure sodium lighting to maintain a 14 h photoperiod. Plants were watered to maintain adequate moisture and trimmed to the soil line four weeks prior to a pathogenicity evaluation.

Selected fungal isolates generated from the 2011 and 2012 fungal collections and reported as graminicolous pathogens were used (Table 3.1). Isolates were transferred to WA, modified PDA (6.0 g PDA + 15.0 g agar (mPDA), or bermudagrass water agar (BWA; 1.5% agar, 1.0% ground bermudagrass (*Cynodon dactylon (*L.) Pers.) tissue) to enhance development of reproductive structures. All cultures used in inoculations were incubated 14 days unless otherwise specified on a laboratory bench top as previously described and subsequently observed for reproductive structure development using light microscopy.

Table 3.1 Selected fungal isolates used in Koch's postulates.

Fungus	Isolate ID	Collection Date	Evaluation Date
Alternaria alternata	DF3, FF11	Aug 2012	Oct 2012
Ascochyta hordei	MLS1	Apr 2012	Mar 2013
Bipolaris cynodontis	VF17, VFA4	Sep 2012	Dec 2012
Bipolaris oryzae	FMXGBO1	Nov 2012	Feb 2012
Bipolaris sorokiniana	FMXGBS1	Sep 2012	Feb 2012
Bipolaris spicifera	DN12	May 2012	Feb 2012
Bipolaris victoriae	DN1	May 2012	Nov 2012
Colletotrichum navitas	VF7, VF30	Sep 2012	Apr 2012
Curvularia lunata	FMXGCV	Mar 2011	Apr, May 2012
Phoma herbarum	DN24	May 2012	Mar 2013
Pithomyces chartarum	VF38, DI10	Nov 2012	Mar 2012
Septoria arundinacea	FS6	Aug 2012	Mar 2013

The inoculation protocols used for Koch's postulates were specific to each fungal isolate based on published literature when available; however, inoculum production was conducted in a similar manner for all isolates. Either a spore or macerated mycelium inoculum (400 ml) was prepared based on the sporulation characteristics of the fungal isolate. To achieve a spore inoculum, axenic cultures of selected fungal isolates were flooded with sdH_2O containing 0.01% Tween 20. The surfaces were scraped with a rubber policeman to dislodge spores and the solution was decanted into a sterile beaker. Spore concentration was calculated using a hemacytometer to meet protocol standards per fungal isolate. To prepare a macerated mycelium solution, one axenic culture grown

on mPDA was macerated in 100 ml of sdH_2O plus 0.01% Tween 20 using a Waring Laboratory Blender (Conair Corporation, Stamford, CT). Control inoculum was sdH2O plus 0.01% Tween 20 or a macerated fungal-free mPDA plate in the same sterile solution. If an inoculum protocol was not available for a specific fungal isolate, spore concentrations were adjusted to 5×10^4 to 1×10^5 spores/ml.

Two incubation approaches were used for Koch's postulates to accommodate the range of environmental conditions experienced in the greenhouse. Initial evaluations (Nov 2011 to Jan 2012) were conducted in the greenhouse, while subsequent evaluations (post Feb 2012) were conducted in growth chambers. In the greenhouse, 15 pots each of Freedom and Illinois Mxg were inoculated with a fungal preparation as previously described and five pots of each cultivar served as the noninoculated control. Clear plastic Sterilite 25 qt storage boxes (Sterilite Corp., Townsend, MA) lined with moistened paper towels served as the incubation chambers and contained five pots per cultivar. The control plants were incubated separately. Plants were misted with a plastic spray bottle with the inoculum until dripping, covered in the storage boxes and placed on top of greenhouse benches. The temperature was maintained between 21 to 32°C; however, by Feb 2012, the temperature in the greenhouse exceeded 32°C.

Subsequent evaluations were conducted using two growth chambers (AR-66L Controlled Environmental Chambers, Percival Scientific, Perry, IA) each containing four storage boxes with five pots each of Freedom or Illinois plants either inoculated or noninoculated controls. As previously described, the storage boxes were lined with moistened paper towels and served as the incubation chamber. Plants were misted with inoculum until dripping, then covered in the storage boxes that each contained a

humidifier (Cool Mist Evaporative Humidifier, Hunter Fan Company, Memphis, TN). Plants were maintained at 28°C in darkness for a period of 16 to 24 h with 100% humidity. After this initial incubation period, plants were then subjected to 28°C with a 12 h photoperiod in the storage boxes for 14 days. Plants were subsequently placed in the greenhouse to observe symptom development.

Selected fungal isolates

Ascochyta hordei var. *hordei* isolate MLS1.

Koch's postulates were conducted in March, 2013 using a conidial suspension (9×10^3 conidia/mL). The inoculum was prepared by dislodging pycnidia from five, 14-day-old cultures of MLS1 grown on BWA. Giant miscanthus cultivars were misted with the *As. hordei* var. *hordei* inoculum (400 ml) until runoff and incubated in the growth chamber as previously described.

Alternaria alternata isolates DF3 and FF11.

Koch's postulates were conducted in October, 2012 using a conidial suspension (1×10^5 conidia/mL). The inoculum (400 ml) was prepared from 14-day-old cultures grown on WA (2 cultures of each isolate) and misted onto the leaves of Mxg cultivars until runoff. The plants incubated in the growth chamber.

Bipolaris cynodontis isolates VF17 and VFA4.

Koch's postulates were conducted in December, 2012 using inoculum procedures described by Pratt (2006) in which 1.5 to 2.8×10^4 conidia/ml were used as the conidial suspension to inoculate bermudagrass and ryegrass. *B. cynodontis* cultures grown on

PDA were used to create an inoculum (4×10^4 conidia/ml) which was used to inoculate Mxg clutivars according to the growth chamber protocol.

Bipolaris oryzae isolate FMXGBO1

In February, 2012 Koch's postulates were conducted to evaluate the pathogenicity of *B. oryzae* on Mxg cultivars. The inoculum production reported by Krupinsky et al. (2004) was followed in which a conidial suspension of 6 to 14×10^3 conidia/ml was inoculated on switchgrass. In this evaluation, 5.6×10^4 conidia/ml was used to inoculate Mxg cultivars. Incubation of plants was according to the greenhouse protocol.

Bipolaris sorokiniana isolate FMXGBS1

Koch's Postulates were conducted in February, 2012 according to a modified inoculum reported by Vu et al. (2011) in which a conidia suspension of 2.4×10^4 conidia/ml was inoculated on switchgrass. In this evaluation, *B. sorokiniana* was inoculated on Mxg cultivars using a solution of 10% potato dextrose broth (PDB), 15% inoculum suspension, and 75% sdH_2O with 0.01% Tween 20. The solution was adjusted to 1×10^5 conidia/ml. Plants were inoculated and incubated according to the growth chamber protocol.

Bipolaris spicifera isolate DN12

In February, 2012 Mxg cultivars were inoculated with *B. spicifera* according to inoculum procedures reported by Vu et al. (2011b) in which a suspension of 4.5×10^6 conidia/ml was used to inoculate switchgrass. Isolate DN12 was inoculated onto Mxg cultivars using an inoculum of 10% PDB, 10% inoculum suspension (6.3×10^4

conidia/ml), and 80% sdH_2O with 0.01% Tween 20. Inoculated Mxg cultivars were incubated based on the growth chamber protocol.

Koch's postulates using *Bipolaris victoriae* was conducted in November, 2012. Isolate DN12 had insufficient sporulation to create a conidial suspension inoculum at time of evaluation, therefore a macerated mycelium solution was created on PDA for inoculations. Inoculations were conducted using the growth chamber protocol.

Colletotrichum navitas isolates VF30 and VF7

Koch's Postulates were conducted in April, 2012 using an inoculum protocol adapted from Crouch et al. (2009). Briefly, a solution of 5×10^4 conidia/ml was suspended in 0.1% PDB and used to inoculate Mxg cultivars. These plants were incubated in the growth chamber.

Curvularia lunata isolate FMXGCV

Koch's postulates were conducted in April and May 2012 using an inoculum production protocol reported by Roberts and Tredway (2008). *C. lunata* conidia were harvested from cultures grown on WA. A conidia suspension consisting of 3×10^5 conidia/ml for the April evaluation, while a solution of 2×10^5 conidia/ml was used in the May evaluation. Giant miscanthus cultivars incubated in the growth chamber.

Phoma herbarum isolate DN24

Koch's postulates were conducted, March 2013, using a conidia inoculum preparation of 5.6×10^4 conidia/ml. The inoculum was prepared from nine *P. herbarum* cultures grown on BWA. Giant miscanthus cultivars incubated under the growth chamber protocol.

Pithomyces chartarum isolates VF38, DI10, DIS9, and DIS24

Koch's postulates were conducted in March, 2012 and January, 2013. The inoculum protocol described by Ahonsi et al. (2010) was followed using isolates VF38 and DI10 with a condia suspension adjusted to 2×10^6 conidia/ml and an incubation schedule for Mxg cultivars consisting of a 48 h darkness period at 26°C, followed by a 15 h day/9 h night photoperiod at 25°C/23°C, respectively. *P. chartarum* inoculations in 2013 were conducted using isolates DIS9 and DIS24 grown on PDA and an inoculum consiting of 5×10^4 conidia/ml. Inoculated Mxg cultivars incubated under the growth chamber protocol.

Septoria arundinacea isolate FS6

Koch's postulates were conducted in March, 2013 using cultures grown on BWA to produce a conidia suspension consisting of 2.6×10^4 conidia/ml. The inoculum was applied to Mxg cultivars and incubated under the growth chamber protocol.

CHAPTER IV

RESULTS

Field Disease Ratings

Symptom characterization

Primary symptoms observed on Mxg were various leaf spots. All initial symptoms began as small (1 to 3 mm), roughly circular to elliptical, red to brown lesions which further progressed, enlarged, or coalesced into different leaf spots, blotches, and blight (Figs 4.1 to 4.3). Leaf spots began as small, reddish-brown, circular lesions which developed into larger (1 to 2 cm), broadly elliptical spots with bleached to tan-colored necrotic centers and reddish-brown to dark-brown to black margins (Fig 4.1 a). Leaf spot distribution was typically isolated on the leaf; however, leaf spots in some instances were observed to enlarge and coalesce (Fig 4.1 b, c). Leaf spot orientation was generally elliptically elongated and parallel to leaf venation; however, in some instances larger, coalescing spots were observed to expand laterally across the leaf blade delimited by the midrib and leaf margins (Fig 4.1 d, e). Leaf blotch lesions initially began as small, scattered red flecks (Fig 4.2 a). Flecks later progressed into large areas of dark brown to black necrosis, with straw colored blotches of necrosis associated with black margins (Fig 4.2 b). Leaf spots that eventually developed into blighted and necrotic leaf areas initially began as small, densely compacted, red or reddish-brown lesions sometimes associated with light chlorosis (Fig 4.3 a, b). Lesions enlarged, elongated, or coalesced

into larger spots with black margins and necrotic areas of blighted tissue that ultimately led to premature desiccation and leaf death (Fig 4.3 c, d).

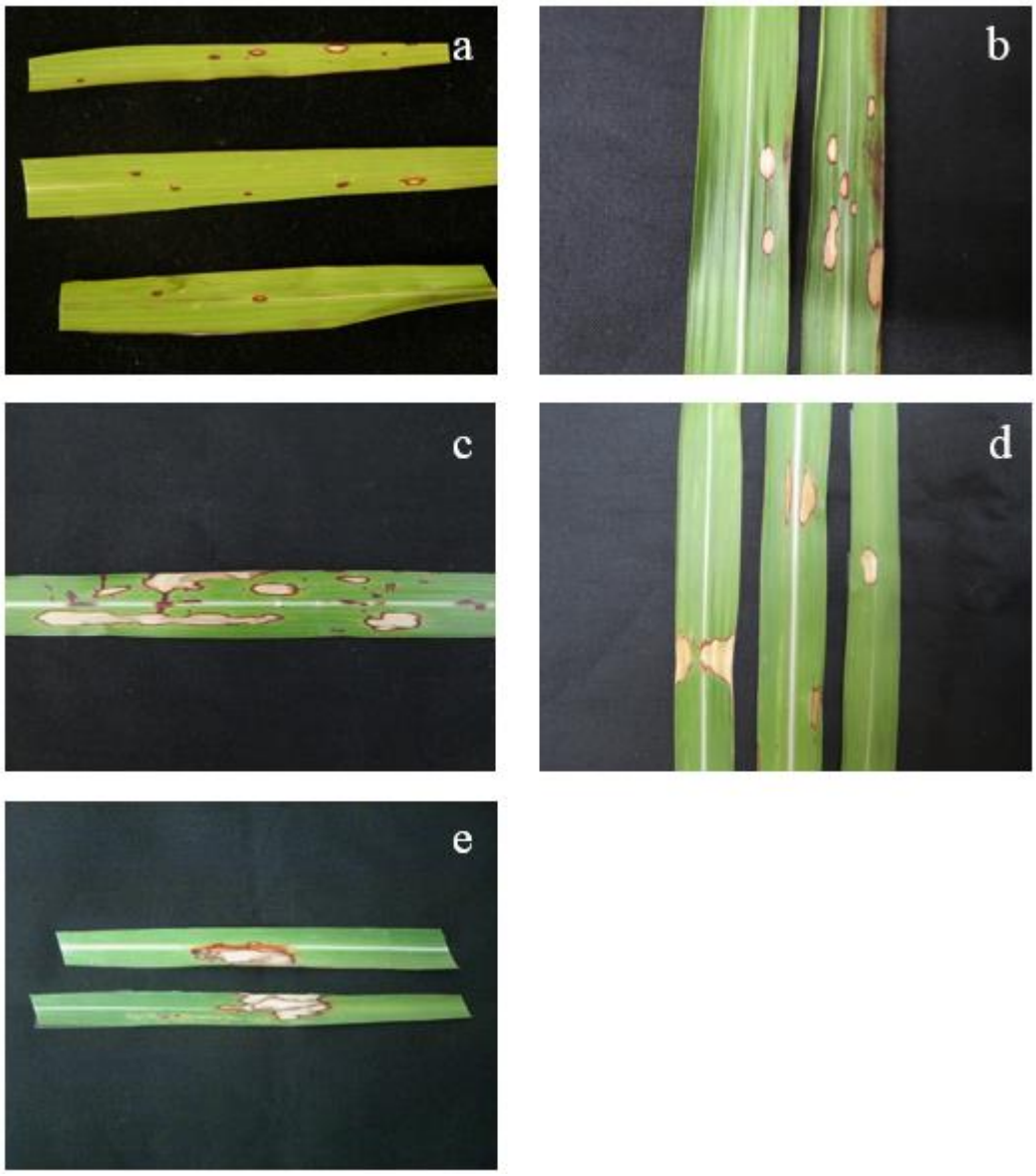

Figure 4.1 Leaf spot symptoms observed on giant miscanthus (*Miscanthus x giganteus*) cultivars throughout the growing season, 2011 and 2012.

(a) Small, reddish-brown lesions of initial leaf spot symptoms. (b,c) Mature, coalesing leaf spot lesions parallel to leaf venation. (d,e) Expanding leaf spots delimited by midrib and leaf margins.

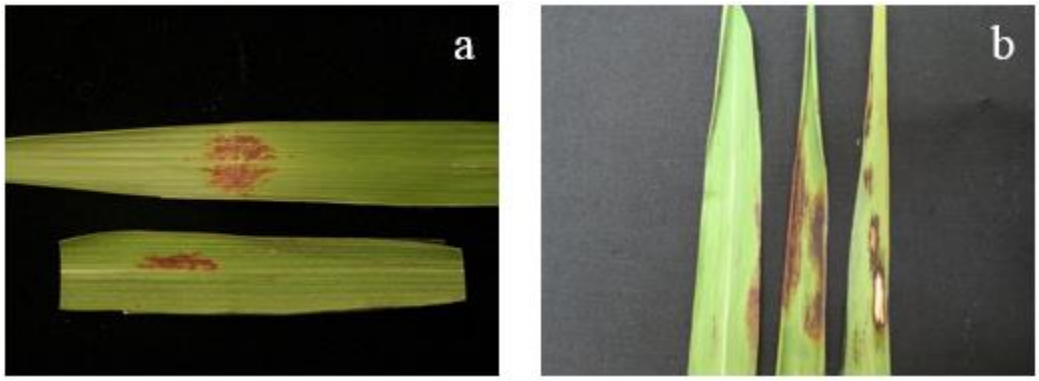

Figure 4.2 Leaf blotch symptoms observed on giant miscanthus (*Miscanthus x giganteus*) cultivars throughout the growing season, 2011 and 2012.

(a) Small, scattered reddish flecks of initial leaf blotch symptoms. (b) Elongated, straw-colored blotches of necrotic tissue with black margins.

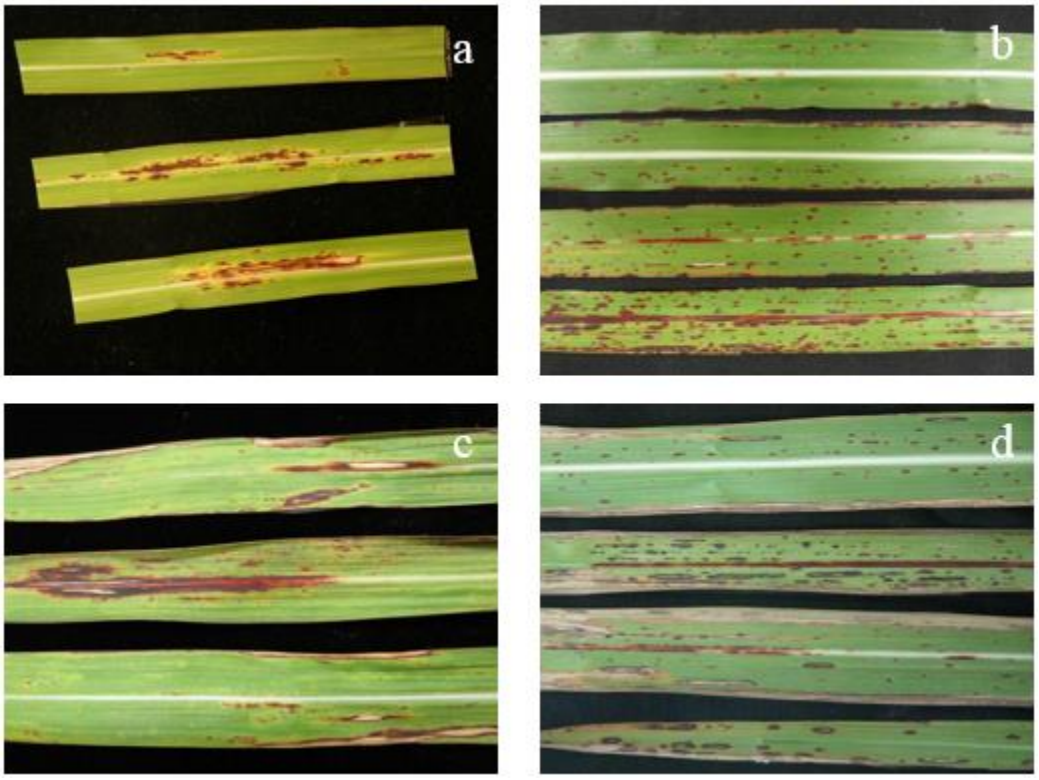

Figure 4.3 Leaf blight symptoms observed on giant miscanthus (*Miscanthus x giganteus*) cultivars throughout the growing season, 2011 and 2012.

(a, b) Densely compacted reddish-brown lesions and light chlorosis associated with initial leaf blight symptoms. (c, d) Expanding, elongated lesions and leaf blight with dark-brown to black necrosis.

Disease Assessment

Freedom, Illinois, and Nagara Mxg stands at the Dairy location were considered juvenile, having been established for two years. Foliar disease incidence (FDI) was similar for the three Mxg cultivars (F=0.3659) over the two year study (Table 4.1). Mean (n = 8) FDI throughout the two growing seasons was relatively low, resulting in 1.8% for Freedom, 2.2 % for Illinois, and 2.7% for Nagara (Table 4.2). Foliar disease incidence response over time (month) was significant ($P = 0.05$), indicating FDI within the three Mxg cultivars changed over time (Table 4.1).

Cultivars of Freedom and Illinois Mxg and EG1101 SG at the Forage location were considered juvenile as well having been established for three years; however, stands were better established at this location due to management practices and reduced weed pressure. Cultivar response to FDI was significant ($P = 0.05$) with the probability of observing a higher F ratio of < 0.0001 (Table 4.1). Foliar disease incidence was significantly higher for EG1101 Swg when compared to Freedom and Illinois. Mean FDI (n = 8) throughout the two growing seasons was less than 2.6% for Freedom and Illinois in comparison to 13.0% in EG1101 SG plots (Table 4.2). When initial assessments were conducted in Apr, FDI was < 1.0% for both Freedom and Illinois Mxg, but 5.0% for EG1101. By mid-season (Jul), FDI had increased by 2.0% for Freedom, 1.4% for Illinois, and 7% for EG1101. At the end of the assessment period (Oct) FDI had escalated by 7.0% for Freedom, 6.0% for Illinois, and 16.0% for EG1101. A best fit model, fitted for each cultivar across monthly FDI assessments, supports the observation of greater disease incidence associated with EG1101 SG as compared to the Mxg cultivars (Fig. 4.4). FDI response over time was also significant (Table 4.1).

Freedom Mxg and Alamo SG established at the Variety location were considered mature, having been established for ten years. Similar to results observed at the Forage location, FDI cultivar response among Freedom and Alamo were significantly different ($P = 0.05$), with the probability of observing a greater F ratio of < 0.0001 (Table 4.1). Foliar disease incidence was significantly greater for Alamo when compared to Freedom therefore best fit models were applied to cultivars and plotted across months (Table 4.2; Fig 4.5). Overall mean FDI (n = 16) was 2.8% for Freedom and 16.7% for Alamo throughout the two growing seasons (Table 4.2; Fig 4.5). Initial observations (April) of FDI were < 1.0% for Freedom in contrast to 9.2% for Alamo. As the season progressed, a slight increase in FDI was observed in Freedom, but Alamo continued to express increased FDI. Foliar disease incidence associated with Alamo increased 63.0% over the growing season and was 58.0% greater than FDI of Freedom. Foliar disease incidence response over time was also significant, indicating FDI between the two grasses changed over the growing season (Table 4.1).

Table 4.1 Mixed procedure analysis of variance tests of hypotheses between subject effects for field disease incidence of giant miscanthus (*Miscanthus x giganteus*) and switchgrass (*Panicum virgatum*) cultivars at three locations in combined years, 2011 and 2012.

	Location		
	Dairy	Forage	Variety
Subject Effects		F Value [z]	
Cultivar (C)	1.02 NS	47.72 ***	534.82 ***
Month (M)	67.52 ***	62.23 **	181.29 ***
C*M	0.05 NS	1.85 NS	12.55 **

[z] (**) Significant at $P = 0.001$; (***) Significant at $P = 0.0001$; (NS) Not significant at $P = 0.05$.

Table 4.2 Mean foliar disease incidence ratings for giant miscanthus (*Miscanthus x giganteus*) and switchgrass (*Panicum virgatum*) cultivars at the Dairy, Forage, and Variety locations, 2011-2012.

Location	Cultivar	Mean foliar disease incidence (%) [z]	*P*-value
Dairy			0.3659
	Freedom	1.8 a	
	Illinois	2.2 a	
	Nagara	2.7 a	
Forage			< 0.0001
	Freedom	2.6 b	
	Illinois	1.9 b	
	EG1101	13.0 a	
Variety			< 0.0001
	Freedom	2.8 b	
	Alamo	16.7 a	

[z] Means within location columns shared by the same letter are not statistically significant according to Fisher's protected LSD at $P = 0.05$.

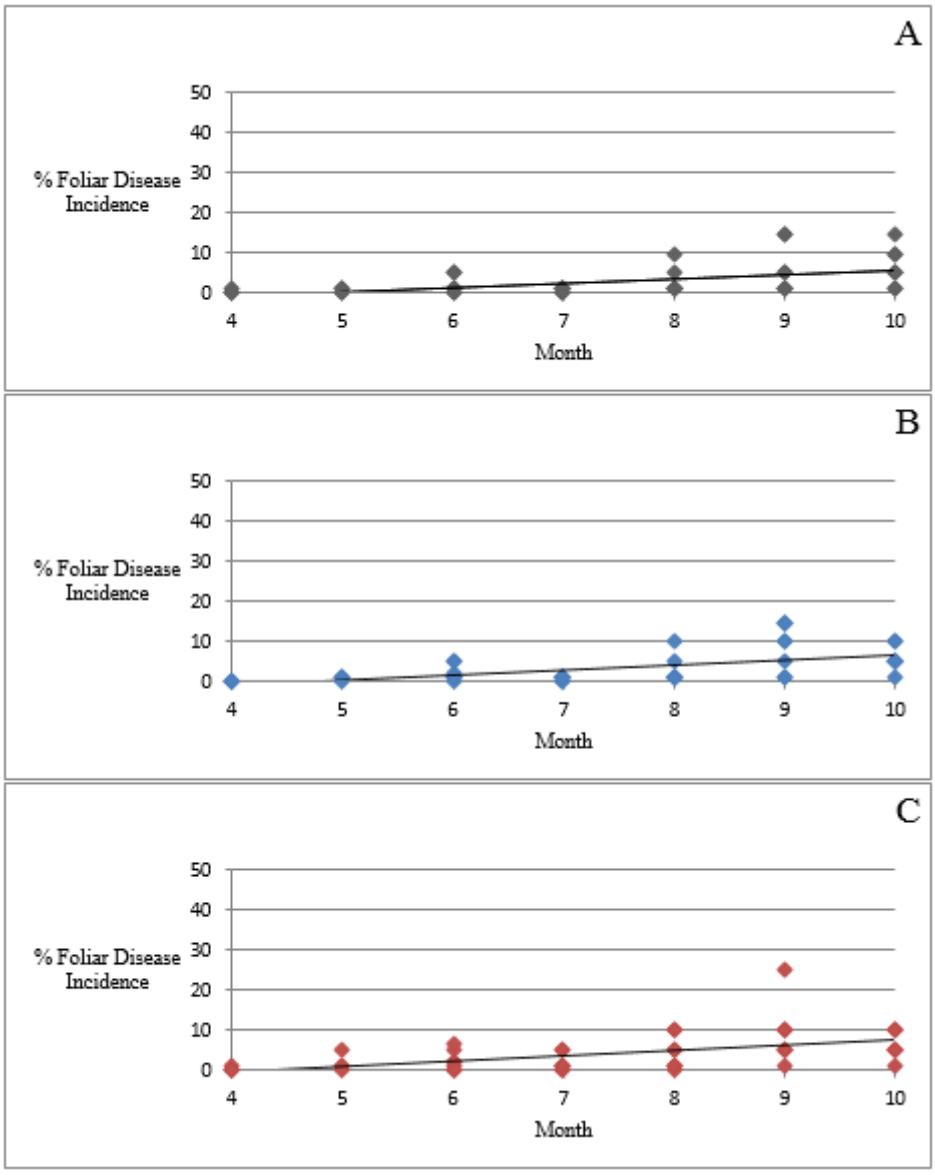

Figure 4.4 Field disease incidence of giant miscanthus (*Miscanthus x giganteus*) throughout two growing seasons, 2011 and 2012, at the Dairy location in Sessums, MS.

A) Freedom, B) Illinois, and C) Nagara; (April=4; October =10)

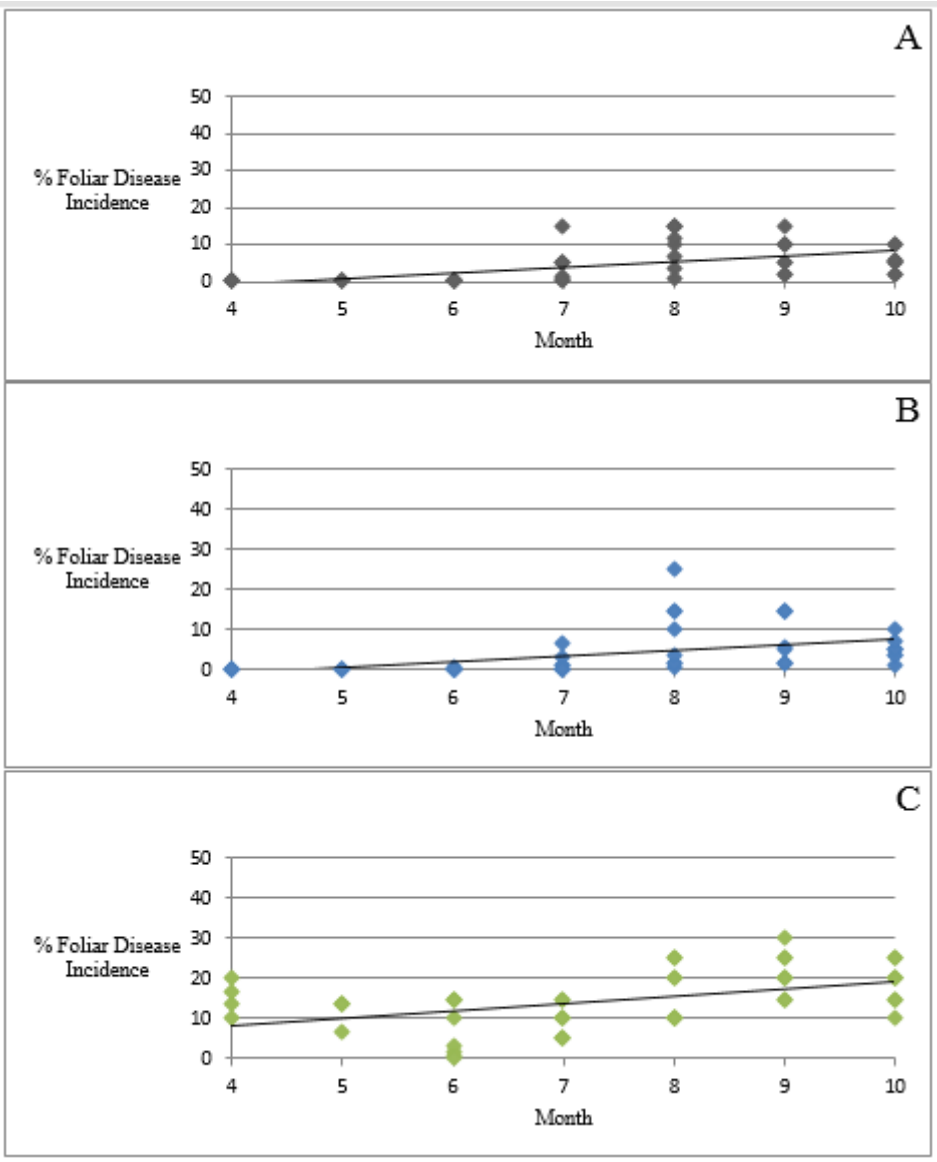

Figure 4.5 Field disease incidence of giant miscanthus (*Miscanthus x giganteus*) and switchgrass (*Panicum virgatum*) throughout two growing seasons, 2011 and 2012, at the Forage location in Starkville, MS.

A) Freedom, B) Illinois, C) EG1101; (April=4; October =10)

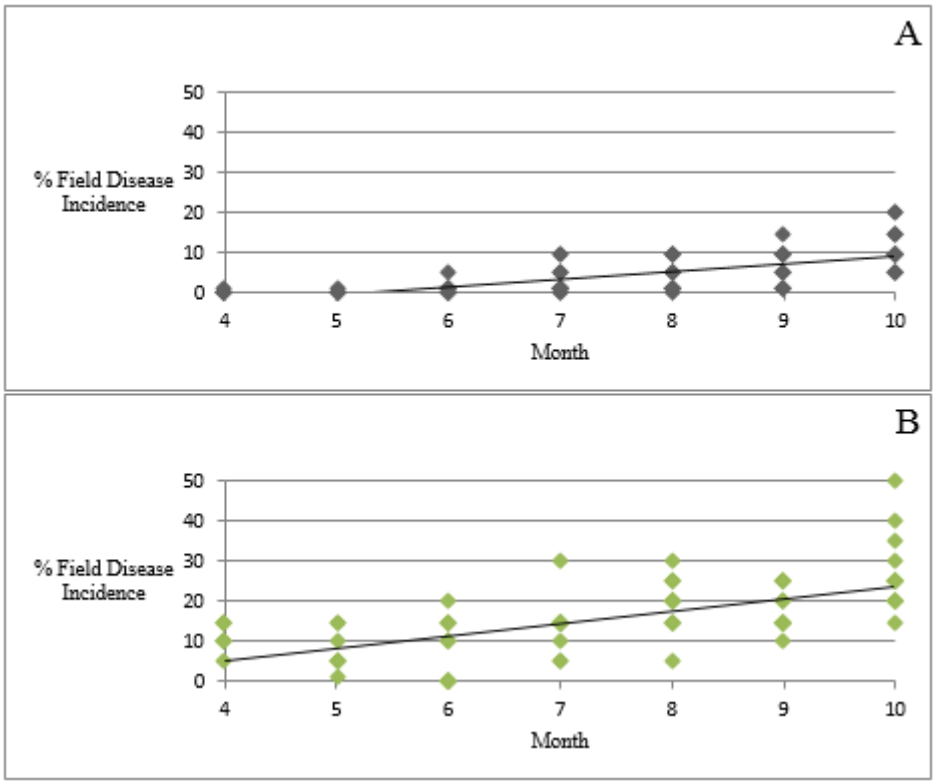

Figure 4.6 Field disease incidence of giant miscanthus (*Miscanthus x giganteus*) and switchgrass (*Panicum virgatum*) throughout two growing seasons, 2011 and 2012, at the Variety location in Starkville, MS.

A) Freedom, B) Alamo; (April=4; October =10)

Fungal Collections

Observed Fungi

Four thousand four hundred (n = 4,400) leaf segments displaying foliar lesions were plated and observed over the 2011 and 2012 growing seasons. Isolates representing 30 fungal genera were collected and described herein. This compilation includes graminicolous pathogens as well as cosmopolitan saprophytes (Farr et al. 1989).

Alternaria (Nees.). DEUTEROMYCOTA. HYPHOMYCETE

The members of this genus are considered parasites or saprophytes on plant material (Barnett and Hunter 1998). *Alternaria* isolates appeared thick and dark grey when cultured on PDA, while colonies cultured on WA were sparse grey with prolific sporulation. Hyphae, conidiophores, and conidia were pigmented, appearing light to dark-brown. Ovoid to ellipsoidal, transverse and longitudinally septate conidia were produced in chains with a broadly rounded base and apical beak (Fig 4.7) (Barnett and Hunter 1998; Domsch et al. 2007). Isolate DF3 was isolated in August, 2012 from Freedom Mxg at the Dairy location. The DF3 isolate was sequenced with ITS primers and subjected to a BLAST search against the NCBI GenBank. The 562 bp ITS fragment shared 99.0% identity and 100% query coverage to *Alternaria alternata* isolate IEIHBT (GenBank Accession Number GQ121322).

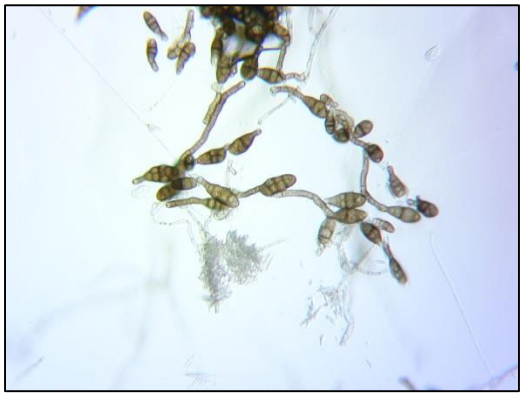

Figure 4.7 Germinating conidia of *Alternaria alternata* with transverse and longitudinal septa.

Ascochyta (Lib). DEUTEROMYCOTA. COELOMYCETE

Primarily causes leaf spots, classifying the genus as parasitic to many plant species (Barnett and Hunter 1998). Hyphae were pale brown, branched, and septate (Sutton 1980). Dark brown to black, globose, ostiolate, immersed pycnidia were observed within leaf segments and in axenic culture. Conidia were thin walled, hyaline, two-celled and oblong to ovoid to irregular (Fig 4.8) (Sutton 1980; Barnett and Hunter 1998). Isolate MLS1 suspected to be *Ascochyta* sp. was isolated in April, 2012 from leaf spot symptoms with visible pycnidia observed on Freedom Mxg. In culture, MLS1 produced grey, fluffy colonies on mPDA and PDA. Mycelia was pale to dark brown, branched, and immersed in culture media. The MLS isolate was sequenced with ITS primers and subjected to a BLAST search against the NCBI GenBank. The 515 bp ITS fragment shared 98% identity and 94% query coverage to *Ascochyta hordei* var. *hordei* strain CBS 544.75 (GenBank Accession Number GU237887).

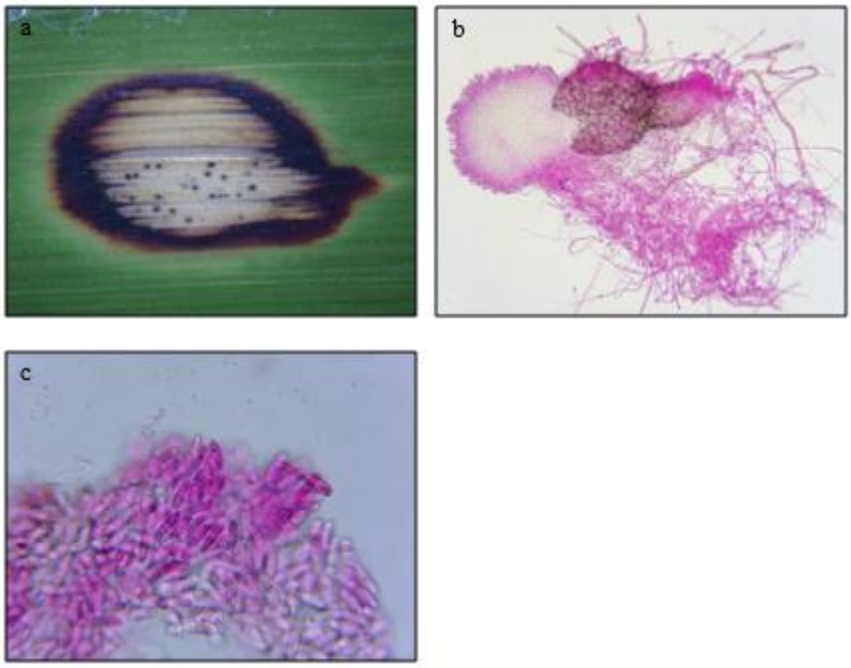

Figure 4.8 Identifying characteristics of *Ascochyta* sp.

a) Leaf spot symptom in which *Ascochyta hordei* isolate MLS was isolated from, with visible pycnidia in center of lesion. b) Pycnidia produced in axenic culture. c) Two-celled conidia of *Ascochyta hordei*.

Aspergillus (Link.). DEUTEROMYCOTA. HYPHOMYCETE

A cosmopolitan fungus with over 260 species found in environments such as soil, compost, decayed plant material, and stored grain (Domsch et al. 2007; Seifert et al. 2011). Erect conidiophores were produced on the surface of leaf segments, ending in a globose or clavate swelling covered in a layer of phialides radiating from the apex (Barnett and Hunter 1998; Domsch et al. 2007). Dry basipetal chains of hyaline, globose, single-celled conidia were produced from the phialides.

Bipolaris (Shoem.). DEUTEROMYCOTA. HYPHOMYCETE

Bipolaris sp. is parasitic, mostly on gramineaceous hosts (Barnett and Hunter 1998). Cultures produced on PDA were brown, grey, or black. Conidiophores were

simple, brown, with sympodial growth and conidia produced from apical pores. Conidia were dematiaceous, multi-septate, elliptical, straight or curved, with bipolar germination (Barnett and Hunter 1998).

Bipolaris cynodontis (Nelson)

Bipolaris cynodontis is a parasite of many agronomic crops, including *Cynodon dactylon, Oryza, Panicum, Pennisetum, Triticum,* and *Zea,* among other genera (Sivanesan 1987), with a nearly worldwide distribution. According to the literature (Sivanesan 1987), conidia of *B. cynodontis* were slightly curved, cylindrical, pale to mid golden-brown, 3 to 9 (commonly 7 to 8) septate, with an average size range of 30 to 75 × 10 to 16 µm (Fig 4.9). Isolate VF17 suspected to be *B. cynodontis* was isolated September, 2012 from Freedom Mxg at the Variety location. Conidia were hyaline to light tan, slightly curved, cylindrical with slightly tapered end cells, 4 to 8 septate, with a size range of 42.5 to 50 × 10 to 12.5 µm (average 46.8 × 11.2 µm). Isolate VF17 was sequenced with ITS primers and subjected to a BLAST search against the NCBI GenBank. The 567 bp ITS fragment shared 100% identity and 100% query coverage to *Cochliobolus cynodontis* strain NBRC 9793 (GenBank Accession Number JN943389).

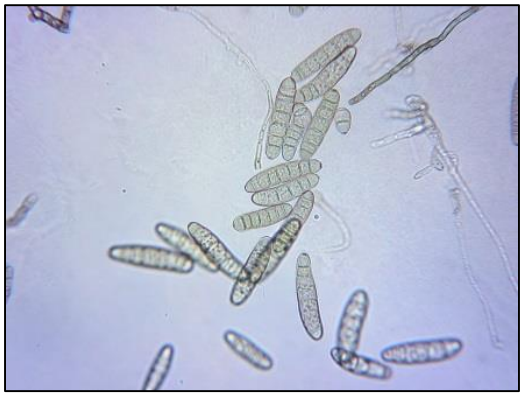

Figure 4.9 Slightly curved, cylindrical conidia of *Bipolaris cynodontis*.

Bipolaris oryzae (Breda de Haan) Shoemaker

B.oryzae is a parasite of *Oryza sativa* L., as well as many other grass genera (Sivanesan 1987). Literature (Sivanesan 1987) describes morphological features as grey to dark grey colonies and conidia which were curved, fusoid, pale to mid golden brown, 6 to 14 distoseptate, with an average size of 63 to 153 × 14 to 22 µm. Isolate DFCA20 was isolated on Nov, 2012 from Freedom Mxg at the Dairy location. Conidia produced in axenic culture were tan to light golden-brown, curved, 5 to 7 septate, and 102 to 127.5 × 20.4 to 25.5 µm in size (Fig 4.10). Isolate DFCA20 was identified as *B. oryzae* based on conidial morphology (conidia size, shape, and color) as compared to the appropriate literature (Sivanesan 1987).

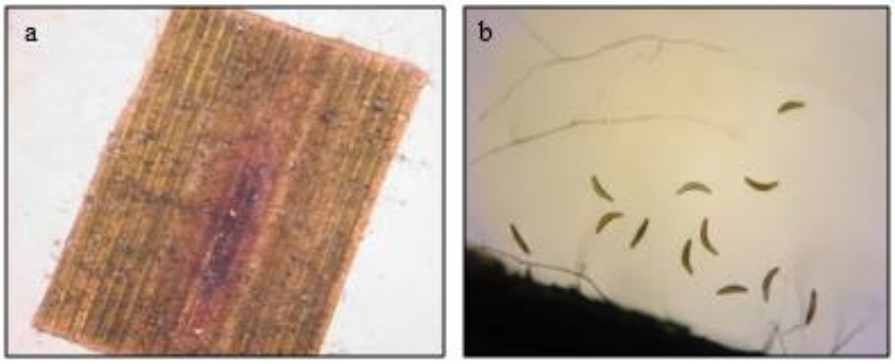

Figure 4.10 Identifying characteristics of *Bipolaris oryzae*.

a) Plated leaf segment on WA with sporulation of *B. oryzae*. b) Curved, pale to mid golden-brown conidia of *B. oryzae*.

<u>*Bipolaris sorokiniana* (Sacc.) Shoemaker</u>

B. sorokiniana is described as a parasite of *Avena*, *Hordeum*, *Secale*, *Triticum*, and other grasses. It has been isolated from a wide variety of other plants with a worldwide distribution (Sivanesan 1987). Cultures described from literature were grey to dark-brown with abundant sporulation (Sivanesan 1987). Conidia produced by *B. sorokiniana* were curved, broadly ellipsoidal, dark olivaceous-brown, smooth, 3 to 12 septate (commonly 6 to 10), and 40 to 120 × 17 to 28 µm (Sivanesan 1987). Isolate DFCS22 suspected to be *B. sorokiniana* was isolated Sep, 2012 from Freedom Mxg at the Dairy location. Axenic cultures produced on PDA were light to dark-grey. Conidia produced on conidiophores were brown to olivaceous, broadly ellipsoidal, had 4 to 8 septa, and were 61.2 to 91.8 × 15.3 to 25.5 µm in size (Fig 4.11). Isolate DFCS22 was tentatively identified as *B. sorokiniana* based on conidia size and color; in addition, ITS sequences were also subjected to a BLASTn search against the NCBI GenBank. The 575 bp fragment shared 99% identity and 100% query coverage with *B. sorokiniana* isolate OTU730 (GenBank Accession Number GU934504).

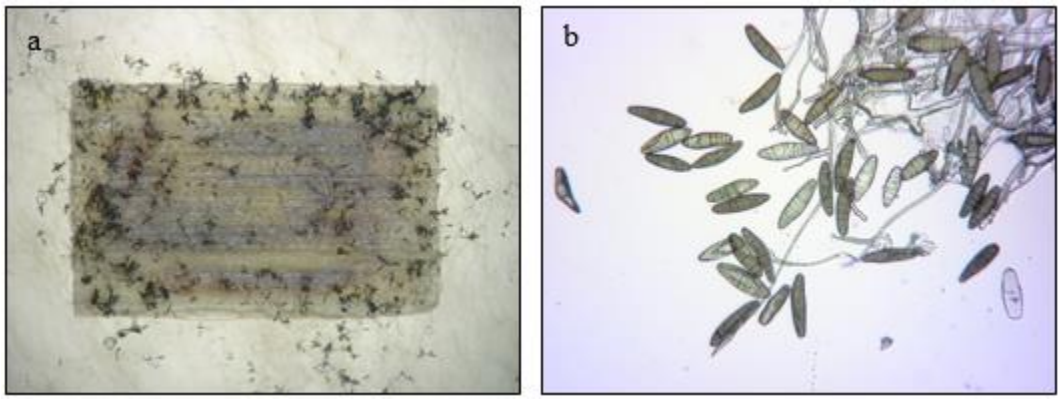

Figure 4.11 Identifying characteristics of *Bipolaris sorokiniana*.

a) Plated leaf segment on WA with sporulation of *B. sorokiniana*. b) Broadly ellipsoid, dark olivaceous-brown conidia of *B. sorokiniana*.

Bipolaris spicifera (Nelson)

B. spicifera has been described to cause leaf spot, root rot, and has also been associated with spring dead spot of *Cynodon dactylon* and foot rot of winter wheat (Sivanesan 1987). Many common agronomic crops such as *Agrostis*, *Avena*, *Cynodon*, *Oryza*, *Panicum*, *Pennisetum*, *Poa*, *Saccharum*, *Sorghum*, *Triticum*, *Zea*, as well as many other grasses have been described as hosts of *B. spicifera* (Sivanesan 1987). Conidia are described in literature as straight, oblong or cylindrical with rounded distal cells, golden brown, always 3-septate, with a size of 20 to 40 × 9 to 14 µm (Sivanesan 1987). Isolate DN12 suspected to be *B. spicifera* was isolated from Nagara Mxg at the Diary location in May, 2012. Cultures produced on PDA were grey. Conidia were produced sympodially on conidiophores, were tan, straight, 2 to 3 septate, and 20 to 22.5 × 7.5 to 10 µm in size (Fig 4.12). Isolate DN12 was identified as *B. spicifera* based on conidia morphology (size); however, ITS sequences were subjected to a BLASTn search against the NCBI

GenBank. The 551 bp fragment shared 100% identity and 100% query coverage with *B. spicifera* strain 2199 (GenBank Accession No. HM195265).

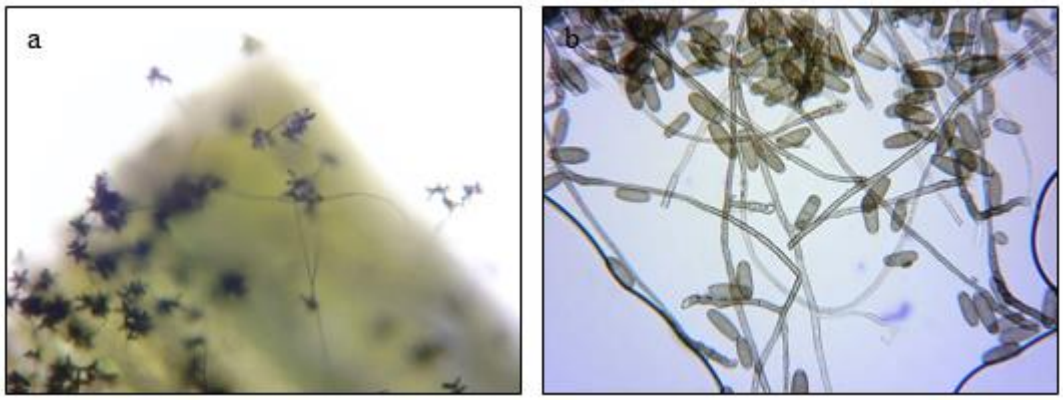

Figure 4.12 Identifying characteristics of *Bipolaris spicifera*.

a) Sporulation of *B. spicifera* on plated leaf segment on WA. b) Golden-brown cylindrical conidia characteristic of *B. spicifera*.

<u>*Bipolaris victoriae* (Meehan and Murphy) Shoem</u>

B. victoriae is a parasite of oat (*Avena* spp.) cultivars specifically related to cv. Victoria in North America, causing seedling blight and culm necrosis (Sivanesan 1987). Major agronomic crops such as *Agropyron*, *Avena*, *Hordeum*, *Panicum*, *Paspalum*, *Oryza*, *Setaria*, *Sorghum*, *Triticum*, and *Zea* are known hosts of this fungus (Sivanesan 1987). According to literature (Sivanesan 1987) conidia are slightly curved, broadly fusiform, pale to mid golden brown, 4 to 11 septate (mostly 8 to 10), with a size of 40 to 120 × 12 to 19 µm. Isolate DN1 suspected to be *B. victoriae* was isolated from Nagara Mxg at the Dairy location in May, 2012. Axenic cultures were light to dark grey. Conidia of DN1 were slightly curved to mostly straight, pale to golden brown, 5 to 7 septate, with a size of 56 to 87 × 15 to 25 µm (Fig 4.13). Isolate DN1 was identified as

B. victoriae based on molecular identification. Internal transcribed spacer sequences were subjected to a BLASTn search against the NCBI GenBank. The 568 bp fragment shared 100% identity and 100% query coverage with *Cochliobolus victoriae* isolate HVW (GenBank Accession No. EF452448).

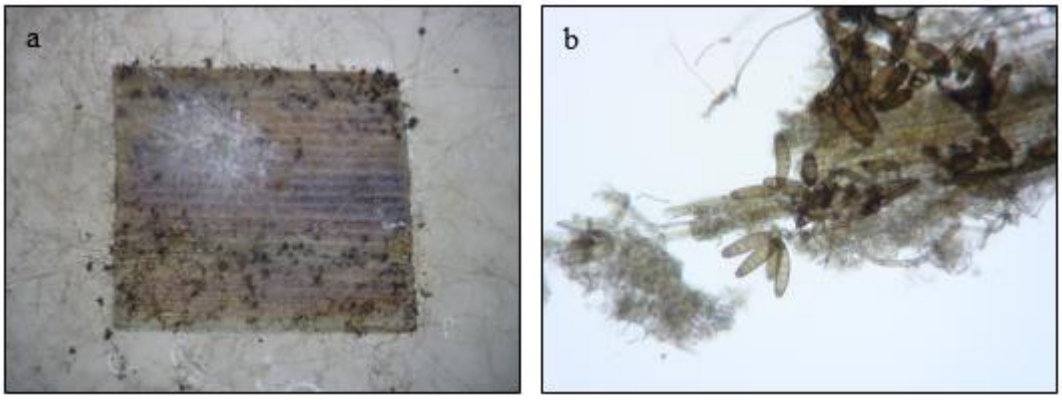

Figure 4.13 Identifying characteristics of *Bipolaris victoriae*.

a) Sporulation of *B. victoriae* on plated leaf segment on WA. b) Slightly curved, golden-brown conidia of *B. victoriae*.

Cephaliophora (Thaxt). DEUTEROMYCOTA. HYPHOMYCETE

Members of the genus *Cephaliophora* exists as a saprophyte on dung or decaying plant material (Barnett and Hunter 1998). Conidiophores were short with an enlarged rounded apical cell bearing numerous blastoconidia on all sides produced simultaneously (Barnett and Hunter 1998; Domsch et al. 2007). Conidia appeared lightly pigmented, 1 to 3 septate, obovoid to elongate and narrower at the base.

Chaetomium (Kunze). ASCOMYCOTA. SORDARIOMYCETES

Commonly found on cellulose substrates as well as on seed, soil, and dung (Domsch et al. 2007). Ascomata were ostiolate perithecia, attached to the colonized leaf

segments by rhizoids (Domsch et al. 2007). Perithecia were superficial, globose or vase-shaped and covered with lateral and terminal, septate, coiled or arcuate, smooth or roughened hairs (Hanlin 1990). Asci were clavate and 4 to 8 spored. Ascospores were aseptate, light olive brown to dark brown, limoniform and apiculate, globose, smooth, and pushed out of the ostiole in a cirrhus (Hanlin 1990).

Colletotrichum (Corda). DEUTEROMYCOTA. COELOMYCETE

Many species in the *Colletotrichum* genus are recognized as plant pathogens causing anthracnose and leaf spots (Domsch et al. 2007). Black pointed sterile setae were produced on edges of cushion shaped, subepidermal acervuli on leaf segments and in axenic culture (Barnett and Hunter 1998). Conidia produced on short conidiophores inside acervuli were hyaline, aseptate, falcate, and sometimes guttulate (Fig 4.14) (Sutton 1980). Rounded or irregular, brown appressoria were produced on leaf segments as well as the bottom of culture plates. Isolate VF 7 suspected to be *Colletotrichum* sp. was isolated Sep 2012 from the Freedom Variety location. The VF7 isolate was sequenced with ITS primers and subjected to a BLAST search against the NCBI GenBank. The 587 bp ITS fragment shared 99% identity and 92% query coverage to *C. navitas* culture-collection CBS: 125086 (GenBank Accession Number JQ005769).

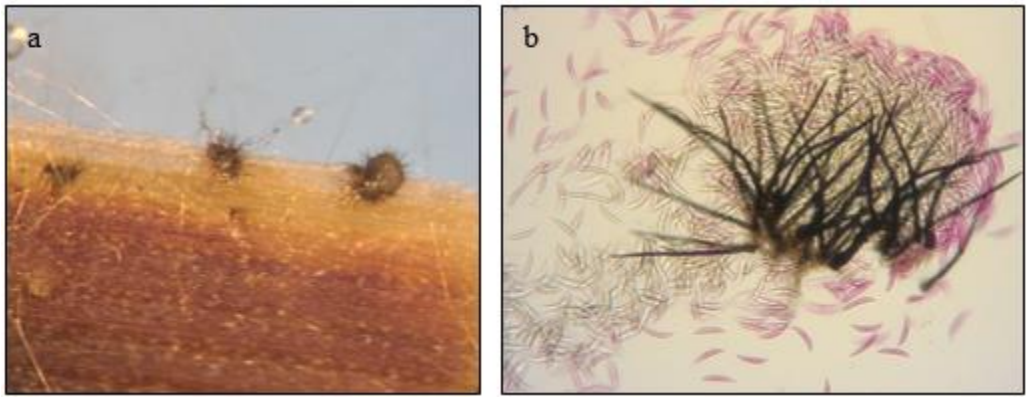

Figure 4.14 Identifying characteristics of *Colletotrichum* sp.

a) Acervuli developing on plated leaf segment on WA. b) Setae and hyaline, falcate, one celled conidia of *Colletotrichum navitas*.

<u>*Coniella* (Höhn). DEUTEROMYCOTA. COELOMYCETE</u>

Coniella sp. produced separate, globose, pale brown, immersed, ostiolate pycnidia on leaf segments as well as in axenic culture (Sutton 1980). Conidia were olivaceous to brown, aseptate, and fusoid with a broad base and an obtuse apex (Fig 4.15) (Sutton 1980).

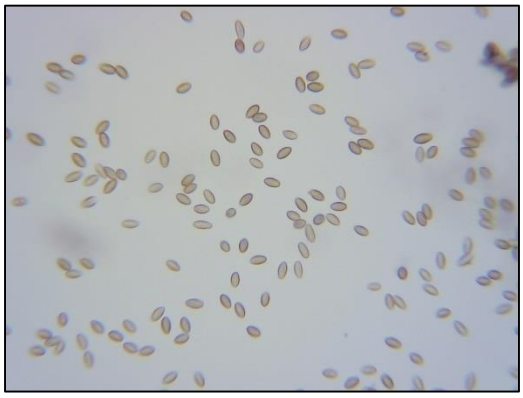

Figure 4.15 Pale-brown, fusoid conidia of *Coniella* sp.

Curvularia (Boedijn). DEUTEROMYCOTA. HYPHOMYCETE

Some species in this genus are located in tropical and subtropical climates existing as facultative parasites or saprophytes; however, some species are ubiquitous on plant and other organic substrates (Barnett and Hunter 1998; Domsch et al. 2007). Short, simple, and dark-brown pigmented conidiophores produced conidia at the apex. Conidia were dark, fusiform, had 3 or more transverse septa, and had an enlarged central cell causing spores to be bent (Fig 4.16). Isolate FMXGCV thought to be *Curvularia* was isolated from Freedom Mxg in March, 2011. The fungus was identified as *C. lunata* based on morphological features of conidia such as an enlarged median cell and size consistent with taxonomic keys (Ellis 1971).

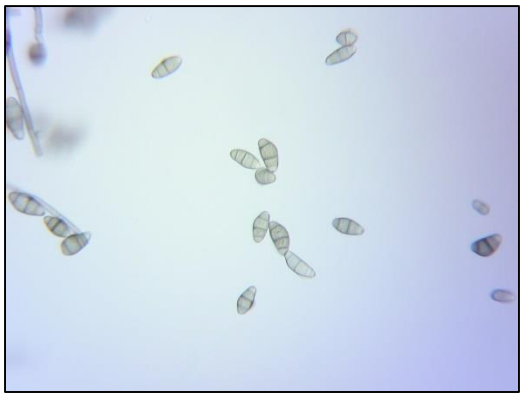

Figure 4.16 Conidia of *Curvularia lunata* with 3 or more transverse septa and enlarged central cell.

Epicoccum (Link). DEUTEROMYCOTA. HYPHOMYCETE

Epicoccum is cosmopolitan and mostly saprophytic on leaves and stems (Barnett and Hunter 1998; Seifert et al. 2011). Dark brown, globose, dictyosporous conidia were

produced on short conidiophores located inside dark colored sporodochia on the surface of leaf segments (Barnett and Hunter 1998).

Exserohilum (Dreschler) Leonard & Suggs. DEUTEROMYCOTA. HYPHOMYCETE

Exserohilum is cosmopolitan on leaves of grasses and contains approximately 30 species (Seifert et al. 2011). Isolates in axenic culture produced dark grey colonies. Sporulation was prolific on the surface of leaf segments. Conidiophores were long, olivaceous to dark-brown, and smooth with sympodial conidiogenesis (Sivanesan 1987). Conidia were light brown, fusiform to obclavate, straight to slightly curved, and multi-distoseptate (Fig 4.17) (Sivanesan 1987). A strongly protuberant hilum is present at both ends, while the septation above this hilum thickened (Domsch et al. 2007; Seifert et al. 2011; Sivanesan 1987).

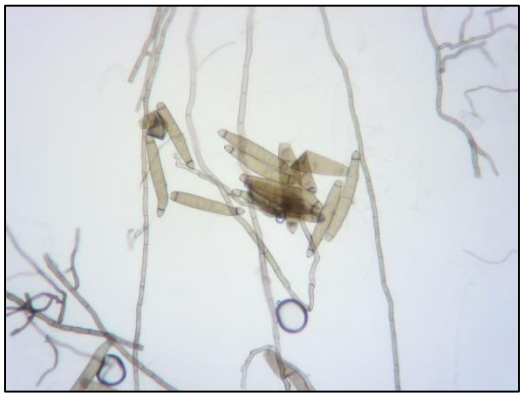

Figure 4.17 Identifying characteristics of *Exserohilum* sp.

Light brown, multi-distoseptate conidia of *Exserohilum* with protuberant hilum at both ends.

Fusarium (Link.). DEUTEROMYCOTA. HYPHOMYCETE

Fusarium is a cosmopolitan cellulose decomposer as well as a plant parasite causing root and stem rot, vascular wilt, and fruit and grain diseases (Domsch et al. 2007). Colonies produced on PDA were white to pink to pale yellow and cotton-like. Conidiophores were produced on hyaline, immersed mycelium singly or in groups (sporodochia) bearing both macro and micro hyaline phialoconidia which form a slimy mass with maturity (Barnett and Hunter 1998). Macroconidia were 1 to 7 septate, and slightly fusiform to curved (Fig 4.18). Microconidia were smaller, aseptate, ovoid or oblong, and produced singly or in chains (Barnett and Hunter 1998; Domsch et al. 2007).

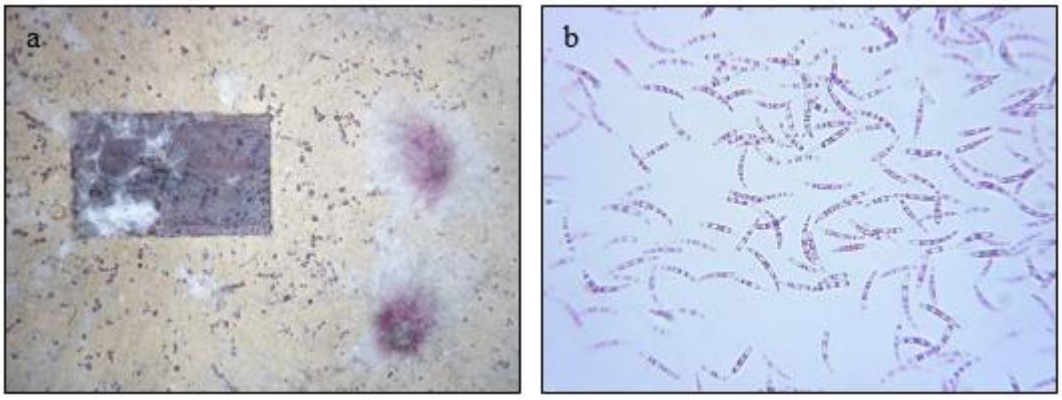

Figure 4.18 Identifying characteristics of *Fusarium* sp.

a) Sporodochia surrounding plated leaf segments in axenic culture. b) Multiseptate macroconidia of *Fusarium* sp.

Lacellinopsis (Subramanian). DEUTEROMYCOTA. HYPHOMYCETE

Lacellinopsis is a cosmopolitan pantropical saprophyte on leaves of many grasses as well as soil litter (Seifert et al. 2011). Simple, septate, and brown setae were produced. Mixed in with setae were conidiophores with a globose apex becoming cupulate with maturity and detachment of conidia (Barnett and Hunter 1998). Conidia

borne on this apex were brown, globose, aseptate, and produced in acropetal chains (Barnett and Hunter 1998).

Leptosphaeria (Ces. & De Not.). ASCOMYCOTA. DOTHIDEOMYCETES

With over 700 described species (Domsch et al. 2007), *Leptosphaeria* sp. is a known parasite and saprophyte on herbaceous dicot leaves and stems (Hanlin 1990). Ascomata were separate or scattered perithecoid pseudothecia which were immersed, glabrous, and dark-brown. Pseudothecia containing bitunicate asci with 4 to 8 spores were produced (Fig 4.19a) (Hanlin 1990). Ascospores were pale brown to almost hyaline, fusiform, cylindrical to filiform, constricted at the median cell, with 3 to many transverse septa (Fig 4.19b). Anamorphs include; *Coniothyrium*, *Phoma*, *Septoria*, and *Stagonospora* (Hanlin 1990).

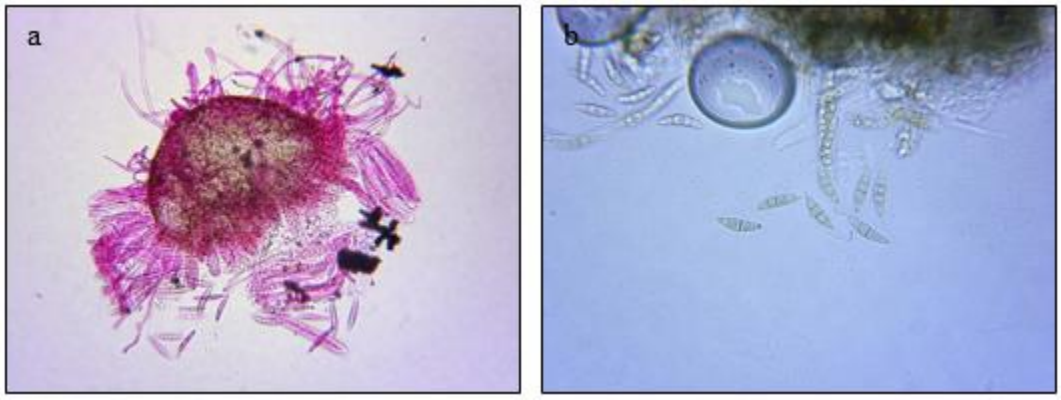

Figure 4.19 Identifying characteristics of *Leptosphaeria* sp.

a) *Leptosphaeria* sp. pseudothecia produced in axenic culture with asci and 4 to 8 ascospores. b) Hyaline, fusiform ascospores with constricted median cell and 3 septa.

Mortierella (Coemans). ZYGOMYCOTA. ZYGOMYCETE

This fungus is a common soil saprophyte (Barnett and Hunter 1998). Sporangiophores produced on leaf segments were black, thick at the base and tapered upward. Sporangiospores were globose and hyaline (Domsch et al. 2007).

Nigrospora (Zimmerm.). DEUTEROMYCOTA. HYPHOMYCETE

Nigrospora is a weak plant parasite and soil saprophyte (Barnett and Hunter 1998). Colonies produced on WA and mPDA were white to pale grey. Broad, peg-like conidiophores were produced directly on mycelia. Conidia were shiny black, smooth, aseptate, globose with an equatorial germ slit (Fig 4.20) (Domsch 2007).

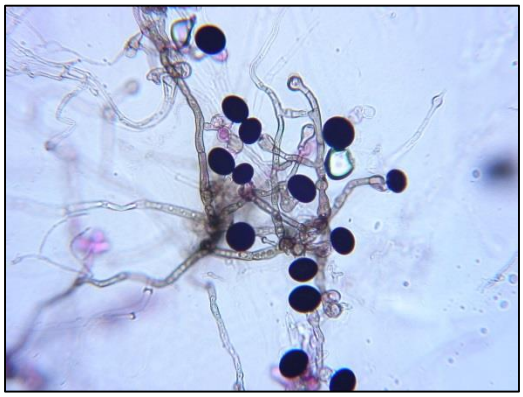

Figure 4.20 Identifying characteristics of *Nigrospora* sp.
Shiny black, aseptate, globose conidia of *Nigrospora* produced in axenic culture.

Paecilomyces (Bainer). DEUTEROMYCOTA. HYPHOMYCETE

This fungus is a common soil saprophyte. Similar in morphology, *Paeciloymyces* is distinguished from *Penicillium* by its irregular branching conidiophores and divergent, long and slender phialides (Domsch et al. 2007). Phialoconidia were produced in dry basipetal chains, aseptate, hyaline, and ovoid to fusoid (Barnett and Hunter 1998).

Paraphaeosphaeria (Westendorp). ASCOMYCOTA. DOTHIDEOMYCETES

Paraphaeosphaeria contains 22 described species (Fukuhara 2002). Ascomata produced on plated segments were scattered, immersed in tissue, brown, globose to erumpent perithecia. Asci were bitunicate, cylindrical with a broadly rounded apex with 8 ascospores. Ascospores were biseptate, broadly elliptical, widest near the central cell and constricted at apical septa, echinulate and dark yellowish brown (Fig 4.21) (Shoemaker and Erikson 1967).

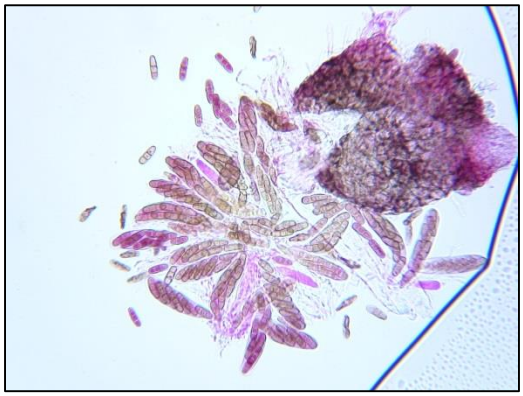

Figure 4.21 Identifying characteristics of *Paraphaeosphaeria* sp.

Paraphaeosphaeria sp. perithecia with emerging asci holding 8 ascospores which were broadly elliptical, biseptate, and widest at central cell with constricted apical septa.

Penicillium (Link). DEUTEROMYCOTA. HYPHOMYCETE

This fungus is a ubiquitous soil saprophyte with over 40 species (Domsch et al. 2007). Conidiophores arose singly from plated segments and branched near the apex ending in a cluster of phialides. Phialoconidia were hyaline, aseptate, globose or ovoid and produced in dry basipetal chains (Barnett and Hunter 1998).

Pestalotia (de Not.). DEUTEROMYCOTA. COELOMYCETE

Pestalotia sp. is a plant parasite as well as saprophyte (Barnett and Hunter 1998). This fungus produced immersed, septate, brown mycelium. Dark, cushion or disc shaped acervuli were produced within mycelium (Sutton 1980). Conidia were dark, 4 to 5 septate, fusiform, straight to slightly curved, hyaline, with 3 to 9 long, thin, simple appendages (Fig 4.22) (Sutton 1980, Barnett and Hunter 1998).

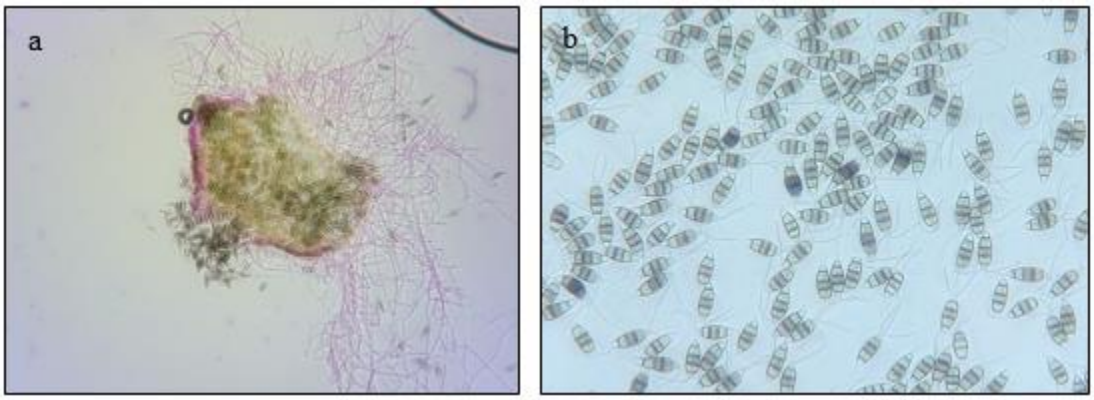

Figure 4.22 Identifying characteristics of *Pestalotia* sp.
a) Acervuli produced in axenic culture. b) Pycnidia with 3 to 9 long, simple appendages.

Phoma (Sacc.). DEUTEROMYCOTA. COELOMYCETE

Phoma sp. is the largest and most widely distributed member of the Sphaeropsidales (Domsch et al. 2007), with over 2,000 described species (Sutton 1980). They are found as parasites of most plant parts (Barnett and Hunter 1998). Mycelium was immersed, septate, and pale brown. Separate, immersed, erumpent with maturity, ostiolate, brown, and globose pycnidia with thin walls were produced on WA and mPDA (Fig 4.23a). Conidia were hyaline, aseptate, guttulate, and ellipsoid to cylindrical to fusiform to pyriform to globose (Fig 4.23b) (Sutton 1980). Isolate DN24 suspected to be

Phoma sp. was isolated in May, 2012 from Nagara Mxg at the Dairy location. The DN24 isolate was sequenced with ITS primers and subjected to a BLAST search against the NCBI GenBank. The 525 bp ITS fragment shared 99% identity and 98% query coverage to *Ph. herbarum* strain C2P21B (GenBank Accession Number JQ936276).

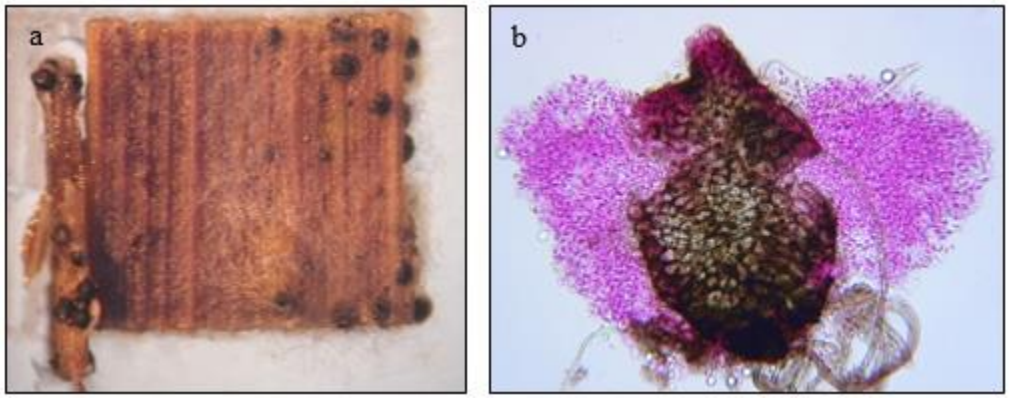

Figure 4.23 Identifying characteristics of *Phoma* sp.

a) Dark-brown to black pycnidia produced on plated leaf segments. b) Pycnidia with hyaline, globose to ellipsoid to cylindrical to fusiform conidia.

<u>*Pithomyces* (Berk. and Broome). DEUTEROMYCOTA. HYPHOMYCETE</u>

This fungus contains 15 species which are common globally as saprophytes on dead leaves and stems of many plants (Domsch et al. 2007). Conidiophores were produced on short lateral pegs from hyaline mycelium on WA and mPDA. Condia were single, broadly ellipsoidal, pale to dark brown, verrucose, 0 to 13 transverse septa and one or more longitudinal septa (Fig 4.24) (Barnett and Hunter 1998). Isolates DIS9 and DIS24 thought to be *Pithomyces* sp. based on conidia morphology observed were isolated in Nov 2012 from Illinois Mxg at the Dairy location. Both isolates were easily identified as *P. chartarum* due to their broadly ellipsoid shape as well as presence of transverse and longitudinal septa (Ellis 1976).

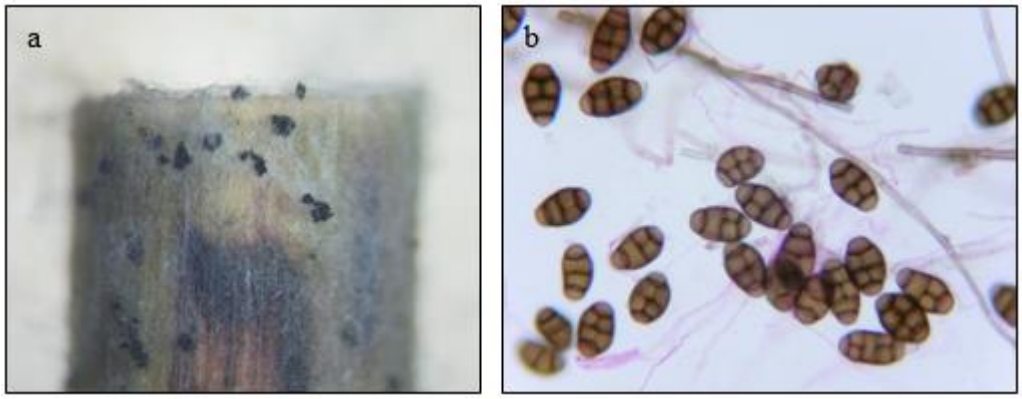

Figure 4.24 Identifying characteristics of *Pithomyces* sp.

a) Conidia produced in mycelium on plated leaf segment. b) Transverse and longitudinal septa indicative of *P. chartarum*.

<u>*Puccinia* (Persoon). BASIDIOMYCOTA. PUCCINIOMYCETES</u>

Species of *Puccinia* cause rust diseases of many vascular plants. There are 3,000 to 4,000 species of *Puccinia* which can be found globally in all environments except the artic. These fungi can be heteroecious or autoecious with many variations in life cycles (Ramachar and Cummins 1965). *Puccinia* produced uredinia which were epidermal on leaf segments and produced variably echinulate spores born singly on small pedicles (Fig 4.25). *Puccinia* telia observed from field collections before surface disinfestation were subepidermal at first then became erumpent with maturity, producing 2-celled spores with a longitudinal septum born on a long hyaline pedicle (Ramachar and Cummins 1965).

Figure 4.25 Identifying characteristics of *Puccinia* sp.

a) Uredinia present on leaf surface of switchgrass. b) Orange echinulate urediniospores with germ pore present.

Rhinocladiella (Nannf.). DEUTEROMYCOTA. HYPHOMYCETE

Rhinocladiella sp. is a common wood saprophyte (Barnett and Hunter 1998). Long brown conidiophores which are simple and elongated by sympodial growth were produced. Conidia were produced apically on new sympodial growth points, and were dry, hyaline to dark, and ovoid to oblong-ellipsoidal (Barnett and Hunter 1998).

Septoria (Sacc). DEUTEROMYCOTA. COELOMYCETES

Septoria sp. with over 2,000 described species (Sutton 1980) is parasitic; however, some species can cause leaf spots (Barnett and Hunter 1998). The fungus produced pale brown, immersed and branching mycelia on plated leaf segments as well as in culture on WA and mPDA. Pycnidia were immersed, separate or aggregated, ostiolate, globose and dark brown to black (Barnett and Hunter 1998). Conidia produced were hyaline, multiseptate, and narrowly elongate to filiform (Fig 4.26) (Sutton 1980). Isolate FS6 suspected to be *Septoria* sp. was isolated in Aug 2012 from EG1101 Swg at the Forage location. The FS6 isolate was sequenced with ITS primers and subjected to a

BLAST search against the NCBI GenBank. The 886 bp ITS fragment shared 98% identity and 98% query coverage to *S. arundinacea* strain 281.72 (GenBank Accession Number AJ496628).

Figure 4.26 Identifying characteristics of *Septoria* sp.

Dark brown to black globose pycnidia of *Septoria* sp. expelling hyaline, narrowly elongated conidia.

<u>*Spegazinnia* (Sacc). DEUTEROMYCOTA. HYPHOMYCETE</u>

Members of this specific genus are common saprophytes on plants and soil (Seifert et al. 2011). *Spegazzinnia* sp. produced small, dark sporodochium bearing 2 different types of conidia: one, a 4-celled, spiny, apically born on a long conidiophore; and another, a 4-celled, smooth spore born on a short conidiophore (Fig 4.27) (Barnett and Hunter 1998).

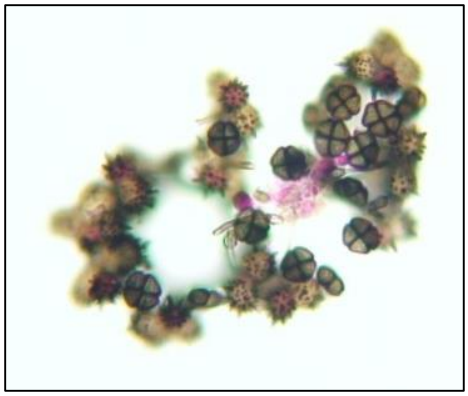

Figure 4.27 Identifying characteristics of *Spegazzinia* sp.

Four-celled spiny conidia as well as smooth four celled conidia of *Spegazzinia*.

<u>*Stagonospora* (Sacc). DEUTEROMYCOTA. COELOMYCETE</u>

Stagonospora sp. is a parasite and saprophyte on leaves and stems of many plants (Barnett and Hunter 1998). Mycelium produced was immersed, branched, septate and brown. Pycnidia produced were separate, immersed, globose, black, and ostiolate (Fig 4.28a). Conidia were hyaline, smooth, with 1 to 4 transverse eusepta, cylindrical to fusiform, straight to slightly curved, and sometimes guttulate (Fig 4.28b) (Sutton 1980). Isolate FIS17 suspected to be *Stagonospora* sp. was isolated in Nov 2012 from Illinois Mxg at the Forage location. The FIS17 isolate was identified as *Stagonospora* sp. based on pycnidia and conidia morphology (Sutton 1980).

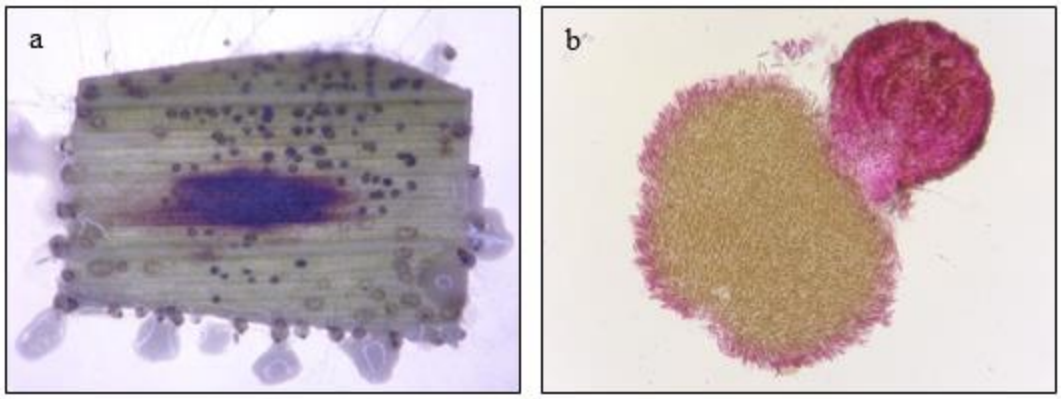

Figure 4.28 Identifying characteristics of *Stagonospora* sp.

a) Pycnidia of different maturation stages present on plated leaf segment. b) Pycnidia of *Stagonospora* with hyaline, cylindrical conidia with 1 to 4 septa.

Tetraploa (Berk. and Broome). DEUTEROMYCOTA. HYPHOMYCETE

The described members of this genus are exclusively considered to be saprophytes (Barnett and Hunter 1998). Conidiophores produced by *Tetraploa* are verrucose, arising from superficial mycelium that branch and anastomose to form an intricate network. Conidia were produced singly, pleurogenous, verruculose to verrucose, with mature conidia having long appendages and shallow furrows between 3 to 4 columns of cells which developed individually (Fig 4.29) (Ellis 1971).

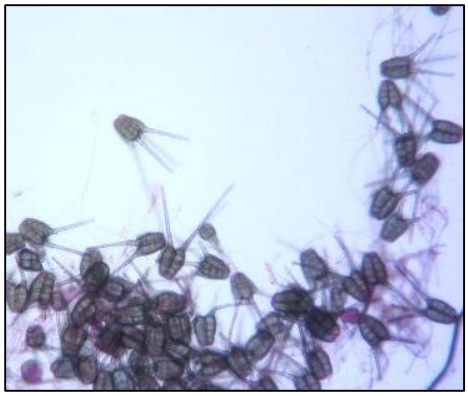

Figure 4.29 Identifying characteristics of *Tetraploa* sp.
Verruculose to verrucose conidia of *Spegazzinia* sp. with long appendages and shallow furrows which create 3 to 4 columns of individually developing cells.

2011 Results

One thousand six hundred (1,600) foliar lesions were plated and observed over the growing season between Jun and Sep 2011. From the infected foliar tissues, isolates representing 19 fungal genera were collected. This compilation includes both known pathogens of gramineaceous hosts as well as cosmopolitan saprophytes (Farr et al. 1989). Fungal frequency of occurrence was recorded monthly and compiled for the growing season.

Of the 17 genera of fungi that were identified on foliar lesions associated with Freedom, Illinois, and Nagara Mxg plots at the Dairy location, 70% were graminicolous parasites. Fourteen fungi identified were Hyphomycetes while two, *Phoma* and *Stagonospora*, were Coelomycetes. When compiled and averaged across all cultivars, *Alternaria* and *Phoma* were the predominant pathogens with isolation frequencies of 38.0% and 56.0%, respectively (Table 4.3). *Curvularia* (10.0%) and *Stagonospora* (12.0%) were also isolated at relatively high frequencies. Overall, fungi most frequently (> 10.0%) identified from Mxg cultivars included *Alternaria*, *Curvularia*, *Phoma*, and

Stagonospora in contrast to those identified at lower frequencies (< 10.0%) *Aspergillus*, *B. cynodontis*, *B. oryzae*, *Cephaliophora*, *Epicoccum*, *Exserohilum*, *Fusarium*, *Leptosphaeria*, *Nigrospora*, *Pithomyces*, *Paecilomyces*, *Spegazinnia*, and *Tetraploa* (Table 4.3).

Seventeen fungi were identified from foliar lesions associated with Freedom and Illinois Mxg and EG1101 Swg at the Forage location in which 65.0% were pathogens of gramineaceous hosts. Of the fungi identified, ten were Hyphomycetes and three were Coelomycetes. Again, *Alternaria* and *Phoma* were the predominant fungi, both having isolation frequencies of 42.0%. *Colletotrichum* (19.0%), *Epicoccum* (15.0%), and *Stagonospora* (15.0%) were also identified at relatively high frequencies. Overall, fungi most frequently (> 10.0%) identified from Mxg and Swg cultivars included *Alternaria*, *Colletotrichum, Epicoccum*, *Phoma*, and *Stagonospora*, in contrast to those identified at lower frequencies (< 10.0%) *Aspergillus*, *Bipolaris cynodontis*, *B. oryzae*, *B. sorokiniana*, *B. spicifera*, *Curvularia*, *Fusarium*, *Leptosphaeria*, *Cephaliophora*, *Nigrospora*, *Paecilomyces*, and *Puccinia*.

Fifteen fungi were identified from foliar lesions associated with Freedom Mxg and Alamo Swg at the Variety location, and of these, 80.0% have previously been reported as pathogens of gramineaceous hosts. Ten of these were Hyphomycetes and four were Coelomycetes. The predominant pathogens observed were *Alternaria* (34.0%), *Colletotrichum* (26.0%), and *Phoma* (41.0%); with *Fusarium* (12.0%) and *Stagonospora* (10.0%) having relatively high frequencies as well. Overall, fungi most frequently (> 10.0%) identified from Mxg and Swg cultivars included *Alternaria*, *Colletotrichum, Fusarium,* and *Phoma*, while those fungi identified at lower frequencies (< 10.0%) were

Aspergillus, *B. cynodontis*, *B. oryzae*, *B. sacchari*, *B. spicifera*, *Curvularia*, *Epicoccum*, *Cephaliophora*, *Septoria*, *Nigrospora*, *Paecilomyces*, *Pithomyces*, *Puccinia*, and *Stagonospora*.

2012 Results

Two thousand and eight hundred (2,800) foliar lesions were collected and plated over the growing season between Apr and Oct 2012. From the infected foliar tissues, isolates representing 28 fungal genera were identified. This compilation includes both known pathogens of gramineaceous hosts as well as cosmopolitan saprophytes (Farr et al. 1989). Fungal frequency of occurrence was recorded monthly and compiled for the growing season.

Of the 20 genera of fungi identified from foliar lesions associated with Freedom, Illinois, and Nagara Mxg at the Dairy location, 55.0% had previously been reported to be pathogens of gramineaceous hosts. From these foliar lesions 15 fungi identified were Hyphomycetes, while three were Coelomycetes. Fungi with the greatest identification frequencies were *Alternaria* (18.0%), *Epicoccum* (12.0%), and *Phoma* (35.0%). Fungi isolated at lower frequencies (< 10.0%) from Mxg cultivars include; *Ascochyta, Aspergillus, B. oryzae, B. spicifera, Cephaliophora, Coniella, Curvularia, Epicoccum, Exserohilum, Fusarium, Mortierella, Nigrospora, Paraphaeosphaeria, Penicillium, Pestalotia, Phoma, Pithomyces, Rhinocladiella, Stagonospora,* and *Tetraploa* (Table 4.4).

A total of 21 fungal genera were identified from foliar lesions associated with Freedom and Illinois Mxg and EG1101 Swg at the Forage location and of these, 57.0% had previously been reported as pathogens of gramineaceous hosts. Eleven fungi were

classified as Hyphomycetes, while five were Coelomycetes. *Alternaria* (28.0%) and *Epicoccum* (23.0%) had the greatest frequency of identification; while *Colletotrichum* (12.0%), *Fusarium* (11.0%), and *Phoma* (15.0%) were relatively high as well. Fungi isolated at low frequencies (< 10.0%) included *Ascochyta*, *B. cynodontis*, *B. oryzae*, *B. spicifera*, *B. victoriae*, *Cephaliophora*, *Coniella*, *Curvularia*, *Glomerella*, *Lacellinopsis*, *Leptosphaeria*, *Mortierella*, *Nigrospora*, *Paecilomyces*, *Paraphaeosphaeria*, *Septoria*, *Stagonospora*, and *Tetraploa* (Table 4.4).

Fewer fungi were identified from foliar lesions collected from Freedom Mxg and Alamo Swg at the Variety location than the others in 2012, with 16 genera observed. Of those, 69.0% have previously been reported as graminicolous pathogens. This includes nine Hyphomycetes and five Coelomycetes. Averaged across both Freedom and Alamo, *Alternaria* (24.0%), *Colletotrichum* (19.0%), *Epicoccum* (12.9%), *Fusarium* (18.0%), and *Phoma* (17.0%) were the predominantly reoccurring pathogens. Fungi isolated at the other locations were at frequencies < 10.0% and include *Ascochyta*, *B. cynodontis*, *B. oryzae*, *Chaetomium*, *Curvularia*, *Nigrospora*, *Paecilomyces*, *Puccinia*, *Rhinocladiella*, *Septoria*, *Stagonospora*, and *Tetraploa* (Table 4.4).

Table 4.3 Fungal frequency (%) associated with field disease collections from giant miscanthus[z] (*Miscanthus x giganteus*) and switchgrass[z] (*Panicum virgatum*) across the June to September, 2011 growing season at research locations in Starkville, MS.

Fungi	Location							
	Dairy			Forage			Variety	
	FMXG	IMXG	NMXG	FMXG	IMXG	ESWG	FMXG	ASWG
Alternaria	42.0	42.0	32.0	56.0	44.0	28.0	32.5	35.0%
Aspergillus	0.5	1.0	0.0	0.0	1.0	0.5	0.0	0.5
Bipolaris cynodontis	0.0	3.0	0.0	0.0	2.0	0.5	0.5	0.0
B. oryzae	3.0	4.0	7.0	5.0	2.0	1.5	0.5	0.5
B. sacchari	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0
B. sorokiniana	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
B. spicifera	0.0	0.0	0.0	5.5	3.5	2.0	2.5	0.0
Cephaliophora	3.5	3.5	0.0	0.0	0.0	0.5	0.0	0.5
Colletotrichum	0.0	0.0	0.0	1.0	0.0	57.0	4.0	47.0
Curvularia	9.5	11.0	10.0	15.0	5.5	2.5	4.5	4.5
Epicoccum	7.5	6.5	5.0	22.5	14.0	8.0	8.5	6.0
Exserohilum	0.0	0.0	2.5	0.0	0.0	0.0	0.0	0.0
Fusarium	1.0	9.0	2.0	3.0	4.0	11.0	4.5	19.0
Leptosphaeria	1.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0
Nigrospora	8.0	7.5	0.0	4.5	4.5	0.5	4.5	1.0
Paecilomyces	1.5	0.5	1.5	3.0	3.0	1.0	1.5	1.0
Phoma	52.5	61.0	57.5	51.5	57.5	17.5	57.5	25.0
Pithomyces	1.5	2.5	1.5	0.0	0.0	0.0	1.0	0.0
Puccinia	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.5
Septoria	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5
Spegazinnia	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Stagonospora	10.5	14.5	9.5	6.0	23.5	14.0	11.0	8.5
Tetraploa	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[z] FMXG= Freedom giant miscanthus; IMXG= Illinois giant miscanthus; NMXG= Nagara giant miscanthus; ESWG= EG1101 switchgrass; ASWG= Alamo switchgrass.

Table 4.4 Fungal frequency (%) associated with field disease collections from giant miscanthus[z] (*Miscanthus x giganteus*) and switchgrass[z] (*Panicum virgatum*) across the April to September, 2012 growing season at research locations in Starkville, MS.

Fungi	Location							
	Dairy			Forage			Variety	
	FMXG	IMXG	NMXG	FMXG	IMXG	ESWG	FMXG	ASWG
Alternaria	17.7	17.7	17.1	27.7	29.7	26.5	16.5	30.5
Ascochyta	0.0	5.5	0.2	0.5	0.5	0.0	0.0	1.0
Aspergillus	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Bipolaris cynodontis	0.0	0.0	0.0	0.2	0.0	0.0	1.7	0.5
B. oryzae	0.0	0.8	1.7	0.0	0.2	0.0	0.0	0.2
B. spicifera	0.0	0.0	1.1	0.5	0.0	0.2	0.0	0.0
B. victoriae	0.0	0.0	0.0	0.2	0.2	0.2	0.0	0.0
Cephaliophora	0.0	0.0	0.2	0.0	0.5	0.0	0.0	0.0
Chaetomium	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8
Colletotrichum	0.0	0.0	0.0	9.0	0.5	27.0	0.0	37.0
Coniella	1.1	0.0	7.0	0.0	0.2	0.0	0.0	0.0
Curvularia	0.2	1.4	0.5	2.2	1.4	0.5	1.7	2.8
Epicoccum	9.0	14.0	14.0	25.0	28.0	15.4	21.0	4.8
Exserohilum	0.0	1.1	0.2	0.0	0.0	0.0	0.0	0.0
Fusarium	8.5	6.2	8.8	6.5	5.7	22.0	11.1	24.2
Glomerella	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0
Lacellinopsis	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0
Leptosphaeria	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0
Mortierella	0.2	0.0	0.0	1.4	0.0	0.2	0.0	0.0
Nigrospora	2.5	8.2	4.8	7.7	4.2	1.7	3.1	0.2
Paecilomyces	0.0	0.0	0.0	0.8	0.8	0.0	0.2	0.0
Paraphaeosphaeria	0.0	0.2	0.5	0.0	0.2	0.8	0.0	0.0
Penicillium	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0
Pestalotia	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0
Phoma	45.4	32.5	28.2	16.0	17.1	12.0	26.8	7.4
Pithomyces	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0
Puccinia	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1
Rhinocladiella	0.0	0.2	0.0	0.0	1.1	0.8	1.4	0.0
Septoria	0.0	0.0	0.0	2.0	0.5	0.8	0.0	0.8
Stagonospora	1.7	2.5	0.0	2.2	2.5	3.7	0.2	2.5
Tetraploa	0.0	0.8	0.0	0.2	0.0	0.0	0.2	0.0

[z] FMXG= Freedom giant miscanthus; IMXG= Illinois giant miscanthus; NMXG= Nagara giant miscanthus; ESWG= EG1101 switchgrass; ASWG= Alamo switchgrass

Koch's postulates

Koch's postulates conducted with *B. oryzae* isolate FMXGBO1 produced foliar symptoms three days post-inoculation (DPI) consisting of small (2 to 5 mm), red to reddish-brown flecks which developed into small lesions with slight chlorotic halos.

Lesions further progressed (7 DPI) into larger eyespot lesions containing straw colored centers with distinct reddish-brown to black margins (Fig. 4.30a). Similar foliar symptoms were observed on both Freedom and Illinois Mxg. No symptoms were observed on the noninoculated controls. Upon investigation of symptoms with light microscopy, conidia were observed germinating in infection centers (Fig. 4.30b). *B. oryzae* was reisolated from diseased material and identified based on conidia morphology (Fig. 30c).

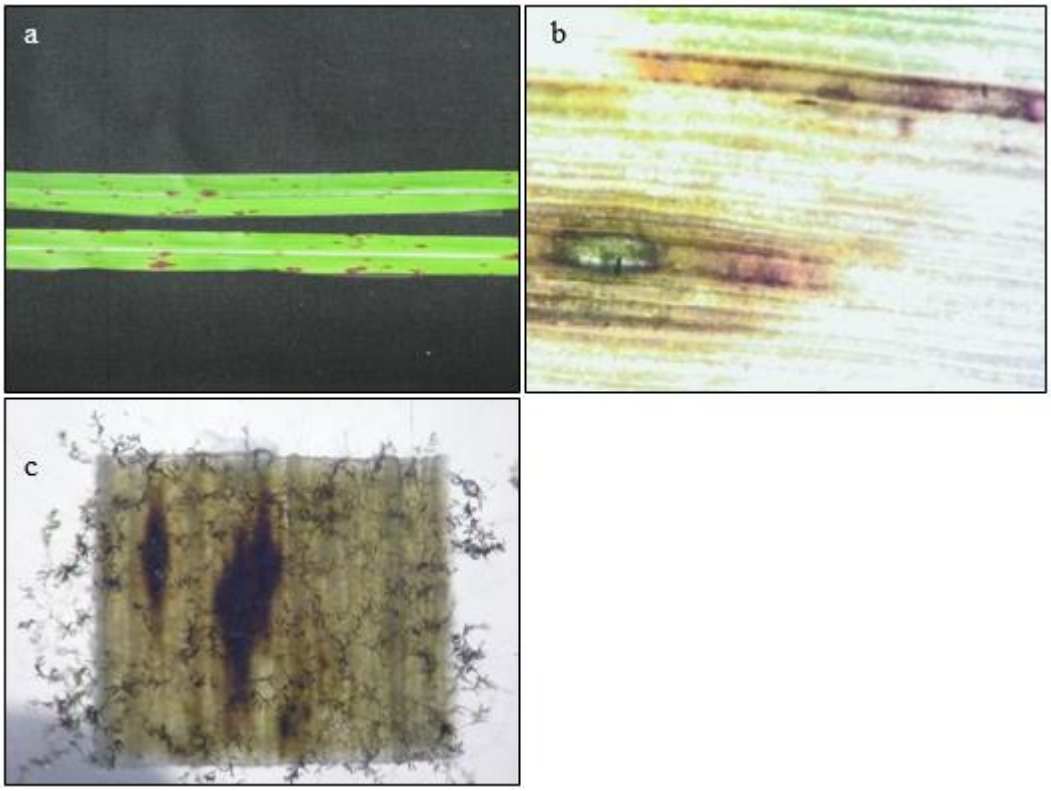

Figure 4.29 Koch's postulates conducted with *Bipolaris oryzae* isolate FMXGBO1 on Freedom and Illinois giant miscanthus (*Miscanthus* x *giganteus*).

a) Red flecking and eyespot symptoms observed 8 DPI on Freedom giant miscanthus b) Germinating conidia of *B. oryzae* on Freedom leaf tissue. c) Curved conidia indicative of *B. oryzae* present on plated leaf segment for pathogen reisolation.

Koch's postulates conducted with *B. sorokiniana* isolate FMXGBS1 was positive on both Mxg cultivars, producing symptoms 7 DPI Foliar symptoms consisted of small (2 to 5 mm), brown, elongated spots with chlorotic halos which progressed into larger (5 mm to 2 cm) elongated lesions surrounded by distinct chlorotic and necrotic areas along leaf veins (Fig. 4.31a). No symptoms were observed on the noninoculated control plants. *B. sorokiniana* was reisolated from disease symptoms and confirmed according to conidia morphology (Fig. 4.31b).

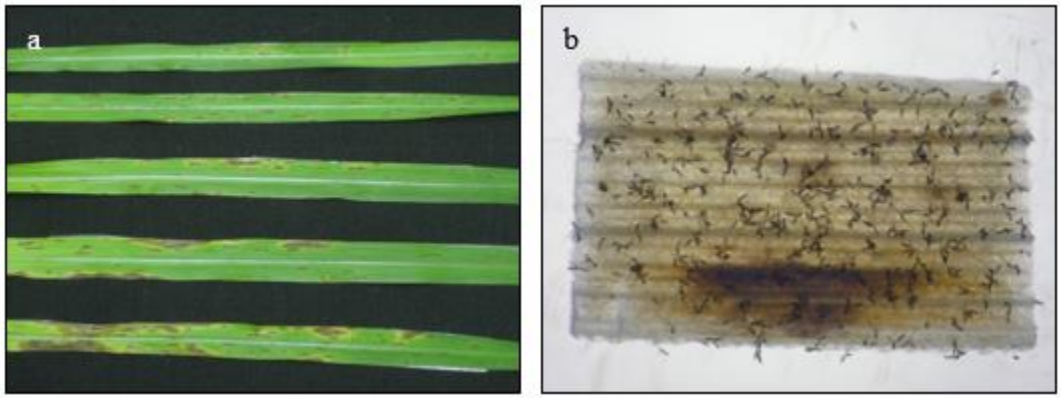

Figure 4.30 Koch's postulates conducted with *Bipolaris sorokiniana* isolate FMXGBS1 on Freedom and Illinois giant miscanthus (*Miscanthus* x *giganteus*).

a) Brown spots and larger, elongated lesions with distinct chlorotic and necrotic halos.
b) Confirmation of *B. sorokiniana* based on conidia morphology colonizing infected giant miscanthus (*Miscanthus* x *giganteus*) tissue.

Koch's Postulates conducted with *B. victoriae* isolate DN1 produced symptoms 7 DPI on all inoculated plants of both Freedom and Illinois Mxg, while no symptoms were observed on the noninoculated control plants. Foliar symptoms initially consisted of small (2 to 5 mm), red lesions with slight chlorotic halos (Fig. 4.32a). Lesions increased in size slightly (5 to 10 mm), and developed necrotic centers creating a characteristic

eyespot lesion with distinct reddish-brown to black margins and straw colored centers (Fig. 4.32b). Chlorotic halos were present on mature symptoms, but not to the extent as that observed in foliar symptoms caused by *B. sorokiniana* . *B. victoriae* was reisolated and confirmed based on conidia morphology as well as ITS sequencing.

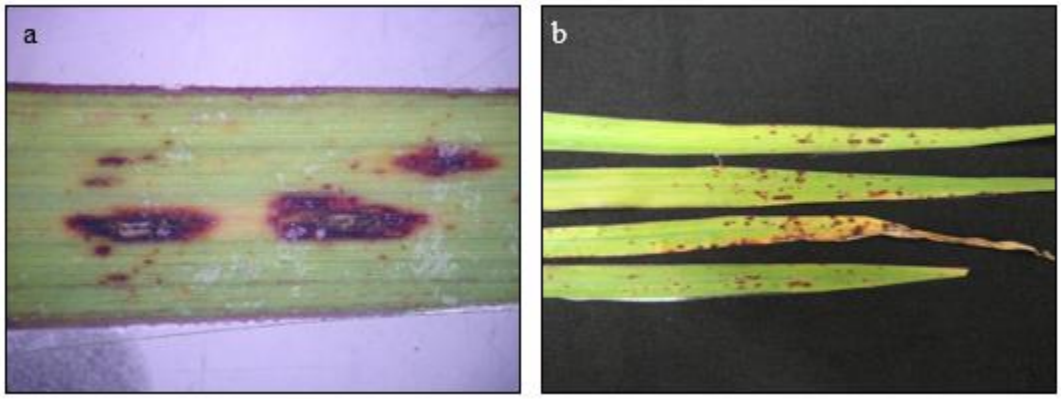

Figure 4.31 Koch's Postulates conducted with *Bipolaris victoriae* isolate DN1 on Freedom and Illinois giant miscanthus (*Miscanthus* x *giganteus*).

a) Small, red lesions progressing into larger lesions with distinct red margins and necrotic centers. b) Red eyespot lesions on foliar tissue of giant miscanthus.

Koch's Postulates conducted with *C. navitas* isolates VF7 and VF30 produced symptoms 10 DPI. Growth chambers were maintained at 30°C with a 16 h photoperiod. During the incubation period, a serendiptious event occurred causing the cooling unit to fail in one of the growth chambers housing half of the experiment. The temperature in this growth chamber exceeded 37°C, causing the lighting system to automatically turn off. Inoculated Mxg plants incubating in this growth chamber became etiolated and stressed due to high temperatures and lack of light. These heat stressed and etiolated plants developed symptoms consisting of elongated elliptical lesions with brown borders mainly on leaf margins. These lesions expanded and sometimes coalesced with age.

Areas of severe chlorosis and necrosis bordered these lesions (Fig 4.33). Signs of acervuli and setae, oriented vertically with leaf veins, were present upon further examination with a compound microscope. Symptoms were observed only on Freedom Mxg plants present in the failed growth chamber; however, symptoms were not present on noninoculated controls nor Illinois plants held in the growth chamber that did not fail. The pathogen was reisolated and identified as *C. navitas* based on conidia morphology.

Figure 4.32 Koch's postulates conducted with *Colletotrichum navitas* on environmentally stressed Freedom giant miscanthus (*Miscanthus* x *giganteus*).

Chlorotic and necrotic elliptical lesions resulting from infection caused by *C. navitas* isolates VF7 and VF30.

Koch's postulates conducted with the remaining fungi, Alternaria alternata, Ascochyta hordei, Bipolaris cynodontis, B. spicifera, Phoma herbarum, Pithomyces chartarum, and Septoria arundinacea did not result in symptom development under inoculation and incubation procedures carried out in this study.

CHAPTER V

DISCUSSION

Field Disease Ratings

Symptom characterization

The symptoms observed on Mxg culitvars over the two year study were consistent with fungal infections of major crops within the Gramineae family such as barley (*Hordeum* spp.), corn (*Zea* spp.), oat (*Avena* spp.) rice (*Oryza* spp.), sorghum (*Sorghum* spp.), sugarcane (*Saccharum* spp.), and wheat (*Tritichum* spp.). Principal symptoms observed on Mxg during this study were categorized as leaf spots, leaf blotches, as well as some that initially began as leaf spots but later developed into leaf blights; all of which are symptoms of diseases previously reported and regularly diagnosed on grasses. Leaf spots like those observed on Mxg (Fig. 4.1) have been described from many grass hosts: rough leaf spot (*Ascochyta sorghina*) and target leaf spot (*Bipolaris sorghicola*) of sorghum (Frederiksen 2000); ring spot (*Leptosphaeria sacchari*) and eye spot (*Bipolaris sacchari*) of sugarcane (Edgerton 1955); Northern leaf spot (*Cochliobolus carbonum*) and grey leaf spot (*Cercospora zeae-maydis*) of corn (White 1999); Ascochyta leaf spot (*Ascochyta tritici*) and tan spot (*Pyrenophora tritici-repentis*) of wheat (Wiese 1977; Murray et al. 2009); as well as brown leaf spot (*Bipolaris oryzae*) of rice (Webster 1992). Leaf blotches (Fig. 4.2) have been previously reported and commonly diagnosed such as spot blotch (*Cochliobolus sativus*) of wheat and barley as well as many other grasses

(Murray et al. 2009), leaf blotch (*Rhynchosporium spp*.) of barley and rye (Murray et al. 2009), as well as Septoria leaf blotch of wheat (*Septoria nodorum, S. tritici*) (Murray et al. 2009). Symptoms of leaf blight (Fig. 4.3) are common diseases of grass hosts as well, including southern corn leaf blight (*B. maydis*) and Northern leaf blight (*E. turcicum*) of corn (White 1999), and Alternaria leaf blight (*A. triticina*) of wheat (Wiese 1977). The fact that these fungi were observed on Mxg is consistent with the published literature of foliar diseases of most gramineaceous hosts.

Disease Assessment

The foliar disease assessment conducted in this study showed results in which significant host differences were observed in locations where both Swg and Mxg cultivars were present (Forage and Variety locations), and under closer examination cultivars of Alamo and EG1101 Swg had an average of 83.0% greater incidence of foliar diseases than cultivars of Freedom and Illinois Mxg at the locations where Swg was present (Table. 4.2) Switchgrass had far more initial disease when compared to Mxg in both growing seasons, suggesting that perennial inoculum levels may be present and may be an increasing problem as the ages of these individual stands of perennial bioenergy crops increase. Switchgrass is native to the grasslands of the United States (Ghimire et al. 2011), and Mxg has been introduced within the last 30 years (Stewart 2009). The age of crop establishment in the U.S. could possibly be a factor in explaining greater foliar disease pressure in Swg that has continually been exposed to pathogens. In contrast, Mxg is a recently introduced crop not having long-term exposure to disease pressure, coinciding with what was observed with the foliar disease assessment. In this study, leaf spot (*B. oryzae*), anthracnose (*C. navitas*), as well as rust (*P. emaculata*) were the

primary diseases observed on switchgrass and may account for higher disease incidence when compared to only leaf spot diseases of Mxg. In addition, anthracnose which has been reported in the states of: Iowa, New Jersey, New York, North Carolina, Pennsylvania, and Tennessee (Crouch et al. 2009), was observed to have severe effects on Swg in stands in Starkville; however, anthracnose symptoms were not observed on Mxg, nor has incidence of Mxg anthracnose been reported anywhere else. The close proximity of inoculum reservoirs such as Swg, as well as the positive Koch's postulates demonstrated through this research, suggests that anthracnose may become a primary disease of Mxg in the near future. Another disease that could potentially be problematic for Mxg is leaf spot (*B. oryzae*). Like anthracnose, leaf spot caused by *B. oryzae* has been previously described as a pathogen of Swg in Mississippi, New York, and North Dakota (Krupinsky et al. 2004; Tomaso-Peterson and Balbalian 2010; Waxman and Bergstom 2011). The presence of this pathogen on Swg has already been confirmed in Mississippi (Tomaso-Peterson and Balbalian 2010), and through this research the pathogen has been isolated and pathogenic capabilities demonstrated on Mxg. The more predominant and drastic foliar disease of Swg, rust, was never observed on Mxg over the two year study period.

At the Dairy location where only the Mxg cultivars Freedom, Illinois, and Nagara were present, no cultivar differences were observed in terms of foliar disease incidence. The similar cultivar response at this location was expected, as little genetic diversity exists among Mxg cultivars. In essence, the recent establishment of Mxg as a second generation biofuel crop is advantageous when considering disease management. The pathogens that incite foliar diseases of traditional grass hosts such as Swg may have not

developed the host-pathogen relationship with Mxg due to the relative short existence of the host crop. One would expect this relationship to shift in favor of the pathogen over time due to continual exposure and the perennial nature of Mxg.

Fungal Collections

Fungi representing 30 genera were collected and identified from foliar disease collections of symptomatic material observed on Swg and Mxg stands located in Starkville, MS, of these, 16 known pathogens of gramineaceous hosts were identified (Farr et al. 1989). Of the fungi identified, many are economically important pathogens of major agronomic crops such as: *Aspergillus*, *Bipolaris*, *Colletotrichum*, *Exserohilum*, *Fusarium*, *Puccinia*, and *Septoria*. However, these pathogens were identified at relatively low frequencies compared to those fungi identified as saprophytes or endophytes such as *Alternaria*, *Curvularia*, and *Epicoccum* (Barnett and Hunter 1998). Of the many fungi that have been reported to be pathogenic on Swg in other states; *Alternaria* (Vu 2012), *B. oryzae* (Krupinsky et al. 2004; Tomaso-Peterson and Balbalian 2010; Waxman and Bergstrom 2011), *B. sorokiniana* (Vu 2011), *B. spicifera* (Vu 2011), *C. navitas* (Crouch et al. 2009; Waxman and Bergstrom 2011), *Curvularia* (Fajolu 2012), and *Puccinia* (Frazier et al. 2013; Hirsch et al. 2010; Zale et al. 2008) have been identified from symptomatic leaves collected from Swg in Mississippi. Of the reported pathogens of *Miscanthus*, such as *P. chartarum* (Ahonsi et al. 2010) and *Stagonospora* (O'Neill and Farr 1996; Pusz and Plaskowska 2010), both were identified from Mxg in Mississippi. The observation of these pathogens from both Swg and Mxg suggests disease issues of these crops may be more prevalent and exacerbated with the increased attention of the bioenergy industry and production of bioenergy grasses.

Differences in the number of fungal genera identified between seasons were observed, with a greater number of genera identified from the 2012 growing season (28 genera) than the 2011 season (19 genera). Possible explanations for this include differences in environmental conditions between the two seasons, the greater amount of time in which samples were collected for the 2012 season, as well as an increase in diagnostic skills, experience, and knowledge in fungal identification. Environmental conditions between the two growing seasons were relatively similar (Table 5.1). However, the slightly lower temperatures observed in 2012 (-1.4°C), as well as the increased rainfall (0.28 cm) and greater humidity (2.0%) could contribute to the greater number of fungal genera observed. The addition of two months to the growing season in 2012, due to the earlier emergence of plant material, could be another possible explanation for the greater number of fungal genera observed as well. Finally, the diagnostic capabilities of the author greatly improved after the experience of recognizing and identifying fungi from the first season, and may have contributed to the increase of fungal genera observed in the 2012 season.

Table 5.1 Monthly environmental conditions associated with the 2011 and 2012 growing seasons in Starkville, MS.

Date	Average Temperature (°C)	Total Rainfall (cm)	Total Humidity (%)
Jun 2011	28.05	11.55	67.0
Jul 2011	28.16	10.66	73.0
Aug 2011	28.50	3.81	66.0
Sep 2011	22.55	6.04	70.0
Oct 2011	16.39	2.10	66.0
2011[z]	24.73	6.83	68.0
Apr 2012	19.22	9.14	66.0
May 2012	23.88	9.88	69.0
Jun 2012	26.61	3.70	62.0
Jul 2012	27.77	18.74	73.0
Aug 2012	25.77	5.94	75.0
Sep 2012	23.27	0.05	72.0
Oct 2012	16.88	2.31	70.0
2012[z]	23.33	7.11	70.0

[z] 2011 and 2012 seasonal total rainfall reported as average monthly rainfall (cm). Climate data obtained from the Department of Geosciences at Mississippi State University at http://geosciences.msstate.edu/ftpdata/wx/data/.

Koch's postulates

Throughout the two year study, leaves displaying foliar symptoms were collected from Swg and Mxg hosts, and fungi identified as graminicolous pathogens were isolated for Koch's postulates. Twelve fungal isolates were selected, representing eight fungal genera. Of the twelve fungal isolates evaluated, four isolates were determined to be pathogenic to Mxg (Fig. 4.7) including *B. oryzae*, *B. sorokiniana*, *B. victoriae*, as well as *C. navitas*. *B. oryzae*, *B. sorokiniana*, and *C. navitas* have all previously been reported as

a pathogen of many grass hosts, including Swg (Crouch et al. 2009; Krupinsky et al. 2004; Tomaso-Peterson and Balbalian 2010; Vu 2011a; Vu 2011b; Waxman and Bergstrom 2011) (Fig. 4.7). However, *B. victoriae* has yet to be described as a pathogen of either Swg or Mxg. The symptoms incited by *B. oryzae* and *B. victoriae* were quite similar in the fact that small brown to red eye spot lesions formed on the leaf surface, with little to no chlorosis or necrosis in contrast to those symptoms incited by *B. sorokiniana* or *C. navitas*. Both *B. sorokiniana* and *C. navitas* incited symptoms on Mxg cultivars of chlorosis and necrosis; associated with either an elliptical shaped lesion in *Colletotrichum* evaluations, or areas of brown blighted leaves associated with *B. sorokiniana*. Nevertheless, all four pathogens created symptoms that had drastic effects on foliar material, which could have a significant effect on biomass yield if any of these pathogens were able to incite an epidemic in field production situations.

When conducting Koch's postulates with *Alternaria alternata*, *Ascochyta hordei*, *Bipolaris cynodontis*, *B. spicifera*, *Curvularia lunata*, *Phoma herbarum*, *Pithomyces chartarum*, and *Septoria arundinacea*, no symptoms developed within the allotted incubation period. Isolates of *P. chartarum*, previously described as a pathogen of Mxg (Ahonsi et al. 2010), were unsuccessful based on the protocol the authors reported. Koch's postulates using *P. chartarum* were conducted several times without successful demonstration of pathogenicity, suggesting that possibly the correct conditions such as proper environment, a virulent pathogen, or a susceptible host was not present to incite disease.

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