5-2018

Identification, characterization, and genetic comparison of *Fusarium* species isolated from switchgrass (*Panicum virgatum* L.)

Tamara Annette Collins
University of Tennessee, tcolli26@utk.edu

To the Graduate Council:

I am submitting herewith a thesis written by Tamara Annette Collins entitled "Identification, characterization, and genetic comparison of *Fusarium* species isolated from switchgrass (*Panicum virgatum* L.)." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

Bonnie H. Ownley, Major Professor

We have read this thesis and recommend its acceptance:

Denita Hadziabdic-Guerry, Darrell D. Hensley, Robert N. Trigiano

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

Identification, characterization, and genetic comparison of *Fusarium* species isolated from switchgrass (*Panicum virgatum* L.)

A Thesis Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Tamara Annette Collins
May 2018

Dedication

I dedicate this research to my family. To my remarkable parents, Timothy and Susan Collins for being there for me throughout my life to encourage me to strive for the best, never settle, and never give up. To my precious children, Gideon and Killian Collins-Figgs for giving me a reason to work hard to make the world better for generations to come. To my incredible boyfriend, Tyler and his parents Keith and Leslie Marcum for being extra strength and support through my graduate school process.

Acknowledgments

Thank you to my advisor Dr. B. Ownley for giving me the opportunity to be part of the Entomology and Plant Pathology graduate program. Her guidance and avocation for diversity, specifically women in science related fields, gave me the extra confidence and drive I needed to succeed. Thank you to Dr. D. Hensley for hiring me as a graduate assistant on the cornelian cherry dogwood grant. I would like to thank my other committee members, Dr. D. Hadziabdic, Dr. R. Trigiano, for their assistance and insight during this project, and Dr. K. Gwinn for extra encouragement and guidance above and beyond. Thank you to my lab mates Sara Collins, Alex Bruce, Shalini Yerukala, Mary Dee, and adopted lab mates from the Dr. R. Trigiano's Molecular Genetics Lab: Sarah Boggess, Chris Wyman, Tyler Edwards, Marcin Nowicki, and Dr. Romina Gazis for all your valuable insight and humorous moments. For her aid in bioinformatics and computer software, I would like to thank Dr. Margaret Staton. I would like to thank the Tennessee Department of Agriculture, the U.S. Department of Agriculture, and The University of Tennessee for funding this research. Finally, to my family, thank you for being here to support me and push me to never give up, and for putting up with the stressful and heartbreaking moments that we encountered the last two years.

Abstract

Fusarium spp. are ubiquitous, soilborne and seedborne pathogens that affect over 100 economically important plant hosts annually, resulting in billions of dollars in economic losses. They cause a variety of plant disease symptoms including, crown, root, and stem rots, wilts, and foliar necrosis. Furthermore, they produce harmful mycotoxins that are detrimental to the health of animals and humans. Members of this genus have been isolated from diseased tissues of switchgrass. The objective is to correctly identify and classify *Fusarium* isolates to species using both classic phenotypic characterization and current genetic technologies of DNA extraction and polymerase chain reaction and create microsatellite loci that will give more insight into the genetic template of Fusarium species. This will enable us to better understand the role these fungi play in agriculture systems and how to control the pathogenic ones more effectively. Whole genome sequences were developed for four *Fusarium* species (*F. equiseti*, *F. graminearum*, *F. oxysporum*, and *F. sporotrichioides*) isolated from commercial switchgrass seed from 11 geographic locations across the United States. From each genome, 15 dinucleotide and 15 trinucleotide (n = 30) microsatellite loci were evaluated for amplification and polymorphism. An additional 30 microsatellite loci were developed for *F. graminearum*. The percentage amplification of the microsatellites from each species was 93% for *F. equiseti*, 42% for *F. graminearum*, 50% for *F. oxysporum* and 36% for *F. sporotrichioides*. The sample size of *F. equiseti* isolates was small (n = 6), therefore this species was not included in further study. Microsatellite loci from the *Fusarium* species were considered polymorphic if they yielded one clear peak and the base pair (bp) length difference among individual alleles was greater than 3 bp. Polymorphic

microsatellite loci were evaluated for cross amplification of *F. graminearum*, *F. oxysporum*, and *F. sporotrichioides*. Microsatellite loci Fg057 (from *F. graminearum*) and Fr006 (*F. sporotrichioides*) did not amplify products from *F. oxysporum* isolates, and Fo006 and Fo012 (from *F. oxysporum*) did not amplify against *F. sporotrichioides*. *Fusarium oxysporum* isolates were nonpathogenic endophytes of switchgrass, while isolates of *F. graminearum* and *F. sporotrichioides* were pathogenic on this host.

Table of Contents

List of Tables

List of Figures

Chapter 1 Literature Review

Switchgrass

General information

Switchgrass (*Panicum virgatum* L.) is native to the U.S. It comprises the highest percentage of the three main native grasses, including big bluestem (*Andropogon gerardii Vitman*) and Indian grass (*Sorghastrum nutans* L. Nash), which are found in North American prairies (MOSER AND VOGEL 1995; VERMERRIS 2008). This perennial is found growing from 20˚ north latitude to 60˚ north latitude, ranging from Southern Canada, throughout the U.S. and into Central Mexico (Fig. 1) (LEWANDOWSKI *et al.* 2003; SMART *et al.* 2004; VOGEL 2004; PARRISH AND FIKE 2005). Two predominant taxonomic ecotypes of switchgrass exist: lowland and highland (PORTER 1966; BRUNKEN AND ESTES 1975; GUNTER *et al.* 1996; SMART *et al.* 2004; VOGEL 2004; SANDERSON *et al.* 2012). Hultquist (1996) confirmed these taxonomic groups using chloroplast DNA. Lowland varieties are tall, and largely found in wet areas with mild winter temperatures. They have longer blue-green leaves and coarser stems. Highland varieties are short, produce less biomass than the lowland types, and are more tolerant of dry conditions (CASLER *et al.* 2007; SANDERSON *et al.* 2012). Healthy mature stands of switchgrass grow as a closed canopy system reaching over 3 m tall (GRAVERT *et al.* 2000; GRAVERT AND MUNKVOLD 2002) and distribute a substantial portion of photosynthetic products to sustain a sizable, active root system that is up to 80% of the total plant biomass (LIEBIG *et al.* 2005). High levels of resource allocation to root production has multiple benefits that include improved capacity to utilize water and nutrients from deeper soils,

augmented enrichment of soil associated with high inputs of soil carbon from root yield, amplified activity of microbial communities, and increased capacity of switchgrass to store and mobilize energy and nutrients needed to regrow following cutting or grazing (STOUT *et al.* 1988; KSZOS 2005). Research by Gebhart *et al.* (1994), Bransby *et al*. (1998), Garten and Wullschleger (2000), and Ma *et al*. (2000a; 2000b) indicated that switchgrass could reduce CO_2 emissions and improve soil quality by carbon sequestration. Switchgrass is caespitose and possesses a dispersed panicle type seed head with two-flowered spikelets at the end of branches (SMART *et al.* 2004). One spikelet contains the fertile reproduction parts where seeds are formed, while the other spikelet holds the stamen. The lemma and palea are indurate and adhere tightly to the caryopsis. Conventional seeding and harvesting equipment can be used to easily integrate this grass into existing crop lands. (VOGEL 2002; LEWANDOWSKI *et al.* 2003). Soil tillage is only necessary in the first growing season, consequently there is a reduced risk of soil erosion (MA *et al.* 2000a). Traditionally the most important aspect is that switchgrass produces high latent biofuel returns leading the U.S. Department of Energy (DOE) to identify switchgrass as one of the main crops that could be dedicated to biofuel production (MCLAUGHLIN 1993; KSZOS 2005; VERMERRIS 2008).

Economic uses

Oil is the most important energy source traded globally, and is classified as a limited resource as there is a finite amount within the earth to be extracted for use (HAUGOM *et al.* 2016). Because scientists and policy makers are aware that oil will eventually become unavailable, and delivers negative environmental impacts, research has shifted to the use of other energy sources, like bioethanol, to supplement and eventually

replace the use of oil. The U.S. produces bioethanol mainly from corn grain, which will not be a sustainable long-term option as increasing demand for corn-based ethanol will have significant arable land requirements (SUN AND CHENG 2002). Increased use of corn for ethanol production will also result in higher corn prices needed for livestock feed, and could negatively affect exports of animal products (ELOBEID *et al.* 2007). Complementary biofuels are needed to supplement energy use and aid in clean burning alternatives that require less land and resource use. Switchgrass is currently planted on a moderate scale for hay, pasture, biofuel, and conservation practices as it has environmental benefits, and is adaptable in marginal croplands, needing very little management to thrive (VERMERRIS 2008; SANDERSON *et al.* 2012). Switchgrass can survive at a pH below 5 and above 7 when mature, and it can produce more than other warm-season grasses of the southern plains region under these conditions. Biomass can be processed into oxygenated and clean solids (bio-char, carbon fiber, bioplastics, and nanotubes), liquids (bioethanol, biodiesel, bio-oil, biobutanol, and biokerosene), and gases (biomethanol, biopropane, biosyngas, and biohydrogen) (DAGGETT *et al.* 2007; DEMIRBAS 2009; FILSON *et al.* 2011; RODDY 2012; JAIN *et al.* 2014; KIM *et al.* 2014a; KIM *et al.* 2014b; LEWIS *et al.* 2015; CHEN *et al.* 2016). In relation to biofuels, studies have considered the importance of high cellulose content for bioethanol production and low ash content for combustion systems (MCLAUGHLIN *et al.* 1999). Scientists continue to discover and develop new methods of utilizing switchgrass in the most efficient and effective ways.

Pathogens of switchgrass

Since the early 2000s several pathogens have been identified that are associated with switchgrass (Table 1). Among these pathogens are species of *Fusarium*. Members of the genus *Fusarium* are known for causing a variety of disease symptoms on various hosts, and are found globally (LESLIE AND SUMMERELL 2006). These fungi are becoming more understood through modern technological advances, but there is still much that is not known about them.

Fusarium

Classification

Link (1809) introduced the genus *Fusarium* as fungi having spindle or canoe shaped conidia*"* (LESLIE *et al.* 2008). Over 1,000 species have been identified since that time based on morphological taxonomy. Various efforts have been made to improve the way in which *Fusarium* species are classified. The Wollenweber and Reinking classification system (1935) contained 16 sections encompassing 65 species. Snyder and Hansen compiled *Fusarium* into nine species (1940). The Snyder and Hansen system was replaced by the Nelson *et al.* (1983b) system, in which the only species that remained unchanged from Snyder and Hansen's (1940) classification scheme were *F. oxysporum* and *F. solani* (Martius) Appel & Wollenweber emend. Snyder and Hansen. In 2006, Leslie and Summerell compiled 70 species of *Fusarium*; this was the first classification scheme for the genus to combine morphological and phylogenetic information.

Classical taxonomy of species in the genus *Fusarium* is based on the structure and abundance of asexual reproductive structures (i.e. chlamydospores, microconidia,

macroconidia, and phialides) and on cultural characteristics (colony texture, pigmentation of mycelial mass, pigmentation of media, and cultural aroma) (BOOTH 1971; NELSON *et al.* 1983b; FU *et al.* 1991; GORDON AND MARTYN 1997; EDEL *et al.* 2000; LORENS *et al.* 2006). Not all spore types are created by all species, and only 20% of *Fusarium* species have a known sexual cycle (MA *et al.* 2013). DNA sequencing technology has become a standard method for confirmation of morphological identification (TAYLOR *et al.* 2000; SITES AND MARSHALL 2004). Further complicating classification is that definitive morphology of *Fusarium* has seldom been studied *in vivo,* and the descriptions and illustrations now widely used are based almost entirely on *in vitro* observations. Many authors have concluded that species in this genus exhibit differing traits based on the media in which they are grown indicating probable differences between wild types and lab grown cultures (BOOTH 1971; NELSON *et al.* 1983a; LESLIE *et al.* 2008). Further complications for classification arise as these fungi exist as various groups of endophytes (LESLIE *et al.* 1990), saprophytes (FRACCHIA *et al.* 2000) and plant pathogens (CHANDRA *et al*. 2008a, 2008b)

Plant pathogens

Windels (2000) estimated that *Fusarium* species are responsible for billions of dollars in losses every year due to lower crop yields and wastes from mycotoxin contamination. The American Phytopathological Society (www.apsnet.org/online/common/search.asp) has listed over 83 of 101 economically important plants that are susceptible to at least one *Fusarium*- associated disease (WULFF *et al.* 2011). Possible disease symptoms caused by members of *Fusarium* include root or stem rots, cankers, wilts, fruit or seed rots, and leaf diseases. These fungi are difficult to identify due to their pleomorphic

tendency and presence of both homothallic and heterothallic strains in the same species (BOOTH 1971). Fusaria are difficult to identify to species because of variation among isolates (SNYDER *et al.* 1940; GAUDET *et al.* 1989). Management of *Fusarium* diseases is difficult as they are both seedborne and soilborne and many strains have been reported as etiologic instruments of infections in humans (RICHARDSON AND LASS-FLORL 2008).

Human and animal health concerns

Fusarium species are common contaminants of cereal crops and other human food sources worldwide (GELDERBLOM *et al.* 1988; HARRISON *et al.* 1990; ROTTER *et al.* 1991a; TOLLESON *et al.* 1996; HAGLER *et al.* 2001; BOTTALICO AND PERRONE 2002; SNIJDERS 2004; SCHOLLENBERGER *et al.* 2005a; LESLIE *et al.* 2008; MARIN *et al.* 2012). *Fusarium* species produce over 20 harmful mycotoxins, such as beauvericin, fumonisin, zearalenone, and the most dominant mycotoxin, deoxynivalenol (DON), that affect the health of animals and humans causing allergic reactions, infections, and in severe cases of immune depressed patients, death (TRENHOLM *et al.* 1989; ROTTER *et al.* 1991b; KWON-CHUNG AND BENNETT 1992; STERNBERG 1994; MCGINNIS *et al.* 1999; PONTON *et al.* 2000; BENNETT AND KLICH 2003; LESLIE *et al.* 2008; RICHARDSON AND LASS-FLORL 2008; MARIN *et al.* 2012). Numerous species of *Fusarium* have been described as the cause of human keratomycosis, mycetoma, and onychomycosis (WINDELS 2000; RICHARDSON AND LASS-FLORL 2008; GURLAR *et al.* 2009). The term mycotoxin was devised in 1962 in London, England, when approximately 100,000 turkey poults died (BLOUT 1961; FORGACS 1962). Since their discovery, mycotoxins have been researched, with the focus on those that severely impact the health of plants, humans, and animals.

Pathogenic *Fusarium* species are categorized as plant pathogens, and both primary and secondary forms of vertebrate pathogens (SWEENEY AND DOBSON 1998). Primary pathogens affect individuals with healthy immune systems, while secondary or opportunistic pathogens take advantage of immuno-compromised systems, especially those with AIDS, and those recovering from chemotherapy and surgeries (BENNETT AND KLICH 2003). Fungal growth on vertebrates are classified as mycoses, while dietary, respiratory, dermal, and other exposures to toxic fungal metabolites produce mycotoxicoses (BENNETT AND KLICH 2003). Most mycotoxicoses result from eating contaminated foods, skin contact by infested substrates, and inhalation of fungal spores (SWEENEY AND DOBSON 1998; BENNETT AND KLICH 2003). Mycotoxins may cause a decrease in food consumption, lowered milk production, and a decrease in body weight gain at low levels of exposure (SWEENEY AND DOBSON 2000). Impaired resistance to infections, increased predisposition to stress, and reduced fertility are common at moderate levels of exposure, and high levels of contamination may produce disease, liver and kidney damage, edema, severe blood clotting and hemorrhaging, and altered digestion (SWEENEY AND DOBSON 1998; FINK-GREMMELS 1999; ZAIN 2011; NICHEA *et al.* 2015). Many countries have instituted maximum allowable levels of mycotoxins in cereal products to protect human and animal welfare (ADAMS 1921; PUGH *et al.* 1933; SNYDER AND NASH 1968; SEAMAN 1982; SUTTON 1982; WANG *et al.* 1982; HART *et al.* 1984; SNIJDERS 1990; WINDELS 2000; ROSSI *et al.* 2001; HORBERG 2002; LANGEVIN *et al.* 2004; PAUL *et al.* 2004; PESTKA *et al.* 2004; SCHOLLENBERGER *et al.* 2005b; CULLER *et al.* 2007; DEL PONTE *et al.* 2007; ZHANG *et al.* 2009; NISHIO *et al.* 2010). Kuiper-Goodman (1998), a prominent researcher in risk assessment, positions mycotoxins higher than synthetic

contaminants, plant toxins, food additives, or pesticide residues in causing chronic dietary risks. The seriousness of these diseases is there are few, if any, ways to treat severe mycotoxin exposure. Fink-Gremmels (1999) described specific methods for management of mycotoxicoses in veterinary medicine, with evidence that selected strains of *Lactobacillus* effectively bind dietary mycotoxins (EL-NEZAMI *et al.* 1998; EL-NEZAMI 2002). Because of the illnesses they can cause, mycotoxins T-2 and diacetoxyscirpenol (DAS) are on the USDA/CDC select agent list and require special permits for study in the U.S. (www.cdc.gov/od/sap/dpcs/saist/pdf). Species complexes of *Fusarium* that produce mycotoxins include *Fusarium oxysporum*, *F. graminearum*, *F. solani*, *F. poae,* and *F. verticillioides* (HERRON *et al.* 2015). The total number of people affected is thought to be less than the number of people affected by bacterial, protozoan, and viral infections, however, fungal diseases are still considered a serious international health problem (BENNETT AND KLICH 2003). Understanding the biology of the organism causing the disease is key to finding treatments and preventing exposure. The following *Fusarium* species were chosen for this study because of their known and potential impacts on human health and damage to many economically important crops including switchgrass.

Fusarium equiseti

Fusarium equiseti is found globally as a soil saprophyte on rotting fruit and other decaying plant matter (BOOTH 1971; MARASAS *et al.* 1984; LESLIE AND SUMMERELL 2006). It serves as a weak pathogen and secondary invader of a wide range of crops and belongs to the *Fusarium incarnatum — F. equiseti* species complex, which contains over 28 strains of two species *F. equiseti* and *F. incarnatum — F. semitectum — F.*

pallidoroseum (O'DONNELL *et al*. 2009*)*. Trichothecenes are known to be produced by over 24 different *Fusarium* species including *F. equiseti* (MARASAS *et al.* 1984; LOGRIECO *et al.* 1992; KOSIAK *et al.* 2005a; ADEJUMO *et al.* 2007). Trichothecenes are a group of mycotoxins that are highly toxic to both plants and animals as they are inhibitors of protein synthesis in eukaryotic cells by interfering with peptidyl transferase activity (MCLAUGHLIN *et al.* 1977; DESJARDINS *et al.* 1993; PRELUSKY 1994; PRELUSKY *et al.* 1994; BROWN *et al.* 2001). Dietary exposure to trichothecenes can increase susceptibility of a host to other microbial infections (PESTKA AND BONDY 1994). These mycotoxins are reported to cause acute to severe diseases based on a variety of factors including type of mycotoxin ingested, the amount ingested, frequency of ingestion, individual's size, fat content, hydration level, and age among other variables (TRENHOLM *et al.* 1989). Several studies indicate intraspecific diversity among *F. equiseti* isolates (KRISTENSEN *et al.* 2005; JURADO *et al.* 2006a). Kosiak *et al.* (2005) reported two distinct groups, with morphological differences and variation in toxin production, from strains from Northern Europe. An introductory phylogenetic analysis on *F. equiseti* strains from Southern and Northern Europe also showed two distinct groupings, but toxigenic profiles were not analyzed in the study (JURADO *et al.* 2006a). There are currently no phylogenetic studies comparing North American strains of *F. equiseti*. Morphology of this species is difficult, as there are two distinct phenotypes. One type has macroconidia with a very long apical cell, while the other has a shorter less tapered apical cell. Mycelia range from white to yellow to peach in color, while sporodochia are cream to bright orange. This *Fusarium* species has not been reported produce polyphialides. Chemical and molecular approaches have all but replaced classical taxonomy when identifying this species as

technologies have advanced (NIRENBERG AND O'DONNELL 1998; O'DONNELL *et al.* 1998a).

Fusarium graminearum

Fusarium graminearum was first described by Petch (1936) from its teleomorph stage *Gibberella zeae*. It is the primary member of the *F. graminearum* complex, a grouping of fusaria that includes at least 20 *Fusarium* species that cause severe economic damage in wheat and cereal crops. It is the most common *Fusarium* species found in nature (CANCER 1993; BAI AND SHANER 1994; PARRY *et al.* 1995; WISNIEWSKA *et al.* 2011). Disease caused by *F. graminearum* is considerably linked with temperature and humidity (PUGH *et al.* 1933; ROSSI *et al.* 2001; EDWARDS 2004; LOGRIECO AND VISCONTI 2004). Studies show that *F. graminearum* is the predominant pathogen causing disease, specifically *Fusarium* head blight (FHB) of wheat, under warmer, humid conditions in China, Canada, Australia, Central Europe and the U.S. *Fusarium culmorum*, *F. avenaceum*, *F. sporotrichioides* and *F. langsethiae* are found more frequently in cool and humid settings, and *F. poae* is found in warmer and drier areas (KIECANA AND PERKOWSKI 1998; ROSSI *et al.* 2001; XU AND NICHOLSON 2009). *Fusarium graminearum* is responsible for disease symptoms on a variety of hosts in including corn (SUTTON 1982), soybean (AGARWAL 1976), switchgrass (COLLINS 2016), and wheat (XUE *et al*. 2004). The pathogen is dispersed primarily by conidia that are carried by wind or splashed by water to new establishment sites (PAUL *et al.* 2004; XU AND NICHOLSON 2009). It has been shown to colonize debris of harvested wheat, corn and other rotation crops (HOFFER *et al.* 1918; ADAMS 1921; SNYDER AND NASH 1968; SEAMAN

1982; SUTTON 1982; COOK 1984; WICKLOW *et al.* 1987; ZHU AND FAN 1989; FERNANDEZ AND FERNANDEZ 1990; MILLER *et al.* 1998).

Most of the mycotoxin studies are focused on the species that compose the FHB complex. Deoxynivalenol, nivalenol, zearalenone, T-2, and HT-2 mycotoxins are just a few products produced by *F. graminearum* (MARASAS *et al.* 1984; ROTTER *et al.* 1991a; BROWN *et al.* 2001; LOGRIECO *et al.* 2002). These toxins are translocated within the wheat head by the xylem and phloem and build up in cells. Deoxynivalenol may cause vomiting, diarrhea, fever, abortion, and other symptoms of severe poisoning in humans and animals (UENO 1977). It has been associated with anemia, immuno-suppression, and cancer (PRELUSKY 1994; PESTKA *et al.* 2004; SCHOLLENBERGER *et al.* 2005b). Many countries have developed health and safety plans that allow a maximum level of DON in cereal and cereal products to prevent human and animal mycotoxicoses (ARTHUR 1891; HOFFER *et al.* 1918; ADAMS 1921; PUGH *et al.* 1933; SNYDER AND NASH 1968; WARREN AND KOMMEDAHL 1973; SEAMAN 1982; SUTTON 1982; WANG *et al.* 1982; HART *et al.* 1984; ZHU AND FAN 1989; MATTHIES AND BUCHENAUER 2000; WINDELS 2000; LU *et al.* 2001; ROSSI *et al.* 2001; HORBERG 2002; PAUL *et al.* 2004; DEL PONTE *et al.* 2007; PAUL *et al.* 2007; BUERSTMAYR *et al.* 2009).

Fusarium graminearum has an optimal growth range of between 24˚C to 30˚C (WHEELER *et al.* 1991). *Gibberella zeae*, the sexual stage of *F. graminearum*, develops fruiting bodies on the mycelium and form ascospores, while macroconidia are formed asexually from the mycelia. *Fusarium graminearum* has been divided into at least 13 different phylogenetic species (O'Donnell *et al.* 2004, 2008*b*; Starkey *et al.* 2007; Yli-Mattila *et al.* 2009). The number of genes used to divide the 13 species is sizable,

however there is not a specific gene that can distinguish all species from one another (Yli-Mattila *et al.* 2009). It is important that DNA sequences be considered as a collection to more accurately divide species based on genetic characterization. This fungus is an important pathogen of switchgrass that can cause leaf spotting, stem streaking, seedling blight, root rot, stunting, and biomass reduction (COLLINS 2016).

Fusarium oxysporum

Fusarium oxysporum Schlechtendahl emend. Snyder and Hansen is one of the most economically important members of the genus *Fusarium*. This species is found globally and contains strains that are pathogenic and nonpathogenic (GORDON AND MARTYN 1997). It is part of a complex that contains numerous genetic lineages. (ARMSTRONG AND ARMSTRONG 1948; WILSON 1995; KISTLER 1997; O'DONNELL AND CIGELNIK 1997; BAAYEN *et. al.* 2000; O'DONNELL *et al*. 2000; O'DONNELL *et al.* 2008a). Over 150 host-specific forms of *F. oxysporum*, known as *formae speciales*, have been described to date, each with its own specific host or group of hosts (ARMSTRONG 1981; HAWKSWORTH *et al.* 1995; O'DONNELL *et al.* 1998b; O'DONNELL AND CIGELNIK 1999; BAAYEN *et al.* 2000; O'DONNELL *et al.* 2009; FOURIE *et al.* 2011; GHAG *et al.* 2015). The basis of host specificity in *F. oxysporum* is currently unknown (BAAYEN *et al.* 2000), therefore, individuals in a given *formae speciales* cannot be presumed closely related or evolved from a common ancestor (O'DONNELL *et al.* 1998b). The majority of the *formae speciales* designations for this species are pathologically useful, but not phylogenetically useful (GORDON AND MARTYN 1997). Available evidence suggests that monophyletic *formae speciales* are the exceptions among *F. oxysporum* (LESLIE AND SUMMERELL 2006) as most contain two or more races.

Fusarium oxysporum f. sp. *cubense* (Foc) is one of the most familiar and damaging *Fusarium* species. Responsible for Panama disease of banana in the 1960s, Foc almost ended banana cultivation (PLOETZ 1990). Over 50,000 hectares of exotic 'Gros Michel' (AAA) plantations were affected (PLOETZ AND PEGG 2000). A genetically resistant cultivar of banana, called Cavendish, was developed to replace cultivars that were susceptible to the disease (DALE *et al.* 2017). Panama disease is still a threat today as new strains of *F. oxysporum* are developing that have adapted to overcome the 'Cavendish' resistance (DALE *et al.* 2017; MOLINA *et al.* 2009; MOLINA *et al.* 2010; GHAG *et al.* 2015; AGUAYO *et al*. 2017).

Fusarium oxysporum has non-septate microconidia formed on short monophialides, 3-septate macroconidia formed from monophialides and on branched conidiophores in sporodochia, as well as chlamydospores with smooth to rough walls that are formed singly or in pairs. Chlamydospores are kidney-shaped resting structures that stay viable in soil for more than 30 years and germinate in the presence of host roots (PLOETZ 2006). No known teleomorph of this species exists as sexual fruiting bodies have never been observed (TAYLOR *et al*. 1999). For reproduction to occur, two mating type (MAT) idiomorphic alleles, specifically MAT-1 and MAT- 2, must be present (METZENBURG AND GLASS 1990; KRONSTAD AND STABEN 1997; ARIE *et al.* 2000). These idiomorphic alleles were found to have high sequence similarity to sexually reproducing *Fusarium* species, such as *Gibberella zeae*, the sexual form of *F. graminearum*, and were functionally expressed based on polymerase chain reaction (PCR) (YUN *et al.* 2000; ARIE *et al.* 2000.). In the case of *Foc*, the causal agent of Panama disease in banana, phylogenetic

studies have supported the possibility of sexual recombination (BENTLY AND BASSAM 1996; GROENWALD *et al*. 2006; KOENIG *et al.* 1997; O'DONNELL *et al.* 1998).

Although isolates of *F. oxysporum* found on switchgrass thus far have not been confirmed as pathogenic, a possibility of mutation, could select for a pathogenic strain based on cases seen in other *formae speciales* of *F. oxysporum*. Nonpathogenic strains of *F. oxysporum* are widespread and genetically more diverse than their pathogenic complements (GORDON AND OKAMOTO 1992); however, they are not as well studied (EDEL *et al.* 2001). After the discovery that nonpathogenic strains play a role in *Fusarium* wilt suppressive soils (ALABOUVETTE 1990), interest in these strains increased considerably (ELIAS *et al.* 1991; KATAN *et al.* 1994).

Fusarium oxysporum isolates can only be differentiated from one another by pathogenicity traits, which is difficult due to the host specificity of *F. oxysporum*. Classification through pathogenicity can be problematic because pathogenicity tests can be influenced by temperature, host age, method of inoculation, and other variables. Tests conducted in different laboratories may produce contrasting results (CORRELL 1991; DAVIS *et al.* 1996). Field testing is time-consuming, expensive, and test locations may not be obtainable for the required interval of time. Large numbers of host species may need to be tested to confirm that the isolate is truly nonpathogenic (ALABOUVETTE *et al.* 2009). *Fusarium oxysporum* was chosen for this study because of this prospective, as well as a comparison to known *Fusarium* pathogens of switchgrass to potentially find a genetic difference among pathogenic versus nonpathogenic strains.

Fusarium sporotrichioides

Fusarium sporotrichioides was first discovered by Link in 1809. Researcher conflicts arise, as to where to place this species, because of variations in morphology among isolates. It is generally placed in the same classification as *Sporotrichiella* section alongside *F. poae* and *F. tricinctum* (WOLLENWEBER AND REINKING 1935; VISCONTI *et al.* 1985). It has also been placed among *Arthrosporiella* section based on morphological characteristics of conidia. This species is responsible for significant economic losses to head blight in wheat (TEKAUZ *et al.* 2009), ear rot in corn, dry rot of potato (KOTAN *et al.* 2009), leaf necrosis of cornelian cherry dogwood (COLLINS *et al.* unpublished data), and spoilage of a variety of improperly stored fruits and vegetables as part of the *F. graminearum* complex (ALABOUVETTE 1990). *Fusarium sporotrichioides* produces type A trichothecenes, scirpentrol (STO), T-2 triol, T-2 tetraol, and diacetoxyscirpenol (DAS) (VISCONTI *et al.* 1985; CHELKOWSKI *et al.* 1989; BROWN *et al.* 2001; MATEO *et al.* 2002; MOSS 2002; PERKOWSKI *et al.* 2003; MOSS AND THRANE 2004; THRANE *et al.* 2004; PERKOWSKI *et al.* 2007), which inhibit protein synthesis (WISNIEWSKA *et al.* 2011). *Fusarium sporotrichioides* and *F. graminearum* are known to produce T-2 toxin, which exhibits phytotoxic, zootoxic, and antibiotic properties making it the most toxic *Fusarium* compound (MARASAS *et al.* 1969; JOFFE 1974; UENO 1977; BOTTALICO *et al.* 1980; BOTTALICO AND LERARJO 1983; VISCONTI *et al.* 1985). Additional, the mycotoxin butanolide, which causes mitochondrial damage in animals and chlorophyll retention in plants, and moniliformin, which causes breakdown of carbohydrates as it inhibits the citric acid cycle are produced (BOTTALICO *et al.* 1980). When *F. sporotrichioides* meets human and animal tissues it is known to cause cutaneous ulceration (KANO *et al.* 2011).

T-2 toxin has been reported to cause cases of poisoning in domestic animals, causing changes in leukocyte counts, weight loss, vomiting, depression, antibody formation, and diarrhea (PASCALE *et al.* 2003). This pathogen was common in the Soviet Union during World War II infecting wheat and millet that caused severe Alimentary toxic Aleukia outbreaks (DESJARDINS 2006; YLI-MATTILA AND GAGKAEVA 2010).

Fusarium sporotrichioides has an optimum growth temperature of between 22.5°C and 27.5°C, a maximum of 35°C and a reported minimum of 22°C (SWEENEY AND DOBSON 1998). It is composed of pink-white mycelia that brown with age. Pink-red pigmentation is produced in potato dextrose agar, and coloration occurs only at the medium surface. Aerial mycelia are floccose forming many pale-to-bright orange sporodochia and polyphialides. Microconidia are oval to pyriform, non-septate, hyaline, and have smooth, thin walls. Microconidia measure 10-15 µm by 2-5 µm. Macroconidia are sickle-shaped, and 3 to 5 septate, moderately curved to straight, hyaline, thick walled, and 40-50 µm in length (KANO *et al.* 2011). *Fusarium sporotrichioides* is difficult to distinguish from *F. armeniacum* as they have significantly similar morphology, differing in the presence or absence of microconidia, and abundance of chlamydospores. When genetic testing is completed they also have identical ITS genetic sequences when comparing species in nucleotide databases.

Plant Pathogen Characterization Techniques

Traditional taxonomy

Virulence and aggressiveness are the standard attributes most frequently used for morphological studies of plant pathogens. They provide a clear picture of race diversity

and distribution. However, these traits do not allow the evolutionary relationships among pathotypes to be distinguished (SCHAAD *et al.* 2003; LOPEZ-DIAZ *et al.* 2017). Morphological character tests can be time consuming and costly. These markers are deemed unreliable, as they are unpredictable, inconstant among individuals, and vary with the environment (O'DONNELL *et al.* 1998b; STEENKAMP *et al.* 2002; MARIN *et al.* 2012). Wild type fungal cultures may degenerate with sub-culture repetition to present pionnotal or mycelial cultures further complicating identification. The frequency of degeneration of *Fusarium* species varies among, and within, species (SNYDER *et al.* 1940; OSWALD 1949; SNYDER AND HANSEN 1954; SNYDER AND TOUSSOUN 1965; TOUSSOUN AND NELSON 1975; PUHALLA 1981; GAUDET *et al.* 1989). Proteins and isozymes have been studied as indicators to relieve some of the time and cost of species characterization (HAMPTON *et al.* 1990; PRIESTLEY *et al.* 1992). These molecular enzymes forms have the same catalytic function, and are used in plant pathology for fingerprinting, genetic diversity, and taxonomy, including variants with unique net charges that are separated by their differential motilities in an electric field (ROTHE 1994). However, due to low levels of polymorphisms and an overall restriction to a specific development stage, they have been reported to have limited use in plant pathology genetic research (HAMRICK 1989; PRIESTLEY *et al.* 1992).

Genetic characterization

In general, genetic markers provide a diagnostic tool that allows direct identification of pathotypes in any developmental stage independent of the original environment and allelic variation at a given locus (POWELL *et al.* 1996; SCHLÖTTERER 2004). Allozyme markers measure variations in the electrophoretic movement of proteins that the DNA

encodes but was not considered sensitive enough. However, DNA-based markers allow the number of mutations of different alleles to be quantified (SCHLÖTTERER 2004). Over the last 20 years, use of genetic markers as genetic mapping tools, has increased significantly across many different scientific studies. A DNA marker is generally classified into one of two groups. Type I markers are visualized by hybridization of the restriction enzyme-digested DNA to a known probe. Random Amplified Length Polymorphisms (RFLP) is considered a Type I marker. Type II markers are associated with anonymous genomic sequences and involve amplification of a certain loci with a specific primer sequence. Random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphism (AFLPs), internal transcribed spacer regions (ITS), and single nucleotide polymorphisms (SNP) are all Type II markers (BENALI *et al.* 2011). Microsatellite loci are widely dispersed in eukaryotic genomes, and are often highly polymorphic (occurring in distinctive forms) due to variation in the number of repeat units (BRUFORD 1993). Polymerase chain reaction primers are designed to recognize flanking sequences. Species identified using phylogenetic methods are generally difficult or impossible to identify using conventional morphological traits (AOKI *et al.* 2003). Caution should be taken when pinpointing a species as identification based on only a single species classification concept relying on only phenotype or only genotype leaves room for identification error.

Molecular techniques for *Fusarium* identification

Genetic analyses that utilize DNA sequence data have significantly contributed to our understanding of the systematics of *Fusarium* and other fungi. Species differentiations are made possible that are critical for understanding inter- and intraspecific relationships

with respect to toxin profiles (MIRETE *et al.* 2004; KRISTENSEN *et al.* 2005; JURADO *et al.* 2006a; O'Donnell *et al.* 2009) and resistance genes. Moreover, these techniques have provided the basis for developing prompt, specific, and accurate diagnostic procedures based on PCR that can be used to predict mycotoxin risk, and provide the information required for early control strategies (KNUTSEN *et al.* 2004; KONSTANTINOVA AND YLI-MATTILA 2004; JURADO *et al.* 2006b). Molecular data has been used throughout *Fusarium* to identify groups in combination with phenotypic, distinctive, characteristics (MARASSAS *et al*. 2001; ZELLER *et al.* 2003). *Fusarium* oxysporum genome sequences have been studied using intron regions of histone coding genes, the b-tubulin gene, the calmodulin gene (O'DONNELL *et al.* 2009; STEENKAMP *et al.* 2002, WULFF *et al.* 2010), ITS region (O'DONNELL AND CIGELNIK 1997), the intergenic spacer region (IGS) (YIL-MATTILIA AND GAGKAEVA 2010) and the translation elongation factor gene EF-1a (O'DONNELL *et al.* 1998b; O'DONNELL *et al.* 2000). ITS does not work well to differentiate between many *Fusarium* species, such as *F. sporotrichioides* and *F. armeniacum*, because the resultant sequences are nonspecific (O'DONNELL *et al.* 2000). The EF-1a gene has been used as a standard for a single-locus identification tool and is a suitable marker for differentiation among *Fusarium* species (GEISER *et al.* 2004). PCR-based assays are used to diagnose and characterize *Fusarium* (DOOHAN 1998; O'DONNELL *et al.* 2000) and provide valuable insights, however more information is needed to develop a complete understanding of phylogenetic relationships within the genus *Fusarium*.

Microsatellite Loci

Microsatellite loci in eukaryotic genomes, also known as simple sequence repeats (SSRs), have been documented since the 1970s, although the substantial number and

almost ubiquitous distribution of such sequences throughout eukaryotes was first highlighted by HAMADA (1982). Microsatellite loci have become a common tool to determine genetic relatedness among fungal species (BRIDGE *et al.* 2003; YOUNG *et al.* 2009; TSUI 2012).

Current uses

Introduction of PCR technology (MULLIS AND FALOONA 1987; KARY 1990) and its common use in research laboratories has increased over the last 30 years allowing for the use of microsatellite loci to be transformed into a highly flexible genetic marker (WEBER AND MAY 1989; LITT AND LUTY 1989; TAUTZ 1989). Microsatellites have proven to be valuable in forensic cases, (HAGELBERG *et al.* 1991; HERBER AND HEROLD 1998; SACCHETTI *et al.* 1999; JAN AND FUMAGALLI 2016) analysis of museum specimens, (ELLEGREN 1991; PEACOCK *et al*. 2017), linkage analysis (GEORGES *et al.* 1993; HAUYU *et al*. 2016), and evolutionary studies (BOWCOCK *et al.* 1994; HATMAKER 2016) and are considered the common marker for parentage and relatedness testing in humans and other species (ELLEGREN 1991; HAGELBERG *et al.* 1991; DIXON *et al.* 1992; BILL AMOS 1993; HUGHES AND QUELLER 1993; LAGERCRANTZ *et al.* 1993; TAYLOR *et al.* 1994; ROBERTS *et al.* 1999; CHAMBERS AND MACAVOY 2000; HATMAKER 2016).

Research Objectives and Justification

Research described in this study has the following objectives:

Objective 1: Characterize and confirm identification of *Fusarium* species isolated from commercial switchgrass seed using morphological and molecular methods.

Justification: Identification of seedborne switchgrass pathogens is essential for

prevention and treatment of infected seed. To design reliable species-specific microsatellite loci, each individual isolate must be correctly identified.

Objective 2: Identify, design and characterize microsatellite loci for *F. equiseti, F. graminearum, F. oxysporum*, and *F. sporotrichioides*. *Justification*: Design of microsatellite loci starts with a baseline genome sequence, which is used in biostatistical analysis software to map sections of the organism's chromosomes and create scaffolds from which microsatellite loci sequences can be selected.

Objective 3: Develop microsatellite loci to test against extracted DNA from individual isolates of each *Fusarium* species and to test for cross amplification of other *Fusarium* species. *Justification*: Knowledge of species relatedness at a molecular level will aid in identifying alleles involved in virulence and pathogenicity. Species-specific microsatellite loci developed in this study will offer rapid detection of specific *Fusarium* species with PCR. Prompt identification is necessary to control fungal pathogens.

Research Definitions

Biofuel: energy derived directly from living matter

Biomass: non-fossilized material from photosynthetic organisms adapted as a renewable energy source

Caespitose: grows in small dense clumps

Caryopsis: the true seed

Ecotype: a definite line of plant or animal occupying a specific habitat

Floret: one of the small flowers making up a multipart flower head

Gel electrophoresis: method for separation and analysis of macromolecules (DNA, RNA and proteins) based on size and charge

Lemma: a phytomorphological term in botany indicating a part of the spikelet of grasses (*Poaceae*); the lowermost of two chaff-like bracts enclosing the grass floret

Microsatellite: di-, tri-, or tetra nucleotide tandem repeats in DNA sequences; the number of repeats is different in alleles of an individual and among populations of DNA; also known as simple sequence repeats (SSRs) or primers.

Mycelial cultures: are classified as having abundant mycelium and lack sporodochia

Mycotoxicoses: animal diseases caused by mycotoxins in the respiratory, digestive, circulatory, and other internal systems of vertebrates

Mycotoxicology: is the study of mycotoxins

Mycotoxins: naturally occurring compounds or secondary metabolites produced by filamentous fungi growing on plants in the field or during storage

Nanotube: tube-like structure made from sheets of carbon, called graphene, and used to conduct and hold electric currents

Panicle: loose, branching cluster of flowers

Pathogenicity: an organism's (microbe's) ability to cause disease

Pathotype: disease-causing variant of a microorganism distinct from other members of its species by its virulence level and molecular markers

Phialides: dilated top part of a conidiophore; projection from a hypha

Phytomorphological: plant characteristic

Phytotoxins: fungal products that are toxic to plants

Pionnotal cultures: classified as having abundant sporodochia and a lack of mycelium

Pleomorphy: fungi grown in different media have different forms

Polymerase chain reaction (PCR): a molecular technique used in biological fields to amplify copies of a selected segment of DNA, generating thousands to millions of copies of the DNA sequence

Polymorphic: occurring in several distinctive forms, specifically regarding species or genetic variation

Race: individual classification using pathogenicity regarding host cultivars

Spikelet: basic unit of a grass flower, consisting of two outer bracts (glumes) at the base and one or more florets above

Virulence: signifies the degree of damage caused by a microbe to its host

Chapter 2 Identification, characterization, and genetic comparison of *Fusarium* spp. isolated from switchgrass (*Panicum virgatum* L.)

A. Vu, O.L. Fajolu, M.M. Dee, and S. Collins developed fungal collections, and initial morphological identification of *Fusarium* species and determination of pathogenicity. D. Hensley, R. Trigiano, D. Hadziabdic, K. D. Gwinn, M. Staton, and B. H. Ownley provided research expertise for the study, and it was funded by the United States Department of Agriculture. I confirmed fungal identifications using morphological characters, and genomic sequences, and performed genetic characterization of isolates with newly developed microsatellite loci. I determined levels of genetic diversity and polymorphisms between microsatellite loci developed from *F. equiseti, F. graminearum*, *F. oxysporum*, and *F. sporotrichioides*.

Abstract

Fusarium species are soil- and seedborne fungi that are known to be pathogens of a variety of plant hosts globally. In previous studies, several *Fusarium* species were isolated from 30 commercial switchgrass seed lots. A total of 172 *Fusarium* isolates were identified to species using phenotypic and molecular characteristics. Pathogenic isolates of *F. acuminatum, F. armeniacum, F. equiseti, F. graminearum, F. sporotrichioides,* and *F. tricinctum,* as well as a nonpathogenic *F. oxysporum* were identified. From this collection, genomic DNA was sequenced from a single pathogenic isolate of four species (*F. equiseti, F. graminearum, F. sporotrichioides*, and *F. oxysporum*) to develop microsatellite loci. A total of 150 microsatellites were designed and tested for amplification of DNA from the derived species and across the other three

Fusarium species. Thirty primers were designed from each microsatellite genome 15 dinucleotide repeats and 15 trinucleotide repeats, for each species. An additional 30 loci were designed for *F. graminearum* after preliminary tests indicated that only 40% of the first 30 designed microsatellites amplified across 30 individuals. Microsatellite loci were screened for amplification against two DNA samples from the species from which they were derived, i.e. *F. equiseti* primers were tested against DNA samples from two *F. equiseti* isolates. If amplification was observed, the primers were tested against DNA from the collected isolates of that species. Base pair lengths were compared across individuals of each species and primers that resulted in >3 bp difference were tested for cross amplification of DNA to other *Fusarium* species. Although 111 primers amplified DNA of the species from which they were derived, only 33 met the base pair length and quality requirements to be tested for cross amplification. The number of isolates in the collection was too small for a robust analysis of the cross-transfer data (*Fusarium graminearum* n = 30, *Fusarium oxysporum* n = 24*, F. sporotrichioides* n = 14*, F. equiseti* n = 6). *Fusarium equiseti* was not tested in the cross-amplification study due to the small number of isolates available. Primers that did not amplify across all three species include *F. graminearum* derived primer Fg0057, which did not amplify against any of the 24 *F. oxysporum* isolates, and *F. oxysporum* derived primers Fo006 and Fo012 that did not amplify against 14 isolates of *F. sporotrichioides.*

Introduction

With the growth of the biofuel industry and other commercial uses of switchgrass derived products, it is important to identify and understand the characteristics of pathogens that impact switchgrass production. Several pathogens of switchgrass have

been described from the southeastern U.S. (Table 1). In 2008, Zale described *Puccinia emaculata* on switchgrass in Tennessee. Studies by Vu (2011), and Vu et al. (2011a, c, d; 2012; 2013a, b) identified several fungi associated with commercial switchgrass seed. Fajolu (2012) and Collins (2016) determined the virulence of several pathogenic fungal isolates from switchgrass. These included *Alternaria alternata*, *Bipolaris* spp., *Curvularia lunata* var. *aeria, Fusarium spp.,* and *Phoma herbarum*. *Bipolaris* and *Fusarium* species can cause significant biomass loss, reduced stand establishment, and changes in chemical components of switchgrass (FAJOLU 2012; COLLINS 2016).

Variability among plant pathogens is an important topic of research, as it directly affects the pathogen's ability to cause disease (ZABALOGEAZCOa 2008). The evolution of fungi is determined by five evolutionary forces: mutation, natural selection, genetic drift, gene flow, and reproductive means (MCDONALD AND LINDE 2002). These mutations may occur at any stage of growth and determine, virulence, host range, infectivity, and phenotypic characteristics (PARKER AND GREGORY 2004) Fusaria have a relatively short growth stage and can evolve quickly when exposed to new environmental conditions. Applying genetic techniques for identification has helped to confirm identification of fungal isolates across research and has provided information concerning species relatedness (SCHILLING *et al.* 1996; WILSON A *et al.* 2004; YERGEAU *et al.* 2005; JURADO *et al.* 2006b; LIEVENS *et al.* 2008; LIN *et al.* 2009). The phylogenetic species concept is considered by researchers to be the best tool for phyletic diversity and evolutionary relationships within *Fusarium* (NIXON AND WHEELER 1990; O'DONNELL AND CIGELNIK 1997). To a degree, limitations inflicted by morphological standards have been replaced using DNA sequences for phylogenetic analyses (TAYLOR *et al.* 1999; SITES AND

MARSHALL 2004). Many techniques have been widely used to classify *Fusarium* spp. at a genetic level. They are valuable because of their economic importance and value as biological research tools. These neutral genetic markers include random fragment length polymorphisms (RFLP); random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP), microsatellite loci or simple sequence repeats (SSR), genetic sequences for the internal transcribed spacer region (ITS), translation elongation factor 1-α (TEF), β-tubulin, and calmodulin, and single nucleotide polymorphisms (SNP) (DALLAS 1992; BRASIER 1997; KOENIG *et al.* 1997; O'DONNELL *et al.* 1998a; ALVES-SANTOS *et al.* 1999; O'DONNELL *et al.* 2000; LEES *et al.* 2006; WULFF *et al.* 2010; FOURIE *et al.* 2011; MCCARTNEY *et al*. 2003). Sequence data can be explored for insertions or deletions, and can be used directly for similarity-based DNA comparisons and/or phylogenetic analyses (FOURIE *et al.* 2011). This will give insight to what genes give the fungi the ability to be pathogens and may provide a way to combat the pathogen in the future.

Genes sequences for the ITS and TEF regions are commonly used to differentiate between *Fusarium* species. These sequences are highly conserved intra-specifically, but are variable among species (SCHOCH *et al*. 2012). Regions ITS1 and ITS2 are non-coding regions that lie between the 18S and 5.8S, and 5.8S and 28S coding regions, respectively. RNA polymerase I transcribes the whole rDNA unit as a single 45S molecule that can be amplified with PCR. The TEF gene is generally a single copy that encodes the translation elongation factor that controls the rate of protein synthesis (BALDAUF 1999). Automated sequencing is used to assemble sequences that can be

compared and matched with sequences in various available nucleotide databases to identify an unknown isolate.

Microsatellites, or simple sequence loci are tandemly repeated DNA sequence units of 2–6 bp (HAMILTON 1999; TAUTZ AND RENZ 1984; TAUTZ 1989; BRUFORD 1993; KELKAR *et al.* 2010; NAG AND MITRA 2017). Variability in length can be demonstrated with PCR and primers designed from the conserved flanking sequences, followed by vitalization as discrete and co-dominant alleles through electrophoretic analysis (TAUTZ 1989; WEBER AND MAY 1989; MATSUOKA 2002). The flanking regions are generally highly conserved, which allows for amplification with PCR primers.

The predominant mutation mechanism in microsatellite tracts is 'slipped-strand mispairing" (LEVINSON AND GUTMAN 1987; SCHLÖTTERER AND TAUTZ 1992; TAUTZ AND SCHLÖTTERER 1994; SCHLÖTTERER 2000b; SCHLÖTTERER 2000a). Tautz and Renz (1984) and Eisen (1999) described the process as a slipped-strand mispairing that occurs within a microsatellite array during DNA synthesis. It results in either the gain of repeat units if the newly synthesized DNA chain loops out, or the loss of repeat units if the template chain loops out. Depending on the sequences making up the array, and in part, whether the event occurs on the leading (continuous DNA synthesis) or lagging (discontinuous DNA synthesis) strand, determines whether the DNA will loop one of the two ways (FREUDENREICH *et al.* 1997). Microsatellites containing longer units evolve faster than those containing shorter units primarily due to inefficient repair of large mismatched sequence fragments. As mutations slowly add and subtract units in microsatellite arrays, alleles of different repeat numbers develop in the population (JIN *et al.* 1996; PETES *et al.* 1997; ESTOUP AND CORNUET 1999). Advantages to this technique

are the reproducibility of microsatellite loci across different laboratories (SAGHAI *et al.* 1994; AGARWAL *et al.* 2008). The mutation rate of microsatellite loci tends to increase as they get larger (WIERDL *et al.* 1997). Di-nucleotide repeats occur much more frequently than tri- or tetra-nucleotide repeats so considerations of these issues need to be accounted for in experimental design.

In this research, microsatellites were chosen based on their ability to show variations among closely related individuals. Microsatellites are considered useful because of their highly polymorphic nature and relative abundance in eukaryotic (WEBER 1990; TOTH *et al.* 2000; KATTI *et al.* 2001; SCHLÖTTERER 2004) and prokaryotic genomes (FIELD AND WILLS 1996; GUR-ARIE *et al.* 2000; Wang *et al.* 1994). Disadvantages are that microsatellites are prone to miscalling and dropped alleles. Miscalling is a result of error in sample loading, low fluorescence, other markers misloaded in the same lane, and human error in genotype calling. Errors can be caused by repeat expansion during PCR (CLARKE *et al.* 2001). Methods have been developed to reduce these errors (LINCOLN AND LANDER 1992; WAITS *et al.* 2001; SOBEL *et al.* 2002).

Microsatellites have been the topic of many articles and reviews (ELLEGREN 1991; TAUTZ AND SCHLÖTTERER 1994; JARNE AND LAGODA 1996; CHAMBERS AND MACAVOY 2000; SCHLÖTTERER 2000b; LI *et al.* 2002; DIERINGER AND SCHLÖTTERER 2003; BUSCHIAZZO AND GEMMELL 2006; CHISTIAKOV *et al.* 2006; OLIVEIRA *et al.* 2006; SELKOE AND TOONEN 2006; SUBIRANA AND MESSEGUER 2008; SUN *et al.* 2009; NAG AND MITRA 2017). Like SNPs, microsatellites can be used for identifying disease genes, clinical genetic testing, forensics, and more (NAG AND MITRA 2017).

Although there are many unique marker methods from which to choose, microsatellite markers were developed for this study to characterize genetic diversity, and relatedness within and among four species of *Fusarium* (*F. equiseti, F. graminearum, F. oxysporum,* and *F. sporotrichioides*) isolated from switchgrass seeds, seedlings, and field plants (Vu 2011; Collins 2016). To have confidence in isolate identification, this study incorporated both classical taxonomy and the phylogenetic species concept to confirm classification of these observed isolates.

This research has the following objectives: 1) Characterize and identify *Fusarium* species isolated from switchgrass using classical morphology and molecular methods; 2) Extract genomic DNA from four *Fusarium* species for whole genome sequencing for use in primer design by creating a baseline genome sequence that is used in biostatistical analysis software to map sections of the organism's chromosomes and develop microsatellite loci; 3) Develop primers to test against extracted DNA from individual isolates of each *Fusarium* species to test for amplification and polymorphisms within and among species: and 4) Test microsatellite loci for amplification against several isolates of the species from which they were derived, and for cross amplification against other *Fusarium* species. Development of species exclusive primers may offer a prompt identification method for these switchgrass pathogens.

Materials and Methods

Culture collection

Fusarium isolates used in this study are from a collection maintained in the laboratory of B. H. Ownley at the University of Tennessee, Knoxville. Development and characterization of this collection has been described by Vu (2011) and Collins (2016).

Phenotypic characterization

Fungal isolates were removed from 4°C storage and cultured onto potato dextrose agar (PDA) amended with 3.45 mg fenpropathrin/liter (Danitol 2.4 EC, Valent Chemical, Walnut Creek, CA) and 10 mg/liter rifampicin (Sigma-Aldrich, St. Louis, MO) to reduce bacterial and mite contamination before transferring to unamended medium. To observe morphological characters of a pure isolate, fungi were transferred to PDA, No. 4 Sythetischer narhrstoffarmer agar and carnation leaf agar (Leslie and Summerell, 2006).

Molecular characterization

DNA was extracted from isolates grown in potato dextrose broth (PDB) with the DNeasy Plant Mini kit (Qiagen, Valencia, CA). A standard PCR protocol was used to amplify ITS region of ribosomal DNA using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC - 3') primers. To amplify the TEF gene region, primers ef1 (5'-ATGGGTAAGGA (A/G)GACAAGAC-3') and ef2 (5-GGA(G/A)GTACCAGT(G/C)ATCATGTT-3) were used (BALDAUF 1999; VU 2011). Each PCR reaction contained 1 µl dimethyl sulfoxide (DMSO; Fisher Scientific, Pittsburgh, PA). Once amplified, 4 µl of ITS and TEF PCR products were visualized with

SYBR®Safe DNA gel stain (Invitrogen, Waltham, MA) on 2% agarose gels using a UVP Benchtop 2UV Transilluminator PhotoDoc-It^{TM} imaging system to determine DNA quality and amplification. DNA was cleaned with ExoSapit (Fisher Scientific) and purified DNA was quantified (Thermo Scientific). The PCR products were sequenced at The University of Tennessee Genomics Core (http;//mbrf.utk.edu/). ABI sequence results were processed through Sequencher 5.3 (Gene Codes, Ann Arbor, MI). Both the Basic Local Alignment Search Tool (BLAST) available from the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/) and *Fusarium*MLST databases were used to confirm species identification to 95-100% certainty.

Polymerase chain reaction amplification

Each PCR reaction was carried out in a 10-µl reaction solution containing 1 µl of 2 ng/µl of diluted template DNA, 1 µl of 2.5 µM dNTP mixture, 1 µM of each primer forward and reverse, 0.08 µl of *Taq* DNA polymerase (AmpliTaq Gold, Perkin Elmer), 1 µl reaction buffer, 1 µl of 25 µM $MgCl_2$ and 4.42 µl of sterile deionized water. The temperature cycling was carried out in 96-well plates in a thermocycler. The cycling conditions were: initial denaturation at 95°C for 2 min, followed by 36 cycles of 95°C for 20s, 20s at 55°C and 1 min at 72°C, with a hold for 2 min at 72°C, and a final hold at 4˚C.

Microsatellite design

A paired-end DNA library (Illumina) was prepared to produce read pairs. Reads were cleaned with Skewer version 0.2.2 (JIANG *et al.* 2014). Sequencing adapters and low quality (mean quality <30), short reads (<30 bases) were trimmed. Quality control of

reads occurred using FastQC (Babraham Bioinformatics, Cambridge, England). Reads were categorized by specifying a minimal quality score of 20 in at least 70% of the bases. Specified reads were assembled using SOAPdenovo2 (LUO *et al.* 2012) with kmer = 53. Microsatellite loci were identified with a custom Perl script (https://github.com/statonlab/disculaSSRs). Di-, tri-, and tetranucleotide perfect repeats were reported if they met the following criteria: dinucleotide motifs with 8-40 repeats, trinucleotide motifs with 7-30 repeats, and tetranucleotide motifs with 6-20 repeats. Sequences were masked for low intricacy regions with DUSTMASKER (MORGULIS *et al.* 2006) and primers were designed using PRIMER3 v2.3.6 (UNTERGASSER *et al.* 2012). Primer3 default settings were set except the maximum primer size was 25; primer product size ranged between 100 and 200 bp (WOUDENBERG *et al.* 2015); and primer minimum and maximum annealing temperature was 55℃ and 65℃, respectively. The primer minimum GC percentage was between 40 and 60%; the maximum allowable length of a mononucleotide repeat (mx poly-x) was set to 3; and primer GC clamp was set to 2. Sequence data was mined by Dr. M. Staton (The University of Tennessee). Primer design was chosen using ten repeats of dinucleotide, ten repeats of trinucleotide and ten repeats of tetranucleotides. This library was screened for a variety of microsatellites: ten repeats of di-, tri- and tetranucleotides from each of the four unique *Fusarium* species. Primers (n = 150) were chosen at the University of Tennessee and purchased from Integrated DNA Technologies (Coralville, IA). Desalted custom synthesized oligonucleotides were returned as dehydrated powders, which were hydrated to create working primers to test for amplification of DNA fragments from *Fusarium* species.

Microsatellite screening

PCR products were run against all Fusaria isolates and analyzed on a QIAxcel Capillary Electrophoresis System (QIAGEN) using an internal 25-bp size standard. Reactions that did not produce a product were repeated for a total of three repetitions of each microsatellite locus. Microsatellite loci that did not amplify were removed from further analyses.

Diversity

To determine allelic classes of fusaria, FLEXIBIN v2 (AMOS 2006) was used to automatically bin raw allele length data into classes. A 2-3 bp standard error was used to determine allele size classes. Binned data was used in all the following analyses. Identical multiple locus *Fusarium* species haplotypes were identified using POPPR v2.1.1 (KAMVAR *et al.* 2014) a package for R v3.3.1 (TEAM 2016). All isolates (n = 68) used in this analysis were grouped by species. The program GenAlEx v6.5 (PEAKALL AND SMOUSE 2006; PEAKALL AND SMOUSE 2012) and POPPR (KAMVAR *et al*. 2014) were used to estimate measures of genetic diversity across all isolate loci. Measures included numbers of multilocus haplotypes (MLG), average number of alleles per locus (Na), the average number of effective alleles per population (Ne), the Shannon-Wiener index of MLG diversity (H), and Nei's genotypic diversity corrected to sample size (Hexp). The standardized index of association ($\overline{r}_d$) (AGAPOW AND BURT 2001) was estimated using 10,000 permutations, and the null hypothesis: $r_d = 0$ (linkage equilibrium) was tested in POPPR. The index of association detects signatures of multiple loci linkage and possible clonal reproduction within populations. The standardized index of association, r_d corresponds to the index of association (I_A), but is less biased as it is independent of

the number of loci used in the study. The value of r_d is expected to be zero in panmictic populations, whereas clonally reproducing organisms should have values considerably greater than zero (SCHOEBEL *et al.* 2014). All analyses were calculated using 10,000 combinations at $P < 0.05$ significance level.

Results

Identified *Fusarium* species

Previous identifications of isolates within the *Fusarium* collection from switchgrass were confirmed or corrected. For example, isolate AK104 was previously identified as *F. equiseti* but was corrected to *F. sporotrichioides* based on genetic sequences and morphology. In all, only 6 isolates were determined to be *F. equiseti* from the original set of 27 "*F. equiseti"* isolates, while 19 isolates were no longer viable (Table 2). *Fusarium graminearum* contained the largest number of identified isolates. The initial identification of 250 isolates yielded 105 confirmed isolates, and 3 isolates that were misidentified as *F. oxysporum* (Table 2). Initially, there were 55 isolates of *F. oxysporum,* but only 39 were accounted for and confirmed as *F. oxysporum* (Table 2). In addition, there were isolates that were not identified in the original studies conducted by Vu (2011) and Collins (2016) that were identified in this study (Table 2).

Microsatellite development

One hundred and fifty (15 di- and 15 trinucleotide repeat motifs from each species, with an additional 15 di- and 15 trinucleotides for *F. graminearum*) microsatellite loci were designed. Primer pairs were tested for amplification against two DNA samples extracted from the three *Fusarium* species from which they were derived (i.e. *F. equiseti* loci were

tested against DNA from two *F. equiseti* isolates). For each species, primers that led to amplification of a PCR product of the expected size range were further tested against 30 isolates of *F. graminearum*, 28 isolates of *F. oxysporum*, or 14 isolates of *F. sporotrichioides* (Table 11). Isolates of *F. equiseti* were removed from the study due to small sample size (n = 6). Primers that did not amplify or had multiple stutter peaks were not used for further analyses.

Fusarium equiseti – Microsatellites from *F. equiseti* indicated that the sizes of clone inserts ranged from 100 to 342 bp, with an average size of 198 bp. Of the 98,350 sequences analyzed at 10X coverage, trinucleotides were the most common type of microsatellite repeat, with AGC motif (20.54%) appearing most frequently. Repeats consisting of two or three adjacent blocks of different dinucleotide repeats made up 40.56% of the dinucleotide repeats. There were 3,442 of the 98,350 microsatellite loci (0.35%) sequenced that were suitable for primer design (Table 3).

Fusarium graminearum – Microsatellites from *F. graminearum* indicated that the sizes of clone inserts ranged from 99 to 412 bp. Of the 125,011 sequences analyzed, dinucleotides were the most common microsatellite repeat type, with AC motif (21.49%) appearing most frequently. Repeats consisting of two or three adjacent blocks of different dinucleotide repeats made up 43.92% of the dinucleotide repeats. Of the 125,011 microsatellites sequenced, 12,274 microsatellites (0.98%) were suitable for primer design (Table 4).

Fusarium oxysporum – Microsatellites from of *F. oxysporum* indicated that the sizes of clone inserts ranged from 86 to 368 bp. Of the 20,923 sequences analyzed,

dinucleotides were the most common microsatellite repeat type, with the CG motif (22.89%) appearing most frequently. Repeats consisting of two or three adjacent blocks of different dinucleotide repeats made up 45.81% of the dinucleotide repeats. Only 166 microsatellites of the 20,923 microsatellites (0.01%) sequenced were suited for primer design (Table 5).

Fusarium sporotrichioides – Microsatellites from of *F. sporotrichioides* indicated that the sizes of clone inserts ranged from 129 to 426 bp. Of the 19,696 sequences analyzed, tetranucleotides were the most common microsatellite repeat type (41.12%) followed by dinucleotides (40.35%) with AG (15.99%) and CG motifs (3.64%) appearing most frequently. Only 1,182 microsatellites of the 19,696 microsatellites (0.60%) sequenced were suited for primer design (Table 5).

Microsatellite amplification analysis

Fusarium equiseti - Thirty primers were developed from *F. equiseti* genomic DNA, and fifteen were tested across six isolates of *F. equiseti* before the microsatellites were removed from further study, due to a small number of available isolates for testing. Allele sizes resulted in a range of 100 to 342 bp across twelve of the loci (Table 8). Microsatellite loci Fe007 and Fe014 did not amplify across any of the six *F. equiseti* isolates. *Fusarium equiseti* loci were not used in cross amplification tests.

Fusarium graminearum - Thirty microsatellite loci were originally developed from *F. graminearum* genomic DNA (Table 11) and were tested for amplification against two isolates of *F. graminearum*. If loci amplified they were tested against a DNA from 29 *F. graminearum* isolates. Seventeen loci did not amplify or resulted in three or more

peaks and were not used for further testing. Because only a small number of microsatellite loci amplified, an additional 30 loci were developed for *F. graminearum* (Table 11); eighteen loci from the new set did not amplify or produced multiple peaks. A total of 25 microsatellite loci from the total of 60 were used in further testing against *F. graminearum* isolates. Statistical analysis showed that there is a single population of 29 individuals with 29 multilocus genotypes. Thompson's diversity index (H) was 3.37, expected heterozygosity was 67% indicating that there is a relatively high diversity among individuals, r_d was 0.10 indicating a low chance of clonality, with and a P-value of 0.91 indicating no significance among haplotype diversity across *F. graminearum* isolates and that genetic recombination was occurring (Table 7). Twelve loci were tested for cross amplification of DNA from 24 *F. oxysporum* and 14 *F. sporotrichioides* isolates. Amplification of the 12 loci was 49.39% across isolates from all three *Fusarium* species, with a percent amplification range for each locus between 33.82% (Fg057) and 72.06% (Fg056) across 68 DNA samples. Locus Fg057 did not amplify against any of the *F. oxysporum* DNA isolates (Table 15).

Fusarium oxysporum - Thirty microsatellite loci were developed from *F. oxysporum* genomic DNA (Table 12) and were tested for amplification against two isolates of *F. oxysporum*. Loci that amplified were tested against DNA from 24 *F. oxysporum* isolates. Statistical analysis showed that there is a single population of 24 individuals with 24 multilocus genotypes. Thompson's diversity index H was 3.18, expected heterozygosity was 62% and rbard was 0.05, with a *P* value of 0.002, indicating a significant difference among loci across *F. oxysporum* individuals and a low rate of genetic recombination

(Table 8). Fifteen loci did not amplify against DNA from two isolates of *F. oxysporum*. Ten of the fifteen loci that did amplify against *F. oxysporum* were used in cross amplification tests against DNA from thirty *F. graminearum* and fourteen *F. sporotrichioides* isolates. Five of the loci were removed from further testing as they did not meet the single peak and >3 bp criteria. Amplification of the ten loci was 59.15% across isolates from all three *Fusarium* species, with a percent amplification range for each locus between 36.77% (Fo019) and 83.82% (Fo001) across 68 DNA samples. Loci Fo006, Fo012, and Fo017 did not amplify against DNA from any of the 14 *F. sporotrichioides* isolates (Table 15).

Fusarium sporotrichioides - Thirty microsatellite loci were developed from genomic DNA of *F. sporotrichioides* (Table 13) and were tested for amplification against two isolates of *F. oxysporum*. If loci amplified, they were tested against DNA from 14 *F. sporotrichioides* isolates. Statistical analysis showed that there is a single population of 14 individuals with 14 multilocus genotypes. Thompson's diversity index H was 2.64, expected heterozygosity was 57% and rbard was 0.06, with a *P* value of 0.40 indicating no significant difference among loci across *F. sporotrichioides* individuals and genetic recombination (Table 9). Fifteen loci were tested and amplified against DNA from two isolates of *F. sporotrichioides*. Twelve of the fourteen loci that did amplify against *F. oxysporum* were used in cross amplification tests against thirty *F. graminearum* and twenty-four *F. oxysporum* isolates. One of the loci was removed from further testing as it did not meet the single peak and >3 bp criteria. Amplification of the 12 loci was 49.75% across isolates of all three *Fusarium* species, with a percent amplification range for each locus between 32.35% (Fr006) and 73.53% (Fr003) across 68 DNA samples. All

12 microsatellite loci from *F. sporotrichioides* amplified against DNA from isolates of all three *Fusarium* species (Table 15).

Discussion

This microsatellite study analyzed the genetic diversity of *Fusarium* isolates of four species (*F. equiseti, F. graminearum, F. oxysporum*, and *F. sporotrichioides*). It was determined through the standardized index of association (r_{d}) that there is high gene flow between individuals of the same species indicating genetic exchange and/or mutation through sexual and asexual reproduction. A high level of diversity implies that *Fusarium* species have the ability to rapidly change over a short period of time to adapt to changing environments. Breeding of resistant plant varieties takes several years to develop and new virulent strains of fungal pathogens rise to overcome genetic resistance of plants within a few years after the variety is released to the public (ELLIS *et al*. 2014). Furthermore, a previously avirulent pathogen race may mutate to become virulent (ELLIS *et al*. 2014). In populations with high genetic diversity and the possibility for sexual reproduction, the chances of genetic recombination leading to a virulent strain are increased.

Rapidly developing molecular technology has enabled scientists to develop controls to combat virulent species through rapid diagnosis and an improved understanding of virulence genes. The markers commonly used for species-level phylogenetics in fungi are portions of protein-coding genes that are intron-rich and evolve at a higher rate than other frequently used markers such as internal transcribed spacer (ITS) regions of the nuclear ribosomal RNA gene (O'DONNELL *et al.* 1998a; O'DONNELL *et al.* 2000; GEISER

2003). ITS is useful for identification of fungi to genus level and is used for initial identification purposes in many studies. However, fusaria within the *Gibberella* clade retain nonorthologous copies of the ITS2, which can lead to incorrect phylogenetic inferences (O'DONNELL AND CIGELNIK 1997; O'DONNELL *et al.* 1998a). Unlike ITS, the TEF gene encodes an essential part of the protein translation mechanism, as has high phylogenetic utility (O'DONNELL *et al.* 1998a). It is informative at the species level, nonorthologous copies of the gene have not been detected, and universal primers have been designed which work for the entire genus. TEF primers were first developed for fungi to investigate lineages within the *F. oxysporum* complex (O'DONNELL *et al.* 1998b). This gene has a high level of sequence polymorphism among closely related species, even in comparison to the genes such as calmodulin, beta-tubulin and histone H3. TEF is currently the chosen marker for single-locus identification in *Fusarium* and is often paired with ITS for identification of species. This study and the prior studies by Vu (2011) and Collins (2016) utilized ITS as the initial identifier and TEF for classification conformation.

The *Fusarium* MLST server (http://*fusarium*.cbio.psu.edu) contains a BLAST search tool that allows users to query unknown sequences against the database. GenBank is another database that is available for fungal identification, and can be accessed via the Entrez website at the US National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/Entrez/). GenBank database allows anyone to enter sequences and does not provide quality control leaving potential for misidentified accessions. *FUSARIUM*-ID contains curated, well-characterized sequences that can be used for confirmation (GEISER *et al.* 2004). Using both databases in unison, aids in

conformation of the correct identification. Caution should be used in relying solely on these databases as there is potential for sequence matching error and the possibility that the species sequence will be one that has not yet been entered in the databases. Positive confirmation of the isolates used in this study by morphological traits and genetic sequencing is highly important for accurate use in future studies and for reporting purposes.

Contamination by insects, fungi, bacteria, and viruses is highly probable when storing fungi for extended periods. Frequent transfer of fungi prevents death of the fungal culture and the establishment of fungal pests. Virulence and pathogenicity are known to degrade over time when isolates are grown solely on artificial substrate. Sterile carnation leaf agar is commonly used to slow fungal mutation to adaptation to media. Care should be taken in transferring mycelia and spores, isolation of isolates from possible contaminants is crucial. Fusaria spores can spread by various means causing concern for contamination to other containers and areas. Caution should be used when handling these fungi to prevent contamination to and from the fungal isolates. Light, elevated temperatures, and a high rate of airflow increase the rate of fungal isolate degradation. Storage in a cool, humid and darkened environment in closed containers will aid in prolonged storage of fusaria.

Microsatellite markers offer faster, accurate, and more efficient identification and early detection of plant pathogens (Bridge *et al.* 2003; Schaad *et al.* 2003). Many research facilities have already adopted many different molecular techniques for fungal plant disease detection (Schaad *et al.* 2003; Lopez-Diaz *et al.* 2017). Among these practices are genetic characterization, genome mapping, gene mapping, genome evolution

studies, population diversity studies, and phylogenic relationship comparisons. Attention should be paid to the limits of bioinformatics software, DNA extraction methods, and DNA sequence cloning as each technology has their own level of error. Microsatellite primers developed for a single species may be used to detect polymorphisms in other related individuals. The repeat sequence and the flanking regions must be conserved across taxa to achieve this successfully. Evolutionary distance between the source and the target species will determine PCR amplification. Higher genomic similarities would indicate a greater conservation of SSR-flanking regions. Comparable sized amplification products must be obtained with DNA from related individuals. Caution should be used when comparing similar PCR products obtained across different species. Two equally sized products amplified in different species might include mutations, deletions, and reorganizations in the flanking region or changes in the repeat (PEAKALL *et al.* 1998; SEFC *et al.* 1999; CHAMBERS AND MACAVOY 2000; ROSSETTO *et al.* 2000). Statistical errors and misinterpretation of data may be a result of variations used by different researchers complicating the use of microsatellites in phylogenetic studies.

Questions to consider in future research are: How many nucleotide differences constitute a species divergence in fusaria and other organisms. How much error is the result of DNA movement through the electrophoresis system? How much error is from mutations and statistical binning? Ultimately, the best approach is to determine the techniques that result in the least error and use them as a standard across all microsatellite studies for consistency and replication purposes.

Research Definitions

Array: entire sections of DNA sequence containing multiples of core repeats (CHAMBERS AND MACAVOY 2000)

Biallelic: two alleles or two forms of part of a gene

Bootstrap: a procedure where a random subset of the data is re-run through the phylogenetic analysis, the reported value is the percentage of bootstrap replicates in which the subset appeared. A value of 100% means that the subset is well-supported because it appeared in all bootstrap replicates.

Panmictic: a population where all individuals are potential partners without mating restrictions and all recombination are possible

Orthologs: genes in different species that evolved from a common ancestral gene by speciation (O'DONNELL AND CIGELNIK 1997)

Nonorthologs: genes in different species that evolved independently rather than from a common ancestral gene (O'DONNELL AND CIGELNIK 1997)

Mycotoxins: naturally occurring compounds or secondary metabolites produced by fungi growing on plants in the field or during storage (NICHEA *et al.* 2015)

Random fragment length polymorphisms (RFLP): is a PCR-based molecular method used in genetics research, DNA fingerprinting, and genetic engineering.

References

Adams, J. F. 1921. Observations on wheat scab in Pennsylvania and its pathological histology. Phytopathology 11: 115-125.

Adejumo, T. O., U. Hettwer and P. Karlovsky. 2007. Occurrence of *Fusarium* species and trichothecene in Nigerian maize. International Journal of Food Microbiology 116: 350-357.

Agapow, P.-M., and A. Burt. 2001. Indices of multilocus linkage disequilibrium. Molecular Ecology Notes 1: 101-102.

Agarwal, D. K. 1976. *Fusarium* root rot of soybean - a new record from India. Indian Phytopathology 29: 471.

Agarwal, M., N. Shrivastava, and H. Padh. 2008. Advances in molecular techniques and their applications in plant sciences. Plant Cell Replication 27: 617-631.

Aguayo, J., D. Mostert, C. Fourier-Jeandel, I. Cerf-Wendling, B. Hostachy, A, Viljoen, R. Loos. 2017. Development of a hydrolysis probe-based real-timer assay for the detection of tropical strains of *Fusarium oxysporum* f. sp. *cubense* race 4.

Alabouvette, C. 1990. Biological control of *Fusarium* wilt pathogens in suppressive soils. Pages 27-43 In: Biological Control of Soil-Borne Plant Pathogens. D. Hornby, D., ed. CAB International, Wallingford, Oxon, UK.

Alabouvette, C., C. Olivain, Q. Migheli, and C. Steinberg, 2009. Microbiological control of soil-borne phytopathogenic fungi with special emphasis on wilt-inducing *Fusarium oxysporum*. New Phytologist 184: 529-544.

Alves-Santos, F. M., E. P. Benito, A. Eslava, and J. M. Diaz-Minguez. 1999. Genetic diversity of *Fusarium oxysporum* strains from common bean fields in Spain. Applied and Environmental Microbiology 92: 3335-3340.

Amos, W. J. I. H., A. Frodsham, L. Zhang, S. Best, and A. V. S. Hill. 2006. Automated binning of microsatellite alleles problems and solutions. Molecular Ecology Notes 7**:** 10-14.

Amos, B., C. Schlötterer, and D. Tautz. 1993. Social structure of pilot whales revealed by analytical DNA typing. Science 260: 670-672.

Armour, J. A. L., R. Neuman, S. Gobert, and A. J. Jeffreys. 1994. Isolation of human simple repeat loci by hybridization selection. Human Molecular Genetics 3**:** 599-605.

Aoki, T., K. O'Donnell, Y. Homma, and A. R. Lattanzi. 2003. Sudden-death syndrome of soybean is caused by two morphologically and phylogenetically distinct species within the *Fusarium solani* species complex – *F. virguliforme* in North America and *F. tucumaniae* in South America. Mycologia 95**:** 660-684.

Arie, T., I. Kaneko, T. Yoshida, M. Noguchi, Y. Nomura, *et al.* 2000. Mating-type genes from asexual phytopathogenic ascomycetes *Fusarium oxysporum* and *Alternaria alternata*. Molecular Plant-Microbe Interactions 13**:** 1330-1339.

Armstrong, G. M. and J. K. Armstrong. 1981. *Formae speciales* and races in *Fusarium oxysporum* causing wilt diseases. Pages 391-399 In: *Fusarium*: Diseases, Biology, and Taxonomy. P. E. Nelson, T. A. Toussoun, and R. J. Cook, eds. Pennsylvania University Press, PA.

Armstrong, G. M., and J. K. Armstrong. 1948. Nonsusceptible hosts as carriers of wilt *Fusaria*. Phytopathology 38**:** 808-826.

Arthur, J. C., 1891. Wheat scab. Indiana Agricultural Experimental Station Bulletin 36**:** 129-138.

Baayen, R. P., K. O'Donnell, P. J. M. Bonants, E. Cigelnik, L. P. N. M. Kroon, *et al.* 2000. Gene genealogies and AFLP analyses in the *Fusarium oxysporum* complex identify monophyletic and non-monophyletic formae speciales causing wilt and rot disease. Phytopathology 90**:** 891-900.

Bai, G. H., and G. Shaner. 1994. Scab of wheat: prospects for control. Plant Disease 78**:** 760-766.

Baldauf, S. L. 1999. A search for the origins of animals and fungi: comparing and combining molecular data. The American Naturalist 154**:** 178-188.

Benali, S., B. Mohamed, H. J. Eddine, and C. Neema. 2011. Advances of molecular markers application in plant pathology research. European Journal of Scientific Research 50**:** 110-123.

Bennett, J. W., and M. Klich. 2003. Mycotoxins. Clinical Microbiology Reviews 16**:** 497-516.

Blount, W. P. 1961. Turkey "X" disease. Turkeys 9**:** 52, 55-58, 61-71, 77.

Booth, C. 1971. The Genus *Fusarium*. Commonwealth Agricultural Bureaux [for the] Commonwealth Mycological Institute, CABI Publishing, Kew, Surrey, 237 pp.

Bottalico, A., P. Lerario, and A. Visconti. 1983. Production of mycotoxins (zearalenone, trichothecenes and moniliformin) by *Fusarium* species in Italy. Microbiologie Aliments Nutrition 1**:** 133-142.

Bottalico, A., P. Lerario, and A. Visconti. 1980. Some experimental data on the phytotoxicity of mycotoxins. Phytopathologia Mediterranea 19**:** 196-198.

Bottalico, A., and G. T. Perrone. 2002. Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. European Journal of Plant Pathology 108**:** 611-624.

Bowcock, A. M., A. Ruiz-Linares, J. Tomfohrde, E. Minch, J. R. Kidd, *et al.* 1994. High resolution of human evolutionary trees with polymorphic microsatellites. Nature 368**:** 455-457.

Bransby, D. I., S. B. McLaughlin, and D. J. Parrish. 1998. A review of carbon and nitrogen balances in switchgrass grown for energy. Biomass Bioenergy 14**:** 379-384.

Bridge, P. D., T. Singh, and D. K. Arora. 2003. The application of molecular markers in the epidemology of plant pathogenic fungi. Pages 57-68 In: Fungal Biotechnology in Agricultural, Food, and Environmental Applications. D. K. Arora, ed. CRC Press, Boca Raton, FL. Marcel Dekker Inc. Basel, New York.

Brown, D. W., S. P. McCormick, N. J. Alexander, R. H. Proctor, and A. E. Desjardins. 2001. A genetic and biochemical approach to study trichothecene diversity in *Fusarium sporotrichioides* and *Fusarium graminearum*. Fungal Genetics and Biology 32**:** 121-133.

Brstmayr, H., T. Ban, and J. A. Anderson. 2009. QTL mapping and marker-assisted selection for *Fusarium* head blight resistance in wheat: a review. Plant Breeding 128**:** 1-26.

Bruford, M. W., and R. K. Wayne. 1993. Microsatellites and their application to population genetic studies. Current Opinion in Genetics and Development 3**:** 939-943.

Brunken, J. N., and J. R. Estes. 1975. Cytological and morphological variation in *Panicum virgatum* L. Southwestern Naturalist 19**:** 379-385.

Bruvo, R., N. K. Michiels, T. G. D'Souza, and H. Schulenburg. 2004. A simple method for the calculation of microsatellite genotype distances irrespective of ploidy level. Molecular Ecology Notes 13**:** 2101-2106.

Burgess, L. W., and G. A. Forbes. 1993. Characterization and distribution of *Fusarium acuminatum* subsp*. Armeniacum s*ubsp. *nov.* Mycologia 85**:** 119-124.

Buschiazzo, E., and N. J. Gemmell. 2006. The rise, fall and renaissance of micro-satellites in eukaryotic genomes. Bioassays 28**:** 1040–1050.

Carris, L. M., L. A. Castlebury, and J. Zale. 2008. First report of *Tilletia pulcherrima* bunt on switchgrass (*Panicum virgatum*) in Texas. Plant Disease 92**:** 1707.

Casler, M. D., K. P. Vogel, C. M. Taliaferro, N. J. Ehlke, J. D. Berdahl, *et al.* 2007. Latitudinal and longitudinal adaptation of switchgrass populations. Crop Science 47**:** 2249-2260.

Chambers, G. K., and E. S. MacAvoy. 2000. Microsatellites: consensus and controversy. Comparative Biochemistry and Physiology 126**:** 455-476.

Chandra, N. S., U. A. C. Shankar, S. R. Niranjana, M. S. Reddy, H.S. Prakash. 2008a. Molecular detection and characterization of *Fusarium verticillioides* in maise (*Zea mays*. L.) grown in southern India. Annual Microbiology 58: 359-367.

Chandra, N. S., U. A. C. Shankar, S. R. Niranjana, M. S. Reddy, H.S. Prakash, C. N. Mortensen. 2008b. Control of *Fusarium verticillioides* causing ear rot of maize by *Pseudomonas fluorescens*. Pest Management Science 65: 769-775.

Chelkowski, J., M. Manka, H. Kwasna, A. Visconti, and P. Golinski. 1989. *Fusarium sporotrichioides* Sherb., *Fusarium tricinctum* (Peck) Sacc. and *Fusarium poae* (Peck) Wollenw. cultural characteristics, toxigenicity and pathogenicity towards cereals. Journal of Phytopathology 124**:** 155-161.

Chen, T., R. Liu, and N. R. Scott. 2016. Characterization of energy carriers obtained from the pyrolysis of white ash, switchgrass and corn stover — Biochar, syngas and bio-oil. Fuel Processing Technology 142**:** 124-134.

Chistiakov, D. A., B. Hellemans, and F. A. M. Volckaert. 2006. Microsatellites and their genomic distribution, evolution, function and applications: a review with special reference to fish genetics. Aquaculture 255**:** 1-29.

Clarke, L. A., C. S. Rebelo, J. Goncalves, M. G. Boavida, and P. Jordan. 2001. PCR amplification introduces errors into mononucleotide and dinucleotide repeat sequences. Molecular Pathology 54**:** 351–353.

Collins, S. 2016. Characterization of *Fusarium* species and *Alternaria alternata*, and their effects on switchgrass health and chemical components. M.S. Thesis, The University of Tennessee, Knoxville.

Cook, R. J. 1984. *Fusarium* diseases of wheat and other small grains in North America. Pages 39-52 In: Toxigenic *Fusarium* Species. W. F. O. Marasas, P. E. Nelson, and T. A. Toussoun, eds. Toxigenic *Fusarium* Species. Pennsylvania State University Press, University Park.

Cornuet, J. M., and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144**:** 2001-2014.

Correll, J. C. 1991. The relationship between formae speciales, races, and vegetative compatibility groups in *Fusarium oxysporum*. Phytopathology 81**:** 1061-1064.

Crouch, J. A., L. A. Beirn, L. M. Cortese, S. A. Bonos, and B. B. Clarke. 2010. Anthracnose disease of switchgrass caused by the novel fungal species *Colletotrichum navitas*. Mycological Research 113**:** 1411-1421.

Culler, M. D., J. E. Miller-Garvin, and R. Dill-Macky. 2007. Effect of extended irrigation and host resistance on deoxynivalenol accumulation in *Fusarium*-infected wheat. Plant Disease 91**:** 1464-1472.

Daggett, D. L., R. C. Hendricks, R. Walther, and E. Corporan. 2007. Alternate Fuels for Use in Commercial Aircraft. NASA STI Technical Memorandum. 20 pp.

Dallas, J. F. 1992. Estimation of microsatellite mutation rates in recombinant inbred strains of mouse. Mammaology Genome 3**:** 452-456.

Davis, R. D., N. Y. Moore, and J. K. Kochman. 1996. Characterisation of a population of *Fusarium oxysporum* f. sp. *vasinfectum* causing wilt of cotton in Australia. Australian Journal of Agriculture Research 47**:** 1143-1156.

Del Ponte, E. M., J. M. C. Fernandes, and G. C. Bergstrom. 2007. Influence of growth stage on *Fusarium* head blight and deoxynivalenol production in wheat. Journal of Phytopathology 155**:** 577-581.

Demirbas, A. 2009. Biofuels securing the planet's future energy needs. Energy Conversion and Management 50**:** 2239-2249.

Desjardins, A. E., T. M. Hohn, and S. P. McCormick. 1993. Trichothecene biosynthesis in *Fusarium* species: chemistry, genetics, and significance. Microbiological Reviews 57**:** 595-604.

Desjardins, A. E. 2006. Fusarium mycotoxins: chemistry, genetics and biology. American Pathological Society. St. Paul.

Dieringer, D., and C. Schlötterer. 2003. Two distinct modes of microsatellite mutation processes: evidence from the complete genomic sequences of nine species. Genome Research 13**:** 2242–2251.

Dixon, M. J., J. Dixon, D. Raskova, M. M. Le Beau, R. Williamson, *et al.* 1992. Genetic and physical mapping of the Treacher Collins syndrome locus: refinement of the localization to chromosome 5q32-33.2. Human Molecular Genetics 1**:** 249-253.

Earl, D. A., and B. M. von Holdt. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4**:** 359-361.

Edel, V., C. Steinberg, N. Gautheron, and C. Alabouvette. 2000. Ribosomal DNA-targeted oligonucleotide probe and PCR assay specific for *Fusarium oxysporum*. Mycological Research 104**:** 518-526.

Edel, V., C. Steinberg, N. Gautheron, G. Recorbet, and C. Alabouvette. 2001. Genetic diversity of *Fusarium oxysporum* populations isolated from different soils in France. FEMS Microbiology Ecology 36**:** 61-71.

Edwards, S. G. 2004. Influence of agricultural practices on *fusarium* infection of cereals and subsequent contamination of grain by trichothecene mycotoxins. Toxicology Letters 153**:** 29-35.

Edwards, A., H. A. Hammond, L. Jin, C. T. Caskey, and R. Chakraborty. 1992. Genetic variation of five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics 12**:** 241-253.

Ellegren, H. 1991. DNA typing of museum birds. Nature 354: 113.

El-Nezami, H., P. E. Kankaanpaa, S. Salminen, and J. Ahokas. 1998. Physicochemical alterations enhance the ability of dairy strains of lactic acid bacteria to remove aflatoxins from contaminated media. Journal of Food Protection 61: 466-468.

El-Nezami, H., N. Polychronaki, S. Salminen, and H. Mykkanen. 2002. Binding rather than metabolism may explain the interaction of two food-grade *Lactobacillus* strains with zearalenone and its derivative-zearalenol. Applied and Environmental Microbiology 68: 3545-3549.

Elias, K. S., R. W. Schneider, and M. M. Lear. 1991. Analysis of vegetative compatibility groups in nonpathogenic populations of *Fusarium oxysporum* isolated from symptomless tomato roots. Canadian Journal of Botany 69: 2089-2094.

Ellegren, H., 1991. DNA typing of museum birds. Nature 354: 113.

Ellis, M. L., M. M. Díaz Arias, L. F. Leandro and G. P. Munkvold. 2012. First report of *Fusarium armeniacum* causing seed rot and root rot on soybean (*Glycine max*) in the United States. Plant Disease 96: 1693.

Elobeid, A., S. Tokgoz, and C. Hart. 2007. The ethanol outlook for Brazil and the United States and implications for livestock. International Sugar Journal 109: 174-177.

Estoup, A., and J. M. Cornuet. 1999. Microsatellite evolution: inferences from population data. Pages 49-65 In: Microsatellites: Evolution and Applications, D. B. Goldstein and C. Schlötterer, eds. Oxford University Press, Oxford.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology Notes 14: 2611-2620.

Excoffier, L., and H. E. Lischer. 2010. Arlequin suite ver. 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10**:** 564-567.

Fajolu, O. L. 2012. Characterization of *Bipolaris* species, their effects on switchgrass biomass yield and chemical components. PhD diss. The University of Tennessee, Knoxville.

Fajolu, O. L., A. L. Vu, M. M. Dee, J. Zale, K. D. Gwinn, *et al.* 2012. First report of leaf spot and root rot of switchgrass caused by *Curvularia lunata* var. *aeria* in the United States. Plant Disease 96**:** 1372.

Fernandez, M. R., and J. M. C. Fernandes. 1990. Survival of wheat pathogens in wheat and soybean residues under conservation tillage systems in southern and central Brazil. Canadian Journal of Plant Pathology 12**:** 289-294.

Field, D., and C. Wills. 1996. Long, polymorphic microsatellites in simple organisms. Proceeding of the Royal Society of London B. Biological Science 263**:** 209-215.

Filson, P., N. Labbé, S. W. Jackson, E. Doukhanina, P. Radhakrishnan, *et al.* 2011. Variability of switchgrass, a dedicated bioenergy crop. Proceedings of the 2011 Annual Meeting of the American Institute of Chemical Engineers.

Fink-Grernmels, J. 1999. Mycotoxins: their implications for human and animal health. Veterinary Quarterly 21**:** 115-120.

Fischer, D., and K. Bachmann. 1998a. Microsatellite enrichment in organisms with large genomes (*Allium cepa* L.). Biotechniques 24**:** 796-802.

Forgacs, J., 1962. Mycotoxicoses—the neglected diseases. Feedstuffs 34**:** 124-134.

Forgacs, J., and W. T. Carll. 1962. Mycotoxicoses. Advances in Veterinary Science 7: 273-382.

Fourie, G., E.T. Steenkamp, R.C. Ploetz, T.R. Gordon, and A. Viljoen. 2011. Current status of the taxonomic position of *Fusarium oxysporum* formae specialis cubense within the *Fusarium oxysporum* complex. Infection, Genetics and Evolution 11: 533-542.

Francchia S., I. Garcia-Romera, A. Godeas. J. A. Ocampo. 2000. Effect of the saprophytic fungus Fusarium oxysporum on arbuscular mycorrhizal colonization and growth of plants in greenhouse and field trials. Plant Soil 223:175-184.

Francis, R. 2016. POPHELPER: an R package and web app to analyse and visualize population structure. Molecular Ecology Resources: 1-6.

Freudenreich, C. H., J. B. Stavenhagen, and V. A. Zakian. 1997. Stability of a CTG/CAG trinucleotide repeat in yeast is dependent on its orientation in the genome. Molecular and Cellular Biology 17: 2090.

Fu, Y.-H., D. P. Kuhl, A. Pizzuti, M. Pieretti, J. S. Sutcliffe, *et al.* 1991. Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. Cell 67: 1047-1058.

Garten, C. T., Jr. and S. D. Wullschleger. 2000. Soil carbon dynamics beneath switchgrass as indicated by stable isotope analysis. Journal of Environmental Quality 29: 645-653.

Geiser, D. M. 2003. Practical molecular taxonomy of fungi. Pages 3-14 In: Advances in Fungal Biotechnology for Industry, Medicine and Agriculture, L. Lange and J. Tkacz, eds. Kluwer Academic Pub., Dordrecht, The Netherlands.

Geiser, D. M., M. M. Jiménez-Gasco, S. Kang, I. Makalowska, N. Veeraraghavan, *et al.* 2004. *FUSARIUM*-ID v. 1.0: a DNA sequence database for identifying *Fusarium*. European Journal of Plant Pathology 110**:** 473-479.

Glaubitz, J. C., O. E. Rhodes, and J. A. Dewoody. 2003. Prospects for inferring pairwise relationships with single nucleotide polymorphisms. Molecular Ecology 12**:** 1039-1047.

Guadet, J., J. Julien, J. F. Lafay and Y. Brygoo. 1989. Phylogeny of some *Fusarium* species, as determined by large-subunit rRNA sequence comparison. Molecular Biology and Evolution 6**:** 227-242.

Gebhart, D. L., H. B. Johnson, H. S. Mayeux, and H. W. Polley. 1994. The CRP increases soil organic carbon. Journal of Soil Water Conservation 49**:** 488-492.

Geiser, D. M., M. M. Jiménez-Gasco, S. Kang, I. Makalowska, N. Veeraraghavan, *et al.* 2004 *FUSARIUM*-ID v. 1.0: a DNA sequence database for identifying *Fusarium*. European Journal of Plant Pathology 110**:** 473-479.

Gelderblom, W. C. A., K. Jaskiewicz, W. F. O. Marasas, P. G. Thiel, R. M. Horak, *et al.* 1988. Fumonisins - novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. Applied and Environmental Microbiology 54**:** 1806-1811.

Georges, M., A. B. Dietz, A. Mishra, D. Nielsen, L. S. Sargeant, *et al.* 1993. Microsatellite mapping of the gene causing weaver disease in cattle will allow the study of an associated quantitative trait locus. Proceedings of the National Academy of Science USA 90**:** 1058-1062.

Ghag, S. B., U. K. S. Shekhawat, and T. R. Ganapathi. 2015. *Fusarium* wilt of banana: biology, epidemiology and management. International Journal of Pest Management 61: 250-263.

Gordon, T. R., and R. D. Martyn. 1997. The evolutionary biology of *Fusarium oxysporum*. Annual Review of Phytopathology 35**:** 111-128.

Gordon, T. R., and D. Okamoto. 1992. Population structure and the relationship between pathogenic and non-pathogenic strains of *Fusarium oxysporum*. Phytopathology 82**:** 73-77.

Graniti, A. 1972. The evolution of the toxin concept in plant pathology. Pages 1-18 In: Phytotoxins in Plant Diseases. R. K. Wood, A. Ballio, and A. Graniti, eds. Academic Press, New York, NY.

Gravert, C. E., and G. P. Munkvold. 2002. Fungi and diseases associated with cultivated switchgrass in Iowa. Journal of the Iowa Academy of Science 109**:** 30-34.

Gravert, C. E., L. H. Tiffany, and G. P. Munkvold. 2000. Outbreak of smut caused by *Tilletia maclaganii* on cultivated switchgrass in Iowa. Plant Disease 84**:** 596.

Grove, J. F., and M. Pople. 1980. The insecticidal activity of beauvericin and the enniatin complex. Mycopathologia 70**:** 103-105.

Gunter, L. E., G. A. Tuskan, and S. D. Wullschleger. 1996. Diversity among populations of switchgrass based on RAPD markers. Crop Science 36**:** 1017-1022.

Gupta, S., S. B. Krasnoff, N. L. Underwood, J. A. A. Renwick, and D. W. Roberts. 1991. Isolation of beauvericin as an insect toxin from *Fusarium semitectum* and *Fusarium moniliforme* var. *subglutinans*. Mycopathologia 115**:** 185-189.

Gur-Arie, R., C. J. Cohen, Y. Eitan, L. Shelef, E. M. Hallerman, *et al.* 2000. Simple sequence repeats in *Escherichia coli*: abundance, distribution, composition, and polymorphism. Genome Research 10**:** 62-71.

Gurjar, G. S., M. P. Barve, A. P. Giri and V. S. Gupta. 2009. Identification of Indian pathogenic races of *Fusarium oxysporum* f. sp. *ciceris* with gene specific ITS and random markers. Mycologia 101**:** 484-495.

Gustafson, D. M., A. Boe, and Y. Jin, 2003. Genetic variation for *Puccinia emaculata* infection in switchgrass. Crop Science 43**:** 755-759.

Hagelberg, E., I. C. Gray, and A. J. Jeffreys. 1991. Identification of the skeletal remains of a murder victim by DNA analysis. Nature 352**:** 427-429.

Hagler, W. M., Jr, N. R. Towers, C. J. Mirocha, R. M. Eppley, and W. L. Bryden 2001. Zearalenone: mycotoxin or mycoestrogen? Pages 321-331 In: *Fusarium*: Paul E. Nelson Memorial Symposium. B. A. Summerell, J. F. Leslie, D. Backhouse, W. L. Bryden, and L. W. Burgess, eds. APS Press, St. Paul, MN.

Hamada, H., M. G. Petrino, and T. Kakunaga. 1982. A novel repeated element with Z-DNA-forming potential is widely found in evolutionarily diverse eukaryotic genomes. Proceedings of the National Academy of Sciences USA 79**:** 6465-6469.

Hamilton, M. B., E. L. Pincus, A. Di Fiore, and R. C. Fleischer. 1999. Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. BioTechniques 27**:** 500.

Hampton, R., E. M. Ball, and S. De Boer, eds. 1990. Serological methods for detection and identification of viral and bacterial plant pathogens: a laboratory manual. American Phytopathological Society, St. Paul, MN.

Hamrick, J. L. 1989. Isozymes and the analysis of genetic structure in plant populations. Pages 87-105 In: Isozymes in Plant Biology. D. E. Soltis and P. S. Soltis, eds. Dioscorides Press, Portland, OR.

Hansen, H. N. 1938. The dual phenomenon in imperfect fungi. Mycologia 30**:** 442-455.

Harrison, L. R., B. M. Colvin, J. T. Greene, L. E. Newman, and J. R. Cole. 1990. Pulmonary edema and hydrothorax in swine produced by fumonisin B1 a toxic metabolite of *Fusarium moniliforme*. Journal of Veterinary Diagnostic Investigation 2**:** 217-221.

Hart, L. P., J. Pestka, and M. J. Liu. M. T. 1984. Effect of kernel development and wet periods on production of deoxynivalenol in wheat infected with *Gibberella zeae*. Phytopathology 74**:** 1415-1418.

Hatmaker, A. 2016. Population and genomics within the genus Pityopsis. M. S. Thesis. University of Tennessee, Knoxville.

Haugom, E., O, Mydland, and A. Pichler. 2016. Long term oil prices. Energy Economics 58: 84-94.

Hawksworth, D. L. 1974. Mycologist's Handbook: an introduction to the principles of taxonomy and nomenclature in the fungi and lichens. Commonwealth Mycological Institute, Kew, U.K.

Hawksworth, D. L., P. M. Kirk, B. C. Sutton, and D. N. Pegler. 1996. Ainsworth and Bisby's Dictionary of the Fungi. 8th edition. CAB International, Wallingford, U.K.

Herber, B., and K. Herold. 1998. DNA typing of human dandruff. Journal of Forensic Sciences 43**:** 648–656.

Herron, D. A., M. J. Wingfield, B. D. Wingfield, C. A. Rodas, S. Marincowitz, *et al.* 2015. Novel taxa in the *Fusarium fujikuroi* species complex from *Pinus* spp. Studies in Mycology 80**:** 131-150.

Hirsch, R. L., D. O. Tebeest, B. H. Bluhm, and C. P. West. 2010. First report of rust caused by *Puccinia emaculata* on switchgrass in Arkansas. Plant Disease 94**:** 381.

Hoffer, G. N., A. G. Johnson and D. Atanasoff. 1918. Corn root rot and wheat scab. Journal of Agriculture Research 14**:** 611-612.

Horberg, H. 2002. Patterns of splash dispersed conidia of *Fusarium poae* and *Fusarium culmorum*. European Journal of Plant Pathology 108**:** 73-80.

Huayu, Z., G. Luquin, S. Pengyao, L. Feshi, H. Jianbin, S. Xiaofen, and Y. Luming. 2016. Development of genome-wide SSR markers in melon with their cross- species transferability analysis and utilization in genetic diversity study. Molecular Breeding 36: 1-14.

Hughes, C. R., and D. C. Queller. 1993. Detection of highly polymorphic microsatellite loci in a species with little allozyme polymorphism. Molecular Ecology 2**:** 131-137.

Hultquist, S. J., K. P. Vogel, D. J. Lee, K. Arumuganathan, and S. Kaeppler. 1996. Chloroplast DNA and nuclear DNA content variations among cultivars of switchgrass, *Panicum virgatum* L. Crop Science 36**:** 1049-1052.

Ito, T., C. L. Smith, and C. R. Cantor. 1992. Sequence-specific DNA purification by triplex affinity capture. Proceedings of the National Academy of Sciences USA 89**:** 495-498.

Jain, A., R. E. Hammonds, J. L. Kerrigan, and J. M. Henson. 2014. Characterization of a *Trichoderma atroviride* strain isolated from switchgrass bales and its use to

saccharify ammonia-pretreated switchgrass for biobutanol production. Biomass and Bioenergy 64: 299-308.

James, D., A. James, P. Jean-Yves, K. Harjeet, M. Smith, S. Peeraza-Echeverria, F. Garcia-Bastidas, G. Kema, P. Waterhouse, K. Mengerson, and R. Harding. 2017. Transgenic Cavendish bananas with resistance to *Fusarium* wilt tropical race 4. Nature Communications. 8: 1-8.

Jan C. and L. Fumagalli. 2016. Polymorphic DNA microsatellite markers for forensic individual identification and parentage analyses of seven threatened species of parrots (family *Psittacidae*). Peer Journal 9: 1-7.

Jarne, P., and P. J. L. Lagoda. 1996. Microsatellites, from molecules to populations and back. Trends in Ecology and Evolution 11: 424-429.

Jiang, H., R. Lei, S. W. Ding, and S. Zhu. 2014. Skewer: a fast and accurate adapter trimmer for next-generation sequencing paired-end reads. BMC Bioinformatics 15: 182.

Jin, L., C. Macaubas, J. Hallmayer, A. Kimura, and E. Mignot. 1996 Mutation rate varies among alleles at a microsatellite locus: Phylogenetic evidence. Proceedings of the National Academy of Sciences USA 93: 15285-15288.

Joffe, A. Z. 1974. Toxicity of *Fusarium poae* and *F. sporotrichioides* and its relation to alimentary toxic aleukia. Pages 229-262 In: Mycotoxins. I. F. H. Purchase, ed. Elsevier, Amsterdam.

Jurado, M., C. Vázquez, C. Callejas, and M. T. González-Jaén. 2006a. Occurrence and variability of mycotoxigenic *Fusarium* species associated to wheat and maize in the South of Spain. Mycotoxin Research 22: 87-91.

Jurado, M., C. Vázquez, S. Marín, V. Sanchis, and M. T. González-Jaén. 2006b. PCR-based strategy to detect contamination with mycotoxigenic *Fusarium* species in maize. Systematic and Applied Microbiology 29**:** 681-689.

Jurado, M., C. Vázquez, S. Marín, V. Sanchis, and M. T. González-Jaén. 2006b. PCR-basedstrategy to detect contamination with mycotoxigenic *Fusarium* species in maize. Systematic and Applied Microbiology 29**:** 681-689.

Kamvar, Z. N., J. F. Tabima, and N. J. Grunwald. 2014. Poppr: An R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. Peer Journal 2**:** 281.

Kandapal, R. P., G. Kandapal, and S. M. Weissman. 1994. Construction of libraries enriched for sequence repeats and jumping clones, and hybridization selection for region-specific markers. Proceedings of the National Academy of Sciences USA 91**:** 88-92.

Kano, R., H. Maruyama, M. Kubota, A. Hasegawa, and H. Kamata. 2011. Chronic ulcerative dermatitis caused by *Fusarium sporotrichioides*. Medical Mycolology 49**:** 303-305.

Katan, T., R. Berliner, and J. Katan. 1994. Vegetative compatibility in populations of *Fusarium oxysporum* from wild carnation. Mycological Research 98**:** 1415-1418.

Katti, M. V., P. K. Ranjekar, and V. S. Gupta. 2001. Differential distribution of simple sequence repeats in eukaryotic genome sequences. Molecular Biology Evolution 18**:** 1161-1167.

Kelkar, Y. D., N. Strubczewski, and S. E. Hile, *et al.* 2010. What is a microsatellite: a computational and experimental definition based upon repeat mutational behavior at A/T and GT/AC repeats. Genome Biology and Evolution 2**:** 620-635.

Kiecana, I., and J. Perkowski. 1998. Colonization of oat (*Avena sativa* L.) grain by toxigenic fungi *Fusarium poae* (Peck.) Wr. i *Fusarium sporotrichioides* Sherb. Zeszyty Naukowe Akademii Rolniczej w Krakkowie 333**:** 881-884 (in polish).

Kijas, J. M. H., J. C. Folwer, C. A. Garbett, and M. R. Thomas. 1994. Enrichment of microsatellites from the citrus genome using biotinylated oligonucleotide sequences bound to streptavidin-coated magnetic particles. Biotechniques 16**:** 656-662.

Kim, P., D. Hensley, and N. Labbé. 2014a. Nutrient release from switchgrass-derived biochar pellets embedded with fertilizers. Geoderma 232-234: 341-351.

Kim, P., S. Weaver, K. Noh, and N. Labbé. 2014b. Characteristics of bio-oils produced by an intermediate semipilot scale pyrolysis auger reactor equipped with multistage condensers. Energy and Fuels 28**:** 6966-6973.

Kistler, H. C. 1997. Genetic diversity in the plant-pathogenic fungus *Fusarium oxysporum*. Phytopathology 87**:** 474-479.

Knutsen, A. K., M. Torp and A. Holst-Jensen. 2004. Phylogenetic analyses of *Fusarium poae, Fusarium sporotrichioides* and *Fusarium langsethiae* species complex based on partial sequences of translation elongation factor-1 alpha gene. International Journal of Food Microbiology 95**:** 287-295.

Koenig, R. L., R.C. Ploetz, and H. C. Kistler. 1997. *Fusarium oxysporum f.sp. cubense* consists of a small number of divergent and globally distributed clonal lineages . Phylopathology 87**:** 915-923.

Konstantinova, P. S. and T. Yli-Mattila. 2004. IGS-RFLP analysis and development of molecular markers for identification of *Fusarium poae, Fusarium langsethiae, Fusarium sporotrichioides* and *Fusarium kyushuense*. International Journal of Food Microbiology 95**:** 321-331.

Kosiak, E. B., A. Holst-Jensen, T. Rundberget, M. T. González-Jaén and M. Torp. 2005a. Morphological, chemical and molecular differentiation of *Fusarium equiseti* isolated from Norwegian cereals. International Journal of Food Microbiology 99**:** 195-206.

Kotan, R., F. Sahin, E. Demirci, and C. Eken. 2009. Biological control of the potato dry rot caused by *Fusarium* species using PGPR strains. Biological Control 50**:** 194-198.

Kristensen, R., M. Torp, B. Kosiak, and A. Holst-Jensen. 2005. Phylogeny and toxigenic potential is correlated in *Fusarium* species as revealed by partial translation elongation factor 1 alpha gene sequences. Mycological Research 109**:** 173-186.

Kronstad, J. W., and C. Staben. 1997. Mating type in filamentous fungi. Annual Review of Genetics 31**:** 245-276.

McLaughlin, S. B., and L. A. Kszos. 2005. Development of switchgrass (*Panicum virgatum*) as a bioenergy feedstock in the United States. Biomass and Bioenergy 28**:** 515-535.

Kuiper-Goodman, T. 1998. Food safety: mycotoxins and phycotoxins in perspective. Pages 25-48 In: Mycotoxins and Phycotoxins—Developments in Chemistry, Toxicology and Food Safety. M. Miraglia, H. van Edmond, C. Brera, and J. Gilbert, eds. Alaken Inc., Fort Collins, CO.

Kwon-Chung, K. J., and J. E. Bennett. 1992. Medical Mycology. Journal of the institute of Tropical Medicine Sao Paulo 34: 504.

Lagercrantz, U., H. Ellegren, and L. Andersson. 1993. The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates. Nucleic Acids Research 21**:** 1111-1115.

Langevin, F., F. Eudes, and A. Comeau. 2004. Effect of trichothecenes produced by *Fusarium graminearum* during *Fusarium* head blight development in six cereal species. European Journal of Plant Pathology 110**:** 735-746.

Layton, C. N., and G. C. Bergstrom. 2011. Outbreaks of Smut Caused by *Tilletia maclaganii* on Switchgrass in New York and Pennsylvania. Plant Disease 95**:** 1587.

Lees, A. K., R. Wattier, D. S. Shaw, L. Sullivan, N. A. Williams, *et al.* 2006. Novel microsatellite markers for the analysis of *Phytophthora infestans* populations. Plant Pathology 55**:** 311-319.

Lench, N. J., A. Norris, A. Bailey, A. Booth, and A. F. Markham. 1996. Vectorette PCR isolation of microsatellite repeat sequences using anchored dinucleotide repeat primers. V Nucleic Acids Research 24**:** 2190-2191.

Leslie F. J., C. A. S. Pearson, P. E. Nelson, T. A. Toussoun. 1990. *Fusarium* spp. from corn, sorghum, and soybean fields in the central and eastern United States. Phytopathology 80: 343-350.

Leslie, J. F., B. A. Summerell. 2006. The *Fusarium* Laboratory Manual. Blackwell Publishing, Ames, IA.

Levinson, G., and G. A. Gutman. 1987. Slipped-strand mispairing: a major mechanism for DNA sequence evolution. Molecular Biology Evolution 4**:** 203-221.

Lewandowski, I., J. M. O. Scurlock, E. Lindvall and M. Christou. 2003. The development and current status of perennial rhizomatous grasses as energy crops in the U.S. and Europe. Biomass Bioenergy 25**:** 335-361.

Lewis, A. J., S. Ren, X. Ye, P. Kim, N. Labbé, *et al.* 2015. Hydrogen production from switchgrass via an integrated pyrolysis–microbial electrolysis process. Bioresource Technology 195**:** 231-241.

Liebig, M. A., H. A. Johnson, J. D. Hanson, and A. B. Frank. 2005. Soil carbon under switchgrass stands and cultivated cropland. Biomass Bioenergy 28**:** 347-354.

Li, Y. C., A. B. Korol, T. Fahima, A. Beiles, and E. Nevo. 2002. Microsatellites: genomic distribution, putative functions and mutational mechanisms: a review. Molecular Ecology 11**:** 2453–2465.

Lievens, B., M. Rep, and B. P. H. J. Thommas. 2008. Recent developments in the molecular discrimination of formae speciales of *Fusarium oxysporum.* Pest Managagement Science 64**:** 781-788.

Lin, Y. H., J. Y. Chang, E. T. Liu, C. P. Chao, J. W. Huang, *et al.* 2009. Development of molecular marker for specific detection of *Fusarium oxysporum* f. sp. *cubense* race 4. European Journal of Plant Pathology 123**:** 535-365.

Lincoln, S. E., and E. S. Lander. 1992. Systematic detection of errors in genetic linkage data. Genomics 14**:** 604-610.

Litt, M., and J. A. Luty. 1989. A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. American Journal of Human Genetics 44**:** 397-401.

Lopez-Diaz, C., V. Rahjoo, M. Sulyok, V. Ghionna, A. Martin-Vicente, *et al.* 2017. Fusaric acid contributes to virulence of *Fusarium oxysporum* on plant and mammalian hosts. Molecular Plant Pathology 1: 1-14.

Logrieco, A., C. Altomare, A. Moretti, and A. Bottalico. 1992. Cultural and toxigenic variability in *Fusarium acuminatum*. Mycological Research 96**:** 518-523.

Logrieco, A., G. Mulè, A. Moretti, and A. Bottalico. 2002. Toxigenic *Fusarium* species and mycotoxins associated with maize ear rot in Europe. European Journal of Plant Pathology 108**:** 597-609.

Logrieco A. and A. Visconti, eds. 2004. An Overview on Toxigenic Fungi and Mycotoxins in Europe. Springer, The Netherlands. 268 pp.

López-Diaz, C., V. Rahjoo, M. Sulyok, V. Ghionna, A. Martin-Vicente, *et al.* 2017. Fusaric acid contributes to virulence of *Fusarium oxysporum* on plant and mammalian hosts. Molecular Plant Pathology 18: DOI: 10.1111/mpp.12536.

Llorens, A., M. J. Hinojo, R. Mateo, M. T. González-Jaén, F. M. Valle-Algarra, *et al.* 2006. Characterization of *Fusarium* spp. isolates by PCR-RFLP analysis of the intergenic spacer region of the rRNA gene (rDNA). International Journal of Food Microbiology 106**:** 297-306.

Lu, W. Z., S. H. Chen, and Y. Z. Wang. 2001. Research on wheat scab. Science Publication House Press Inc., Beijing.

Luo, R., B. Liu, Y. Xie, Z. Li, W. Huang, *et al.* 2012. SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. Giga Science 1**:** 18.

Ma, Z., C. W. Wood, and D. I. Bransby. 2000b. Carbon dynamics subsequent to establishment of switchgrass. Biomass Bioenergy 18**:** 93-104.

Ma, L.-J., D. M. Geiser, R. H. Proctor, A. P. Rooney, K. Donnell *et al.* 2013. *Fusarium* Pathogenomics. Annual Review of Microbiology 67**:** 399-416.

Marasas, W. F. O., R. J. Bamburg, E. B. Smalley, F. M. Strong, W. L. Ragland, *et al.* 1969. Toxic effect on trout, rats and mice of T-2 toxin produced by the fungus *Fusarium tricinctum* (Cd.). Snyd. et Hans. Toxicology and Applied Pharmacology 15:471-482.

Marasas, W. F. O., L. W. Burgess, R. Y. Anelich, S. C. Lamprecht and D. J. van Schalkwyk, 1988. Survey of *Fusarium* species associated with plant debris in South African Soils. South African Journal of Botany 54**:** 63-71.

Marasas, W. F. O., P. E. Nelson, and T. A. Tousson. 1984. Toxigenic *Fusarium* species: Identity and Mycotoxicology. Pennsylvania State University Press, University Park, PA.

Marin, P., A. Moretti, A. Ritieni, M. Jurado, C. Vázquez, and M. T. González-Jaén. 2012. Phylogenetic analyses and toxigenic profiles of *Fusarium equiseti* and *Fusarium acuminatum* isolated from cereals from Southern Europe. Food Microbiology 31**:** 229-237.

Mateo, J. J., R. M. Mateo, and M. Jiménez. 2002. Accumulation of type A trichothecenes in maize, wheat and rice by *Fusarium sporotrichioides* isolates under diverse culture conditions. International Journal of Food Microbiology 72**:** 115-123.

Matthies, A. and H. Buchenauer. 2000. Effect of tebuconazole (Folicur) and prochloraz (Sportak) treatments on *Fusarium* head scab development, yield and deoxynivalenol (DON) content in grains of wheat following artificial inoculation with *Fusarium culmorum*. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 107**:** 33-52.

McDonald, B. A., and C. Linde. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. Annual Review of Phytopathology 40**:** 349-379.

McGinnis, M. R., L. Sigler, and M. G. Rinaldi. 1999. Some medically important fungi and their common synonyms and names of uncertain application. Clinical Infectious Diseases 29**:** 728-730.

McLaughlin, C. S., M. H. Vaughan, I. M. Campbell, C. M. Wei, M. E. Stafford *et al.* 1977. Inhibition of protein synthesis by trichothecenes. Pages 263-273 In: Mycotoxins in Human and Animal Health. J. V. Rodricks, C. W. Hesseltine, and M. A. Hehlman, eds. Pathotox Publishers, Park Forest South, IL.

McLaughlin, S. B. 1993. New switchgrass biofuels research program for the southeast. Pages 111-115 In: Proceedings 1992 Annual Automotive Development Technology Contractors' Coordination Meeting, Dearborn, MI.

McLaughlin, S. B., J. Bouton, D. Bransby, B. V. Conger, W. R. Ocumpaugh *et al.* 1999. Developing switchgrass as a bioenergy crop. Pages 282-299 In: Perspectives on New Crops and New Uses. J. Janick, ed. ASHS Press, Alexandria, VA.

Metzenberg, R. L. and N. L. Glass. 1990. Mating type and mating strategies in *Neurospora*. BioEssays 12**:** 53-59.

Milgroom, M.G and C. M. Brasier. 1997. Potential diversity in vegetative compatibility types of *Ophiostoma novo-ulmi* in North America. Mycologia 89**:** 722-726.

Miller, J. D., J. Cully, K. Fraser, S. Hubbard, F. Meloche, *et al.* 1998. Effect of tillage practice on *fusarium* head blight of wheat. Canadian Journal of Plant Pathology 20**:** 95-103.

Mirete, S., C. Vázquez, G. Mulè, M. Jurado, and M. T. González-Jaén. 2004. Differentiation of *Fusarium verticillioides* from banana fruits by IGS and EF-1a sequence analyses. European Journal of Plant Pathology 110**:** 515-523.

Molina, A. B., E. Fabregar, V. G. Sinohin, G. Yi and A. Viljoen, 2009. Recent occurrence of *Fusarium oxysporum* f. sp*. cubense* tropical race 4 in Asia. Acta Hortic. 828**:** 109-116.

Molina, A. B., R. C. Williams, C. Hermanto, K. Birte, and P. Kokoa. 2010. Mitigating the threat of banana *Fusarium* wilt: understanding the agroecological distribution of pathogenic forms and developing disease management strategies [Project number HORT/2005/136]. Canberra: ACIAR.

Morgante, M., H. Hanafey, and W. Powell. 2002. Microsatellites are preferentially associated with nonrepetative DNA in plant genome. Nature Genetics 30**:** 194-200.

Morgulis, A., E. M. Gertz, A. A. Schäffer, and R. Agarwala. 2006. A fast and symmetric DUST implementation to mask low-complexity DNA sequences. Journal of Computational Biology 13**:** 1028-1040.

Moser, L. E. and K. P. Vogel. 1995. Switchgrass, big bluestem, and indiangrass. Pages 409-420 In: Forages: An Introduction to Grassland Agriculture (5th edition). R. F. Barnes, C. J. Nelson, M. Collins, and K. J. Moore, eds. Iowa State University Press, Ames, IA.

Moss, M. O. 2002. Mycotoxin review – 2. *Fusarium*. Mycologist 16**:** 156-160.

Moss, M. O. and U. Thrane. 2004. *Fusarium* taxonomy with relation to trichothecene formation. Toxicology Letters 153:23-28.

Mullis, K. B. 1990. The unusual origin of the polymerase chain reaction. Scientific American 262**:** 56-61, 64-65.

Mullis, K. B. and F. A. Faloona. 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods in Enzymology 155**:** 350.

Nayaka, S. C., E. G. Wulff, A. C. Udayashankar, B. P. Nandini, S. R. Niranjana, C. N. Mortensen, H.S. Prakash. 2011. Prospects of molecular markers in Fusarium species diversity. Applied Microbilogy Biotechnology 90: 1625-1639.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89**:** 583-590.

Nelson, P. E., T.A. Toussoun, and W. F. O. Marasas. 1983. *Fusarium* Species: An Illustrated Manual for Identification. Pennsylvania State University, University Park, USA. 206 pp.

Nichea, M. J., S. A. Palacios, S. M. Chiacchiera, M. Sulyok, R. Krska, *et al.* 2015. Presence of multiple mycotoxins and other fungal metabolites in native grasses from a wetland ecosystem in Argentina intended for grazing cattle. Toxins 7**:** 3309-3329.

Nirenberg, H. I. and K. O'Donnell. 1998. New *Fusarium* species and combinations within the *Gibberella fujikuroi* species complex. Mycologia**:** 434-458.

Nishio, Z., K. Takata, M. Ito, M. Tanio, T. Tabiki, *et al.* 2010. Deoxynivalenol distribution in flour and bran of spring wheat lines with different levels of *Fusarium* head blight resistance. Plant Disease 94**:** 335-338.

Nixon, K. C., and Q. D. Wheeler. 1990. An amplification of the phylogenetic species concept. Cladistics 6**:** 211-223.

O'Donnell, K., and E. Cigelnik. 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Molecular and Phylogenetic Evolution 7**:** 103-116.

O'Donnell, K. and E. Cigelnik. 1999. A DNA sequenced-based phylogenetic structure for the *Fusarium oxysporum* complex. Phytoparasitica 27**:** 69.

O'Donnell, K., E. Cigelnik, and H. I. Nirenberg. 1998a. Molecular systematics and phylogeography of the Gibberella fujikuroi species complex. Mycologia 90**:** 465-493.

O'Donnell, K., H. C. Kistler, E. Cigelnik, and R. C. Ploetz. 1998b. Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. Proceedings of the National Academy of Sciences USA 95**:** 2044-2049.

O'Donnell, K., H. C. Kistler, B. K. Tacke, and H. H. Casper. 2000. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. Proceedings of the National Academy of Sciences USA 97**:** 7905-7910.

O'Donnell, K., D. A. Sutton, M. G. Rinaldi, C. Gueidan, P. W. Crous, *et al.* 2009. Novel multilocus sequence typing scheme reveals high genetic diversity of human pathogenic members of the *Fusarium incarnatum — F. equiseti*, and *F. chlamydosporum* species complexes within the United States. Journal of Clinical Microbiology 47**:** 3851-3861.

O'Donnell, K., Sutton D. A., A. Fothergill, D. McCarthy, M. G. Rinaldi, *et al.* 2008a. Molecular phylogenetic diversity, multilocus haplotype nomenclature, and in vitro

antifungal resistance within the *Fusarium solani* species complex. Journal of Clinical Microbiology 46**:** 2477-2490.

Ohashi, J., and K. Tokunaga. 2003. Power of genome-wide linkage disequilibrium testing by using microsatellite markers. Journal of Human Genetics 48**:** 487–491.

Oliveira, E. J., J. G. Pa´dua, M. I. Zucchi, R. Vencovsky, and M. L. C. Vieira, 2006 Origin, evolution and genome distribution of microsatellites. Genetics and Molecular Biology 29**:** 294-307.

Ostrander, E. A., P. M. Jong, J. Rine, and G. Duyk. 1992. Construction of small-insert genomic DNA libraries highly enriched for microsatellite repeat sequences. Proceedings of the National Academy of Sciences USA 89**:** 3419-3423.

Oswald, J. W. 1949. Cultural variation, taxonomy and pathogenicity of *Fusarium* species associated with cereal root rots. Phytopathology 39**:** 359-376.

Parker, I. M., and S. G. Gregory. 2004. The evolutionary ecology of novel plant-pathogen interactions. Anuual Review of Ecological Systems 35: 675-700.

Parrish, D. and J. Fike. 2005. The biology and agronomy of switchgrass for biofuels. Critical Reviews in Plant Sciences 24**:** 423-459.

Parry, D. W., P. Jenkinson, and L. Mcleod. 1995. *Fusarium* ear blight (scab) in small grain cereal — A review. Plant Pathology Journal 44**:** 207-238.

Pascale, M., M. Haidukowski and A. Visconti. 2003. Determination of T-2 toxin in cereal grains by liquid chromatography with fluorescence detection after immunoaffinity column clean-up and derivatization with 1-anthroylnitrile. Journal of Chromatography A 989**:** 257-264.

Paul, P. A., S. M. El-Allaf, P. E. Lipps, and L. V. Madden. 2004. Rain splash dispersal of *Gibberella zeae* wheat canopies in Ohio. Phytopathology 94**:** 1342-1349.

Paul, P. A., P. E. Lipps, D. E. Hershman, M. P. McMullen, M. A. Draper *et al.* 2007. A quantitative review of tebuconazole effect on *Fusarium* head blight and deoxynivalenol content in wheat. Phytopathology 97**:** 211-220.

Peacock, M. M., E. R. Hekkala, V. S. Kirchoff, L. G. Heki. Return of a giant: DNA archibal museum samples helps to identify a unique cutthroat trout lineage formerly thought to be extinct. Royal Society Open Science 4: 1-19.

Peakall, R., S. Gilmore, W. Keys, M. Morgante, and A. Rafalski. 1998. Cross-species amplification of soybean (*Glycine max*) simple sequence repeats (SSRs) within the genus and other legume genera: Implications for the transferability of SSRs in plants. Molecular Biology and Evolution 15**:** 1275-1287.

Peakall, R., and P. E. Smouse. 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6**:** 288-295.

Peakall, R., and P. E. Smouse. 2012. GenAlEx 6.5: Population genetic software for teaching and research-an update: Genetic analysis in Excel. Bioinformatics 28**:** 2537-2539.

Perkowski, J., I. Kiecana, J. Stachowiak, and T. Basiński. 2003. Natural occurrence of scirpentriol in cereals infected by *Fusarium* species. Food Additives and Contaminants 20**:** 572-578.

Perkowski, J., M. Wiwart, M. Buśko, M. Laskowska, F. Berthiller, *et al.* 2007. *Fusarium* toxins and fungal biomass indicators in naturally contaminated wheat samples from northeastern Poland in 2003. Food Additives and Contaminants 24**:** 1292-1298.

Pestka, J. J. and G. S. Bondy. 1994. Immunotoxic effects of mycotoxins. Pages 339-359 In: Mycotoxins in Grain. Compounds other than Aflatoxins. J. D. Miller and H. L. Trenholm, eds. Eagan Press. St. Paul, MN.

Pestka, J. J., H. R. Zhou, Y. Moon, and Y. J. Chung. 2004. Cellular and molecular mechanisms for immune modulation by deoxynivalenol and other trichothecenes: unraveling a paradox. Toxicology Letters 153**:** 61-73.

Petch, T. 1931. *Gibberella saubinetii* (Mont.) Sacc. Annales of Applied Biology 34**:** 257-260.

Petes, T. D., P. W. Greenwell, and M. Dominska. 1997. Stabilization of microsatellite sequences by variant repeats in the yeast *Saccharomyces cerevisiae*. Genetics 146**:** 491.

Ploetz, R. C. 1990. Variability in *Fusarium oxysporum* f. sp. *cubense.* Canadian Journal of Botany 68**:** 1357-1363.

Ploetz, R. C. 2006. *Fusarium* wilt of banana is caused by several pathogens referred to as *Fusarium oxysporum* f. sp. *cubense*. Phytopathology 96**:** 653-656.

Ploetz, R. C. and K. G. Pegg. 2000. *Fusarium* wilt. Pages 143-159 In Diseases of Banana, Abaca and Enset. CABI Publishing, Wallingford, U.K.

Pontón, J., R. Ṙchel, K. V. Clemons, D. C. Coleman, R. Grillot, *et al*. 2000. Emerging pathogens. Medical Mycology 38**:** 225-236.

Porter, C. L. 1966. An analysis of variation between upland and lowland switchgrass, *Panicum virgatum* L., in central Oklahoma. Ecology 47**:** 980-992.

Powell, W., M. Morgante, C. Andre, et al. 1996. The comparison of RFLP, RAPD, AFLP, and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding 2**:** 225-238.

Prelusky, D. B. 1994. Residues in food products of animal origin. Pages 405-419 In: Mycotoxins in Grain. Compounds other than Aflatoxins. J. D. Miller and H. L. Trenholm, eds. Eagan Press. St. Paul, MN.

Prelusky, D. B., B. A. Rotter, and R. G. Rotter. 1994. Toxicology of mycotoxins. Pages 359-403 In: Mycotoxins in Grain. Compounds other than Aflatoxins. J. D. Miller, and H. L. Trenholm, eds. Eagan Press. St. Paul, MN.

Priestley, R. A., F. M. Dewey, P. Nicholson, and H. N. Rezanoor. 1992. Comparison of isoenzyme and DNA markers for differentiating W-, R- and C- pathotypes of *Pseudocercosporella herpotrichoides*. Plant Pathology 41**:** 591-599.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155**:** 945-959.

Pugh, G. W., H. Johann, and J. G. Dickson. 1933. Factors affecting infection of wheat heads by *Gibberella saubinetii*. Journal of Agriculture Research 46: 771-797.

Puhalla, J. E. 1981. Variation in *Fusarium* cultures. Pages 293-305 In: *Fusarium*; Diseases, Biology and Taxonomy, P. E. Nelson, T. A. Toussoun, and R. J. Cook, eds. The Pennsylvania State University Press: University Park, PA. 67-373.

Rabie, C. J., E. W. Sydenham, P. G. Thiel, A. Lubben, and W. F. O. Marasas. 1986. T-2 toxin production by *Fusarium acuminatum* isolated from oats and barley. Applied and Environmental Microbiology 52**:** 594-596.

Richardson, M. and C. Lass-Florl, 2008. Changing epidemiology of systemic fungal infections. Clinical Microbiology and Infection 14**:** 5-24.

Roberts, L. J., T. M. Baldwin, T. P. Speed, E. Handman, and S. J. Foote. 1999. Chromosomes X, 9 and the H2 locus interact epistatically to control Leishmania major infection. European Journal of Immunology 29**:** 3047-3050.

Roddy, D. J. 2012. Biomass and biofuels - Introduction. Comprehensive Renewable Energy. Biomass and Biofuel Production 5: 1-9.

Rossetto, M., F. C. L. Harriss, A. Mclauchlan, R. J. Henry, P. R. Baverstock, *et al.* 2000. Interspecific amplification of tea tree (*Malaleuca alternifolia-Myrtaceae*) microsatellite loci-potential implications for conservation studies. Australian Journal of Botany 48**:** 3 Rossi, V., A. Ravanetti, E. Pattori, and S. Giosue. 2001. Influence of temperature and humidity on the infection of wheat spikes by some fungi causing *Fusarium* head blight. Journal of Plant Pathology 83**:** 189-198.

Rothe, G. M. 1994. Electrophoresis of Enzymes: Laboratory Methods. Springer, Berlin/Heidelberg. 305 pp.

Rotter, B. A., B. K. Thompson, D. B. Prelusky, and H. L. Trenholm 1991a. Evaluation of potential interactions involving trichothecene mycotoxins using chick embryotoxicity bioassay. Archives of Environmental Contamination and Toxicology 21**:** 621-624.

Sacchetti, L., G. Calcagno, I. Coto, N. Tinto, E. Vuttariello, *et al.* 1999. Efficiency of two different nine-loci short tandem repeat systems for DNA typing purposes. Clinical Chemistry 45**:** 178-183.

Saghai Maroof, M. A., R. M. Biyashev, G. P. Yang, Q. Zhang, and R. W. Allard. 1994. Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations, and population dynamics. Proceedings of the National Academy of Sciences USA 91**:** 5466-5470.

Sanderson, M. A., M. R. Schmer, V. Owens, P. Keyser, and W. Elbersen. 2012. Crop management of switchgrass. Pages 87-112 In: Switchgrass, Green Energy and Technology, A. Monti, ed. Springer-Verlag, London.

Schaad, N. W., R. D. Frederick, J. Shaw, W. L. Schneider, Robert Hickson, *et al.* 2003. Advances in molecular-based diagnostics in meeting crop biosecurity and phytosanitary issues. Annual Review of Phytopathology 41: 305-324.

Schilling, A., E. Möller, and H. H. Geiger. 1996. Polymerase chain reaction-based assays for species-specific detection of *Fusarium culmorum*, *F. graminearum*, and *F. avenaceum*. Molecular Plant Pathology 85**:** 515-523.

Schlötterer, C. 2004. The evolution of molecular markers-just a matter of fashion? Nature 5**:** 63-69.

Schlötterer, C. 2000a. Evolutionary dynamics of microsatellite DNA. Chromosoma 109**:** 365-371.

Schlötterer, C., and D. Tautz. 1992. Slippage synthesis of simple sequence DNA. Nucleic Acids Research 20**:** 211.

Schoebel, C. N., J. Stewart, N. J. Gruenwald, D. Rigling, and S. Prospero. 2014. Population history and pathways of spread of the plant pathogen *Phytophthora plurivora*. PloS One 9**:** e85368.

Schollenberger, M., H. M. Muller, M. Rufle, S. Suchy, S. Planck, *et al.* 2005. Survey of *Fusarium* toxins in foodstuffs of plant origin marketed in Germany. International Journal of Food Microbiology 97**:** 317-326.

Seaman, W. L. 1982. Epidemiology and control of mycotoxigenic fusaria on cereal grains. Canadian Journal of Plant Pathology 4**:** 187-190.

Sefc, K. M., F. Regner, E. Turetscheck, J. Glössl, and H. Steinkellner. 1999. Identification of microsatellite sequences in *Vitis riparia* and their applicability for genotyping of different *Vitis* species. Genome 42**:** 367-373.

Selkoe, K. A., and R. J. Toonen. 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecology Letters 9**:** 615-629.

Sites, J. W., and J. C. Marshall. 2004. Operational criteria for delimiting species. Annual Review of Phytopathology 35**:** 199-277.

Smart, A. J., L. E. Moser, and K. P. Vogel. 2004. Morphological characteristics of big bluestem and switchgrass plants divergently selected for seedling tiller number. Crop Science 44**:** 607-613.

Snijders, C. H. A. 1990. *Fusarium* head blight and mycotoxin contamination of wheat: A review. Netherlands Journal of Plant Pathology 96**:** 187-198.

Snijders, C. H. A. 2004. Resistance in wheat to *Fusarium* infection and trichothecene formation. Toxicology Letters 153**:** 37-46.

Snyder, W. C., and H. N. Hansen. 1954. Variation and speciation in the genus *Fusarium*. Annals of the New York Academy of Sciences 60**:** 16-23.

Snyder, W. C., H. N. Hansen, *et al*. 1940. The species concept in *Fusarium*. American Journal of Botany 27**:** 64-67.

Snyder, W. C., and S. M. Nash. 1968. Relative incidence of *Fusarium* pathogens of cereals in rotation plots at Rothamsted. Transactions of the British Mycological Society 51**:** 417-425.

Snyder, W. C., and T. A. Toussoun. 1965. Current status of taxonomy in *Fusarium* species and their perfect states. Phytopathology 55**:** 833-837.

Sobel, E., J. C. Papp, and K. Lange. 2002. Detection and integration of genotyping errors in statistical genetics. American Journal of Human Genetics 70**:** 496-508.

Steenkamp, E. T., B. D. Wingfield, A. E. Desjardins, W. F. O. Marasas, and M. J. Wingfield. 2002. Cryptic speciation in *Fusarium subglutinans*. Mycologia 94**:** 1032-1043.

Stepień, L., and J. Chelkowski. 2010. *Fusarium* head blight of wheat: pathogenic species and their mycotoxins. World Mycotoxin Journal 3**:** 107-119.

Sternberg, S. 1994. The emerging fungal threat. Science 266**:** 1632-1634.

Stout, W. L., G. A. Jung, and J. A. Shaffer. 1988. Effects of soil and nitrogen on water use efficiency of tall fescue and switchgrass under humid conditions. Soil Science Society of America Journal 52**:** 429-4334.

Subirana, J. A., and X. Messeguer. 2008. Structural families of genomic microsatellites. Gene 408**:** 124-132.

Sun, Y., and J. J. Cheng. 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresource Technology 83**:** 1-11.

Sun, J. X., J. C. Mullikin, N. Patterson, and D. E. Reich. 2009. Microsatellites are molecular clocks that support accurate inferences about history. Molecular Biology and Evolution 26**:** 1017-1027.

Sutton, J. C. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Canadian Journal of Plant Pathology 4**:** 195-209.

Sweeney, M. J. and A. D. W. Dobson. 1998. Mycotoxin production by *Aspergillus, Fusarium* and *Penicillium* species. International Journal of Food Microbiology 43**:** 141-158.

Sweeney, M., S., White, and A. Dobson. 2000. Mycotoxins in Agriculture and Food Safety. Irish Journal of Agricultural and Food Research 39: 235-244.

Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Research 17**:** 6463.

Tautz, D., and M. Renz. 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Research 12**:** 4127-4138.

Tautz, D., and C. Schlötterer. 1994. Simple sequences. Current Opinion in Genetics and Development 4**:** 832-837.

Taylor, J. W., D.J. Jacobson, and M. C. Fisher. 1999. The evolution of asexual fungi: reproduction, speciation and classification. Annual Review of Phytopathology 37**:** 197-246.

Taylor, A. C., W. B. Sherwin, and R. K. Wayne. 1994. Genetic variation of microsatellite loci in a bottlenecked species: the northern hairy-nosed wombat *Lasiorhinus krefftii*. Molecular Ecology 3**:** 277-290.

Taylor, J. W., D. J. Jacobson, S. Kroken, T. Kasuga, D. M. Geiser, *et al.* 2000. Phylogenetic species recognition and species concepts in fungi. Fungal Genetics and Biology 31**:** 21-32.

Team, R. C. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Tekauz, A., B. McCallum, and J. Gilbert. 2009. Review: *Fusarium* head blight of barley in western Canada. Canadian Journal of Plant Pathology 22**:** 9-16.

Thrane, U., A. Adler, P. E. Clasen, Galvano. F., W. Langseth, *et al.* 2004. Diversity in metabolite production by *Fusarium langsethiae, F. poae*, and *F. sporotrichioides*. International Journal of Food Microbiology 95**:** 257-266.

Tolleson, W. H., K. L. Dooley, W. G. Sheldon, J. D. Thurman, T. J. Bucci, *et al.* 1996. The mycotoxin fumonisin induces apoptosis in cultured human cells and in livers and kidneys of rats. Pages 237-250 In: Fumonisins in Food, Advances in Experimental Medicine and Biology, Vol. 392. L. S. Jackson, J. W. DeVries, and L. B. Bullerman, eds. Springer, Boston, MA.

Tomoda, H., X. H. Huang, H. Nishida, R. Nagao, S. Okuda, *et al.* 1992. Inhibition of acyl-CoA: cholesterol acyltransferase activity by cyclodepsipeptide antibiotics. Journal of Antibiotics 45**:** 1626-1632.

Toth, G., Z. Gaspari, and J. Jurka. 2000. Microsatellites in different eukaryotic genome: survey and analysis. Genome Research 10**:** 1967-1981.

Toussoun, T. A. and P. E. Nelson. 1975. Variation and speciation in the fusaria. Annual Review of Phytopathology 13**:** 71-82.

Trenholm, H. L., D. W. Friend, R. M. G. Hamilton, D. B. Prelusky, and B. C. Foster. 1989. Lethal toxicity and nonspecific effects. Pages 107-114 In: Trichothecene Mycotoxicosis: Pathophysiologic effects. V. R. Beasley, ed. Vol I. CRC Press, Boca Raton, FL.

Tsui, C. K., A. D. Roe, Y. A. El-Kassaby, A. V. Rice, S. M. Alamouti, F. A. Sperling, *et al.* 2012. Population structure and migration pattern of a conifer pathogen, *Grosmannia clavigera*, as influenced by its symbiont, the mountain pine beetle. Molecular Ecology 21**:** 71-86.

Ueno, Y. 1977. Trichothecenes. Overview Address. Pages 189-205 In: Mycotoxins in Human and Animal Health. J. V. Rodricks, C. W. Hesseltine, and M.A. Mehlman, eds. Pathotox Publishers, Inc. Park Forest South, IL.

Untergasser, A., I. Cutcutache, T. Koressaar, J. Ye, B. C. Faircloth, *et al.* 2012. Primer3— new capabilities and interfaces. Nucleic Acids Research 40**:** 115.

U.S.D.A. NRCS National Plant Data Team. 2016. (https://plants.usda.gov/core/profile?symbol=PAVI2). Accessed December 1, 2016.

Van Wyk, P. S., O. Los, G. D. C. Pauer, and W. F. O. Marasas. 1987. Geographic distribution and pathogenicity of *Fusarium* species associated with crown rot of wheat in the Orange Free State, South Africa. Phytophylactica 19**:** 271-274.

Vermerris, W. 2008. Genetic Improvement of Bioenergy Crops, Springer-Verlag, New York, NY. 450 pp.

Visconti, A., C. J. Mirocha, A. Bottalico, and J. Chelkowski. 1985. Trichothecene

mycotoxins produced by *Fusarium sporotrichioides* strain P-11. Mycotoxin Research 1: 3-10.

Vogel, K. P. 2002. The challenge: high quality seed of native plants to ensure successful establishment. Seed Technology 24: 9-15.

Vu, A. L. 2011. Identifying pathogens of switchgrass and investigating antimicrobial activity of switchgrass-derived extractives. M.S. thesis, The University of Tennessee, Knoxville.

Vu, A. L., M. M. Dee, R. J. Gualandi Jr., S. Huff, J. Zale, *et al.* 2011a. First report of leaf spot caused by *Bipolaris spicifera* on switchgrass in the United States. Plant Disease 95: 1191.

Vu, A. L., M. M. Dee, K. D. Gwinn, and B. H. Ownley. 2011b. First report of spot blotch and common root rot caused by *Bipolaris sorokiniana* on switchgrass in Tennessee. Plant Disease 95: 1195.

Vu, A. L., K. D. Gwinn, and B. H. Ownley. 2011c. First report of *Sclerotinia homoeocarpa* causing dollar spot on switchgrass in the United States. Plant Disease 95: 1585.

Vu, A. L., M. M. Dee, T. Russell, J. Zale, K. D. Gwinn, *et al.* 2012. First report of leaf spot caused by *Alternaria alternata* on switchgrass in Tennessee. Plant Disease 96: 763.

Vu, A. L., M. M. Dee, T. Russell, J. Zale, K. D. Gwinn, *et al.* 2013a. First report of leaf spot caused by *Bipolaris oryzae* on switchgrass in Tennessee. Plant Disease 97: 1654.

Vu, A. L., K. D. Gwinn, and B. H. Ownley. 2013b. First report of leaf spot on switchgrass caused by *Pithomyces chartarum* in the United States. Plant Disease 97**:** 1655.

Waits, L. P., G. Luikart, and P. Taberlet. 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Molecular Ecology 10**:** 249-256.

Wang, Y. Z., X. N Yong, and Q. P. Xiao. 1982. The improvement of identification techniques of scab resistance of wheat and development of resistant sources. Scientica Agricultura Sinica 5**:** 67-77.

Warren, H. L., and T. Kommedahl. 1973. Fertilization and wheat refuse effects on *Fusarium* species associated with wheat roots in Minnesota. Phytopathology 63**:** 103-108.

Waxman, K. D., and G. C. Bergstrom. 2011. First report of anthracnose caused by *Colletotrichum navitas* on switchgrass in New York. Plant Disease 95**:** 1032.

Weber, J. L. 1990. Informativeness of human poly(GT)n polymorphisms. Genomics. 7**:** 524-530.

Weber, J. L., and P. E. May. 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. American Journal of Human Genetics 44**:** 388-396.

Wheeler, K. A., B. F. Hurdman, and J. I. Pitt. 1991. Influence of pH on the growth of some toxigenic species of *Aspergillus*, *Penicillium* and *Fusarium*. International Journal of Food Microbiology 12**:** 141-150.

Wicklow, D. T., G. A. Bennett, and O. L. Shotwell. 1987. Secondary invasion of soybean by *Fusarium graminearum* and resulting mycotoxin contamination. Plant Disease 71**:** 1146.

Wierdl, M., M. Dominska, and T. D. Petes. 1997. Microsatellite instability in yeast: dependence on the length of the microsatellite. Genetics 146**:** 769-779.

Wilson, D. 1995. Endophyte – the evolution of a term, and clarification of its use and definition. Oikos 73**:** 274-276.

Wilson A., D. Simpson, E. Chandler, P. Jennings, and P. Nicholson. 2004. Development of PCR assays for the detection and differentiation of *Fusarium sporotrichioides* and *Fusarium langsethiae*. FEMS Microbiol Letters 233**:** 69-76.

Windels, C. E. 2000. Economic and social impacts of *Fusarium* head blight: Changing farms and rural communities in the northern Great Plains. Phytopathology 90**:** 17-21.

sporotrichioides Sherb. toxins evaluated in cereal grain with *Trichoderma harzianum*. Journal of Plant Protection Research 51(2):1-6.

Wollenweber, H. W., and O. A. Reinking. 1935. Die Fusarien, ihre Beschreibung. Schadwirkung und Bekampfung. Verlag Paul Parey, Berlin, Germany.

World Health Organization and International Agency for Research on Cancer. 1993. Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. Monograph 56. World Health Organization, Geneva Switzerland.

Woudenberg, J., M. Seidl, J. Groenewald, M. de Vries, J. Stielow, *et al.* 2015. *Alternaria* section *Alternaria*: Species, *formae speciales* or pathotypes? Studies in Mycology 82**:** 1-21.

Wulff, E. G., J. L. Sørensen, M. Lübeck, K. F. Nielsen, U. Thrane, *et al.* 2010. *Fusarium* spp. associated with rice Bakanae: ecology, genetic diversity, pathogenicity and toxigenicity. Environmental Microbiology 12**:** 649-657.

Xu, X. M., and P. Nicholson. 2009. Community ecology of fungal pathogens causing wheat head blight. Annual Review of Phytopathology 47**:** 83-103.

Xue, A. G., K. C. Armstrong, H. D. Voldeng, G. Fedak, and C. Babcock. 2004. Comparative aggressiveness of isolates of *Fusarium* species causing head blight on wheat in Canada. Canadian Journal of Plant Pathology 26**:** 81-88.

Yergeau, E., M. Filion, V. Vujanovic, and M. St-Arnaud. 2005. A PCR-denaturing gradient gel electrophoresis approach to assess *Fusarium* diversity in asparagus. Journal of Microbiological Methods 60**:** 143-154.

Yli-Mattila, T. and Gagkaeva, T. 2010. Molecular chemotyping of *Fusarium graminearum*, *F. culmorum* and *F. cerealis* isolates from Finland and Russia. Pages 159-177 In: Molecular Identification of Fungi. Y. Gherbawy, and K. Voight, eds. Springer, Berlin.

Young, J. P., L. Ju Kyong, K. Nam-Soo. 2009. Simple sequence repeat polymorphisms (SSRPs) for evaluation of molecular diversity and germplasm classification of minor crops. Molecules 14: 4546-4569.

Yun, S.-H., T. Arie, I. Kaneko, O. C. Yoder, and B. G. Turgeon. 2000. Molecular organization of mating type loci in heterothallic, homothallic, and asexual *Gibberella*/ *Fusarium* species. Fungal Genetic Biology 31**:** 7-20.

Zabalgogeazcoa, I. 2008. Fungal endophytes and their interaction with plant pathogens. Spanish Journal of Agriculture Research 6: 138-146.

Zain, M. I. 2011. Impact of mycotoxins on humans and animals. Journal of Saudi Chemical Society. 15: 129-144.

Zale, J., L. Freshour, S. Agarwal, J. Sorochan, B. H. Ownley *et al.* 2008. First report of rust on switchgrass (*Panicum virgatum*) caused by *Puccinia emaculata* in Tennessee. Plant Disease 92: 1710.

Zhang, Y. J., P. S. Fan, X. Zhang, C. J. Chen, and M. G. Zhou. 2009. Quantification of *Fusarium graminearum* in harvested grain by real-time polymerase chain reaction to assess efficacies of fungicides on *Fusarium* head blight, deoxynivalenol contamination, and yield of winter wheat. Phytopathology. 99: 95-100.

Zhu, H., and Y. Fan. 1989. Wheat scab epidemics and efficacy of testing for resistance in field in northern Fujiang Province. Journal of Jiangsu Agriculture Science 1: 75-78.

Appendix

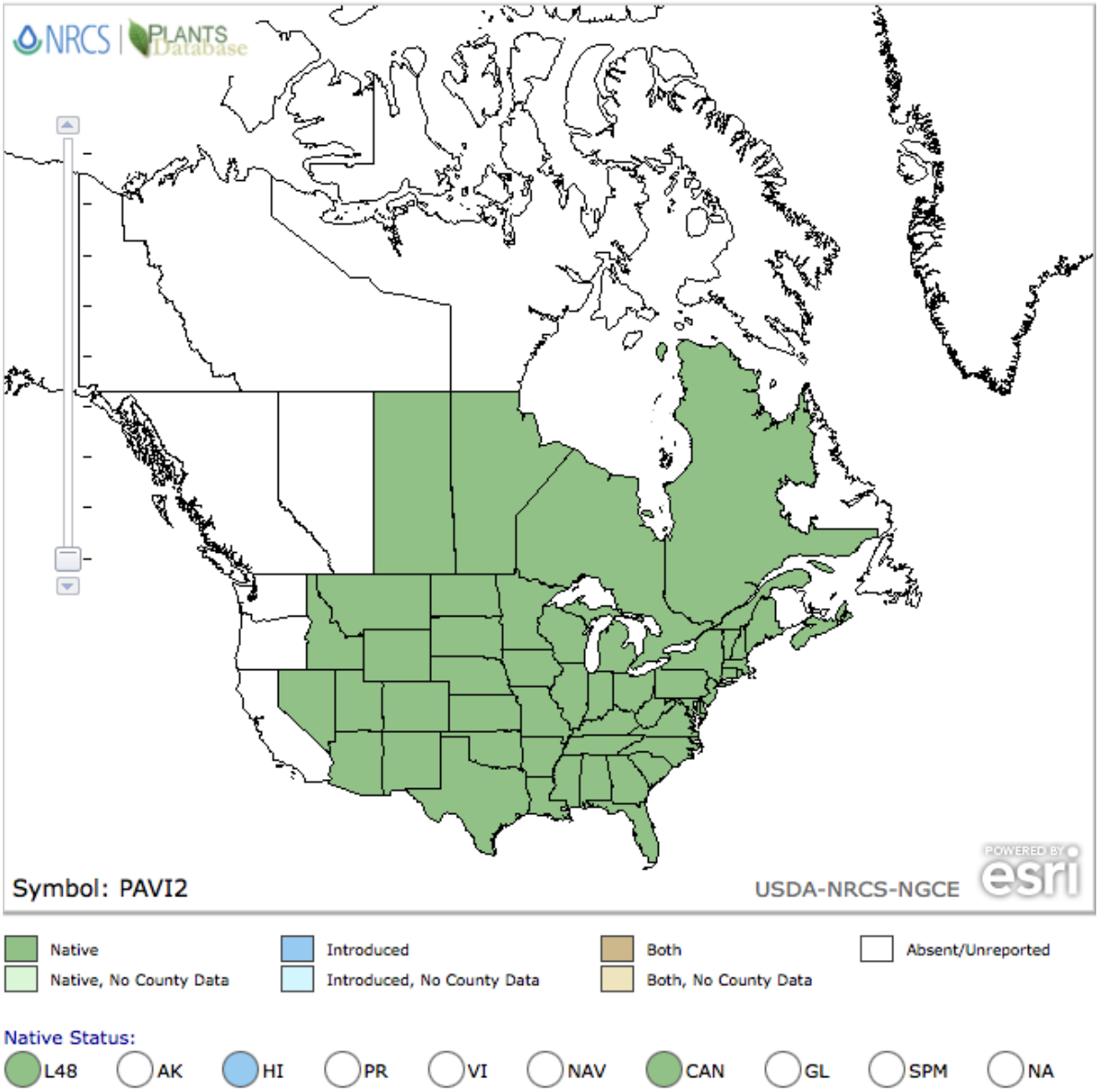

Figure 1 Map of switchgrass (Panicum virgatum L.)

native growth range. Map native status bar indicates that switchgrass has been introduced to the islands of Hawaii (U.S.D.A. N.R.C.S. National Plant Team 2016).

Table 1. Pathogens isolated from commercial switchgrass seed or field plants

Common name	Scientific name	Location	Reference
Smut	*Tilletia maclaganii*	Iowa	Gravert *et al*. 2000
Rust	*Puccinia emaculata*	Tennessee	Zale *et al*. 2008
Bunt	*Tilletia pulcherrima*	Texas	Carris *et al*. 2008
Anthracnose	*Colletotrichum navitas*	New Jersey	Crouch *et al*. 2010
Smut	*Tilletia maclaganii*	New York, Pennsylvania	Layton and Bergstrom 2011
Anthracnose	*Colletotrichum navitas*	New York	Waxman and Bergstrom 2011
Spot Blotch	*Bipolaris sorokiniana*	Tennessee	Vu *et al*. 2011b
Dollar Spot	*Sclerotinia homoeocarpa*	Tennessee	Vu *et al*. 2011c
Leaf Spot	*Bipolaris spicifera*	Tennessee	Vu *et al*. 2011a
Leaf Spot	*Bipolaris victoriae*	Tennessee	Fajolu 2012
Leaf Spot	*Alternaria alternata*	Tennessee	Vu *et al*. 2012
Leaf Spot	*Curvularia lunata* var. *aeria*	Tennessee	Fajolu *et al*. 2012
Leaf Spot	*Fusarium acuminatum*	Tennessee	Vu 2011
Leaf Spot	*Fusarium armeniacum*	Tennessee	Vu 2011
Leaf Spot	*Bipolaris oryzae*	Tennessee	Vu *et al*. 2013a
Leaf Spot	*Pithomyces chartarum*	Tennessee	Vu *et al*. 2013b
Leaf Spot	*Fusarium equiseti*	Tennessee	Vu 2011
Leaf Spot	*Fusarium graminearum*	Tennessee	Vu 2011
Leaf Spot	*Fusarium tricinctum*	Tennessee	Collins 2016
Leaf Spot	*Fusarium sporotrichioides*	Tennessee	This report

Table 2. Identification of putative *Fusarium* spp. isolated from commercial seed or infected plants of switchgrass, or infected wheat plants

Seed company or field location	Switchgrass cultivar or wheat	Isolate	Species identity (Vu 2011)	Species identity (Collins 2016)	Species identity[a] (This study)	Location of seed company/ production field
Applewood	Blackwell	ABW1	*Fusarium equiseti*	*F. equiseti*	*F. equiseti*	CO/ OK
Applewood	Blackwell	ABW5	*F. equiseti*	*F. equiseti*	nd[b]	CO/ OK
Applewood	Blackwell	ABW8	*F. equiseti*	*F. equiseti*	nd[b]	CO/ OK
Applewood	Blackwell	ABW11	*Fusarium* sp.	*Fusarium oxysporum*	*F. oxysporum*	CO/ OK
Applewood	Blackwell	ABW14	*F. equiseti*	*F. equiseti*	*F. equiseti*	CO/ OK
Applewood	Blackwell	ABW18	*Fusarium* sp.	*F. oxysporum*	*F. oxysporum*	CO/ OK
Applewood	Blackwell	ABW19	*Fusarium* sp.	*F. oxysporum*	*F. oxysporum*	CO/ OK
Applewood	Blackwell	ABW16	*F. equiseti*	*F. equiseti*	nd[b]	CO/ OK
Applewood	Blackwell	ABW17	*F. equiseti*	*F. equiseti*	nd[b]	CO/ OK
Applewood	Cave-N-Rock	ACNR128	*Fusarium* sp.	*F. oxysporum*	*F. oxysporum*	CO/ OK
Applewood	Cave-N-Rock	ACNR132	*Fusarium* sp.	*F. oxysporum*	*F. oxysporum*	CO/ OK
Applewood	Cave-N-Rock	ACNR138	*Fusarium* sp.	*F. oxysporum*	*F. oxysporum*	CO/ OK
Applewood	Cave-N-Rock	ACNR139	*F. oxysporum*	*F. oxysporum*	*F. oxysporum*	CO/ OK
Applewood	Cave-N-Rock	ACNR140	*F. oxysporum*	*F. oxysporum*	*F. oxysporum*	CO/ OK
Applewood	Cave-N-Rock	ACNR141	*F. oxysporum*	*F. oxysporum*	*F. oxysporum*	CO/ OK
Applewood	Cave-N-Rock	ACNR176	*F. oxysporum*	*F. oxysporum*	*F. oxysporum*	CO/ OK
Applewood	Cave-N-Rock	ACNR178	*Rhizopus oryzae*	*F. oxysporum*	*F. oxysporum*	CO/ IL
Applewood	Cave-N-Rock	ACNR179	*F. oxysporum*	*F. oxysporum*	*F. oxysporum*	CO/ IL

Table 2. Continued

Seed company or field location	Switchgrass cultivar or wheat	Isolate	Species identity (Vu 2011)	Species identity (Collins 2016)	Species identity[a] (This study)	Location of seed company/ production field
Applewood	Cave-N-Rock	ACNR195	*F. oxysporum*	*F. oxysporum*	nd[b]	CO/ IL
Applewood	Cave-N-Rock	ACNR180	*F. oxysporum*	*F. oxysporum*	*F. oxysporum*	CO/ IL
Applewood	Cave-N-Rock	ACNR183	*F. oxysporum*	*F. oxysporum*	*F. oxysporum*	CO/ IL
Applewood	Cave-N-Rock	ACNR184	*F. oxysporum*	*F. oxysporum*	*F. oxysporum*	CO/ IL
Applewood	Cave-N-Rock	ACNR198	*F. oxysporum*	*F. oxysporum*	nd[b]	CO/ IL
Applewood	Cave-N-Rock	ACNR207	*F. oxysporum*	*F. oxysporum*	nd[b]	CO/ IL
Applewood	Cave-N-Rock	ACNR223	*F. oxysporum*	*F. oxysporum*	nd[b]	CO/ IL
Applewood	Cave-N-Rock	ACNR224	*F. oxysporum*	*F. oxysporum*	nd[b]	CO/ IL
Applewood	Cave-N-Rock	ACNR226	*F. oxysporum*	*F. oxysporum*	nd[b]	CO/ IL
Applewood	Cave-N-Rock	ACNR228	*F. oxysporum*	*F. oxysporum*	nd[b]	CO/ IL
Applewood	Cave-N-Rock	ACNR231	*F. oxysporum*	*F. oxysporum*	nd[b]	CO/ IL
Applewood	Cave-N-Rock	ACNR244	*F. oxysporum*	*F. oxysporum*	nd[b]	CO/ IL
Applewood	Kanlow	AK61	*F. tricinctum*	*F. tricinctum*	*F. tricinctum*	CO/ OK
Applewood	Kanlow	AK109	*Fusarium acuminatum*	*F. acuminatum*	*F. acuminatum*	CO/ OK
Applewood	Kanlow	AK60	*Fusarium graminearum*	*F. graminearum*	*F. graminearum*	CO/ OK
Applewood	Kanlow	AK104	*F. equiseti*	*F. equiseti*	*Fusarium sporotrichioides*	CO/ OK
Applewood	Kanlow	AK25	*F. equiseti*	*F. equiseti*	nd[b]	CO/ OK
Applewood	Kanlow	AK101	*F. equiseti*	*F. equiseti*	nd[b]	CO/ OK
Applewood	Kanlow	AK102	*F. equiseti*	*F. equiseti*	nd[b]	CO/ OK

Table 2. Continued

Seed company or field location	Switchgrass cultivar or wheat	Isolate	Species identity (Vu 2011)	Species identity (Collins 2016)	Species identity[a] (This study)	Location of seed company/ production field
Applewood	Kanlow	AK103	*F. equiseti*	*F. equiseti*	nd[b]	CO/ OK
Applewood	Kanlow	AK17	*F. graminearum*	*F. graminearum*	*F. graminearum*	CO/ OK
Applewood	Kanlow	AK58	*F. tricinctum*	*F. tricinctum*	*F. tricinctum*	CO/ OK
Curtis & Curtis	Alamo	CA12	*F. graminearum*	*F. graminearum*	*F. graminearum*	NM/ SD
Curtis & Curtis	Alamo	CA 5	*Fusarium* sp.	*F. equiseti*	nd[b]	NM/ SD
Curtis & Curtis	Blackwell	CBW10	*F. graminearum*	*F. graminearum*	nd[b]	NM/ SD
Curtis & Curtis	Blackwell	CBW11	*F. graminearum*	*F. graminearum*	*F. oxysporum*	NM/ SD
Curtis & Curtis	Cave-N-Rock	CNR127	*Fusarium sp.*	*Fusarium sp.*	*F. graminearum*	NM/ SD
Ernst Seed	Alamo	EA38	*F. equiseti*	*F. equiseti*	nd[b]	PA/ MO
Ernst Seed	Alamo	EA23	*F. equiseti*	*F. equiseti*	nd[b]	PA/ MO
Ernst Seed	Alamo	EA63	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ MO
Ernst Seed	Alamo	EA22	*F. equiseti*	*F. equiseti*	nd[b]	PA/ MO
Ernst Seed	Alamo	EA24	*F. equiseti*	*F. equiseti*	nd[b]	PA/ MO
Ernst Seed	Alamo	EA25	*F. equiseti*	*F. equiseti*	nd[b]	PA/ MO
Ernst Seed	Alamo	EA27	*F. equiseti*	*F. equiseti*	nd[b]	PA/ MO
Ernst Seed	Alamo	EA36	*F. equiseti*	*F. equiseti*	nd[b]	PA/ MO
Ernst Seed	Alamo	EA12	*F. graminearum*	*F. graminearum*	nd[b]	PA/ MO
Ernst Seed	Alamo	EA13	*F. graminearum*	*F. graminearum*	nd[b]	PA/ MO
Ernst Seed	Alamo	EA14	*F. graminearum*	*F. graminearum*	nd[b]	PA/ MO
Ernst Seed	Alamo	EA20	*F. graminearum*	*F. graminearum*	nd[b]	PA/ MO
Ernst Seed	Alamo	EA21	*F. graminearum*	*F. graminearum*	nd[b]	PA/ MO

Table 2. Continued

Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location
Ernst Seed	Alamo	EA28	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ MO
Ernst Seed	Alamo	EA31	*F. graminearum*	*F. graminearum*	nd[b]	PA/ MO
Ernst Seed	Alamo	EA49	*F. graminearum*	*F. graminearum*	nd[b]	PA/ MO
Ernst Seed	Alamo	EA61	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ MO
Ernst Seed	Alamo	EA62	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ MO
Ernst Seed	Alamo	EA64	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ MO
Ernst Seed	Alamo	EA65	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ MO
Ernst Seed	Alamo	EA66	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ MO
Ernst Seed	Alamo	EA67	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ MO
Ernst Seed	Alamo	EA68	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ MO
Ernst Seed	Alamo	EA69	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ MO
Ernst Seed	Alamo	EA70	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ MO
Ernst Seed	Blackwell	EBW1	*Fusarium* sp.	*Fusarium* sp.	nd[b]	PA/ MO
Ernst Seed	Cave-N-Rock	ECNR140	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR90	*F. graminearum*	*Fusarium* sp.	nd[b]	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR33	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR44	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR111	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR119	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR127	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR143	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR39	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA

Table 2. Continued

Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location
Ernst Seed	Cave-N-Rock	ECNR29	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR141	*F. graminearum*	*F. graminearum*	nd[b]	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR142	*F. graminearum*	*F. graminearum*	nd[b]	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR136	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR93	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR138	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR99	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR18	*F. graminearum*	*F. graminearum*	nd[b]	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR19	*F. graminearum*	*F. graminearum*	nd[b]	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR20	*F. graminearum*	*F. graminearum*	nd[b]	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR21	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR25	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR26	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR27	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR30	*F. graminearum*	*F. graminearum*	nd[b]	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR31	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR32	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR34	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR35	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR36	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR37	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR38	*F. graminearum*	*F. graminearum*	*F. oxysporum*	PA/ PA

Table 2. Continued

Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location
Ernst Seed	Cave-N-Rock	ECNR40	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR96	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR97	*F. graminearum*	*F. graminearum*	nd[b]	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR98	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR100	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR101	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR102	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR103	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR104	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR107	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR109	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR110	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR112	*F. graminearum*	*F. graminearum*	nd[b]	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR118	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR120	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR121	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR122	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR123	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR124	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR125	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR126	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR128	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA

Table 2. Continued

Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location
Ernst Seed	Cave-N-Rock	ECNR129	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR130	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR131	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR132	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR133	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR134	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR135	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR137	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR139	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR144	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR153	*F. graminearum*	*F. graminearum*	nd[b]	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR60	*F. graminearum*	*Fusarium* sp.	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR93	*F. graminearum*	*Fusarium* sp.	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	ECNR28	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Ernst Seed	Cave-N-Rock	EWR70	*F. graminearum*	*F. graminearum*	*F. graminearum*	PA/ PA
Milan, TN	Wheat	F.G.1	nd[b]	*F. graminearum*	*F. graminearum*	TN/ TN
Milan, TN	Wheat	F.O.1	nd[b]	*F. oxysporum*	*F. oxysporum*	TN/ TN
Johnson & Johnson	Cimarron	JCIM1	*Fusarium* sp.	*Fusarium armeniacum*	*F. sporotrichioides*	PA/ PA
Johnson & Johnson	Cimarron	JCIM151	*Fusarium* sp.	*F. oxysporum*	*F. oxysporum*	OK/ OK
Johnson & Johnson	Cimarron	JCIM2	*Fusarium* sp.	*Fusarium* sp.	*F. oxysporum*	OK/ OK

Table 2. Continued

Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location
Johnson & Johnson	Cimarron	JCIM3	*Fusarium* sp.	*Fusarium* sp.	*F. oxysporum*	OK/ OK
Johnson & Johnson	Cimarron	JCIM126	*Fusarium* sp.	*Fusarium* sp.	*F. equiseti*	OK/ OK
Johnson & Johnson	Cimarron	JCIM40	*F. graminearum*	*F. graminearum*	*F. graminearum*	OK/ OK
Johnson & Johnson	Cimarron	JCIM64	*F. graminearum*	*F. graminearum*	*F. graminearum*	OK/ OK
Johnson & Johnson	Cimarron	JCIM5	*Fusarium* sp.	*Fusarium* sp.	*F. oxysporum*	OK/ OK
Johnson & Johnson	Cimarron	JCIM98	*F. graminearum*	*F. graminearum*	*F. graminearum*	OK/ OK
Johnson & Johnson	Cimarron	JCIM7	*Fusarium* sp.	*F. armeniacum*	*F. sporotrichioides*	OK/ OK
Johnson & Johnson	Cimarron	JCIM66	*F. graminearum*	*F. graminearum*	nd[b]	OK/ OK
Johnson & Johnson	Cimarron	JCIM67	*F. graminearum*	*F. graminearum*	*F. graminearum*	OK/ OK
Johnson & Johnson	Cimarron	JCIM68	*F. graminearum*	*F. graminearum*	nd[b]	OK/ OK
Johnson & Johnson	Cimarron	JCIM69	*F. graminearum*	*F. graminearum*	*F. graminearum*	OK/ OK
Johnson & Johnson	Cimarron	JCIM70	*F. graminearum*	*F. graminearum*	*F. graminearum*	OK/ OK
Johnson & Johnson	Cimarron	JCIM71	*F. graminearum*	*F. graminearum*	nd[b]	OK/ OK

Table 2. Continued

Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location
Johnson & Johnson	Cimarron	JCIM96	*F. graminearum*	*F. graminearum*	*F. graminearum*	OK/ OK
Johnson & Johnson	Cimarron	JCIM97	*F. graminearum*	*F. graminearum*	nd[b]	OK/ OK
Johnson & Johnson	Cimarron	JCIM139	*F. graminearum*	*F. graminearum*	*F. graminearum*	OK/ OK
Johnson & Johnson	Cimarron	JCIM140	*F. graminearum*	*F. graminearum*	*F. graminearum*	OK/ OK
Johnson & Johnson	Cimarron	JCIM144	*F. graminearum*	*F. graminearum*	*F. graminearum*	OK/ OK
Johnson & Johnson	Cimarron	JCIM146	*F. graminearum*	*F. graminearum*	*F. graminearum*	OK/ OK
Johnson & Johnson	Cimarron	JCIM34	*F. oxysporum*	*F. oxysporum*	*F. oxysporum*	OK/ OK
Johnson & Johnson	Cimarron	JCIM35	*F. oxysporum*	*F. oxysporum*	*F. oxysporum*	OK/ OK
Johnson & Johnson	Cimarron	JCIM125	*Fusarium* sp.	*F. oxysporum*	*F. oxysporum*	OK/ OK
Johnson & Johnson	Cimarron	JCIM127	*F. oxysporum*	*F. oxysporum*	*F. oxysporum*	OK/ OK
Johnson & Johnson	Cimarron	JCIM4	*Fusarium* sp.	*Fusarium* sp.	*F. sporotrichioides*	OK/ OK
Johnson & Johnson	Cimarron	JCIM6	*Fusarium* sp.	*Fusarium* sp.	*F. oxysporum*	OK/ OK
Johnson & Johnson	Cimarron	JCIM11	*Fusarium* sp.	*Fusarium* sp.	*F. oxysporum*	OK/ OK

Table 2. Continued

Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location
Johnson & Johnson	Cimarron	JCIM12	*Fusarium* sp.	*Fusarium* sp.	*F. oxysporum*	OK/ OK
Johnson & Johnson	Cimarron	JCIM17	*Fusarium* sp.	*Fusarium* sp.	*F. oxysporum*	OK/ OK
Johnson & Johnson	Cimarron	JCIM32	*Fusarium* sp.	*Fusarium* sp.	*F. oxysporum*	OK/ OK
Johnson & Johnson	Cimarron	JCIM127	*Fusarium* sp.	*Fusarium* sp.	*F. equiseti*	OK/ OK
Johnson & Johnson	Cimarron	JCIM145	*F. graminearum*	*Fusarium* sp.	*F. graminearum*	OK/ OK
Johnson & Johnson	Cimarron	JCIM148	*F. graminearum*	*F. graminearum*	*F. graminearum*	OK/ OK
Roundstone	Alamo	RSA90	*Fusarium* sp.	*F. armeniacum*	*F. sporotrichioides*	KY/ TN
Roundstone	Alamo	RSA97	*Fusarium* sp.	*F. armeniacum*	*F. sporotrichioides*	KY/ TN
Roundstone	Alamo	RSA101	*Fusarium* sp.	*F. armeniacum*	*F. sporotrichioides*	KY/ TN
Roundstone	Alamo	RSA91	*Fusarium* sp.	*F. armeniacum*	*F. sporotrichioides*	KY/ TN
Roundstone	Alamo	RSA93	*Fusarium* sp.	*F. armeniacum*	*F. sporotrichioides*	KY/ TN
Roundstone	Alamo	RSA92	*Fusarium* sp.	*F. armeniacum*	*F. sporotrichioides*	KY/ TN
Roundstone	Alamo	RSA12	*F. armeniacum*	*F. armeniacum*	*F. sporotrichioides*	KY/ TN
Roundstone	Alamo	RSA99	*Fusarium* sp.	*Fusarium* sp.	*F. sporotrichioides*	KY/ TN
Roundstone	Alamo	RSA36	*F. oxysporum*	*F. oxysporum*	*F. oxysporum*	KY/ TN
Roundstone	Alamo	RSA42	*F. armeniacum*	*F. armeniacum*	*F. sporotrichioides*	KY/ TN
Roundstone	Alamo	RSA103	*Fusarium* sp.	*F. armeniacum*	*F. sporotrichioides*	KY/ TN

Table 2. Continued

Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location
Roundstone	Alamo	RSA94	*Fusarium* sp.	*F. armeniacum*	*F. sporotrichioides*	KY/ TN
Roundstone	Alamo	RSA117	*Fusarium* sp.	*F. armeniacum*	*F. sporotrichioides*	KY/ TN
Roundstone	Alamo	RSA35	*F. armeniacum*	*F. armeniacum*	*F. sporotrichioides*	KY/ TN
Roundstone	Alamo	RSA49	*F. armeniacum*	*F. armeniacum*	*F. sporotrichioides*	KY/ TN
Roundstone	Alamo	RSA96	*Fusarium* sp.	*F. armeniacum*	*F. sporotrichioides*	KY/ TN
Roundstone	Alamo	RSA100	*Fusarium* sp.	*F. armeniacum*	*F. sporotrichioides*	KY/ TN
Roundstone	Alamo	RSA102	*Fusarium* sp.	*F. armeniacum*	*F. sporotrichioides*	KY/ TN
Roundstone	Alamo	RSA36	*F. graminearum*	*F. graminearum*	*F. graminearum*	KY/ TN
Roundstone	Alamo	RSA40	*F. graminearum*	*F. graminearum*	*F. graminearum*	KY/ TN
Roundstone	Alamo	RSA44	*F. graminearum*	*F. graminearum*	*F. graminearum*	KY/ TN
Roundstone	Alamo	RSA47	*F. graminearum*	*F. graminearum*	nd[b]	KY/ TN
Roundstone	Alamo	RSA95	*Fusarium* sp.	*F. graminearum*	*F. graminearum*	KY/ TN
Roundstone	Alamo	RSA3	*F. oxysporum*	*F. oxysporum*	*F. oxysporum*	KY/ TN
Roundstone	Alamo	RSA4	*F. oxysporum*	*F. oxysporum*	*F. oxysporum*	KY/ TN
Roundstone	Alamo	RSA5	*F. oxysporum*	*F. oxysporum*	*F. oxysporum*	KY/ TN
Roundstone	Alamo	RSA6	*F. oxysporum*	*F. oxysporum*	*F. oxysporum*	KY/ TN
Roundstone	Alamo	RSA43	*F. oxysporum*	*F. oxysporum*	*F. oxysporum*	KY/ TN
Roundstone	Alamo	RSA45	*F. oxysporum*	*F. oxysporum*	nd[b]	KY/ TN
Roundstone	Alamo	RSA46	*F. oxysporum*	*F. oxysporum*	nd[b]	KY/ TN
Roundstone	Alamo	RSA48	*F. oxysporum*	*F. oxysporum*	nd[b]	KY/ TN
Roundstone	Alamo	RSA49	*F. oxysporum*	*F. oxysporum*	*F. oxysporum*	KY/ TN
Roundstone	Alamo	RSA50	*F. oxysporum*	*F. oxysporum*	*F. oxysporum*	KY/ TN

Table 2. Continued

Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location
Roundstone	Alamo	RSA51	*F. oxysporum*	*F. oxysporum*	*F. oxysporum*	KY/ TN
Roundstone	Alamo	RSA52	*F. oxysporum*	*F. oxysporum*	*F. oxysporum*	KY/ TN
Roundstone	Alamo	RSA94	*Fusarium* sp.	*Fusarium* sp.	*F. sporotrichioides*	KY/ TN
Roundstone	Alamo	RSA87	*Fusarium* sp.	*Fusarium* sp.	*F. graminearum*	KY/ TN
Roundstone	Alamo	RSA89	*Fusarium* sp.	*Fusarium* sp.	*F. graminearum*	KY/ TN
Roundstone	Blackwell	RSBW89	*Fusarium* sp.	*Fusarium* sp.	*F. graminearum*	KY/ TN
Roundstone	Blackwell	RSBW62	*Fusarium* sp.	*F. graminearum*	*F. graminearum*	KY/ TN
Roundstone	Blackwell	RSBW49	*Fusarium* sp.	*F. foetans*	*F. sporotrichioides*	KY/ TN
Roundstone	Blackwell	RSBW50	*Fusarium* sp.	*F. graminearum*	*F. graminearum*	KY/ TN
Roundstone	Blackwell	RSBW4	*F. graminearum*	*F. graminearum*	nd[b]	KY/ TN
Roundstone	Blackwell	RSBW7	*F. graminearum*	*F. graminearum*	nd[b]	KY/ TN
Roundstone	Blackwell	RSBW8	*F. graminearum*	*F. graminearum*	nd[b]	KY/ TN
Roundstone	Blackwell	RSBW9	*F. graminearum*	*F. graminearum*	nd[b]	KY/ TN
Roundstone	Blackwell	RSBW7	*Fusarium* sp.	*F. graminearum*	*F. graminearum*	KY/ TN
Roundstone	Blackwell	RSBW56	*Fusarium* sp.	*F. graminearum*	*F. graminearum*	KY/ TN
Roundstone	Blackwell	RSBW34	*F. oxysporum*	*F. oxysporum*	nd[b]	KY/ TN
Roundstone	Blackwell	RSBW52	*Fusarium* sp.	*Fusarium* sp.	*F. graminearum*	KY/ TN
Roundstone	Blackwell	RSBW63	*Fusarium* sp.	*Phoma herbarum*	*P. herbarum*	KY/ TN
Roundstone	Kanlow	RSK21	*F. graminearum*	*F. graminearum*	nd[b]	KY/ MO
Sharpe	Cave-N-Rock	SCNR75	*Fusarium* sp.	*F. oxysporum*	*F. oxysporum*	KS/ CO
Sharpe	Kanlow	SK230	*Fusarium* sp.	*F. equiseti*	*F. equiseti*	KS/ CO
Sharpe	Kanlow	SK228	*Fusarium* sp.	*Fusarium* sp.	*F. sporotrichioides*	KS/ CO

Table 2. Continued

Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location	Seed company or field location
Sharpe	Kanlow	SK105	*F. graminearum*	*F. graminearum*	nd[b]	KS/ CO
Sharpe	Kanlow	SK106	*F. graminearum*	*F. graminearum*	nd[b]	KS/ CO
Sharpe	Kanlow	SK116	*F. graminearum*	*F. graminearum*	nd[b]	KS/ CO
Sharpe	Kanlow	SK117	*F. graminearum*	*F. graminearum*	nd[b]	KS/ CO
Sharpe	Kanlow	SK118	*F. graminearum*	*F. graminearum*	nd[b]	KS/ CO
Sharpe	Kanlow	SK121	*F. graminearum*	*F. graminearum*	nd[b]	KS/ CO
Sharpe	Kanlow	SK145	*F. graminearum*	*F. graminearum*	nd[b]	KS/ CO
Sharpe	Kanlow	SK192	*F. graminearum*	*F. graminearum*	nd[b]	KS/ CO
Sharpe	Kanlow	SK193	*F. graminearum*	*F. graminearum*	nd[b]	KS/ CO
Sharpe	Kanlow	SK194	*F. graminearum*	*F. graminearum*	nd[b]	KS/ CO
Sharpe	Kanlow	SK195	*F. graminearum*	*F. graminearum*	nd[b]	KS/ CO
Sharpe	Kanlow	SK197	*F. oxysporum*	*F. oxysporum*	nd[b]	KS/ CO
Turner	Alamo	TA13	*F. graminearum*	*F. graminearum*	*F. graminearum*	TX/ TX
Turner	Blackwell	TBW16	*F. graminearum*	*F. graminearum*	*F. graminearum*	TX/ TX
Turner	Blackwell	TBW17	*F. graminearum*	*F. graminearum*	*F. graminearum*	TX/ TX
Turner	Blackwell	TBW39	*F. graminearum*	*F. graminearum*	*F. graminearum*	TX/ TX
Turner	Blackwell	TBW40	*F. graminearum*	*F. graminearum*	*F. graminearum*	TX/ TX
Turner	Blackwell	TBW41	*F. graminearum*	*F. graminearum*	*F. graminearum*	TX/ TX
Turner	Blackwell	TBW107	*F. graminearum*	*F. graminearum*	*F. graminearum*	TX/ TX
Warner Bros.	Alamo	WA3	*Fusarium* sp.	*F. acuminatum*	*F. acuminatum*	OK/ OK
Warner Bros.	Alamo	WA4	*Fusarium* sp.	*Fusarium* sp.	*F. graminearum*	OK/ OK

[a] This study confirmed the classification of 183 isolates of the original 250 isolates identified in studies by Vu (2011) and Collins

(2016). Isolates AK104, CBW11, ECNR38, and RSBW49 were identified as a species differing from the original identifications. All isolates originally identified as *F. armeniacum* were reclassified as *F. sporotrichioides.*

[b] nd = not determined.

Table 3. Characterization of a microsatellite loci library for *Fusarium equiseti*

	Class of repeat[a]						
	Dinucleotide		Trinucleotide		Tetranucleotide		
Number of microsatellites/	1,396	(40.56%)	1,650	(47.94%)	396	(11.50%)	various
Motif	617	(17.93%)	43	(1.25%)			
		$(AC)_n$ or $(CA)_n$ or $(GT)_n$ or $(TG)_n$		$(ACG)_n$ or $(CGA)_n$ or $(GAC)_n$ or $(CGT)_n$ $(GTC)_n$ or $(TCG)_n$			
	611	(17.75%)	147	(4.27%)			
		$(AG)_n$ or $(GA)_n$ or $(CT)_n$ or $(TC)_n$		$(ATG)_n$ or $(TGA)_n$ or $(GAT)_n$ or $(CAT)_n$ or $(ATC)_n$ or $(TCA)_n$			
	6	(0.74%)	158	(4.59%)			
		$(AT)_n$ or $(TA)_n$		$(AAG)_n$ or $(AGA)_n$ or $(GAA)_n$ or $(CTT)_n$ or $(TTC)_n$ or $(TCT)_n$			
	162	(4.71%)	32	(0.93%)			
		$(CG)_n$ or $(GC)_n$		$(AGT)_n$ or $(GTA)_n$ or $(TAG)_n$ or $(ACT)_n$ or $(CTA)_n$ or $(TAC)_n$			
			3	(0.08%)			
				$(AAT)_n$ or $(ATA)_n$ or $(TAA)_n$ or $(ATT)_n$ or $(TTA)_n$ or $(TAT)_n$			
			93	(2.71%)			
				$(GGC)_n$ or $(GCG)_n$ or $(CGG)_n$ or $(GCC)_n$ or $(CCG)_n$ or $(CGC)_n$			

Table 3. Continued

		Class of repeat[a]		
		Dinucleotide	Trinucleotide	Tetraucleotide
Number of microsatellites/			707 (20.54%)	
Motif			$(AGC)_n$ or $(GCA)_n$ or $(CAG)_n$ or $(GCT)_n$ or $(CTG)_n$ or $(TGC)_n$	
			94 (2.73%)	
			$(AAC)_n$ or $(ACA)_n$ or $(CAA)_n$ or $(GTT)_n$ or $(TTG)_n$ or $(TGT)_n$	
			134 (3.89%)	
			$(AGG)_n$ or $(GAG)_n$ or $(GGA)_n$ or $(CCT)_n$ or $(CTC)_n$ or $(TCC)_n$	
			239 (6.94%)	
			$(CCA)_n$ or $(CAC)_n$ or $(ACC)_n$ or $(TGG)_n$ or $(GTG)_n$ or $(GGT)_n$	
Range of Repeats (n)		6 - 20	6 - 21	4 - 10
Total sequences	98,350			
Identified[a]	3,442			
Perfect[b]	1,800			
Compound[b]	50			

[a] 3,442 unique SSRs identified; duplicated SSRs were not included. [b]Classification of SSR motif as described by Gupta (1996).

Table 4. Characterization of a microsatellite loci library for *Fusarium graminearum*

	Class of repeat[a]						
	Dinucleotide		Trinucleotide		Tetranucleotide		
Number of microsatellites/	5,391	(43.92%)	5,137	(41.85%)	1,746	(14.23%)	various
Motif	2,613	(21.29)	151	(1.2%)			
		$(AC)_n$ or $(CA)_n$ or $(GT)_n$ or $(TG)_n$		$(ACG)_n$ or $(CGA)_n$ or $(GAC)_n$ or $(CGT)_n$ $(GTC)_n$ or $(TCG)_n$			
	2,637	(21.49%)	550	(4.48%)			
		$(AG)_n$ or $(GA)_n$ or $(CT)_n$ or $(TC)_n$		$(ATG)_n$ or $(TGA)_n$ or $(GAT)_n$ or $(CAT)_n$ or $(ATC)_n$ or $(TCA)_n$			
	99	(0.81%)	558	(4.55%)			
		$(AT)_n$ or $(TA)_n$		$(AAG)_n$ or $(AGA)_n$ or $(GAA)_n$ or $(CTT)_n$ or $(TTC)_n$ or $(TCT)_n$			
	42	(0.34%)	105	(0.86%)			
		$(CG)_n$ or $(GC)_n$		$(AGT)_n$ or $(GTA)_n$ or $(TAG)_n$ or $(ACT)_n$ or $(CTA)_n$ or $(TAC)_n$			
			33	(0.27%)			
				$(AAT)_n$ or $(ATA)_n$ or $(TAA)_n$ or $(ATT)_n$ or $(TTA)_n$ or $(TAT)_n$			
			169	(1.38%)			
				$(GGC)_n$ or $(GCG)_n$ or $(CGG)_n$ or $(GCC)_n$ or $(CCG)_n$ or $(CGC)_n$			
			1,900	(15.48%)			

Table 4. Continued

	Class of repeat[a]		
	Dinucleotide	Trinucleotide	Tetranucleotide
Number of microsatellites/	1,900	(15.48%)	
Motif		$(AGC)_n$ or $(GCA)_n$ or $(CAG)_n$ or $(GCT)_n$ or $(CTG)_n$ or $(TGC)_n$	
	352	(2.87%)	
		$(AAC)_n$ or $(ACA)_n$ or $(CAA)_n$ or $(GTT)_n$ or $(TTG)_n$ or $(TGT)_n$	
	424	(3.46%)	
		$(AGG)_n$ or $(GAG)_n$ or $(GGA)_n$ or $(CCT)_n$ or $(CTC)_n$ or $(TCC)_n$	
	895	(7.29%)	
		$(CCA)_n$ or $(CAC)_n$ or $(ACC)_n$ or $(TGG)_n$ or $(GTG)_n$ or $(GGT)_n$	
Range of Repeats (n)	6 - 20	6 - 21	4 - 10
Total sequences	125,011		
Identified[a]	12,274		
Perfect[b]	8,273		
Compound[b]	245		

[a] 12,519 unique SSRs were identified; duplicate SSRs were not included.

[b] Classification of microsatellite motif as described by Gupta (1996).

Table 5. Characterization of a microsatellite loci library for *Fusarium oxysporum*

	Class of repeat[a]						
	Dinucleotide		Trinucleotide		Tetranucleotide		
Number of microsatellites	75	(45.18%)	30	(18.07%)	61	(36.75%)	various
Motif	12	(7.23%)	0	(0%)			
		$(AC)_n$ or $(CA)_n$ or $(GT)_n$ or $(TG)_n$		$(ACG)_n$ or $(CGA)_n$ or $(GAC)_n$ or $(CGT)_n$ $(GTC)_n$ or $(TCG)_n$			
	17	(10.24%)	5	(3.01%)			
		$(AG)_n$ or $(GA)_n$ or $(CT)_n$ or $(TC)_n$		$(ATG)_n$ or $(TGA)_n$ or $(GAT)_n$ or $(CAT)_n$ or $(ATC)_n$ or $(TCA)_n$			
	8	(4.82%)	7	(4.22%)			
		$(AT)_n$ or $(TA)_n$		$(AAG)_n$ or $(AGA)_n$ or $(GAA)_n$ or $(CTT)_n$ or $(TTC)_n$ or $(TCT)_n$			
	38	(22.89%)	2	(1.21%)			
		$(CG)_n$ or $(GC)_n$		$(AGT)_n$ or $(GTA)_n$ or $(TAG)_n$ or $(ACT)_n$ or $(CTA)_n$ or $(TAC)_n$			
			0	(0%)			
				$(AAT)_n$ or $(ATA)_n$ or $(TAA)_n$ or $(ATT)_n$ or $(TTA)_n$ or $(TAT)_n$			
			1	(0.6%)			
				$(GGC)_n$ or $(GCG)_n$ or $(CGG)_n$ or $(GCC)_n$ or $(CCG)_n$ or $(CGC)_n$			

Table 5. Continued

	Class of repeat[a]		
	Dinucleotide	Trinucleotide	Tetranucleotide
Number of microsatellites		8 (4.82%)	
Motif		$(AGC)_n$ or $(GCA)_n$ or $(CAG)_n$ or $(GCT)_n$ or $(CTG)_n$ or $(TGC)_n$	
		2 (1.21%)	
		$(AAC)_n$ or $(ACA)_n$ or $(CAA)_n$ or $(GTT)_n$ or $(TTG)_n$ or $(TGT)_n$	
		4 (2.41%)	
		$(AGG)_n$ or $(GAG)_n$ or $(GGA)_n$ or $(CCT)_n$ or $(CTC)_n$ or $(TCC)_n$	
		1 (0.6%)	
		$(CCA)_n$ or $(CAC)_n$ or $(ACC)_n$ or $(TGG)_n$ or $(GTG)_n$ or $(GGT)_n$	
Range of Repeats (n)	6 - 20	6 - 21	4 - 10
Total sequences	20,923		
Identified[a]	167		
Perfect[b]	166		
Compound[b]	1		

[a] 167 unique SSRs were identified; duplicated SSRs were not included.

[b] Classification of microsatellite motif as described by Gupta (1996).

Table 6. Characterization of a microsatellite loci library for *Fusarium sporotrichioides*

	Class of repeat[a]							
	Dinucleotide		Trinucleotide		Tetranucleotide			
Number of microsatellites	477	(40.35%)	219	(18.53%)	486	(41.12%)	various	
Motif	112	(9.48%)	14	(1.18%)				
		$(AC)_n$ or $(CA)_n$ or $(GT)_n$ or $(TG)_n$		$(ACG)_n$ or $(CGA)_n$ or $(GAC)_n$ or $(CGT)_n$ $(GTC)_n$ or $(TCG)_n$				
	189	(15.99%)	45	(3.81%)				
		$(AG)_n$ or $(GA)_n$ or $(CT)_n$ or $(TC)_n$		$(ATG)_n$ or $(TGA)_n$ or $(GAT)_n$ or $(CAT)_n$ or $(ATC)_n$ or $(TCA)_n$				
	133	(11.25%)	44	(3.72%)				
		$(AT)_n$ or $(TA)_n$		$(AAG)_n$ or $(AGA)_n$ or $(GAA)_n$ or $(CTT)_n$ or $(TTC)_n$ or $(TCT)_n$				
	43	(3.64%)	9	(0.76%)				
		$(CG)_n$ or $(GC)_n$		$(AGT)_n$ or $(GTA)_n$ or $(TAG)_n$ or $(ACT)_n$ or $(CTA)_n$ or $(TAC)_n$				
			10	(8.5%)				
				$(AAT)_n$ or $(ATA)_n$ or $(TAA)_n$ or $(ATT)_n$ or $(TTA)_n$ or $(TAT)_n$				
			13	(1.10%)				
				$(GGC)_n$ or $(GCG)_n$ or $(CGG)_n$ or $(GCC)_n$ or $(CCG)_n$ or $(CGC)_n$				
			26	(2.20%)				
				$(AGC)_n$ or $(GCA)_n$ or $(CAG)_n$ or $(GCT)_n$ or $(CTG)_n$ or $(TGC)_n$				

Table 6. Continued

	Class of repeat[a]			
	Dinucleotide	Trinucleotide		Tetranucleotide
Number of microsatellites		29	(2.45%)	
Motif			$(AAC)_n$ or $(ACA)_n$ or $(CAA)_n$ or $(GTT)_n$ or $(TTG)_n$ or $(TGT)_n$	
		18	(1.52%)	
			$(AGG)_n$ or $(GAG)_n$ or $(GGA)_n$ or $(CCT)_n$ or $(CTC)_n$ or $(TCC)_n$	
		11	(0.93%)	
			$(CCA)_n$ or $(CAC)_n$ or $(ACC)_n$ or $(TGG)_n$ or $(GTG)_n$ or $(GGT)_n$	
Range of Repeats (n)	6 - 20	6 - 20		4 - 13
Total sequences	196			
Identified[a]	1,217			
Perfect[b]	1,182			
Compound[b]	35			

[a] Classification of microsatellite motif as described by Gupta (1996).

[b] 1,182 unique SSRs were identified; duplicated SSRs were not included.

Table 7. *Fusarium graminearum* microsatellite loci amplification analysis

Population[a]	Number of individuals (N)	Number of multilocus genotypes (MLG)	Thompson's diversity index (H)	Expected haploid diversity (Hexp)	rbard
1	29	29	3.37	0.67	0.10 NS

[a] Values are based on characterization of 30 isolates of *F. graminearum.*

Table 8. *Fusarium oxysporum* microsatellite loci amplification analysis

Population[a]	Number of individuals (N)	Number of multilocus genotypes (MLG)	Thompson's diversity index (H)	Expected haploid diversity (Hexp)	rbard
1	24	24	3.18	0.62	0.05 *

[a] Values are based on characterization of 28 isolates of *F. oxysporum.*

Table 9. *Fusarium sporotrichioides* microsatellite loci amplification analysis

Population[a]	Number of individuals (N)	Number of multilocus genotypes (MLG)	Thompson's diversity index (H)	Expected haploid diversity (Hexp)	rbard
1	14	14	2.64	0.57	0.06 NS

[a] Values are based on characterization of 14 isolates of *F. sporotrichioides.*

Table 10. Characterization of microsatellite loci developed from *Fusarium equiseti*

SSR ID/Locus	Motif	Lab ID	Primer sequences (5'-3')	Allele size range (bp)[a]	Number of alleles	GenBank Accession No.
80755_ssr88	$(CA)_6$	Fe001	F: GTCACGCTGATTTGGAGTTCC	128 - 138	5	MG751108
			R: ACAGTTGAAGTTGAGTTGGGC			
102416_ssr193	$(GT)_6$	Fe002	F: GTTGTATGTGCGGTTGTGAGG	175 - 177	1	MG751109
			R: AGAGTCATCGGACAAGACTGC			
64739_ssr42	$(CA)_6$	Fe003	F: ACCACTCACTAACGATTCAATTCG	201 - 202	1	MG751110
			R: AACAGTTGCGACAGTTTCAGC			
16589_ssr120	$(TG)_6$	Fe004	F: TCACACGTACTAGCCAACAGC	315 - 317	1	MG751111
			R: CTCGGCGAGAAGATTTCATGC			
57389_ssr96	$(GT)_6$	Fe005	F: ATGCTACTGATGTGAGCTGCC	149 - 153	2	MG751112
			R: GTTTCGAAACTCAGCGTTTGC			
35477_ssr28810	$(GC)_6$	Fe006	F: CCTTCACCTACGACTACGAGC	110 - 111	1	MG751113
			R: CATTCGATCTTCTCCATGCGC			
60702_ssr109	$(TG)_6$	Fe008	F: CCACTTTCTCGCTCTCACTCC	202 - 204	2	MG751115
			R: CTCGTCTTTGCCAATCTTCGC			
44818_ssr35	$(GT)_7$	Fe009	F: TGACTAGAGGATAAGATAAGACGC	333 - 342	6	MG751116
			R: TCTTAACGTGGACACACCTGG			
83722_ssr662	$(GC)_6$	Fe010	F: TGCCCTTATCGAAGAAGGTCG	222	1	MG751117
			R: TAAAGACAACGCCACACTTGC			

Table 10. Continued

SSR ID/Locus	Motif	Lab ID	Primer sequences (5'-3')	Allele size range (bp)[a]	Number of alleles	GenBank Accession No.
73062_ssr652	$(GC)_6$	Fe011	F: ACGGCTTCGGAGATCAATTCG	457	1	MG751118
			R: ATGTCAACTTCAACACCGTGC			
6490_ssr260	$(CA)_6$	Fe012	F: GATCAATCAGTTCGTGGTGGC	100	1	MG751119
			R: CAATGGCGGAATCTTGCAGG			
50895_ssr46	$(TC)_6$	Fe013	F: TTACTGGTTCACTTTATGAGGG	136 - 137	1	MG751120
			R: CACTTTATCCTTCAGCGCTCG			
85455_ssr87	$(GT)_6$	Fe015	F: AGTAACAGTGACAGGGCACG	200	1	MG751122
			R: GTTGTGCCCTGTGTGTAAAGC			
108464_ssr136	$(CCA)_6$	Fe016	F: ACGATGACGACGATGAAGAGG	103	1	MG751114
			R: TGCTGGCCAGTGCACTTTCC			

[a] Range is based on six isolates of *F. equiseti*.

Table 11. Characterization of microsatellite loci developed from *Fusarium graminearum*

SSR ID/Locus	Motif	Lab ID	Primer sequences (5'-3')	Allele size range (bp)[a]	Number of alleles	GenBank Accession No.
126678_ssr468	$(TG)_6$	Fg008	F: GGGTGTCACAACGATTTCAGC	472 – 491	6	MG751123
			R: AATCAACTGAACCACACTGCG			
51570_ssr33	$(TG)_6$	Fg011	F: AATACACCGTCACTCTGTCC	183 – 395	5	[b]
			R: CCTTATCCCTCCACAGCATCC			
25706_ssr50835	$(GA)_7$	Fg012	F: GAAATGGCATCCCTCAAGTGC	233 – 390	7	MG751124
			R: AGTCCATCCATTCATCCAGCC			
8080_ssr150	$(CA)_6$	Fg013	F: TTCCCACTGTATTTCGGTCGG	293 – 412	8	MG751125
			R: CAGTAGTCATGTACGGCGACC			
109690_ssr181	$(AG)_8$	Fg014	F: ACTCTCTGGAAACACTAGCCG	216 – 231	7	MG751126
			R: TGATGCTCATCTCCACACTGG			
115947_ssr948	$(AG)_6$	Fg015	F: GTAACCTCGAAGTAGGTGAGC	312 – 337	7	MG751127
			R: AGGCTTAGTGTAAATCAACGG			
63114_ssr646	$(AAG)_6$	Fg017	F: AACTCTGGTGAATGGTGGTCG	227 – 260	7	MG751128
			R: AATTGCATCAACCGGAAGTGC			
137751_ssr513	$(GCA)_6$	Fg026	F: AGCACTGGAAGGTTGATCAGG	249 – 261	4	MG751129
			R: CACTGTCGCCAGCTAATTACC			
68771_ssr230	$(AAG)_6$	Fg033	F: AGTCTAAACAAGTAGTCGAGCGG	110 – 173	4	MG751130
			R: GTAAGCGGTTTGAGAGAAGGC			

Table 11. Continued

SSR ID/Locus	Motif	Lab ID	Primer sequences (5'-3')	Allele size range (bp)[a]	Number of alleles	GenBank Accession No.
125455_ssr245	$(ACA)_6$	Fg034	F: TCACTGTTCACCATCACCACC	217 – 286	2	MG751131
			R: CTACTTGCGTTGTTCTTGGCG			
95632_ssr69	$(AAT)_6$	Fg035	F: TGACTGATATGGGAGTGAAAGC	121 – 127	2	MG751132
			R: TGTTACATGAATTGGGCAGGC			
85967_ssr182	$(ACC)_6$	Fg036	F: ATGATGAATGGTAGGGCGAGG	162 – 167	3	MG751133
			R: GCCTCATCGAGAAGAACCAGG			
6187_ssr58	$(ACG)_6$	Fg037	F: AGATGACTGGGAGCACAATGG	99 – 105	2	MG751134
			R: TGCCAGAATGATGATCGATGG			
51450_ssr173	$(AGG)_7$	Fg041	F: GTGGTAGTGGTCAGAAGTGGG	119 – 122	2	MG751135
			R: CGATATCAGCCACAGAGGTCC			
120532_ssr92	$(ACT)_6$	Fg043	F: AAGGTGGAAAGTGGGTGTAGG	113 – 325	5	MG751136
			R: GCTGTTGGATCAATCTGCACC			
124551_ssr42	$(AG)_6$	Fg047	F: AGACTAACAATGACAAAGCAGG	100 – 111	6	MG751137
			R: AGAATCTTTGTTGTCGATGGCC			
44663_ssr45	$(GT)_8$	Fg052	F: GTCTATTTGTGCTGGTGCTGG	173 – 190	3	MG751138
			R: TTCCTTGCCTGACTGTCATCC			
118230_ssr158	$(TG)_6$	Fg056	F: ACTAGACAAAGGTGCTGAGCG	113 – 247	4	MG751139
			R: CCATTGTTACCTGCTGCTGC			

Table 11. Continued

SSR ID/Locus	Motif	Lab ID	Primer sequences (5'-3')	Allele size range (bp)[a]	Number of alleles	GenBank Accession No.
105063_ssr581	$(TG)_6$	Fg057	F: CAGAGAAGCAGCAAAGTGAGC	185 – 190	3	MG751140
			R: GGCAGCAGTGACATGTTACC			
60310_ssr87	$(TG)_6$	Fg059	F: AAGGGAGTGGGAGTGACTGAGG	137 – 139	2	[b]
			R: AATGAGATCAAGTGTGTGCGC			

[a] Range is based on 30 isolates of *F. graminearum.*

[b] *awaiting accession numbers*

Table 12. Characterization of 30 microsatellite loci developed from *F. oxysporum*

SSR ID/Locus[a]	Motif	Lab ID	Primer sequences (5'-3')	Allele size range (bp)[a]	Number of alleles	GenBank Accession No.
20469_ssr24452	$(GC)_6$	Fo001	F: TATTGCACCAGCGTGTCTTCG	115	1	MG751158
			R: GACCTGTATTTCCGCATTGCC			
8125_ssr25189	$(GC)_6$	Fo005	F: CCGAGATCTATTCGGTGGACG	315 - 320	3	MG751161
			R: TTTCGGACTCAATCACCTCGC			
5262_ssr128	$(AG)_6$	Fo006	F: AGAACAGGTACTCAATGGCCC	233 - 263	6	MG751162
			R: AAAGGCAGCCGAGAGATATGG			
21652_ssr55814	$(GC)_6$	Fo008	F: GTCTTCTCGGTACTGGATGCG	236 - 300	6	MG751163
			R: AGGGCATCGACGTGTATTTCG			
6619_ssr6366	$(GC)_6$	Fo010	F: GGACAGATCTTTCGGTGGAGG	340 - 344	5	MG751164
			R: TAGTGCATCGAGAAGCCATCG			
14016_ssr786	$(CG)_6$	Fo012	F: GGTCCGTATCCATTAGCTCCG	352 - 358	3	MG751165
			R: GCCAAAGATGACAATGACGGG			
17082_ssr124626	$(GC)_6$	Fo013	F: GGTCATTGTCGTGGTTCTGGG	242 - 254	6	MG751166
			R: GAACGGCGTTCTTCATCATCG			
14159_ssr19216	$(CG)_6$	Fo014	F: CTCTCATGCTGATGCTGACCG	299 - 305	7	MG751167
			R: GAGATCGATACCCATGACGCG			
17707_ssr74	$(TGC)_7$	Fo017	F: ATAAGACCAGCTGATCTCACG	86 - 107	5	MG751168
			R: GAGCAGAACATCAATAGCGGC			

Table 12. Continued

SSR ID/Locus[a]	Motif	Lab ID	Primer sequences (5'-3')	Allele size range (bp)[a]	Number of alleles	GenBank Accession No.
21937_ssr917	$(TCA)_8$	Fo019	F: CGTCCTCTATGATCCCAACCG	318 - 329	5	MG751169
			R: GATGCAGCCGTGTTAATGTCG			
3266_ssr560	$(AAC)_8$	Fo021	F: AACTATGGTGCCCGTCAATCC	330 - 353	7	MG751170
			R: AGGAACCTCATGACCAGAAGC			
13139_ssr93	$(AGC)_6$	Fo024	F: CAGCACGAACTATTAACGGCG	173 - 186	4	MG751171
			R: CTATCGATTCCCAGCTCCTCC			
20656_ssr631	$(TGC)_7$	Fo025	F: AGACATGGGCTGAGAACATCC	345 - 360	3	MG751172
			R: TACCCGAATTGACCGATCTCG			

[a] Range is based on 24 isolates of *F. oxysporum*.

Table 13. Characteristics of 30 microsatellite loci developed from *F. sporotrichioides* and transferability to related species

SSR ID/Locus[a]	Motif	Lab ID	Primer Sequences (5'-3')	Allele size range (bp)[a]	Number of alleles	GenBank Accession No.
15946_ssr620	$(CT)_{10}$	Fr001	F: CGAATCTCACGGTACTCCTCC	340 - 349	3	MG751143
			R: GGTACTCTGTACGTACTGCGG			
8975_ssr76719	$(AT)_{11}$	Fr002	F: TCTCACCGACAACAAGAACCC	298 - 316	5	MG751144
		Fr002	R: CCAACGGTGAATACTCCTCCC			
8797_ssr21260	$(CT)_{7}$	Fr003	F: GCAGTCGAGATAGAGCTACGG	129 - 246	5	MG751145
		Fr003	R: ACAACCATGACATCGAGACCC			
5186_ssr56752	$(CA)_{11}$	Fr004	F: GTATATGGCGACTCCGAGTGG	381 - 389	3	MG751146
			R: TCTGATCTTCAAGTCCGTCCG			
14709_ssr38221	$(TG)_{6}$	Fr005	F: CAACCTGTTTCACCTCAGTGC	254 - 256	2	MG751147
			R: GTTCAACACAAGACACGAGGC			
9894_ssr2726	$(TG)_{8}$	Fr011	F: TGAAGTGAAGTGAGAGGCTGC	0	4	MG751152
			R: GACCCTTAGCTCATCCATCCG			
18060_ssr8144	$(TA)_{6}$	Fr012	F: ACTTAGGGTATATAGCAGTAGCCC	0	5	MG751153
			R: ATAGGATATTGAGCCCGACCC			
8975_ssr73658	$(CT)_{9}$	Fr013	F: AAACGACCCGTACATCTTCGG	360 - 366	3	MG751154
			R: GAGGAGGGCATCATTACCAGG			
13559_ssr48129	$(GA)_{10}$	Fr014	F: CGTAGCACTTGCCATTATGCC	235 - 330	5	MG751155
			R: ATTGACGTCAACATTGGTCGC			

Table 13. Continued

SSR ID/Locus[a]	Motif	Lab ID	Primer Sequences (5'-3')	Allele size range (bp)[a]	Number of alleles	GenBank Accession No.
17172_ssr3508	$(GAA)_8$	Fr015	F: TGACGTCAAATGTGAGGGAGG	200 - 222	5	MG751156
			R: CACCTCACTTCACCTCAGACC			
23251_ssr61642	$(TTA)_7$	Fr016	F: CTGGATGAGATTTGGAACGGC	356 - 358	2	MG751157
			R: AAGAAGCTCCAAACCGATCCC			

[a] Range is based on 14 isolates of *F. sporotrichioides*.

Table 14. Summary of PCR amplification of designed microsatellite loci across four *Fusarium* species

Species	Number of fungal isolates (n)	Number of loci designed	Number of loci that amplified	Number of polymorphic loci	Number of loci used for cross amplification
F. equiseti	6	30	28	*sample too small	0
F. graminearum	28	60	25	19	12
F. oxysporum	24	30	29	14	9
F. sporotrichioides	14[a]	30	30	14	12

[a] Two isolates were collected from diseased cornelian cherry leaves and twelve were from switchgrass seeds.

Table 15. Cross amplification of microsatelite loci from *Fusarium graminearum* derived primers to isolates of other *Fusarium* species

	Primer[a]											
	Fg033	Fg034	Fg035	Fg036	Fg037	Fg041	Fg043	Fg047	Fg052	Fg056	Fg057	Fg059
Species/ isolate	Amplicon size[b]											
Fusarium oxysporum												
ACNR140	-	-	-	-	-	-	-	-	-	-	-	-
ACNR128	174	103	165	-	123	123	142	99	187	242	-	138
ACNR138	-	-	-	-	-	-	-	-	-	196	-	-
ACNR139	-	-	-	265	-	-	-	-	-	110	-	-
ACNR141	127	103	116	265	123	119	133	99	185	116	-	137
ACNR176	-	-	-	261	-	-	133	-	-	107	-	-
ACNR178	-	103	165	-	123	119	139	101	186	116	-	138
ACNR184	-	-	-	-	-	-	-	-	-	-	-	-
MilanO	174	-	169	-	-	-	-	-	-	110	-	138
TBW41	174	103	169	-	-	215	-	-	189	259	-	-
CBW11	174	121	169	-	-	215	-	-	-	107	-	-
ECNR140	-	103	116	261	123	116	132	98	185	116	-	135
RSA3	-	-	114	261	-	-	109	-	179	-	-	136
RSA4	174	103	169	261	123	119	139	-	187	125	-	-
RSA5	127	103	116	261	123	119	139	101	186	116	-	136
RSA43	-	103	116	166	123	119	133	101	186	116	-	136
RSA51	174	103	169	310	-	-	-	-	-	102	-	-
JC3	174	103	169	166	-	-	-	-	-	108	-	-
JC4	176	-	174	-	-	-	-	-	-	96	-	-
JC6	174	-	-	-	-	-	-	-	-	-	-	-
JC11	-	-	-	122	-	-	-	-	-	-	-	-
JC12	174	-	169	-	-	-	-	109	-	-	-	-
JC71	-	-	-	-	-	-	-	142	-	-	-	-
JC151	-	-	-	-	-	-	-	-	-	-	-	-

Table 15. Continued

	Primer[a]											
	Fg033	Fg034	Fg035	Fg036	Fg037	Fg041	Fg043	Fg047	Fg052	Fg056	Fg057	Fg059
Species/ isolate	Amplicon size[b]											
Fusarium sporotrichioides												
AK104	-	99	123	116	104	-	106	104	-	-	101	104
CR	111	-	-	108	167	-	-	-	190	-	-	-
CW	-	-	-	-	-	-	156	-	-	187	-	-
RSA90	137	-	-	165	186	121	124	265	122	-	101	-
RSA91	131	99	186	116	184	121	115	265	122	164	101	132
RSA95	-	-	186	120	-	-	-	-	-	164	-	-
RSA96	137	99	186	116	187	121	115	265	122	164	101	139
RSA100	137	99	186	116	184	121	115	265	122	188	101	-
RSA101	131	99	186	116	184	121	115	265	122	164	101	137
RSA117	106	-	-	108	-	-	171	-	-	-	110	-
RSBW49	173	102	169	265	-	-	171	188	-	107	110	-
JC1	-	-	-	-	-	-	112	127	-	-	-	137
SK228	-	-	131	111	-	-	-	-	-	187	-	-
SK230	-	99	-	-	-	-	109	-	-	187	-	-
Fusarium graminearum												
AK60	173	-	-	-	-	-	319	111	-	247	188	-
CA12	-	286	-	-	102	-	-	104	183	119	188	137
CCNR127	117	-	-	167	-	119	142	102	190	119	188	137
ECNR39	-	268	121	-	102	-	319	100	-	113	-	137
ECNR93	-	-	-	-	-	-	319	102	190	119	188	137
ECNR109	110	268	121	-	99	-	319	-	-	113	-	-
ECNR120	117	-	-	-	-	-	319	100	190	119	-	-
ECNR127	173	-	-	-	-	-	319	110	-	233	-	-
ECNR130	173	217	-	-	105	-	-	100	-	113	-	139
ECNR131	173	-	-	-	-	-	-	110	-	247	-	-

Table 15. Continued

	Primer[a]											
	Fg033	Fg034	Fg035	Fg036	Fg037	Fg041	Fg043	Fg047	Fg052	Fg056	Fg057	Fg059
	Amplicon size[b]											
ECNR136	173	256	-	-	102	-	319	102	190	233	185	-
ECNR139	-	256	-	-	102	-	-	100	190	233	-	-
EA61	-	286	121	167	99	-	133	102	190	-	188	137
EA64	-	-	-	-	-	-	-	-	-	-	-	-
EA67	-	268	121	-	102	-	139	102	-	119	-	-
JC140	126	268	127	167	102	-	325	102	183	119	188	-
JC144	117	268	127	167	105	12	142	104	190	119	190	139
JC148	117	268	121	167	102	119	139	100	190	119	188	137
MilanG	117	268	127	167	105	122	142	105	190	119	188	139
RSA4	117	-	-	162	-	122	142	105	190	119	188	139
RSA87	110	-	-	-	-	-	-	102	-	-	-	-
RSA89	110	268	-	-	-	-	-	-	-	-	-	-
RSBW36	-	-	-	-	-	-	325	-	-	233	-	-
RSBW52	117	268	121	167	105	-	133	100	183	119	185	139
RSBW56	-	-	-	-	105	-	-	102	-	-	-	137
RSBW63	-	-	-	-	102	-	319	-	-	113	-	-
TBW16	117	268	121	167	102	119	133	102	190	119	188	137
TBW17	117	-	121	167	99	-	319	100	-	119	188	137
TBW39	-	-	121	167	102	-	319	100	173	119	188	137
WA4	-	-	-	-	-	-	-	104	-	-	-	-

[a] Primer codes located in Table 11.

[b] Numbers equal amplicon size in base pairs; (-) indicates no amplification.

Table 16. Cross amplification of loci from *Fusarium oxysporum* derived primers to isolates of other *Fusarium* species

	Primer[a]									
	Fo001	Fo005	Fo006	Fo008	Fo012	Fo017	Fo019	Fo021	Fo024	Fo025
Species/ isolate	Amplicon size[b]									
Fusarium oxysporum										
ACNR128	153	314	233	287	352	93	329	345	184	357
ACNR138	153	316	233	295	352	93	326	345	184	357
ACNR139	153	318	261	287	352	86	329	330	173	345
ACNR140	153	316	261	287	352	86	329	330	173	345
ACNR141	153	318	-	287	352	86	329	330	173	-
ACNR176	153	316	237	290	352	102	326	348	182	357
ACNR178	153	316	235	287	352	102	326	348	182	357
ACNR184	153	-	237	298	352	102	326	348	184	357
MilanO	153	320	235	236	355	107	-	339	184	360
TBW41	153	318	235	298	352	105	320	336	184	357
CBW11	153	316	235	298	352	105	318	336	184	357
ECNR140	153	314	249	295	352	105	-	336	-	345
RSA3	153	314	237	295	352	86	-	336	173	345
RSA4	153	314	263	295	352	86	323	330	173	345
RSA5	153	314	261	295	352	86	-	330	173	345
RSA43	153	314	235	295	352	105	318	336	182	357
RSA51	-	314	237	295	352	105	318	336	184	357
JC3	153	316	233	298	352	102	323	339	182	357
JC4	153	318	235	298	352	105	320	336	184	357
JC6	153	320	-	298	358	-	-	353	186	357
JC11	153	-	237	290	355	102	329	341	182	357
JC12	153	316	235	298	352	102	326	339	182	357
JC71	153	316	235	298	352	105	320	336	-	357
JC151	153	314	235	300	352	102	326	348	182	360

Table 16. Continued

	Primer[a]									
	Fo001	Fo005	Fo006	Fo008	Fo012	Fo017	Fo019	Fo021	Fo024	Fo025
Species/ isolate	Amplicon size[b]									
Fusarium sporotrichioides										
AK104	-	-	-	-	-	-	-	-	-	-
CR	-	-	-	-	-	-	-	-	-	-
CW	-	-	-	-	-	-	-	-	-	-
RSA90	153	-	-	-	-	-	-	-	-	-
RSA91	153	-	-	-	-	-	-	-	-	-
RSA95	-	-	-	-	-	-	323	-	269	361
RSA96	153	-	-	-	-	-	-	-	-	-
RSA100	153	-	-	-	-	-	323	339	184	361
RSA101	153	-	-	-	-	-	-	339	184	361
RSA117	-	-	-	-	-	-	-	-	-	-
RSBW49	153	-	-	-	-	-	-	-	-	-
JC1	-	-	-	-	-	-	-	339	186	361
SK228	-	-	-	-	-	-	-	-	-	-
SK230	-	319	-	293	-	-	-	-	-	-
Fusarium graminearum										
AK60	153	318	237	291	256	-	-	354	-	-
CA12	153	320	237	291	358	122	-	-	194	-
CCNR127	153	318	236	290	356	122	-	-	194	364
ECNR39	-	-	-	-	-	-	-	-	-	374
ECNR93	153	318	-	291	-	-	-	-	-	-
ECNR109	-	320	-	293	-	-	-	-	-	-
ECNR120	153	318	236	290	356	119	330	-	194	-
ECNR127	153	318	236	291	356	122	330	341	194	-
ECNR130	153	318	237	291	356	122	-	-	-	-
ECNR131	153	318	-	297	-	104	-	-	-	-
ECNR136	153	318	236	290	356	-	-	-	-	-

Table 16. Continued

	Primer[a]									
	Fo001	Fo005	Fo006	Fo008	Fo012	Fo017	Fo019	Fo021	Fo024	Fo025
Species/ isolate	Amplicon size[b]									
ECNR139	153	318	237	290	-	-	-	-	-	-
EA61	153	318	-	291	-	-	-	-	-	-
EA64	153	322	-	291	-	119	-	-	-	-
EA67	153	318	236	290	-	119	337	-	-	-
JC140	153	320	-	291	-	-	-	-	-	-
JC144	156	322	-	293	-	-	-	-	-	-
JC148	153	318	237	291	358	116	330	-	-	-
MilanG	156	322	239	293	359	122	-	-	199	368
RSA4	156	322	-	293	359	119	-	347	189	368
RSA87	156	322	-	293	-	155	-	-	-	352
RSA89	154	320	-	293	371	162	-	-	-	350
RSBW36	-	322	-	291	358	122	-	-	196	364
RSBW52	152	218	-	291	356	122	-	-	-	-
RSBW56	153	218	-	291	-	-	-	-	-	374
RSBW63	153	320	293	-	-	-	-	-	-	-
TBW16	153	320	236	290	356	122	-	-	194	-
TBW17	153	218	-	291	-	-	-	-	-	-
TBW39	153	218	236	291	356	122	-	-	-	374
WA4	154	320	239	291	-	122	-	-	-	-

[a] Primer codes located in Tables 12.

[b] Numbers equal amplicon size in base pairs; (-) indicates no amplification.

Table 17. Cross amplification of loci from *Fusarium sporotrichioides* derived primers to isolates of other *Fusarium* species

	Primer[a]											
	Fr001	Fr002	Fr003	Fr004	Fr005	Fr006	Fr007	Fr009	Fr013	Fr014	Fr015	Fr016
Species/ isolate	Amplicon size[b]											
Fusarium oxysporum												
ACNR140	203	-	-	-	-	359	-	-	-	-	218	-
ACNR128	-	-	-	-	-	-	-	248	364	256	-	
ACNR138	344	303	136	387	261	-	314	-	256	365	268	328
ACNR139	-	-	136	388	261	-	-	-	-	-	328	-
ACNR141	344	-	-	-	261	-	-	-	206	-	-	-
ACNR176	-	-	-	-	-	-	-	-	290	364	-	-
ACNR178	185	-	136	-	261	-	-	-	249	362	-	328
ACNR184	-	-	-	-	-	-	-	-	-	-	251	-
MilanO	344	301	137	388	261	-	315	-	249	362	-	328
TBW41	-	-	137	387	288	-	315	-	248	364	-	-
CBW11	-	-	-	-	-	-	-	-	248	-	-	-
ECNR140	-	-	-	388	-	-	-	-	248	-	-	-
RSA3	349	-	-	-	-	-	-	-	260	-	267	-
RSA4	-	-	-	-	-	-	-	-	247	-	-	328
RSA5	-	-	136	386	-	-	314	-	248	364	207	328
RSA43	345	301	136	386	288	-	314	308	243	366	208	328
RSA51	339	-	-	-	-	-	-	-	248	-	-	328
JC3	-	301	136	389	288	-	314	307	248	363	207	328
JC4	-	-	139	371	284	-	-	-	249	342	251	351
JC6	-	300	-	-	261	-	-	-	249	-	249	-
JC11	-	-	-		-	-	314	-	-	-	-	-
JC12		-	-	-	-	-	-	-	248	-	248	-
JC71	-	-	-	-	-	-	219	-	249	352	-	184
JC151	-	-	-	-	-	-	-	-	-	-	-	-

Table 17. Continued

	Primer[a]											
	Fr001	Fr002	Fr003	Fr004	Fr005	Fr006	Fr007	Fr009	Fr013	Fr014	Fr015	Fr016
Species/ isolate	Amplicon size[b]											
Fusarium sporotrichioides												
AK104	340	308	-	-	-	362	-	-	-	303	-	-
CR		298	129	381	256	353	314	-	-	308	200	356
CW	346	303	138	389	-	361	320	313	362	325	222	358
RSA90	346	308	138	387	254	-	320	313	362	325	209	358
RSA91	346	308	-	387	254	-	320	311	360	327	209	358
RSA95	346	314	138	387	254	361	-	311	362	327	209	258
RSA96	346	316	138	387	254	361	320	314	360	327	207	358
RSA100	346	303	138	387	256	-	320	313	362	325	209	358
RSA101	346	303	146	387	254	-	-	314	366	327	209	358
RSA117	346	316	138	387	254	361	320	314	360	327	207	358
RSBW49	349	308	138	387	254	361	320	313	360	327	209	358
JC1	340	303	138	387	-	362	321	313	360	327	209	358
SK228	340	308	-	-	-	362	-	-	-	330	-	-
SK230	340	303	141	381	-	-	-	-	366	325	209	-
Fusarium graminearum												
AK60	-	242	-	-	255	359	318	308	359	344	-	-
CA12		-	132	386	242	351	332	-	-	-	-	-
CCNR127	-	-	132	385	241	359	-	305	356	-	-	-
ECNR39	372	-	136	221	243	-	-	-	-	-	-	-
ECNR93	-	360	135	363	243	235	360	-	-	342	-	-
ECNR109	-	-	135	362	243	351	-	340	-	-	163	254
ECNR120	-	-	148	-	244	-	-	-	-	-	-	-
ECNR127	-	-	136	363	245	-	360	-	-	343	-	-
ECNR130	-	380	135	363	243	-	-	-	-	-	-	-
ECNR131	-	-	136	363	243	-	-	-	358	-	-	-

Table 17. Continued

	Primer[a]											
	Fr001	Fr002	Fr003	Fr004	Fr005	Fr006	Fr007	Fr009	Fr013	Fr014	Fr015	Fr016
Species/ isolate	Amplicon size[b]											
ECNR136	-	-	-	-	-	-	358	-	-	-	-	-
ECNR139	-	-	149	-	244	-	-	-	-	-	-	254
EA61	-	-	132	-	-	-	310	-	-	359	-	-
EA64	-	-	-	-	-	-	-	-	-	360	-	254-
EA67	374	-	132	-	242	350	-	-	-	-	-	-
JC140	-	-	134	386	-	352	-	-	-	-	-	-
JC144	-	316	139	386	256	362	320	315	362	-	216	-
JC148	358	-	133	386	243	-	-	-	-	-	-	-
MilanG	348	310	139	387	256	361	320	315	362	330	-	-
RSA4	-	-	134	387	243	-	332	-	254	-	-	-
RSA87	352	308	139	386	256	361	320	315	-	-	-	-
RSA89	250	307	138	384	255	359	320	312	264	-	219	-
RSBW36	-	-	-	-	-	-	325	306	357	-	218	226
RSBW52	374	-	135	362	243	350	-	-	-	-	163	-
RSBW56	374	-	144	-	255	235	-	-	357	-	218	-
RSBW63	-	-	149	-	255	-	-	-	-358	-	219	-
TBW16	117	268	121	167	102	119	133	102	190	119	188	137
TBW17	117	-	121	167	99	-	319	100	-	119	188	137
TBW39	-	-	121	167	102	-	319	100	173	119	188	137
WA4	-	-	-	-	-	-	-		-	-	-	-

[a] Primer codes located in Table 13.

[b] Numbers equal amplicon size in base pairs; (-) indicates no amplification

Cornelian Cherry as a Potential Specialty Crop in Eastern Tennessee

Abstract

In 2016, trees of cornelian cherry, ‘Aurea’, ‘Golden Glory’, ‘Pioneer’, ‘Sunrise’, ‘Variegated’, and ‘Yellow Fruit’ were planted at The University of Tennessee Forest Resources AgResearch and Education Center in Oak Ridge, TN. ‘Pioneer’, ‘Red Dawn’, ‘Red Stone’, ‘Sunrise’, ‘Variegated’, and ‘Yellow Fruit’ were planted at The University of Tennessee Plateau Research and Education Center in Crossville, TN. Trees were planted in a random block design, 13 ft apart, using a 24-in auger. In April 2017, trees were planted to replace those that did not survive through the 2016 growing season. At the Oak Ridge site, three ‘Aurea’, three ‘Pioneer’, one ‘Sunrise’, and all six ‘Variegated’ trees were replaced. All the original trees planted at the Crossville location survived the 2016 and 2017 growing seasons. Six additional trees of ‘Elegant’ were added to both sites in April 2017. For each site, height, trunk diameter, and percent survival of trees were calculated for the 2016 and 2017 growing seasons. Following the 2016 growing season, none of the ‘Variegated’ trees survived at the Oak Ridge site due to full sun exposure, high temperatures, small tree size, high wind, and insufficient water. Insect pests included mites, scales, thrips, and lepidopteran insects, which have potential to cause significant damage in large numbers on small trees specifically in a greenhouse setting. Beneficial arachnid species, predatory beetles, mantis, and various pollinators were observed on the trees in the field. Twenty unique fungi were isolated from leaves with disease symptoms. The fungi were grown in pure culture and screened for pathogenicity in a detached leaf assay. Six fungi caused symptoms on detached leaves, however, only two fungi caused disease in both the screening assay and whole plant

assay. Koch's postulates were completed for those two fungi: *Botrytis cinerea* and *Fusarium sporotrichioides*, which confirmed that they are pathogens on cornelian cherry dogwoods in east Tennessee.

Research Objectives and Justifications

This research had the following main objectives:

Objective 1: Determine stem diameter, height, and survival rate of different cornelian cherry cultivars grown in Oak Ridge and Crossville, TN over a period of two years. *Justification*: Determining growth rate and survivability of cornelian cherry cultivars will provide growers with the information needed to determine if growing these trees in Tennessee is feasible for their crop management plan.

Objective 2: Characterize and identify plant pathogens and pests of cornelian cherry dogwood isolated from saplings grown in eastern Tennessee, using classical morphology and molecular methods. *Justification*: Identification of cornelian cherry pathogens and pests is essential for disease and pest prevention and treatment of infected trees in order for growers to successfully produce this specialty crop.

Introduction

There are approximately 58 species of dogwood (*Cornus*) embodying a morphologically diverse group of predominantly woody trees and shrubs (XIANG, 2006). They are widely distributed throughout the temperate regions of the northern hemisphere, originating in eastern Asia, eastern and northwest North America, and the mountains of Central America, South America, and east Africa (AL-HATMI *et al.* 2017). Dogwoods are divided

by molecular data into two genera that diverged from a common ancestor. There is a blue- or white-fruited group containing the U.S. native northern swamp dogwood *C. racemosa,* and the red-fruited group containing the popular flowering dogwood (*Cornus florida*) and the European cornelian cherry dogwood (*Cornus mas*) (XIANG *et al.* 1996; XIANG et al. 1998; FAN AND XIANG 2001).

Cornelian cherry dogwoods are native to Europe and Southwest Asia (MARKOVIC *et al.* 2014). In the native range and areas of Serbia and Turkey, these trees are cultivated for their edible fruit, wood, and aesthetic value. Wild type plants develop from seedlings and these genotypes vary considerably in time to maturity, fruit size, color, shape, taste, and the nutrient value of fruits. The fruit generally contains a single seed, is oval to oblong, and is 10- to 20-mm long (MARKOVIC *et al.* 2014). Average fruit weight ranges from 5.0 to 8.0 g with the stone comprising 7.5 to 11.0% of the total fruit weight (KLIMENKO 2004). These trees can be found up to an altitude of 1300 m and grow best on medium-deep limestone soils. Cornelian cherry is tolerant of high levels of air pollution and varying levels of soil pH ranging from slightly acidic, to neutral, to alkaline. It grows on different soil types, from sandy to clay, and shallow soils. Many cornelian cherry dogwood cultivars are also drought-resistant. Cornelian cherry trees suitable for hedges, anti-erosion protection, and bonsai growth, and are an important pollination source because they bloom in early spring (DOKUPIL AND ŘEZNIČEK 2012).

Cornelian cherries were historically gathered from bushes growing in nature. The wild type bushes bear irregular yields of small, dry fruits, especially in times of drought (KLIMENKO 2004). Cornelian cherry can be propagated using generative or vegetative methods (MARKOVIC *et al.* 2014). Generative propagation is difficult as the seeds have a

complex dormancy pattern. Germination occurs during the second or third spring after seed dispersal. The seeds must be separated from the pulp of the fruits because the pulp contains germination inhibitors. Stratification includes 12 to 20 weeks of warm and 6 to 16 weeks of cold temperatures. Even after these temperature treatments, seed germination is only 50 to 60% (PIOTTO AND DI NOI 2003). Vegetative propagation is typically implemented by cuttings, sprouts, grafting, or tissue culture (KLIMENKO, 2004). The main technique used in the eastern hemisphere is budding, with a 90 to 98% success rate and propagation by offsets with a 85 to 90% success rate, followed by propagation of green grafts with a success rate of 75 to 78% (KLIMENKO 2004).

Cornelian cherry dogwoods bear a determinate umbel with four expanded, nonpetaloid, involucral bracts (FENG et al. 2011). Cornelian cherry are open pollinated and self-reproducing (BROWICZ 1986). Their genetic diversity has significant potential for use in breeding programs (ERCISLI 2004). With the recognition of cornelian cherry dogwood fruit as a potentially important food source, intensive research was started in the 1960s (RUDKOVSKY 1960; IMAMALIEV 1977). Cornelian cherry breeding programs began in Ukraine (KLIMENKO, 2004), Slovakia (BRINDZA, 2006), Turkey (PIRLAK *et al*. 2003), Czech Republic, Bulgaria, Poland, Austria, and Serbia (NINIC-TODOROVIC *et al.* 2005; BIJELIC *et al*. 2008a; BIJELIC *et al.* 2008b). In the Vojvodina Province, several attempts at Cornelian cherry selection were made at the Faculty of Agriculture in Novi Sad before the 1990s (KLIMENKO 2004). A new program of Cornelian cherry selection was started at the Department for Pomology, Viticulture, Horticulture and Landscape Architecture in 2006. The objectives of this program were: to multiply the previously selected genotypes, identify new genotypes, and establish a collection and an experimental

Cornelian cherry orchard. The main collection of Cornelian cherry genotypes is located at the Hryshko National Botanical Gardens in Kiev, operated by the Ukrainian National Academy of Sciences. The collection contains 14 officially registered cultivars as well as numerous hybrids developed during the breeding and selection program (REIESTR SORTIV ROSLIN UKRAINY NA 2001; KLIMENKO 2004). Regular selection of the cornelian cherry, and research into natural populations, have resulted in establishment of various breeding programs (BIJELIC 2011; BRINDZA *et al.* 2009; BROWICH 1986; ERCICLI 2004; HOFMAN 1954; KLIMENKO 1990; KARADENIZ 2002; SIMMONS 1972; TYSKIEWICH 1977). There has been additional research in Europe and the U.S. on Cornelian cherry cultivation and on developing new cultivars (REICH 1996; KLIMENKO 2004).

Plantings of Cornelian cherry trees are occurring at a small scale in the U.S. The Northwest ornamental industry uses it as a specialty tree in addition to other native and nonnative dogwood species, including flowering dogwood (*Cornus florida*) and Kousa dogwood (*Cornus kousa*). Cornelian cherry dogwoods bloom in the spring and produce moderate to large red, purple, or yellow drupes. The fruit can be eaten whole from the tree, and is used in food production including; jams, marmalades, wines, liqueurs, syrups, and soups (BEJILIC 2009; BEJILIC 2011). In Asian countries, the cornelian cherry is the main ingredient of herbal preparations used to treat diabetes (JIA *et al.* 2003; JAYAPRAKASAM *et al.* 2005). Bio-oils can be produced by hydrothermal processing of cornelian cherry stones (AKALIN *et al.* 2012). The wood is hard, dense, and used for various carpentry from tool handles, to furniture, and jewelry (CHATFIELD 2006). Wild trees grow in inaccessible landscapes, while domesticated cornelian cherry is found mainly in home gardens, and is extensively grown in mixed orchards in indigenous

areas (BEJILIC 2011). Cornelian cherry trees have many environmental benefits, which include an early pollination source, reduced erosion, improved water quality, wildlife habitat and forage, increased soil organic matter, and carbon sequestration. The cornelian cherry is valuable for its high potential for organic production, as it can be grown without chemical application and has good yield potential without special care (DEMIR AND KALYONCU 2003; CAKMAKCI AND TOSUN 2010).

Although these trees are used for a broad range of commodities in Europe, the Middle East, and Asia, and are known to need little maintenance and are tolerant to most abiotic and biotic factors (MARKOVIC *et al.* 2014), additional research is needed to discover what pathogens cause negative results to this crop in the U.S. According to the USDA Fungal Database, 112 fungal isolates have been reported from cornelian cherry dogwoods worldwide (FARR AND ROSSMAN 2013). Of the reported isolates, only *Monilia fructicola* has been described on cornelian cherry dogwood in the U.S. The current study focuses on identification and confirmation of pathogenicity of two fungal pathogens isolated from *Cornus mas* in eastern Tennessee, *Fusarium sporotrichioides* and *Botrytis cinerea.*

Materials and Methods

Establishment of field plots

Two fields of cornelian cherry dogwoods were planted in Tennessee. The first field was planted April 29, 2016 at the Tennessee Forest Resources AgResearch and Education Center in Oak Ridge, TN. Six 'Aurea', 'Golden Glory', 'Pioneer', 'Sunrise', 'Variegated', and 'Yellow Fruit' varieties were planted in a randomized block design for a total of 36 saplings. Holes were drilled with a 24-in auger connected to a tractor, and trees were

placed and covered by hand. This site did not have irrigation installed, but trees were hand watered using an electric pump attached to a Kawasaki four-wheel drive vehicle. The trees were mulched at the beginning of the growing season and toward the end of summer in both 2016 and 2017. Solar panel electric fencing was placed around the field to discourage browsing by wildlife. The second field was planted April 29, 2016 at The University of Tennessee Plateau Research and Education Center in Crossville, TN. Six 'Golden Glory', 'Pioneer', 'Red Dawn', 'Red Stone', 'Sunrise', 'Variegated', and 'Yellow Fruit' varieties were planted in a random block design for a total of 36 saplings. Holes were drilled with a 24-in auger connected to a tractor and trees were placed and covered by hand. The Crossville site had irrigation using a pond water source, and weeds were controlled around the trees with herbicides, mechanical weed eaters, and mowers. In April 2017, six trees of 'Elegant' were added to each field. Three 'Aurea', three 'Pioneer', one 'Sunrise', and six 'Variegated' trees died in 2016 due to high temperatures, long periods of direct sunlight, high wind speeds, poor soil, and inadequate irrigation. The dead trees were replaced in April 2017.

Arthropods

During April to August in 2016 and 2017, trees were scouted for beneficial and pest arthropods. Insect and arachnid samples were collected, photographed, and identified to genus and species.

Potential pathogen identification

During the 2016 and 2017 growing seasons, trees were observed for disease symptoms. Leaves, stems, and buds exhibiting lesions and/or fungal mycelium were collected and plant tissues were surfaced sterilized in 50% bleach for 1 min, rinsed in

sterile water for 30 s, air-dried, and placed on water agar amended with 10 mg/liter rifampicin (Sigma-Aldrich, St. Louis, MO) and 8 mg fenpropathrin/liter (Danitol 2.4 EC, Valent Chemical, Walnut Creek, CA). Plates were incubated for 7 days at 24 ± 1°C. All sampled tissues developed fungal colonies. Blocks of agar (4-mm^3) that contained the developing hyphae of each colony were transferred to potato dextrose agar (PDA) and carnation leaf agar (LESLIE AND SUMMERELL 2006) and incubated for 7 days at 24 ± 1°C. Pure cultures of each colony were developed from a single conidium. Conidia and other phenotypic structures were observed with microscopy to identify each colony to genus or species. Identification of each isolate was confirmed with molecular characterization of the Internal transcribed spacer region and the translation elongation factor 1α region. DNA was amplified with PCR and the PCR products were sequenced at The University of Tennessee Genomics Core. Sequences were aligned using Sequencher 5.4.6 software (Gene Codes Corporation). Aligned sequences were entered into GenBank and *Fusarium* MLST nucleotide search databases.

Detached leaf assays

A screening tests for pathogenicity was conducted by placing 4-mm^2 plugs of mycelial tissue from PDA onto two surface-sterilized cornelian cherry leaves (4 saplings x 8 leaves/sapling = 28 leaves total). Negative controls were 4-mm^2 plugs of sterile PDA on cornelian cherry leaves. Each repetition of inoculated cornelian cherry leaves was kept at 25 ± 1°C in Petri dishes with sterile filter paper wetted with sterile distilled water for moisture. Leaves were monitored for the onset of symptoms for 10 days. The test was conducted four times for each fungal colony. If lesions occurred during the screening assay, the fungal colony was tested in whole plant assays.

Whole plant assay

Thirty-six cornelian cherry saplings of eight cultivars were grown in a greenhouse at The University of Tennessee. For each putative pathogen, four saplings were inoculated with a spore suspension (10^5 conidia/ml) and placed in a humidity chamber for 10 days at 70˚C. After the tenth day, the humidity chamber was removed, and leaves were monitored for symptoms for 10 additional days. Control pots of saplings were sprayed with distilled water. If the fungal colony caused disease symptoms, the pathogen was re-isolated from infected tissues, and identified (as described above) to be the same as the isolate used for inoculation, thus fulfilling Koch's postulates.

Results

Growth of cornelian cherry trees

The mean height of cornelian cherry saplings planted in Oak Ridge, TN ranged from 30.9 to 67.2 cm in 2016 and 48.3 to 109.2 cm in 2017 (Table 16). The mean stem diameter of the cornelian cherry saplings ranged from 4.63 to 6.57 mm. These values are based on measurements of six trees taken in August of each year. The mean height and diameter values for ‘Aurea’, ‘Pioneer’, and ‘Sunrise’ are based on the three trees that survived from the original 2016 spring planting. ‘Variegated’ saplings died after the August 2016 measurement date, thus the mean height and diameter values for 2016 are based on the original six trees planted in April 2016. Values for mean height and diameter for ‘Variegated’ saplings for 2017 are based on a new set of six trees planted April 2017. A total of 13 of 36 (36%) trees died in 2016 and 2 of 42 (4.7%) trees died in 2017.

Mean heights of cornelian cherry saplings planted in Crossville, TN ranged from 51.7 to 137.2 cm in 2016, and 68.6 to 128.8 cm in 2017 (Table 17). Mean diameters of cornelian cherry stems ranged from 4.12 to 12.98 mm in 2016, and 7.23 to 18.38 mm in 2017. These means are based on measurements of six trees taken in August of each year. For 2017, the mean height and stem diameter values for 'Aurea', 'Pioneer', and 'Sunrise' are based on the three trees that survived from the original 2016 spring planting. All 36 trees planted in 2016 survived (100%) and 41 of 42 trees (98%) survived in 2017. The tree that was lost was missing from the field prior to the August measurement date. It may have been taken by wildlife or mowed during field maintenance.

Arthropods associated with *Cornus mas*

Four species of insects and four arachnid species were identified (Table 18). Many of the organisms found in the field were spiders and beneficial beetles; however, various lepidopterans were found foraging on the cornelian cherry dogwood leaves. In the greenhouse, where trees were held before planting, thrips spp., calico scale (*Eulecanium cerasorum*), and the two-spotted spider mite (*Tetranychus urticae*) caused significant damage to cornelian cherry sapling leaves, stems, and buds. Greenhouse pest insects were controlled mechanically by hand removal, high pressure water spray, and organic insecticidal soap.

Fungi associated with *Cornus mas*

Twenty unique fungal colonies were identified from isolations from diseased cornelian cherry leaves on trees in the field (Table 19). Many of the pathogens identified are known pathogens of other plant hosts and can significantly affect human and animal

health. Of the identified species, only six caused lesions on cornelian cherry leaves during initial screening (*Alternaria alternata*, *Botrytis cinerea*, *Fusarium sporotrichioides*, *Mucor racemosus*, *Trichoderma citrinoviride*, and *Rhizopus oryzae*). The six fungi were tested in whole plant assays, and only two species caused disease on healthy cornelian cherry dogwood saplings, *Botrytis cinerea* and *Fusarium sporotrichioides* (See First Reports in Appendix).

Discussion

Growers interested in producing cornelian cherry dogwoods can grow these trees in Tennessee with a few management variables to consider. Currently these trees are difficult to purchase in large quantities. Only a small number of nurseries in the northwestern and northeastern U.S. keep them in stock. The trees are small and obtained from graft propagation. They are priced from $25 to $65 for trees ranging from 1 to 6 ft in height. Saplings may be planted in early spring, although it is recommended that they be planted in late fall or winter, after they have gone into dormancy to limit transplant stress. Seeds may be purchased online from other countries, but as seeds are wildtype there is no guarantee that the phenotypic traits will be standard across the seed lot. Germination, even with proper preparation, is also low.

There are several different cultivars available from which to choose, many of which have been discussed in this study. Cultivars of interest are ‘Aurea’ and ‘Variegated’ (Figure 9) because of their unique leaf coloration, and ‘Yellow Fruit’ (Figure 10) for its large yellow drupes. ‘Aurea’ (Figure 2) began the growing season with vibrant yellow foliage that gradually turned to the typical green of other cultivars as the season progressed and temperatures rose. The recommendation for planting ‘Variegated’

(Figure 9) is to ensure that the sapling has adequate irrigation and is planted in partial sun to full shade. This cultivar has a mix of white and green foliage that is unique, but will be scorched during periods of drought, high temperatures, and consistent direct sunlight. ‘Elegant’ (Figure 3) and ‘Pioneer’ (Figure 5) are known for their large (0.5 to 1-in long) dark red, pear-shaped fruit. ‘Golden Glory’ (Figure 4) and ‘Sunrise’ (Figure 8) are known for their abundant, vibrant, yellow blossoms. ‘Red Dawn’ (Figure 6) and ‘Red Stone’ (Figure 7) are valued as trees that produce large, sweet fruits and are said to be particularly disease resistant. All the cultivars will survive in Tennessee, but as the summer temperatures rise, they are prone to leaf scorch and wilting if not properly irrigated. Smaller trees have difficulty producing new leaves to replace those that have been damaged by environmental factors, such as sun and heat damage, insect and wildlife forage, and microbial diseases. Larger, more established trees tend to be more capable of repairing damaged tissues and tolerating environmental stresses.

Insect pests are predominantly a problem in a greenhouse setting. Various thrips, mites, and scales can potentially cause significant damage to cornelian cherry dogwoods, if not controlled. Environmental factors such as consistent warm temperatures and high humidity in the greenhouse facilitates longer and more frequent life cycle repetitions, resulting in a rapid increase of pest populations. Compounding the problem is the lack of rain and natural predators to keep the populations at an acceptable threshold. Fortunately for growers, there are several cultural methods as well as commercially available biological and chemical control products to combat these pests.

To control insects, prune and dispose infested plant tissues. Low numbers of scales may be removed by hand. Release of biological control predator insects, such as lady

beetle larvae and adults, will control thrips and scale instars. Insecticidal soaps, d-limonene, horticultural oils, and oil based insecticides will aid in control, but will need reapplication throughout the insect life cycle. These methods should be used as part of an integrated pest management program to prevent resistance and provide the best possible control of the pest problem with the least impact to the plant host and surrounding environment.

During this study, 20 different fungal species were collected from diseased cornelian cherry dogwood tissues (Table 20). Of these fungi, two species, *Botrytis cinerea* and *Fusarium sporotrichioides*, were found to cause disease on cornelian cherry dogwood saplings. Signs and symptoms of *B. cinerea* included gray-white mycelia and black sclerotia encompassing necrotic tissues of fruit, stems, and leaves. Lesions started at the buds and fruit, and spread to other plant parts as the disease progressed. Symptoms of *F. sporotrichioides* infection included small, circular pink to red-brown spots throughout the expanse of the leaf. Overtime, necrotic tissues coalesced to encompass the entire leaf surface. Both these pathogens are reliant upon high humidity that is typical of the southeastern U.S. *Botrytis cinerea* grows in cooler temperatures and lives as a saprophyte until the environmental conditions are favorable for disease. *Fusarium sporotrichioides* can survive in soil and on plant parts and debris for long periods, and is a known pathogen on a variety of plant hosts.

Planting resistant cornelian cherry dogwood cultivars and choosing disease-free trees are the best methods for control of fungal pathogens. Controlling humidity and plant stress along with sterilization of pruning and cultivation equipment, and proper disposal of diseased plant tissues and weedy plant hosts that may harbor fungal spores are

other important control methods. Copper solutions may aid in prevention of fungal diseases, although chemical overuse may give rise to fungal resistance and a buildup of heavy metals in the soil.

Table 16. Growth and survival of cornelian cherry dogwood (*Cornus mas* L.) in Oak Ridge, Tennessee, 2016-2017

Cultivar[a]	Nursery	Mean height 2016 (cm)	Mean height 2017 (cm)	Mean stem diameter 2016 (mm)	Mean stem diameter 2017 (mm)	Survival 2016 (%)	Survival 2017 (%)
Aurea	Forest Farms, OR	67.2	109.2[b]	6.57	9.94[c]	50	100[c]
Golden Glory	Whitman Farms, OR	51.7	67.0	4.83	7.54	100	100
Pioneer	Forest Farms, OR	30.9	94.8[b]	4.91	13.25[c]	50	100[c]
Sunrise	Forest Farms, OR	58.5	60.5[b]	5.18	8.12	67	83
Variegated	Forest Farms, OR	38.5	64.8[c]	4.63	8.61[b]	0	83
Yellow Fruit	Whitman Farms, OR	44.5	48.3	5.03	6.74	100	100
Elegant	Whitman Farms, OR	N/A	69.4[d]	N/A	14.31[d]	100	100

[a] Values are based on 6 trees of each of the 7 cultivars, unless otherwise noted.

[b] Values for 'Aurea', 'Pioneer', and 'Sunrise' are based on the 3 trees planted in April 2016.

[c] Values for 'Variegated' are based on 6 replacement trees planted in April 2017 because all trees in the 2016 planting died.

[d] 'Elegant' was planted only in April 2017.

Table 17. Growth and survival of cornelian cherry dogwood (*Cornus mas* L.) in Crossville, Tennessee, 2016-2017

Cultivar[a]	Nursery	Mean height 2016 (cm)	Mean height 2017 (cm)	Mean stem diameter 2016 (mm)	Mean stem diameter 2017 (mm)	Survival 2016 (%)	Survival 2017 (%)
Red Dawn	Rolling River, CA	106.3	128.8	12.98	18.38	100	100
Red Stone	Rain Tree, NY	62.2	79.3	6.87	14.78	100	100
Pioneer	Rolling River, CA	73.2	92.0	7.30	17.61	100	100
Sunrise	Rolling River, CA	137.2	104.5[b]	11.61	16.21	100	100
Variegated	Rain Tree, NY	51.7	68.6	4.12	7.23	100	100
Yellow Fruit	Rain Tree, NY	59.7	90.6[c]	5.22	12.00[c]	100	83
Elegant	One Green World, OR	N/A	88.1[d]	N/A	16.92[d]	N/A	100

[a] Values are based on six trees of seven cultivars, unless otherwise noted.

[b] Two 'Sunrise' trees had lower heights in 2017 due to animal browsing and wind damage.

[c] One 'Yellow Fruit' tree died in May 2016; values for 2017 are based on the five remaining trees planted in 2016.

[d] 'Elegant' was planted only in April 2017.

Figure 2. Cornelian cherry dogwood (*Cornus mas* L.) 'Aurea'.

Left to right: 1) April 2016 – Fruit; 2) June 2016 - 'Aurea' had vibrant yellow foliage at the beginning of spring that changed to green as air temperature increased. 3) Mid-May 2016 - Trees developed leaf scorch due to hot temperatures and constant direct sunlight. 'Aurea' was only planted in Oak Ridge, TN.

Figure 3. Cornelian cherry dogwood (*Cornus mas* L.) 'Elegant'.

Left to right: 1) Oak Ridge, TN - April 2016; 2) Oak Ridge, TN. 'Elegant' was vibrant at the beginning of spring; 3) By mid-May trees developed leaf scorch due to hot temperatures and constant direct sunlight. 'Elegant' was only planted in Oak Ridge, TN.

Figure 4. Cornelian cherry dogwood (*Cornus mas* L.) 'Golden Glory'.

Left to right: 1) Oak Ridge, TN-April 2016; 2) Oak Ridge, 'Golden Glory' was vibrant at the beginning of spring but by mid-May trees developed leaf scorch due to hot temperatures and constant direct sunlight. 'Golden Glory' was only planted in Oak Ridge, TN.

Figure 5. Cornelian cherry dogwood (*Cornus mas* L.) 'Pioneer'.

Left to right: 1) Oak Ridge, TN-April 2016; 2) Oak Ridge, TN-June 2016; 3) Crossville, TN-April 2016; and 4) Crossville, TN-June 2016. 'Pioneer' was vibrant at the beginning of spring, but by mid-May trees developed leaf scorch due to hot temperatures and constant direct sunlight. Trees planted in Crossville, TN were larger and were irrigated; leaf scorch was less severe and trees were able to develop new growth.

Figure 6. Cornelian cherry dogwood (*Cornus mas* L.) ‘Red Dawn’.

Left to right: 1) Crossville, TN-April 2016; 2) Crossville, TN-June 2016. ‘Red Dawn’ was vibrant at the beginning of spring, but by mid-May trees developed leaf scorch due to hot temperatures and constant direct sunlight. ‘Red Dawn’ was only planted in Crossville, TN.

Figure 7. Cornelian cherry dogwood (*Cornus mas* L.) ‘Red Stone’ variety.

Left to right: 1) Crossville, TN-April 2016; 2) Crossville, TN-June 2016. ‘Red Stone’ was vibrant at the beginning of spring, but by mid-May trees developed leaf scorch due to hot temperatures and constant direct sunlight. ‘Red Stone’ was only planted in Crossville, TN.

Figure 8. Cornelian cherry dogwood (*Cornus mas* L.) 'Sunrise'.

Left to right: 1) Oak Ridge, TN-April 2016; 2) Oak Ridge, TN-June 2016; 3) Crossville, TN-April 2016; 4) Crossville, TN-June 2016. 'Sunrise' was vibrant at the beginning of spring by mid-May trees developed leaf scorch due to hot temperatures and constant direct sunlight. Trees planted in Crossville, TN were larger and on irrigation; leaf scorch was less severe and trees were able to develop new growth. 'Sunrise' appeared to be the most heat and drought tolerant cultivar.

Figure 9. Cornelian cherry dogwood (*Cornus mas* L.) 'Variegated'.

Left to right: 1) Oak Ridge, TN-April 2016; 2) Oak Ridge, TN June 2016; 3) Crossville TN June 2016; 4) Jackson, TN 2016. 'Varigated' was vibrant at the beginning of spring, by mid-May trees developed leaf scorch due to hot temperatures and constant direct sunlight. Varigated trees planted in partial sun are able to withstand high temperatures and remain vibrant throughout the growing season.

Figure 10. Cornelian cherry dogwood (*Cornus mas* L.) 'Yellow Fruit'.

Left to right: 1) Oak Ridge, TN-April 2016; 2) Oak Ridge, TN June 2016; 3) Crossville TN June 2016; 4) Jackson, TN 2016. 'Yellow Fruit' was vibrant at the beginning of spring, but by mid-May trees developed leaf scorch due to hot temperatures and constant direct sunlight. Trees planted in Crossville, TN were larger and were irrigated; leaf scorch was less severe and trees were able to develop new growth.

Table 18. Arachnids and insects observed on cornelian cherry dogwood (*Cornus mas* L.) in east Tennessee

Common Name	Scientific name
Asian multicolored lady bug	(*Harmonia axyridis*)
Brown Marmorated Stink Bug	(*Halyomorpha halys*)
Bold Jumping Spider	(*Phidippus audax*)
Calico Scale	(*Eulecanium cerasorum*)
Crab Spider	(*Mecaphesa* spp.)
Lepidopteran spp.	
Mantis	Mantis (*Mantis religiosa)*
Starbellied Orb Weaver	(*Acanthepeira stellate*)
Thrips spp.	
Two Spotted Spider Mite	(*Tetranychus urticae*)

Table 19. Fungal species associated with cornelian cherry dogwood (*Cornus mas* L.)

Scientific name of fungus	Known potential as a human pathogen	Known potential as a plant pathogen	Pathogen on cornelian cherry
Alternaria alternata	N	Y	N
Arthrinium phaeospermum	N	Y	N
Aspergillus ochraceous	Y	N	N
Botrytis cinerea	N	Y	Y
Cladosporium cucumerium	N	Y	N
Discula quercina	N	Y	N
Epicoccum nigrum	N	Y	N
Eutypella vitis	N	Y	N
Fusarium sporotrichioides	Y	Y	N
Mucor racemosus	Y	Y	N
Nigrospora sp.	Y	Y	N
Penicillium citrinum	Y	Y	N
Peniphora sp.	N	Y	N
Phoma herbarum	N	Y	N
Peyronellaea glomerata	N	Y	N
Schizophyllum commune	Y	Y	N
Trametes versicolor	N	Y	N
Trichoderma citrinoviride	N	N	N
Trichoderma virens	Y	N	N

Research Definitions

Gel electrophoresis: method for separation and analysis of macromolecules (DNA, RNA and proteins) based on their size and charge

Inflorescence: group of flowers arranged on a stem or the modified shoot of seed plants where flowers are formed

Involucre: whorl or rosette of bracts surrounding an inflorescence at the base of an umbel

Mycelial cultures: classified as having abundant mycelium and lacking sporodochia (HANSEN 1938)

Mycotoxicoses: animal diseases caused by mycotoxins in the respiratory, digestive, circulatory, and other internal systems of vertebrates (FORGACS AND CARLL 1962)

Mycotoxicology: study of mycotoxins (FORGACS AND CARLL 1962)

Mycotoxins: naturally occurring compounds or secondary metabolites produced by filamentous fungi growing on plants in the field or during storage (NICHEA *et al.* 2015)

Pathogenicity: organism's (microbe's) ability to cause disease

Pathotype: disease-causing variant of a microorganism distinct from other members of its species by its virulence level and molecular markers

Phytotoxins: Fungal products that are toxic to plants as the term is used by plant pathologists; can also refer to toxins made by plants (GRANITI 1972)

Pionnotal cultures: classified as having abundant sporodochia and a lack of mycelium (HANSEN 1938).

Pleomorphy: fungi grown on different media have different forms

Polymerase chain reaction (PCR): molecular technique used in biological fields to amplify copies of a selected segment of DNA, generating thousands to millions of copies of the DNA sequence

Polymorphic: occurring in several distinctive forms, specifically regarding species or genetic variation

Race: individual classification using pathogenicity on host cultivars (FOURIE *et al.* 2011).

Umbel: inflorescence comprised of short flower stalks that spread from a common point- "umbrella like"

Virulence: signifies the degree of damage caused by a microbe to its host

References

Akalin, M. K., K. Tekin, and S. Karagoz. 2012. Hydrothermal liquefaction of cornelian cherry stones for bio-oil production. Bioresource Technology 110: 682-687.

Bijelić, S., B. Gološin, J. Ninić Todorović, S. Cerović, and B. Bogdanović. 2012. Promising cornelian cherry (*Cornus mas* L.) genotypes from natural populations in Serbia. Agriculturae Conspectus Scienticus 77**:** 5-10.

Booth, C. 1971. The Genus *Fusarium*. Commonwealth Agricultural Bureaux [for the] Commonwealth Mycological Institute. University of Michigan. 237 pp.

Browicz, K. 1986. Chronology of trees and shrubs in south–west Asia and adjacent regions. Bogucki Publishers, Poznan.

Bosnjaković, D. I., V. Ognjanov, M. Ljubojevic, G. Barac, M. Predojevic, *et al.* 2012. Biodiversity of wild fruit species of Serbia. Genetika 44**:** 81-90.

Cakmakci, S., and M. Tosun. 2010 Characteristics of mulberry pekmez with cornelian cherry. International Journal of Food Properties 13**:** 713-722.

Demir, F., and I. H. Kalyoncu. 2003. Some nutritional, pomological and physical properties of cornelian cherry (*Cornus mas* L.). Journal of Food Engineering 60**:** 335-341.

Dokupil, L., and V. Řezniček. 2012. Production and use of the Cornelian cherry – *Cornus mas* L. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis LX**:** 49-58.

Ďurkovič, J. 2008. Micropropagation of mature *Cornus mas* 'Macrocarpa'. Trees 22 597-602.

Ďurkovič, J., and J. Bukovska. 2009. Adventitious rooting performance in micropropagated *Cornus mas*. Biologia Plantarum 53**:** 715-718.

Ercisli, S. 2004. Cornelian cherry germplasm resources of Turkey. Fruit and Ornamental Plant Research, Special edition 12**:** 87-92.

Farr, D. F., G. F. Bills, G. P. Chamuris, and A. Y. Rossman. 1995. Fungi on Plants and Plant Products in the United States. American Phytopathological Society, St. Paul. MN.

Jayaprakasam, B., K. S. Vareed, L. K. Olson, and N. G. Muraleedharan. 2005. Insulin secretion by bioactive anthocyanins and anthocyanidins. Journal of Agricultural and Food Chemistry 53**:** 28-31.

Jia, W., W. Gao, and L. Tang. 2003. Antidiabetic herbal drugs officially approved in China. Phytotherapy Research 17**:** 1127-1134.

Klimenko, S. 2004. The cornelian cherry (*Cornus mas* L.): Collection, preservation and utilization of genetic resources. Protection of genetic resources of pomological plants and selection of genitors with traits valuable for sustainable fruit production. Journal of Fruit and Ornamental Plant Research (Special edition) 12**:** 93-98.

Markovic, M., M. Grbic, and M. Djukic. 2014. Effects of type and a method of IBA application on rooting of softwood cutting from elite tree of cornelian cherry (*Cornus mas* L.) from Belgrade Area. Silva Balcanica 15: 30-37.

Mratinić, E., and M. Kojić. 1998. Wild fruit species of Serbia. Institute for agricultural research ‘Serbia’, Belgrade (In Serbian). 1: 125-131.

Ninić-Todorović, J., S. Cerović, B. Gološin, S. Bijelić, R. Miletić. 2005. Pomological biochemical characteristics of selected Cornelian cherry genotypes. Journal of Pomology 39: 395–399.

Piotto, B., and A. Di Noi, eds. 2003. Seed propagation of Mediterranean trees and shrubs. ANPA, Roma 24.

Sequencher® version 5.4.6 DNA sequence analysis software, Gene Codes Corporation, Ann Arbor, MI.

First Report of *Botrytis cinerea* Causing Gray Mold on Cornelian Cherry (*Cornus mas* L.) in the United States

Cornelian cherry dogwood (*Cornus mas* L.) is a potential specialty crop in Tennessee. Trees are prized for their early blossoms, appearance, and edible fruit in Europe and Southeast Asia (BEJILIC 2012). In April 2017, necrotic cornelian cherry tissues ('Aurea' and 'Pioneer') were collected from Crossville and Oak Ridge, TN. Gray-white mycelia and black sclerotia encompassed necrotic buds, stems, and leaves. Lesions initiated on buds and spread to other plant parts as the disease progressed. Symptomatic tissues were surface disinfected (1 min 50% bleach; 30 s sterile distilled water), placed on water agar amended with 10 mg/liter rifampicin (Sigma-Aldrich, St. Louis, MO) and 8 mg fenpropathrin/liter (Danitol 2.4 EC, Valent Chemical, Walnut Creek, CA), and incubated for 7 days at 24 ± 1°C. A 4-mm^2 mycelial plug was transferred to potato dextrose agar (PDA) and incubated for 7 days at 24 ± 1°C. Colonies grew rapidly, with white to dark gray mycelia. Abundant, spherical-ovoid, hyaline conidia were observed on branching conidiophores. Conidia were 10-15 × 8-10 µm. Resting spores (sclerotia) were formed in older colonies. Morphological characteristics were identical to the description of *Botrytis cinerea* (ELLIS 1971). Fungal identification was confirmed through polymerase chain reaction of the ITS region of ribosomal DNA with ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC - 3') primers, and the TEF gene region with ef1 (5'-ATGGGTAAGGA(A/G)GACAAGAC-3') and ef2 (5-GGA(G/A)GTACCAGT(G/C)ATCATGTT-3) primers (BALDAUF 1999). A sequence was deposited in GenBank (Accesssion No. MG719362) Tests for pathogenicity were conducted by placing a 4-mm^2 PDA mycelial plug on two surface-

sterilized cornelian cherry leaves. Negative controls were inoculated with plugs of sterile PDA. Inoculated leaves were held at 25 ± 1°C in a moist chamber. After necrotic lesions appeared in the detached leaf assay, the fungus was tested in a disease assay on saplings in the greenhouse. Four replicates of two cultivars ('Aurea' and 'Pioneer') were inoculated with a spore suspension (10^5 conidia/ml) and covered with a large, clear, plastic bag. After 10 days, the bag was removed, and leaves were monitored for symptoms for an additional 10 days. The pathogen was re-isolated from diseased tissues, and identification was confirmed with PCR and morphological features, thus fulfilling Koch's postulates. *Botrytis cinerea* has a large host range and occurs worldwide (JARVIS 1977; ELAD *et al*. 2007). There has been a report of *B. cinerea* on cornelian cherry in Turkey (ÖZER AND BAYRAKTAR 2014), but to our knowledge, this is the first report of *Botrytis cinerea* causing gray mold on cornelian cherry in the United States FARR AND ROSSMAN 2017).

References

Bijelić, S., B. Gološin, J. Ninić Todorović, S. Cerović, and B. Bogdanović. 2012. Promising cornelian cherry (*Cornus mas* L.) genotypes from natural populations in Serbia. Agriculturae Conspectus Scienticus 77**:** 5-10.

Elad, Y. B. Williamson, P. Tudzynski, and N. Delen, eds. 2007. *Botrytis* spp. and the diseases they cause in agricultural systems - an introduction. Pages 1-8 In: *Botrytis*: Biology, Pathology, and Control. Springer, New York, NY.

Farr, D. F., and A. Y. Rossman. 2017. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved March 15, 2017. https://nt.ars-grin.gov/fungaldatabases.

Jarvis, W. R. 1977. *Botryotinia* and *Botrytis* species: Taxonomy, physiology and pathogenicity. A guide to the Literature. Ottawa, Canada, Department of Agriculture, Monograph No 15.

Ozer, G., and H. Bayraktar. 2014. Australasian Plant Disease Notes 9: 126 pp.

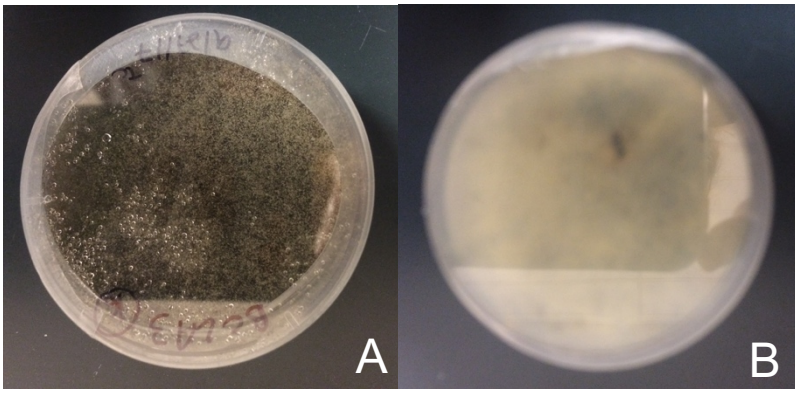

Figure 11. *Botrytis cinerea* on cornelian cherry dogwood (*Cornus mas* L.).

B. cinerea on potato dextrose agar A) Top of plate, B) Bottom of plate.

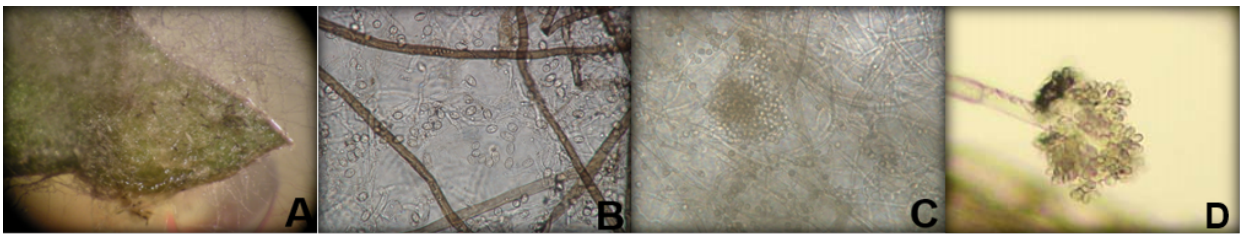

Figure 12. Morphological characteristics of *Botrytis cinerea* on cornelian cherry dogwood (*Cornus mas* L.).

B. cinerea on water agar A) gray mycelia on cornelian cherry dogwood leaf B) conidia, C) conidia cluster D) conidia on branching mycelia.

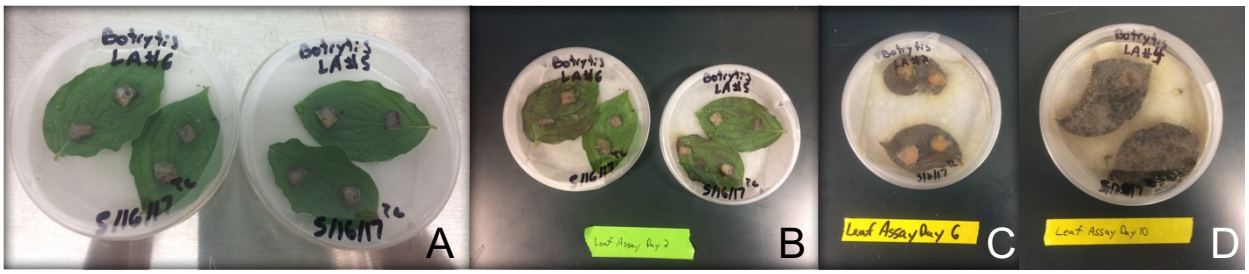

Figure 11. Detached leaf assay *Botrytis cinerea* on cornelian cherry dogwood (*Cornus mas* L.).

Day 1, B) Day 2, C) Day 6, D) Day 10.

Figure 12. Whole plant assay *Botrytis cinerea* on cornelian cherry dogwood (*Cornus mas* L.).

A) Day 10 detached leaf, B) whole plant gray mold on leaves, C) Gray mold on stems and buds, D) *B. cinerea* infected tree in Crossville, TN.

First Report of *Fusarium sporotrichioides* Causing Foliar Necrosis on Cornelian Cherry (*Cornus mas* L.) in the United States

In Europe and Southeast Asia, cornelian cherry (*Cornus mas* L.) has few known pathogens, and is prized for its early blossoms, wood, and edible fruit (Bijelić et al. 2012). In April 2016, necrotic cornelian cherry sapling leaves, were collected from Crossville and Oak Ridge, Tennessee. Symptoms included small, circular pink to red-brown spots on leaves that coalesced to cover the entire leaf surface. Small sections of symptomatic leaf tissue were surface disinfected (1 min 50% bleach, 30 s sterile distilled water), air dried, placed on water agar amended with 10 mg/liter rifampicin (Sigma-Aldrich, St. Louis, MO) and 8 mg fenpropathrin/liter (Danitol 2.4 EC, Valent Chemical, Walnut Creek, CA) (aPDA), and incubated for 7 days at 24 ± 1°C. A 4-mm^3 block of agar with hyphae was transferred to potato dextrose agar (PDA) and incubated for 7 days at 24 ± 1°C. Colonies grew rapidly, with pink mycelia that browned with age. Pink-red pigmentation occurred at the medium surface. Aerial mycelia are floccose with pale to bright orange sporodochia, monophialides, and polyphialides. Microconidia were oval to pyriform, with smooth, thin, hyaline walls, and measured 5.5 to 12.2 × 2.0 to 3.2 µm. Macroconidia were pyriform, 3 to 5 septate, moderately curved to straight, hyaline, with thick walls, and 18.8 to 36.3 × 2.4 to 7.1 µm in size. Colonies were morphologically identical to the description of *Fusarium sporotrichioides* Sherb. (LESLIE *et al.* 2008). Genomic DNA was extracted from mycelia grown on PDA and identification was confirmed with sequences of the internal transcribed spacer region with primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC - 3'),

and sequences of the translation elongation factor gene region, with primers ef1 (5'-ATGGGTAAGGA(A/G)GACAAGAC-3') and ef2 (5-GGA(G/A)GTACCAGT(G/C)ATCATGTT-3) (BALDAUF 1999). The ITS sequence was deposited in GenBank (Accession No. MG719361). The TN isolate had XX% to XX% identity with several isolates of *F. sporotrichioides* from GenBank and *Fusarium* MLST databases. Pathogenicity screening was tested by placing a 4-mm^2 mycelial plug of PDA on two surface-sterilized cornelian cherry leaves (4 repetitions, 8 leaves = 24 leaves total). Controls were inoculated with sterile PDA plugs. Inoculated leaves were kept at 25 ± 1°C in a moist chamber. After the appearance of lesions, the isolate was tested in a greenhouse disease assay with Cornelian cherry saplings. Four saplings were inoculated with a spore suspension (10^5 conidia/ml), placed under a humidity chamber for 10 days at 70˚C, and leaves were monitored for symptoms for 10 additional days. Control saplings were sprayed with distilled water. The pathogen was re-isolated from infected tissues, and PCR was done to confirm molecular identification, fulfilling Koch's postulates. *Fusarium sporotrichioides* is a serious pathogen of various plant hosts, and isolates can produce trichothecene mycotoxins that cause toxicosis in animals and humans (FARR *et al.* 1995). This disease could denote an economic concern for potential nursery growers and homeowners. To our knowledge, this is the first report of *F. sporotrichioides* causing foliar necrosis on cornelian cherry in the United States.

References

Baldauf, S. L. 1999. A search for the origins of animals and fungi: comparing and combining molecular data. The American Naturalist 154**:** 178-188.

Bijelić, S., B. Gološin, J. Ninić Todorović, S. Cerović, and B. Bogdanović. 2012. Promising cornelian cherry (*Cornus mas* L.) genotypes from natural populations in Serbia. Agriculturae Conspectus Scienticus 77**:** 5-10.

Farr, D. F., and A. Y. Rossman. 2017. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved March 15, 2017. https://nt.ars-grin.gov/fungaldatabases.

Leslie, J. F., and B. A. Summerell. 2008. The *Fusarium* Laboratory Manual. Blackwell Publ., Ames IA.

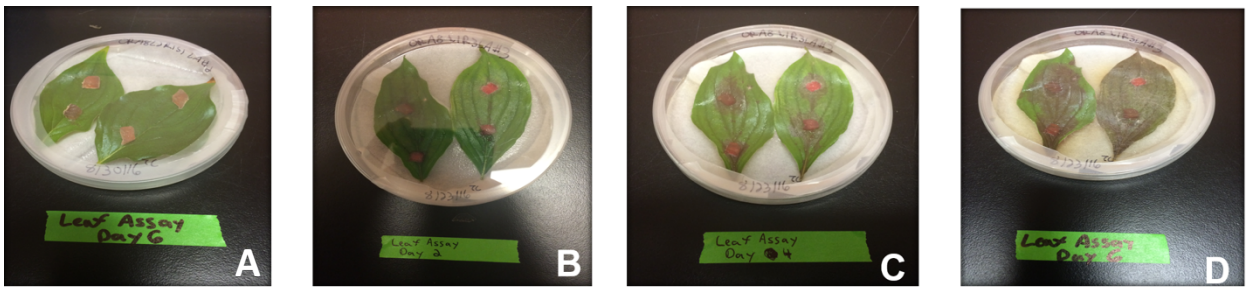

Figure 13. *Fusarium sporotrichioides* detached leaf assay on cornelian cherry dogwood (*Cornus mas* L.).

Potato dextrose agar plugs on cornelian cherry dogwood leaves A) Control, Day 6, *F. sporotrichioides* B) Day 2, C) Day 4, D) Day 6.

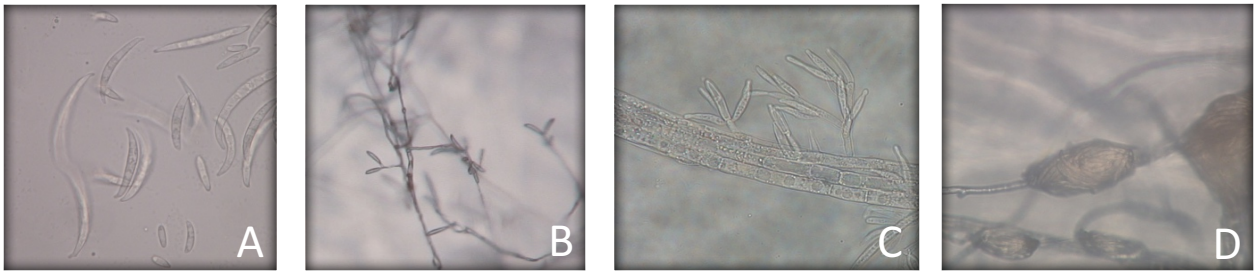

Figure 14. Morphological Characteristics of *Fusarium sporotrichioides* isolated from cornelian cherry dogwood.

Morphological characteristics of F. *sporotrichioides* cultured on carnation leaf agar A) conidia, B) monophialides, C) polyphialides, D) sporodochia.

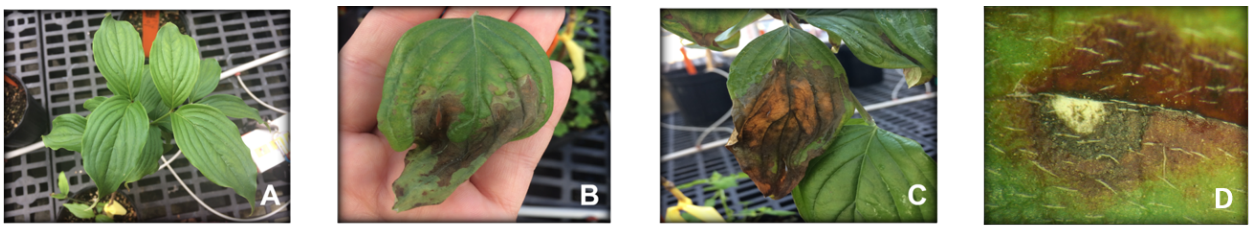

Figure 15. *Fusarium sporotrichioides* whole plant assay on cornelian cherry dogwood (*Cornus mas* L.).

Experiment repetition 1: A) Healthy sapling, B) Day 2, C) Day 6, D) Leaf lesion.

Vita

Tamara Collins was born in Denver, Colorado March 5, 1986. She received a Bachelor of Science degree in Wildlife and Fisheries Management from Lincoln Memorial University at Harrogate, TN in May 2010. After taking five years off from academics, Tamara further pursued an education in Plant Pathology. In December 2017, she earned a Master of Science degree in Entomology and Plant Pathology under the direction of Dr. Bonnie Ownley. Tamara wrote her thesis “Identification, characterization, and genetic comparison of *Fusarium* species isolated from switchgrass (*Panicum virgatum* L.) and Appendix: The potential of cornelian cherry dogwood (*Cornus mas* L.) grown in east Tennessee” January 2015-August 2017.