GENETIC DIVERSITY AND PATHOGENICITY OF SORGHUM-ASSOCIATED *FUSARIUM* SPECIES

by

VUYISWA SYLVIA BUSHULA

B.S., Stellenbosch University, South Africa, 2005
M.S., Stellenbosch University, South Africa, 2008

AN ABSTRACT OF A DISSERTATION

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Abstract

Understanding the genetic structure of fungal pathogens enables the prediction of evolutionary forces that drive pathogen evolution, which assists informed decision-making regarding disease management. The genetic structure of *Fusarium thapsinum* and *F. andiyazi*, two important pathogens that cause grain mold and stalk rot of sorghum (*Sorghum bicolor*), are little understood.

The genetic structure and pathogenicity of a *F. thapsinum* population from sorghum in Kansas were evaluated with amplified fragment length polymorphisms (AFLPs), vegetative compatibility groups (VCGs), sexual cross-fertility, and seedling pathogenicity. Two sympatric populations and a genetically intermediate "hybrid" group were identified in Kansas. Seedling pathogenicity of strains ranged from non-pathogenic to pathogenic, which may be partially attributable to genetic variability in the *F. thapsinum* populations.

Genetic relatedness between populations of *F. thapsinum* from sorghum in Kansas, Australia, Thailand, and three African countries (Cameroon, Mali, and Uganda) were evaluated with AFLP markers and sexual crosses. Genetic diversity was high in all locations, but female fertility is very low. These results are consistent with the hypothesis that both sexual and asexual modes of reproduction are important components of the life cycle of *F. thapsinum* in these populations. More strains from Kansas and Africa were available for analysis than from Australia and Thailand, so the Kansas and Africa populations dominated the genetic structure observed. The two smaller populations from Australia and Thailand were more closely related to the Kansas population than they were to the African population. The three non-African populations contained information from the African population and from other, as yet unidentified, source population(s). Identifying the population(s) from which this genetic diversity originated is an important unanswered question.

Stalk rot of sorghum was evaluated by inoculating stalk rot sensitive and stalk rot resistant sorghum lines with six genetically diverse *F. thapsinum* strains from Kansas under field and greenhouse conditions. One susceptible line (Tx7000) and two resistant lines (SC599 and BTx399) were evaluated in the field but only Tx7000 and SC599 were evaluated in the greenhouse. Disease severity was measured by major lesion length and the number of nodes crossed by the lesion. There were differences in aggressiveness amongst the *F. thapsinum* strains in both the greenhouse and field evaluations. This study provides the first evidence for differences in stalk rot aggressiveness amongst *F. thapsinum* strains and highlights the importance of challenging germplasm with well-characterized strains that represent the genetic spectrum of the entire population.

The genetic diversity within *F. andiyazi* populations and some closely related strains was evaluated with AFLP markers. Phylogenetic and STRUCTURE analyses of the AFLP markers grouped the 81 *F. andiyazi* strains into three distinct clusters. The clusters were not based on the geographic origin of the strains. These results indicate the presence of at least one and possibly two undescribed sister taxa of *F. andiyazi*. More work is needed to further characterize these sister species of *F. andiyazi* and to understand their role in sorghum pathogenicity.

There is genetic variation in global populations of *F. thapsinum* and the observed variation could be associated with variation in both seedling and adult plant pathogenicity. The study of *F. andiyazi* populations validated the need to properly identify and characterize *Fusarium* spp. associated with sorghum from different regions of the world.

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Major Professor
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Dedication

This dissertation is dedicated to my loving husband Martins Mbah Njah, my beautiful daughter Iwhumni Wongalophezulu Njah, and my darling mother Nomthandazo Selina Kanunu.

Chapter 1 - Literature Review

Grain sorghum (*Sorghum bicolor*)

Sorghum bicolor originated in Africa and is globally the fifth most important grain crop after wheat (*Triticum aestivum*), rice (*Oryza sativa*), maize (*Zea mays*), and barley (*Hordeum vulgare*) (Maunder, 2002). In Sub-Saharan Africa and southern Asia, grain sorghum is an important subsistence crop and is used for numerous foods including leavened-breads, couscous, fermented and unfermented-porridges, and traditionally brewed African beers (Rooney and Waniska, 2000; Taylor and Dewar, 2000). In countries such as the United States, Australia, China, Brazil, and Argentina, grain sorghum is used as livestock feed and for bio-ethanol production (Maunder, 2002). Common to all sorghum-growing regions of the world are diseases on sorghum that are caused by diverse microorganisms. Disease severity depends upon the consortium of species present and the environmental conditions during the growing season (Bramel-Cox et al., 1988). Grain mold and stalk rot of sorghum are both yield-limiting diseases and reduce seed quality and vigor (Little and Magill, 2003).

Grain mold of sorghum is caused by members of the fungal genera *Alternaria*, *Colletotrichum*, *Curvularia*, *Fusarium*, and *Phoma* (Little et al., 2012), while sorghum stalk rot is caused by members of the fungal genera *Alternaria*, *Cephalosporium*, *Colletotrichum*, *Fusarium*, *Macrophomina*, *Nigrospora*, *Periconia*, and *Trichoderma*. Globally, charcoal rot caused by *Macrophomina phaseolina* and *Fusarium* stalk rot caused by *F. thapsinum* and *F. andiyazi* are regarded as the most prevalent diseases of sorghum stalks (Tesso et al., 2012).

The importance of *Fusarium* species

Members of the genus *Fusarium* are important fungal plant pathogens that cause diverse diseases on important crops that serve as staple foods worldwide (Leslie and Summerell, 2006). In addition to their phytopathogenicity, *Fusarium* species synthesize numerous secondary metabolites and mycotoxins, which have adverse effects on humans and domestic animals that consume the agricultural commodities infected by these fungi (Desjardins, 2006). *Fusarium* species also are opportunistic human pathogens that cause death and blindness (Leslie and Summerell, 2006). *Fusarium* species are therefore of importance as direct causes of plant, animal, and human diseases as well as indirect causes of nutritional diseases in humans and domesticated animals that result from the secondary metabolites that they produce.

Three species concepts in *Fusarium*

Three species concepts commonly are used in *Fusarium* taxonomy – morphological, biological, and phylogenetic (Leslie and Summerell, 2006). The morphological species concept is based on similarities between observable morphological characteristics, e.g., shape and size of macroconidia and microconidia, shape of conidiogenous cells, and the presence or absence of chlamydospores, pseudochlamydospores, and coiled hyphae. Traditional morphological characters often are insufficient for definitive species identifications in the absence of additional information such as host or geographic location (Summerell et al., 2003; Leslie and Summerell, 2006).

The biological species concept is based on sexual cross-fertility between fungal strains. The progeny of a successful cross need to be both viable and fertile (Perkins, 1994). This species concept is the basis for the mating populations (MP) in *Fusarium* (Leslie, 1991). The biological

species concept, however, can be applied only to sexually reproducing species and cannot be used with species with no known sexual phase, *e.g.*, the *F. oxysporum* species complex.

Phylogenetic species rely on molecular markers such as partial DNA sequences of the translation elongation factor, calmodulin, β-tubulin, two DNA-directed RNA polymerase II subunits (RPB1 and RPB2), the nuclear large subunit 28S rDNA, and the ribosomal internal transcribed spacer (ITS) region (O'Donnell and Cigelnik, 1997; O'Donnell et al., 2000, 2013). The use of multiple gene sequences to identify phylogenetic relationships in *Fusarium* species has facilitated the resolution and discovery of new species within large species complexes such as those in the *F. fujikuroi* species complex (O'Donnell et al., 1998). Within this species complex concordance between independent genes resulted in recognition of 45 species, 26 of which were resolved as new species.

The *Fusarium fujikuroi* Species Complex (FFSC)

The FFSC is a group of more than 40 phylogenetic species that are important pathogens of a number of crops including sorghum (Leslie and Summerell, 2006; Kvas et al., 2009). Members of the FFSC are heterothallic fungi requiring strains of opposite mating types (*MAT-1* or *MAT-2*) for sexual crosses to occur. There are 12 known mating populations (MP A–L) within the FFSC (Leslie and Summerell, 2006; Lima et al., 2012): *F. verticillioides* (MP A) (Kuhlman, 1982); *F. sacchari* (MP B) (Leslie et al., 2005); *F. fujikuroi* (MP C) (Kuhlman, 1982); *F. proliferatum* (MP D) (Kuhlman, 1982); *F. subglutinans* (MP E) (Leslie, 1991); *F. thapsinum* (MP F) (Klittich and Leslie, 1992; Klittich et al., 1997); *F. nygamai* (MP G) (Klaasen and Nelson, 1996); *F. circinatum* (MP H) (Nirenberg and O'Donnell, 1998); *F. konzum* (MP I) (Zeller et al., 2003b); *F. xyla-*

rioides (MP J) (Geiser et al., 2005); *F. temperatum* (MP K) (Scauflaire et al., 2011); and *F. tupiense* (MP L) (Lima et al., 2012).

In grain sorghum, members of the FFSC can cause diseases that range from wilts, root and stem rots, to head blight, and grain mold (Leslie and Summerell, 2006). Fusarium stalk rot and grain mold are the most common diseases in all sorghum-growing regions of the world and *F. thapsinum* (MP F) is an important causative agent of both diseases (Frederiksen and Odvody, 2000; Leslie, 2002; Petrovic et al., 2009; Tesso et al., 2005; 2012).

Taxonomy of *F. thapsinum*

Fusarium thapsinum is a heterothallic fungus that is a member of the *F. fujikuroi* species complex (FFSC). Morphological characteristics of *F. thapsinum* are typical of those of several species in the FFSC. Macroconidia produced by *F. thapsinum* are relatively slender, slightly falcate or straight with thin walls. Microconidia are formed in long chains or as small aggregates of conidia. The microconidia are non-septate, and usually club shaped but occasionally napiform. *F. thapsinum* does not form chlamydospores (Klittich et al., 1997).

Prior to being formally recognized as a separate species, strains of *F. thapsinum* were identified as *F. moniliforme*, but reproductive isolation and the production of yellow pigment in the agar by some strains led to its separation from *F. moniliforme* (Klittich and Leslie, 1992; Klittich et al., 1997). *F. moniliforme* has since been subdivided into more than 40 species that are members of the FFSC (Leslie and Summerell, 2006; Kvas et al., 2009) and the name is no longer accepted for use (Seifert et al., 2003). *F. thapsinum* strains that do not produce the characteristic yellow pigment are morphologically indistinguishable from *F. verticillioides* and *F. andiyazi*. *F. andyazi* can be distinguished from *F. thapsinum* on the basis of production of pseudochlamydospores (Marasas et al., 2001). The production of monophialides with long chains of microconidia

in *F. thapsinum*, and production of both monophialides and polyphialides with short chains of microconidia in *F. proliferatum*, are important morphological characters that distinguish these two species.

Isozyme polymorphisms and electrophoretic chromosome karyotypes also differ between mating populations and are consistent with the observed reproductive isolation between the mating populations of the *F. fujikuroi* species complex (Huss et al., 1996; Xu et al., 1995).

Pathology and ecology of *F. thapsinum*

Fusarium thapsinum is an important pathogen of sorghum causing both grain mold and stalk rot diseases (Leslie, 2002; Klittich et al., 1997). This pathogen is distributed worldwide and is found everywhere sorghum is grown. Spores of this fungus can be recovered from the air and soil associated with sorghum fields (Funnell-Harris and Pedersen, 2011). *F. thapsinum* also has been recovered from native North American tallgrass prairie, bananas (*Musa acuminata*), and maize (*Zea mays*) (Leslie et al., 2004; Leslie, 1995).

Grain mold of sorghum is a disease that causes grain deterioration after prolonged fungal association with spikelet tissues between anthesis and physiological maturity under humid, warm, and rainy conditions. In such conducive environments, grain mold is the greatest constraint for optimum grain yield (Forbes et al., 1992; Bandyopadhyay et al., 2000; Leslie, 2002).

Similar to grain mold, stalk rot of sorghum also can be a yield-limiting disease that greatly reduces seed quality and vigor (Little and Magill, 2003, 2009; Jardine, 2006). In Kansas (USA), stalk rot is the most common disease of sorghum with average losses of 4% but that can be as high as 50 to 90%. Dry conditions early in the season favor the initiation of stalk rot in the field. Subsequent warm, wet conditions favor increased disease incidence and severity (Jardine,

2006). *F. thapsinum* is associated with stalk rot of grain sorghum globally (Petrovic et al., 2009; Tesso et al., 2005, 2010). *F. thapsinum* produces high levels of moniliformin, fusaric acid and trace amounts of fumonisins (Leslie and Summerell, 2006).

Population genetics of *F. thapsinum*

The effective population size (N_e) is an important population genetics parameter commonly used to compare different field populations with one another and with an idealized randomly mating population and to estimate the potential effects of drift and inbreeding (Wright, 1931; Caballero, 1994; Leslie and Klein, 1996). For heterothallic fungi, mating type (*MAT-1*:*MAT-2*) frequencies and the proportion of female fertile strains can be used to estimate N_e (Leslie and Klein, 1996).

N_e of a global population of *F. thapsinum* from sorghum (Leslie and Klein, 1996) was only 32% of the count when estimated by the low level of female-fertility, and was 98% of the count when estimated by the relative frequencies of the mating-type alleles. The low level of female-fertility indicates that asexual reproduction is a significant component of the life cycle of this fungus (Leslie and Klein, 1996). Similar results were observed in a population of *F. thapsinum* from Tanzania (Mansuetus et al., 1997). In the Tanzanian population, female-fertility was higher than that in the global population suggesting that sexual reproduction occurred more frequently in Tanzania than in the global population as a whole. Little else is known about the genetic structure of global populations of *F. thapsinum*.

Taxonomy of *F. andiyazi*

Fusarium andiyazi is an important pathogen of sorghum that can cause both grain mold and stalk rot of sorghum, and is a member of the FFSC (Marasas et al., 2001). Morphologically,

F. andiyazi most closely resembles *F. thapsinum*, *F. proliferatum*, and *F. verticillioides*, but forms pseudochlamydospores which none of the other closely related species do. The combination of morphological and molecular markers such as amplified fragment length polymorphisms (AFLPs), and partial sequences of genes such as the elongation factor and β-tubulin clearly differentiate *F. andiyazi* from other closely related species, e.g. *F. thapsinum* (Leslie and Summerell, 2006; Marasas et al., 2001).

Pathology and ecology of *F. andiyazi*

F. andiyazi has been recovered from most sorghum growing regions of the world including South Africa, Australia, Ethiopia, Nigeria, and the USA (Leslie and Summerell, 2006), and may be recovered from both the air and soil associated with sorghum fields (Funnell-Harris and Pedersen, 2011). In South Africa, *F. andiyazi* also was recovered from sugarcane with the pokkah-boeng disease (Govender et al., 2010). *F. andiyazi* is pathogenic to sorghum seedlings in *in vitro* assays (Leslie et al., 2005) and is associated with stalk rot of grain sorghum (Tesso et al., 2005, 2010; Petrovic et al., 2009). *F. andiyazi* produces neither fumonisins nor moniliformin. Recently, this fungal pathogen has been recognized as an opportunistic human pathogen (Kebabci et al., 2014). In a study characterizing *Fusarium* species attacking maize in Syria, Madania et al. (2013) recovered "*F. andiyazi*-like" strains that were similar to *F. andiyazi* based on TEF1-α sequences but could not be conclusively identified as *F. andiyazi*. This result suggests that there is genetic variability in *F. andiyazi* and *F. andiyazi*-like populations and that additional cryptic species with similar or identical morphological characters may remain to be described.

Fertility of *F. andiyazi*

F. andiyazi strains are not inter-fertile with any of the tester strains of the mating populations A to L of the FFSC, and intra-specific crosses also are sterile under laboratory conditions (Marasas et al., 2001). The lack of a known sexual stage in *F. andiyazi* may result from the lack or rarity of female fertile strains in population sampled or the inadequacy of "standard" crossing conditions for the formation of the sexual stage by this fungus (Marasas et al., 2001).

Genetic structure of fungal pathogen populations

The genetic structure of a population includes a description of the amount and distribution of genetic variation within and among its subpopulations (Milgroom, 1996). Within-species genetic variability in pathogen populations may be associated with variation in pathogenicity (McDonald and Linde, 2002). Therefore, information on the distribution of genetic variation between and within fungal pathogen populations may inform decisions on disease management such as deployment of resistance genes or fungicide applications (McDermott and McDonald, 1993; Chen and McDonald, 1996; McDonald and Linde, 2002).

DNA-based markers such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphisms (RFLP), simple-sequence repeats (SSR), amplified length polymorphisms (AFLP), and variable number tandem repeats (VNTR) are amongst the tools that have been used to elucidate population structure (Milgroom, 1996). Vegetative compatibility groups (VCGs) (Leslie, 1993) have been used to assess genetic variation within and the subdivision of some fungal populations, and are especially useful in *Fusarium oxysporum*.

The type of information discerned in these studies varies. In some cases populations were found to be effectively randomly mating, even if there was extensive asexual reproduction during

the epidemic cycle (Chen and McDonald, 1996; Sun et al., 2013). High levels of genetic variation within a population may be associated with variation in pathogenicity (McDonald and Linde, 2002), but this association does not always hold and needs to be tested on a case by case basis (Yugander et a., 2015). In other cases genetic lineages were detected within a species and little, if any sexual recombination was inferred. The presence of multiple lineages within a geographic region may be interpreted as multiple introductions of the pathogen from a sexually reproducing population at the center of origin (Kiros-Meles et al., 2011). A primarily asexual population also may be inferred from relatively low levels of genetic diversity, even in the absence of a clearly identifiable lineage structure (Rosewich et al., 1998).

Multiple species/populations of *Fusarium* have been the subject of population genetic analyses. For example, Korean rice populations of *F. graminearum* have lower levels of genotypic diversity (Lee et al., 2009) than do populations from wheat in China (Gale et al., 2002; Zhang et al., 2010) and the United States (Zeller et al., 2003a, 2004; Gale et al., 2007). Korean maize populations of *F. graminearum*, however, have levels of variation similar to those seen in the US and Chinese wheat populations (Lee et al., 2012). These maize-originated isolates also showed evidence for gene flow between geographically dispersed fungal populations and between members of the multiple genetic lineages/phylogenetic species found in Korea. N_e cannot be determined for *F. graminearum* because it is a homothallic fungus and its life style does not conform to the assumptions made to calculate N_e. N_e has been calculated for both *F. verticillioides* and *F. proliferatum* (Leslie and Klein, 1996). In a global population, female fertility limited N_e in both species and was higher in *F. verticillioides* (89% of the count) than it was in *F. proliferatum* (75% of the count). Local populations, however, may be considerably lower, e.g., N_e for *F. verticillioides* from no-till maize in Argentina was only 36% of the count even though

high levels of genotypic diversity were observed and the strains from different geographic locations appeared to be part of a single large randomly mating population (Chulze et al., 2000; Reynoso et al., 2009). VCG analysis of these Argentinian populations found genetic variation within the VCGs, indicating that strains in the same VCG were not clones of one another as is commonly the case in *F. oxysporum* (Leslie, 1993). For *Fusarium* pathogens of grain sorghum such as *F. thapsinum* and *F. andiyazi*, little is known about their population structure.

Chapter 2 - Genetic population structure and pathogenicity of *Fusarium thapsinum* from sorghum in Kansas

Abstract

Fusarium thapsinum is an important pathogen of sorghum (*Sorghum bicolor*) causing both grain mold and stalk rot. The genetic structure of *F. thapsinum* subpopulations in Kansas sorghum was evaluated with amplified fragment length polymorphisms (AFLP), vegetative compatibility groups (VCGs), sexual cross-fertility, and sorghum seedling pathogenicity tests. Two sympatric subpopulations within the species termed subpopulation 1 and subpopulation 2, and a genetically intermediate hybrid group detected. Genotypic diversity was high in all these groups suggesting that sexual recombination occurs in this subpopulation, but relatively low female fertility suggests that asexual reproduction also is very important. Strains within VCGs could be genotypically diverse, and members of the same VCG were not necessarily clones. VCG markers were not useful for differentiating subpopulations. Variation in pigmentation occured, with yellow-pigmented strains dominating in subpopulation 1 and the hybrid group, and subpopulation 2 split roughly equally between pigment producers and non-producers. Both pathogenic and non-pathogenic strains occurred in two different seedling assays, with less than 5% of the strains pathogenic in both assays. The genetic basis(es) for these differences is unknown. In conclusion, populations of *F. thapsinum* from Kansas probably were introduced, at least initially, some time ago from two different regions, and have been in the state long enough to begin interbreeding with one another, although the low female fertility observed suggests this process is slow. The considerable variability in and unequal distribution of seedling pathogenicity across the three subpopulation groups suggests that many strains are not major seedling pathogens and could

even be endophytes, and that it should be possible to discern the genetic and molecular bases for these characters. The observed differences in population structure and seedling pathogenicity help explain the irregular pattern for the field occurrence of sorghum seedling blight and stand establishment, and establish the need for those screening for sorghum disease resistance to carefully screen the strains used to inoculate field trials and not to assume that all strains are equally pathogenic.

Introduction

Fusarium thapsinum is a heterothallic fungus that is a member of the *F. fujikuroi* species complex (FFSC), a group of more than 40 biological and phylogenetic species that are important pathogens of a number of crops including maize, rice, and sorghum (Leslie, 1991, 1995; Klittich and Leslie, 1992; Klittich et al., 1997; Leslie and Summerell, 2006; Kvas et al., 2009; Lima et al., 2012). Members of the FFSC are not readily differentiated with morphological characters. Various molecular markers including isozymes, DNA profiles, and partial sequences of housekeeping genes also can distinguish *F. thapsinum* from other species of the FFSC (Huss et al., 1996; Steenkamp et al., 2000; Leslie and Summerell, 2006). Members of the FFSC produce numerous mycotoxins that are detrimental to humans and domesticated animals that consume the substrates infected by these fungi (Desjardins, 2006).

Fusarium thapsinum is an important pathogen of grain sorghum (*Sorghum bicolor*), causing both grain mold and stalk rot (Frederiksen and Odvody, 2000; Jardine and Leslie, 1992; Klittich and Leslie, 1992; Klittich et al., 1997; Leslie and Summerell, 2006). The fungus is globally distributed and is found wherever sorghum is grown. Conidia of this fungus can be recovered from the air and soil associated with sorghum fields (Funnell-Harris and Pedersen, 2011). Other substrates for *F. thapsinum* include bananas, maize, peanuts, native North American prairie grasses (*Andropogon gerardii*, *A. scoparius*, and *Sorghastrum nuttans*), and an Australian indigenous grass (*Austrostipa aristiglumis*) (Leslie et al., 2004; Leslie and Summerell, 2006; Bentley et al., 2007).

Grain mold and stalk rot of sorghum are both yield-limiting diseases and greatly affect seed quality and vigor (Little and Magill, 2003, 2009; Jardine, 2006; Prom and Erpelding, 2009). *F. thapsinum* is a common member of the sorghum grain mold and stalk rot disease complexes.

In Kansas, stalk rot is the most common disease of sorghum with an average annual loss of 5%, and increase to 50%, or more, in a bad year. Dry conditions early in the season favor the initiation of stalk rot in the field. Subsequent, warm, wet conditions favor increased disease incidence and severity (Jardine, 2006). Many sorghum lines are susceptible to stalk rot caused by *F. thapsinum* (Tesso et al., 2010). *F. thapsinum* also is pathogenic to sorghum seedlings in *in vitro* assays (Leslie et al., 2005). In most studies, only a single strain of *F. thapsinum* was used, and comparable pathogenicity evaluations of multiple *F. thapsinum* strains are lacking.

Within-species genetic variation in subpopulations of pathogens may indicate variation in pathogenicity (McDonald and Linde, 2002) and geographic origins of the pathogen population. Therefore, the distribution of genetic variation between and within pathogen subpopulations is important information in disease management strategies, e.g. deployment of resistance genes or fungicide application strategies (McDermott and McDonald, 1993; McDonald and Linde, 2002). The objectives of this study were: (i) to elucidate the subpopulation structure of *F. thapsinum* isolated from Kansas sorghum; and (ii) to evaluate the pathogenicity of these strains towards sorghum seedlings. Our working hypothesis was that *F. thapsinum* populations were similar to other *Fusarium* populations with a known sexual stage. In particular, that the populations would be genetically diverse with relatively few, if, any clones; that strains in the same VCG would be more closely related than those in the population as a whole; that there will be no significant subdivisions within the population; and that pathogenicity will not have an easily identified genetic basis. The current report provides the first evidence of variation in seedling pathogenicity within *F. thapsinum* and of genetic subdivisions within populations of this pathogen.

Materials and Methods

Strains were collected from sorghum fields in thirty counties in Kansas, USA over four years (1986-1989) (Appendix A). Strains were recovered from plant material, including asymptomatic or diseased sorghum seeds and the stalks of plants with stalk rot symptoms, as described by Leslie and Summerell (2006). Cultures were purified by subculturing a single microconidium with a micromanipulator. Pure culture spore suspensions were stored in 15% glycerol at -70°C.

Population structure and genotypic diversity

Culture preparation and DNA extractions

Cultures were grown on complete medium (CM) slants for 7 days (Correll et al., 1987). A spore suspension was made by flooding the culture with 1 ml of an aqueous 2.5% Tween 60 (Sigma-Aldrich Corp., St Louis, MO) solution and scraping the agar surface with the tip of a Pasteur pipette. The spore suspension, approximately 2 ml of 1×10^6 conidia/ ml, was used to inoculate a 125-ml Erlenmeyer flask containing 40 ml CM broth. Flasks were incubated on an orbital shaker (135 rpm) for 2 d at room temperature (22-25°C). Approximately 500 mg of fungal mycelia was recovered per ml of culture broth following filtration through a milk filter disc (Ken AG, Ashland, OH). Mycelia were blotted dry with paper towels, wrapped in aluminum foil, and stored at -70°C until DNA was extracted. DNA was isolated by following a cetyltrimethylammonium bromide (CTAB) protocol (Murray and Thompson, 1980) as modified by Leslie and Summerell (2006). DNA pellets were washed twice with 70% ethanol, air-dried for 5 min in an oven at 60°C, and dissolved in 50 µl of 1× TE buffer (pH 8.0) (10 mM Tris base, 1 mM EDTA). DNA concentrations were determined with an ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) and the extracts were stored at -20°C until used.

Amplified fragment length polymorphisms (AFLPs) reaction and analysis

Three primer-pairs (*EcoRI*+TT/*MseI*+AC, *EcoRI*+GG/*MseI*+CT, and *EcoRI*+AA/*MseI*+TT) were used to generate AFLP profiles (Vos et al., 1995) as described by Leslie and Summerell (2006). To confirm the polymorphisms in the AFLP results, a subset of 24 strains were selected that contained putative private alleles and AFLP assays were repeated. *F. thapsinum* strains KSU4093, Fungal Genetics Stock Center (FGSC 7056), and KSU4094 (FGSC 7057) were included as reference strains in the AFLP analysis. Bands between 200 and 500 base pairs in length were scored manually based upon their presence ("1") or absence ("0"). Bands from different individuals of the same molecular size were assumed to be identical.

Population structure was determined by using the Bayesian clustering method in STRUCTURE v2.2 (Pritchard et al., 2000). Individuals were assigned to possible ancestral groups based on an admixture model with no prior population information. The number of groups, *K*, was set from 1 to 5, and 4 independent runs were carried out with "burn-in" replicates set at 20,000 and a run length of 40,000 steps.

POPGENE version 1.32 (Yeh et al., 1999) was used to analyze allele frequencies of polymorphic loci and determine the number of loci in linkage disequilibrium (LD). Genotypic diversity ($\hat{G}$) for each population was estimated as described by Milgroom (1996), the value obtained was normalized by the number of haplotypes identified in a population.

Effective population size (N_e)

MAT-1 *and MAT*-2

MAT-1 and *MAT*-2 PCR reactions used the primers and amplification conditions of Steenkamp et al. (2000). Amplified DNA fragments were separated in a 2% agarose gel stained with 10 mg/ml

of ethidium bromide. *MAT* alleles were diagnosed based on the size of the DNA fragment amplified (200 bp for *MAT*-1 and 800 bp for *MAT*-2).

Sexual crosses and female fertility

Sexual crosses were made on carrot agar as previously described (Klittich and Leslie, 1988). In tests of male fertility, crosses were made in which one of the *F. thapsinum* tester-strains FGSC 7056 *MAT*-2 or FGSC 7057 *MAT*-1 served as the female parent and a wild-type strain (Appendix A) served as the male parent. Crosses were made between strains of different mating type. Crosses in which the wild-type strains were tested for female-fertility had the wild-type strain as the female parent and the tester strain as the male parent. All crosses were repeated three times.

Effective population size based on mating type [$N_{e(mt)}$] and on female fertility [$N_{e(f)}$] were calculated by using the equations of Leslie and Klein (1996).

Length and range in hermaphrodite frequency for equilibrium cycle

In *F. thapsinum*, hermaphroditic strains can serve as the male or the female parent in crosses, but are self-sterile with fertile crosses resulting when strains of the opposite mating type are crossed. The observed hermaphrodite frequency was used to estimate the length, in asexual generations, and the range of female-fertile frequency that might occur if the subpopulation is in an equilibrium cycle. The mutation rate from hermaphrodite to female-sterile (μ) and the fitness of a hermaphrodite during vegetative growth (θ) were assumed to be constant, and $(1-\mu)\theta$ values of 0.98, 0.99, and 0.999 were used with the equations of Leslie and Klein (1996).

Vegetative compatibility

A subset of 67 strains (Appendix A) was tested for heterokaryon compatibility and the results used to group strains into vegetative compatibility groups (VCGs) (Leslie and Summerell, 2006; Leslie, 1993). Nitrate non-utilizing (*nit*) mutants (*nit*1, *nit*3, and NitM) were generated by growing strains on minimal medium (MM) containing 1.5% (w/v) $KClO_3$ (Correll et al., 1987). Phenotypes of the mutants were identified by growing them on MM with nitrate, nitrite, ammonium or hypoxanthine as the sole nitrogen source (Correll et al., 1987). Complementary mutants (*nit*1 and *nit*3, or *nit*1 and NitM) of each field strain were paired on MM to determine if the field strain was heterokaryon self-compatible (HSC) or heterokaryon self-incompatible (HSI) (Correll et al., 1989). The formation of a heterokaryon by *nit* mutants derived from the same parent indicated that the parental strain was HSC. HSC strains were paired with complementary *nit* mutants from other HSC strains to determine if they were in the same vegetative compatibility group (VCG). A line of robust mycelia growth between HSC strains carrying complementary *nit* mutants indicated that the strains were in the same VCG, while no growth indicated that the strains were in different VCGs. HSI strains cannot form heterokaryons and were excluded from the analysis.

Genetic distance between strains was based on AFLP data and calculated by using the Unweighted Pair Grouping by Mathematical Average (UPGMA) algorithm implemented in SAS (v.9.1.; SAS Institute, Cary, North Carolina).

Pigment formation

Cultures grown on full strength potato dextrose agar (PDA) (Difco, Detroit, MI) were evaluated for formation of the characteristic yellow pigment produced by some strains of this species (Klittich et al., 1997). Pigment production of strains was scored as yellow (Y) or non-pigmented (NP).

Seedling pathogenicity

Seed preparation

A grain-mold susceptible sorghum line, Tx430, was used for tests of *F. thapsinum* pathogenicity in rolled-towel and pot-based greenhouse assays. A hot water treatment developed for maize (Daniels, 1983) was modified and used to disinfect and remove internal fungi from the seeds. In particular, approximately 2 g of seed were hydrated, wrapped in four layers of cheesecloth, and incubated in a 55°C water bath for 15 min. After the heat treatment, the seeds were rinsed with sterile, distilled H_2O, and dried overnight in a laminar flow hood. Disinfested seeds were stored in the refrigerator (4°C) and used within two days of preparation.

Inoculum preparation and seed inoculation for the rolled-towel assay

Spore suspensions were prepared as described for DNA analyses, and 1 ml of the suspension was used to inoculate a 125-ml Erlenmeyer flask containing 40 ml of CM broth. Flasks were incubated on an orbital shaker (135 rpm) at room temperature (22-25°C) for 2 d. Cultures were filtered through four layers of cheesecloth to remove mycelia. Conidia remaining in the filtrate were counted with a hemacytometer and spore density adjusted to 1×10^6 conidia/ml in an aqueous 2.5% Tween 60 solution.

Ten ml of a spore suspension was placed in a 15 ml plastic tube containing approximately 30 heat-treated sorghum seeds and left on the bench top for four hours at room temperature (22-25°C). Seeds incubated in sterile-distilled water (dH_2O) served as the mock-inoculated control. With sterile forceps, 25 seeds were placed on top of two layers of wet germination paper (Warham et al., 1996) and then covered with a third layer of paper. The wet germination paper, containing seeds, was rolled up, and held together with a rubber band (Warham et al., 1996). Rolled

towels were placed in a 5-L cereal container (Rubbermaid Co. model number 1856059, Winchester, VA), which acted as a humidity chamber, and incubated at 30°C for 6 d. Seeds with radicles >2 mm in length were considered germinated. Radicle length and seed germination (%) were measured on the 6th day of incubation. Experiments were repeated four times.

Soil preparation for pot-based greenhouse assay

A 1:1 silt clay loam:vermiculite mixture (10 kg) was steam pasteurized at 71°C for 30 min. The soil was cooled to room temperature and supplemented with 2 g of Osmocote® (Scotts Miracle-Gro, Marysville, OH) per kg of soil mixture. Pots were filled with 500 ml of soil cooled to room temperature.

Seed inoculation and planting in the greenhouse

Seed disinfection, fungal inoculum preparation, controls, and seed inoculation were as described above for the rolled-towel assay. Each pot was planted with 25 seeds at a depth of 2 cm. Pots were watered every second day for 14 d. Average temperature (23.4, ±3.2°C) and relative humidity (19±6.2%) readings in the greenhouse were monitored with a HOBO® temperature and humidity data logger (Onset Computer Corp., Bourne, MA). On day 14, the number of seedlings per pot was counted and % emergence calculated. Seedlings were removed from the soil, washed with water and the roots excised with a scalpel. Leaves were blotted dry on paper towels for one minute at room temperature (22-25°C), and fresh weight (mg) determined immediately. The experiment was repeated four times.

Data collection and statistical analysis

Radicle length and % germination [(number of germinated seeds)/(total number of inoculated seeds) × 100] were measured on day 6 in the rolled-towel assay. For the pot-based assay in the greenhouse, fresh weight and percent emergence [(number of emerged seeds)/(total number of seeds planted) × 100] were measured on day 14. The data were analyzed by using PROC GLM, Dunnett's *t* test (mock-inoculated control = seeds treated with distilled H_2O), and Fisher's Least Significant Difference (LSD) test procedures as implemented in SAS (v.9.1.; SAS Institute, Cary, North Carolina). A strain was scored as pathogenic if the mean for any of the variables (radicle length, % germination, fresh weight, and % emergence) was significantly ($P < 0.05$) less than the mock-inoculated control. A strain was scored as non-pathogenic if none of the means were significantly different from the mock-inoculated control. A strain was scored as stimulatory if the mean for fresh weight was significantly ($P < 0.05$) larger than the mock-inoculated control. Differences in pathogenicity were evaluated between subpopulations.

Results

Population structure and genotypic diversity

Population structure was determined with a subset of 34 polymorphic AFLP loci. Both alleles at all 34 loci were present within the population at a frequency of > 5%. Two subpopulations ($K = 2$) were inferred by using STRUCTURE. All strains were admixed and had some ancestry attributable to both subpopulations. Admixed strains were assigned to inferred subpopulations based on their estimated membership fractions or membership coefficients (Q1 and Q2). When membership fractions of 0.55, 0.60, 0.65, 0.70, and 0.75 were tested, no differences in allele frequencies were found between the resulting inferred subpopulations. Strains in a third intermediate region ("hybrids") were ignored in these analyses.

When a membership fraction of 0.80 was used to assign strains to subpopulations, there were allele frequency differences between the subpopulations. This subpopulation definition is used for the rest of the analysis. Thus, a strain with a cluster 1 membership fraction of ≥ 0.8 was inferred to belong in subpopulation 1, and a strain with a cluster 2 membership fraction of ≥ 0.8 was inferred to belong in subpopulation 2. Strains with membership fractions that were < 0.8 in either cluster were placed in an intermediate group and are referred to as the "hybrid" group for the rest of the analysis.

There were 78 strains in subpopulation 1, 55 in subpopulation 2, and 34 in the hybrid group (Table 2.1; Figure 2.1). With 34 polymorphic loci used in the analysis, $2^{34} = 1.72 \times 10^{10}$ unique haplotypes could occur, of which we observed 142 in the whole population of 167, 56 in subpopulation 1, 52 in subpopulation 2, and 34 in the hybrid group. High levels of genotypic diversity were observed in all three groups and was highest ($\hat{G}/n = 1$) in the hybrid group, was 0.92 in subpopulation 2, and 0.73 in subpopulation 1 (Table 2.1). No private alleles were detected in any of the three groups.

The percentage of locus pairs in significant linkage disequilibrium at $P < 0.05$ in the entire population was 42%, 31% in subpopulation 1, 20% in subpopulation 2, and 13% in the hybrid group. At $P < 0.01$, the percentage of locus pairs in significant linkage disequilibrium declined to 30% in the entire population, 21% in subpopulation 1, 10% in subpopulation 2, and 6% in the hybrid group (Table 2.1).

Effective population size (N_e)

Mating type and female fertility

In the entire subpopulation, mating type segregated 101:66 *MAT-1*:*MAT-2*, and was significantly different from the expected 1:1 segregation ratio ($\chi^2 = 7.335$, df = 1, $P < 0.05$). In subpopulation 1, mating type segregated 51:27 *MAT-1*:*MAT-2* and also was significantly different from the expected 1:1 ratio ($\chi^2 = 7.385$, df = 1, $P < 0.05$). Mating type segregated 33:22 *MAT*-1:*MAT-2* in subpopulation 2 ($\chi^2 = 2.200$, df = 1, $P = 0.138$) and 17:17 *MAT-1*:*MAT-2* in the hybrid group, neither of which differed significantly from the expected 1:1 segregation ratio.

Of the 167 *F. thapsinum* strains tested for female fertility, only four strains (F-01375, F-01377, F-01383, and F-02586) were female fertile (hermaphrodites). Three of these strains, F-01375, F-01377, and F-02586 belonged to subpopulation 2, and the fourth strain, F-01383 to subpopulation 1. All four female-fertile strains were of the same mating type (*MAT*-1). The number of perithecia produced by the female-fertile strains was high, ~80% that of a cross between the mating-type tester strains. Strains that did not produce perithecia as the female parent remained barren even if the incubation time for the crosses was extended from four weeks to eight.

For the whole population, the effective population size based on mating type $N_{e(mt)}$ was 96% of the count (total subpopulation), 91% of the count in subpopulation 1, 96% of the count in subpopulation 2, and 100% of the count in the hybrid group (Table 2.2). For the whole population, the effective population size based on female fertility $N_{e(f)}$ was 2% of the count, 5% of the count in subpopulation 1, and 20% of the count in subpopulation 2, and was 0 in the hybrid group since no female fertile strains occurred (Table 2.2). The average number of asexual generations per sexual generation if $(1 - \mu)\Theta = 0.98$, was between 92 and 185 for the whole subpopulation, between 109 and 215 for subpopulation 1, and between 72 and 144 for subpopulation 2

(Table 2.2). The range for hermaphrodite frequency was <1 to 16% for the whole subpopulation, <1 to 11% for subpopulation 1, and <1 to 24% for subpopulation 2.

Vegetative compatibility

A subset of 67 strains was used for vegetative compatibility group (VCG) analysis and 37 VCGs were identified. Twenty-six strains belonged to distinct single member VCGs, while the remaining 41 strains belonged to one of 10 multi-member VCGs. VCG 7 contained strains of each mating type, while the remaining VCGs contained strains of only a single mating type. When subpopulation differentiation of strains in VCGs was evaluated, all members of VCG 3 and VCG 6 belonged to subpopulation 1. Members of VCG 4 and VCG 10 all belonged to subpopulation 2. Members of VCG 1, VCG 5, VCG 8, and VCG 9 belonged to subpopulation 1 or the hybrid group. Members of VCG 2 belonged to subpopulation 2 or the hybrid group, and members of VCG 7 belonged to both subpopulation 1 and subpopulation 2 (Figure 2.2). Strains within VCG 4 and VCG 6 were each recovered from only a single field and a single plant and could represent multiple recoveries of the same strain. The remaining multi-member VCGs all contained strains from multiple fields and presumably represent independent occurrences of the VCG genotype.

Pigment formation

The ratio of Y:NP strains was significantly different from 1:1 ($P < 0.05$) in the population as a whole, 118:49 ($\chi^2 = 28.5$, $P < 0.00001$), in subpopulation 1 – 74:4 ($\chi^2 = 62.8$, $P < 0.00001$), and in the hybrid group – 23:11 ($\chi^2 = 4.2$, $P = 0.04$). The ratio of Y:NP strains was not significantly different from 1:1 in subpopulation 2 – 21:34 ($\chi^2 = 3.1$, $P = 0.08$). The direction of skewing was not consistent, with the pigmented strains dominating in subpopulation 1 and the nonpigmented strains dominating in subpopulation 2 and the hybrid group.

Seedling pathogenicity

The *F. thapsinum* strains were evaluated for pathogenicity on sorghum seedlings in rolled-towel and pot-based greenhouse assays. In the rolled-towel assay, based on mean radicle length, 46/167 (28%) strains were pathogenic (Figure 2.3A). The remaining 121 strains were non-pathogenic and did not differ significantly from the control. Based on mean % germination, 36/167 (22%) strains were pathogenic (Figure 2.3B). The remaining 131 strains were non-pathogenic and did not differ significantly from the mock-inoculated control. Of the pathogenic strains, 29/82 were pathogenic based on both mean radicle length and mean % germination.

In the greenhouse assay, based on mean fresh weight, 45/167 (27%) strains were pathogenic, 94 (56%) were non-pathogenic, and 28 (17%) were stimulatory (Figure 2.3C). Based on mean % emergence, 71/167 (43%) strains were pathogenic, and 96 (57%) were non-pathogenic and did not significantly differ from the mock-inoculated control (Figure 2.3D). Of the pathogenic strains, 32/116 strains were pathogenic based on both mean fresh weight and mean % emergence. Five strains, F-02484, F-02645, F-02659, F-02671, and F-02699, were pathogenic when all four pathogenicity measurements were considered. In addition, there was a significant ($P < 0.05$) negative Pearson correlation between radicle length and fresh weight (data not shown).

For radicle length and fresh weight there were significant differences ($P < 0.05$) between subpopulation 1, subpopulation 2, and the hybrid group (Table 2.4). There were no significant differences ($P = 0.05$) in % germination or % emergence between subpopulation 1, subpopulation 2, and the hybrid group (Table 2.4). Of the five strains that were pathogenic based on all the pathogenicity measurements, strain F-02645 was from subpopulation 1, and strains F-02484, F-02659, F-02671, and F-02699, were from subpopulation 2 (Appendix B).

No meaningful comparisons of pathogenicity could be made between *MAT*-1 and *MAT*-2 strains or between yellow and non-pigmented strains in any of the subpopulation groups. The strains in the multi-member VCGs were too irregularly distributed and too small in number for a meaningful pathogenicity analysis to be made.

Discussion

Our objective was to discern genetic population structure within and the degree of pathogenicity of a population of *F. thapsinum* from sorghum in Kansas. Two subpopulations were identified that can interbreed with one another as 20% of the population has between 20 and 80% of their genetic material from both of the identified subpopulations. This result is the first record of any significant genetic structure in a population of *F. thapsinum*, although some structure is present in related species. For example, populations of *F. proliferatum* from a non-agricultural ecosystem in Australia (Neumann et al., 2004) had a clonal structure, and *F. verticillioides* populations from commercial maize in Argentina (Reynoso et al., 2009) had high levels of genotypic diversity and were part of a larger, randomly mating population.

In the present study, the inferred subpopulations and the hybrid group all had a high genotypic diversity (Table 2.1). High genotypic diversity combined with the presence of genetically intermediate strains in a population usually suggests that sexual recombination is occuring. The high levels of genotypic diversity are consistent with our working hypothesis that *F. thapsinum* populations are similar to other *Fusarium* populations, such as those of *F. verticil-lioides* (Chulze et al., 2000; Reynoso et al., 2009) and *F. graminearum* (Zeller et al., 2004; Lee et al., 2012) with a known sexual stage as part of their life history. In our study, the highest level of LD was detected in the population as a whole. One explanation is that the two inferred subpopulations and the hybrid group have only partially overlapping genotype sets and that the

common genotypes are present at different frequencies within these groups (Nei and Li, 1973; Slatkin, 2008). The non-uniform distribution of LD also is consistent with the conclusion that there is underlying genetic structure in the Kansas population of *F. thapsinum*. Amongst the inferred subpopulations and the hybrid group, the lowest LD occurred in the hybrid group. This result suggests that the two inferred subpopulations have had sufficient time and opportunities to for some interbreeding to occur. Interbreeding reduces LD through recombination and results in strains with genotypes that are not found in either of the inferred subpopulations.

N_e is used to estimate the effects of drift and inbreeding, and to compare field populations with an idealized population. In the present study, N_e values based on female fertility were lower than N_e values based on the mating-type allele ratio (Table 2.2). The very limited number of strains that can function as the female parent in the whole population limits N_e to 20% or less of the count in the individual subpopulations. In a global population of *F. thapsinum* (Leslie and Klein, 1996), the discrepancies in the mating-type ratios reduced $N_{e(mt)}$ to only 98% of the count, while the limited number of female-fertile strains reduced $N_{e(f)}$ to 32% of the count. In Tanzania (Mansuetus et al., 1997), a local population of *F. thapsinum* had an $N_{e(mt)}$ of 91% of the count, and $N_{e(f)}$ of 53%. Thus, low female fertility in local and global populations of *F. thapsinum* is much more important for reducing N_e than are any observed imbalances in *MAT*-allele frequencies. Subpopulation 2 from the present study had more female-fertile strains, and a higher N_e (20%) than subpopulation 1 (5%) (Table 2.2), but overall the number of female-fertile strains in the entire population was very low. Estimates of N_e based on female-fertile strain frequency reported in this study are the lowest ever reported for *F. thapsinum* (Leslie and Klein, 1996; Mansuetus et al., 1997).

Hermaphrodite (female-fertile strains) frequency is the highest immediately after sexual reproduction and decreases as the amount of asexual reproduction increases. By using the equations of Leslie and Klein (1996) it is possible to calculate an upper and lower limit for the expected number of hermaphrodites in a population. In the present study the frequency of hermaphrodites ranged from <1% to 16% for the whole population and the number of asexual generations per sexual generation is ~3700 (Table 2.3). At such low levels of female fertility it is possible that all of the hermaphrodites could be lost to chance and thereby render the population completely asexual. Such a population would slowly lose its genotypic diversity and its ability to generate new genotypes to respond to environmental changes. The low number of female-fertile strains could also be the reason that we could discern two subpopulations, since if sexual reproduction occurred relatively frequently there might have been enough interbreeding between members of the two populations to obliterate the genetic structure we observed.

Vegetative compatibility has been used as a measure of genetic variation in several populations of the *F. fujikuroi* species complex (Chulze et al., 2000; Carter et al., 2008; Reynoso et al., 2009). In the present study, 67 strains were distributed amongst 37 VCGs. Twenty-seven VCGs contained one strain each. The remaining 40 strains were distributed amongst 10 multi-member VCGs that each contained between two and eight members. At least six *vic* loci must be segregating in the population for 37 VCGs to exist (Leslie, 1993). Given the number of VCGs with members from two of the different subgroups in the population, the number of segregating *vic* loci must be relatively small, as the same VCG genotype apparently has occurred independently on multiple occasions for strains in the two subpopulations. The number of strains within a single VCG is limited and a rigorous test is not possible, but it seems unlikely that strains in the same VCG are much more closely related to one another than are any two strains

selected randomly from the population. Similar to Klittich and Leslie (1992), we found that VCG markers detected genetic diversity in *F. thapsinum*. The utility of these subdivisions in unclear, however, as there was no obvious connection between the VCGs and the population structure detected following analysis of the AFLP markers.

Phenotypic variation based on pigment formation in *F. thapsinum* has been reported (Klittich and Leslie, 1992; Klittich et al., 1997). The presence of a diffusing yellow pigment on PDA is an important morphological characteristic for distinguishing *F. thapsinum* from other closely related species within the *F. fujikuroi* species complex (Leslie and Summerell, 2006). In the population as a whole, yellow strains were more than twice as frequent as non-pigmented strains (118:49), but these frequencies are not consistent across the three population subgroups, with subpopulation 1 strains being almost all yellow (74:4), the hybrid group having a majority of yellow strains (23:11), and subpopulation 2 strains being predominantly non-pigmented (21:34). Thus, while yellow pigmentation can be a useful characteristic for distinguishing *F. thapsinum* from other closely related species, e.g., *F. verticillioides* and *F. andiyazi* (Leslie and Summerell, 2006), this character is not useful for resolving the subpopulations identified in this study.

Variation in pathogenicity in *F. thapsinum* strains appears to have a genetic basis. Seedling pathogenicity of the strains was determined with two different measures in two different assays. Based on all four pathogenicity measurements, *F. thapsinum* strains displayed a continuum of phenotypes from non-pathogenic to pathogenic. This work is the first report of variation in pathogenicity by *F. thapsinum* on sorghum seedlings. Twenty-nine strains were pathogenic in the rolled-towel assay and 32 were pathogenic in the pot-based greenhouse assay. Thus, most of the strains evaluated are either endophytes or latent pathogens. We also identified 28 non-pathogenic

strains with stimulatory effects based on fresh weight compared to the mock-inoculated control (Figure 2.3C). The mechanism(s) responsible for the stimulatory effect are unknown. Strains of *F. graminearum* pathogenic to maize also may enhance emergence and seedling growth relative to the non-inoculated control (Munkvold and O'Mara, 2002). Only five strains (F-02645, F-02484, F-02659, F-02671, and F-02699) were consistently pathogenic (Appendix B) in both assays. These strains are of particular interest for their potential use in screening germplasm resistance to seedling diseases or to adult plant diseases such as stalk rot and grain mold.

In the present study, there were significant differences in the means of radicle length and fresh weight between subpopulation 1, subpopulation 2, and the hybrid group. This variation in pathogenicity may be associated with the within-species genetic variation observed with the AFLP markers. Genetic variation may be associated with variation in pathogenicity (McDonald and Linde, 2002). Significant differences were observed between subpopulation 1, subpopulation 2, and the hybrid group with two measurements, radicle length and fresh weight, but not with two other measurements, mean % germination and mean % emergence. None-the-less, there is sufficient overlap in the distribution of the pathogenicity characters to keep either radicle length or fresh weight from being used as characters to resolve the genetically distinguishable subpopulations, and to keep subpopulation identity from being used to define pathogenicity risk.

In conclusion, two subpopulations and a hybrid group exist in *F. thapsinum* from Kansas with at least some hybridization occurring between the primary subpopulations. The possibility for hybridization is greater when fungi spread beyond their normal geographic ranges (Brasier, 2000) and neither *F. thapsinum* nor its sorghum host are native to Kansas. Some counties in Kansas had strains from subpopulation 1, subpopulation 2 and the hybrid group. Such locations could be considered hybrid zones where strains from subpopulation 1 and subpopulation 2

interbreed and produce “hybrids”. Other possible hybrid zones are locations where commercial sorghum seed is grown. If strains of both subpopulations and the hybrid group are present at these locations, then they could be dispersed as endophytes carried in the seed to commercial sorghum fields. Sorghum originated in Africa and was first exported to the USA around 1853 (Maunder, 2002). The population differentiation we observed in the current study could result from pathogen migration with seeds from different regions of the world. Thus, the two Kansas subpopulations probably reflect the introduction of strains from locations where sorghum has been domesticated. The seed carrying the pathogens may have come with immigrants, imported grain, improved seed (commercial or otherwise), and materials for use in inbreeding programs.

Future work should focus on genetic relationships between *F. thapsinum* populations from Kansas and those from other locations, including different parts of Africa and India, to identify where the Kansas subpopulations may have originated, and to determine the potential subdivision of this species on a broader geographic basis. Since both strain and subpopulation level variation occur in pathogenicity, future work should evaluate a subset of strains representing the two subpopulations and their spectrum of interaction with sorghum plants in the field in terms of their ability to cause adult plant diseases such as grain mold and stalk rot.

The identification of variation in seedling pathogenicity requires grounding in field-based tests for diseases of mature plants such as grain mold and stalk rot. The confirmation of the variation in pathogenicity across different host germplasm also is important. Finally, genetic crosses between pathogenic and nonpathogenic strains could lead to the identification of genetic factors and molecular mechanisms in this fungus that are responsible for the observed variation in pathogenicity.

Table 2.1. Estimates of genotypic diversity and the number of pairs of loci in significant linkage disequilibria in two populations and a hybrid group of *Fusarium thapsinum* from grain sorghum based on AFLP loci

Parameters	Population			
	All Populations	Subpopulation 1	Hybrid	Subpopulation 2
No. of isolates	167	78	34	55
No. of haplotypes	142	56	34	52
No. of polymorphic loci (%) [a]	34 (100)	24 (71)	34 (100)	34 (100)
$\hat{G}$[b]	112	41	34	48
$\hat{G}/n$[c]	0.79	0.73	1	0.92
No. of locus pairs in linkage disequilibria[d] (%)				
At $P < 0.05$	235/561 (42)	86/276 (31)	75/561 (13)	111/561 (20)
At $P < 0.01$	168/561 (30)	57/276 (21)	36/561 (6)	56/561 (10)

[a] Calculated by using the loci for which the frequency of both alleles is > 5%.

[b] The genotypic diversity ($\hat{G}$) was calculated as described in Milgroom (1996) from the comparisons of AFLP allelic data at polymorphic loci. $\hat{G} = 1/\sum p_i^2$, where p_i = the observed frequency of the *i*th multilocus genotype in a population.

[c] $\hat{G}/n$ was calculated by dividing $\hat{G}$ by the number of AFLP haplotypes observed in each population.

[d] Linkage disequilibrium was detected by using POPGENE v1.32. Values are shown as the number of locus pairs in linkage disequilibrium/total number of linkage pairs, with the percentage given in parentheses.

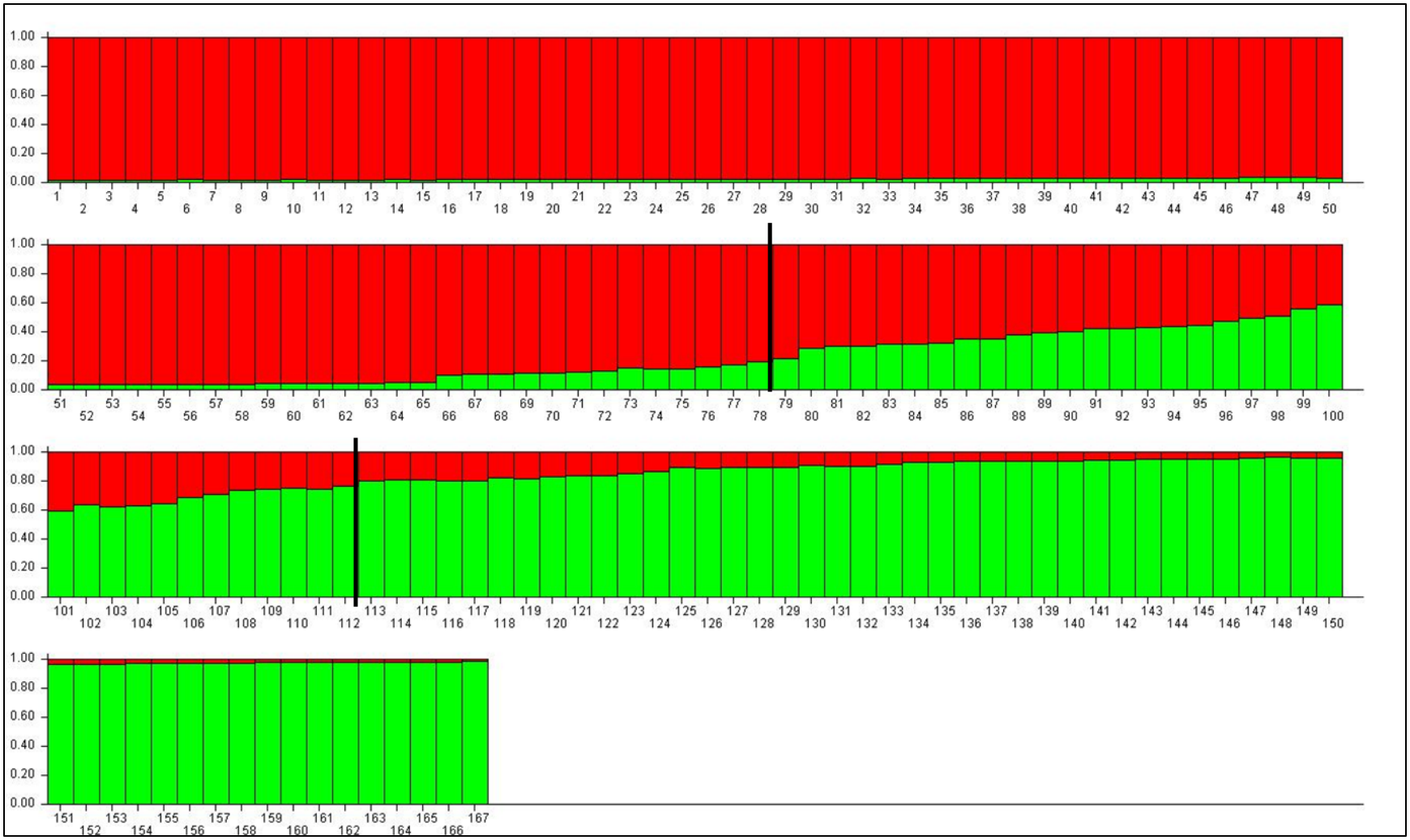

Figure 2.1. STRUCTURE analysis of *F. thapsinum* population from Kansas based on 34 polymorphic AFLP loci. Columns represent individual strains. Strains 1-78 are subpopulation 1, 79-112 are the hybrid group, and 113-167 are subpopulation 2.

Table 2.2 Effective population size parameters for *Fusarium thapsinum* from grain sorghum in Kansas.

Population	Mating type (*MAT-1:MAT-2*)	N_{fs}:N_h	$N_{e(mt)}$[a]	$N_{e(f)}$[b]
Whole population	101:66	163:4	96	9
Population 1	51:27	77:1	91	5
Population 2	33:22	52:3	96	20
Hybrid	17:17	34:0	100	0

N_{fs} – number of female-sterile male fertile strains; N_h – number of hermaphroditic strains;

[a] Inbreeding effective population size based on mating type expressed as a percent of the count based on Equation 4 of Leslie and Klein (1996).

[b] Inbreeding effective population size based on numbers of male and hermaphrodites expressed as a percentage of the actual count based on Equation 6 of Leslie and Klein (1996).

Table 2.3. Length and range in hermaphrodite frequencies for equilibrium cycles for *Fusarium thapsinum* from grain sorghum in Kansas.

Population[a]	Time[b]			Hermaphrodites			Time[c]		
	0.98[d]	0.99[d]	0.999[d]	Maximum[e]	Observed	Minimum[f]	0.98[d]	0.99[d]	0.999[d]
Whole population	92	184	1851	0.157	0.024	<0.001	185	371	3728
Population 1	109	219	2197	0.111	0.013	<0.001	215	432	4341
Population 2	72	144	1447	0.235	0.055	0.003	144	289	2899

[a] No hermaphrodites were observed in the hybrid group, so this group was excluded from the analysis.

[b] Time in asexual generations, to cycle from h_a to the observed frequency given the value of $(1 - \mu)\Theta$ (Leslie and Klein, 1996).

[c] Time in asexual generations, to cycle from the observed frequency to h_b given the value of $(1 - \mu)\Theta$ (Leslie and Klein, 1996).

[d] $(1 - \mu)\Theta$.

[e] Value for h_a if the observed hermaphrodite frequency is used as fs_0 in Equation 3 (Leslie and Klein, 1996).

[f] Value for fs_0 if the observed hermaphrodite frequency is used as h_a in Equation 3 (Leslie and Klein, 1996).

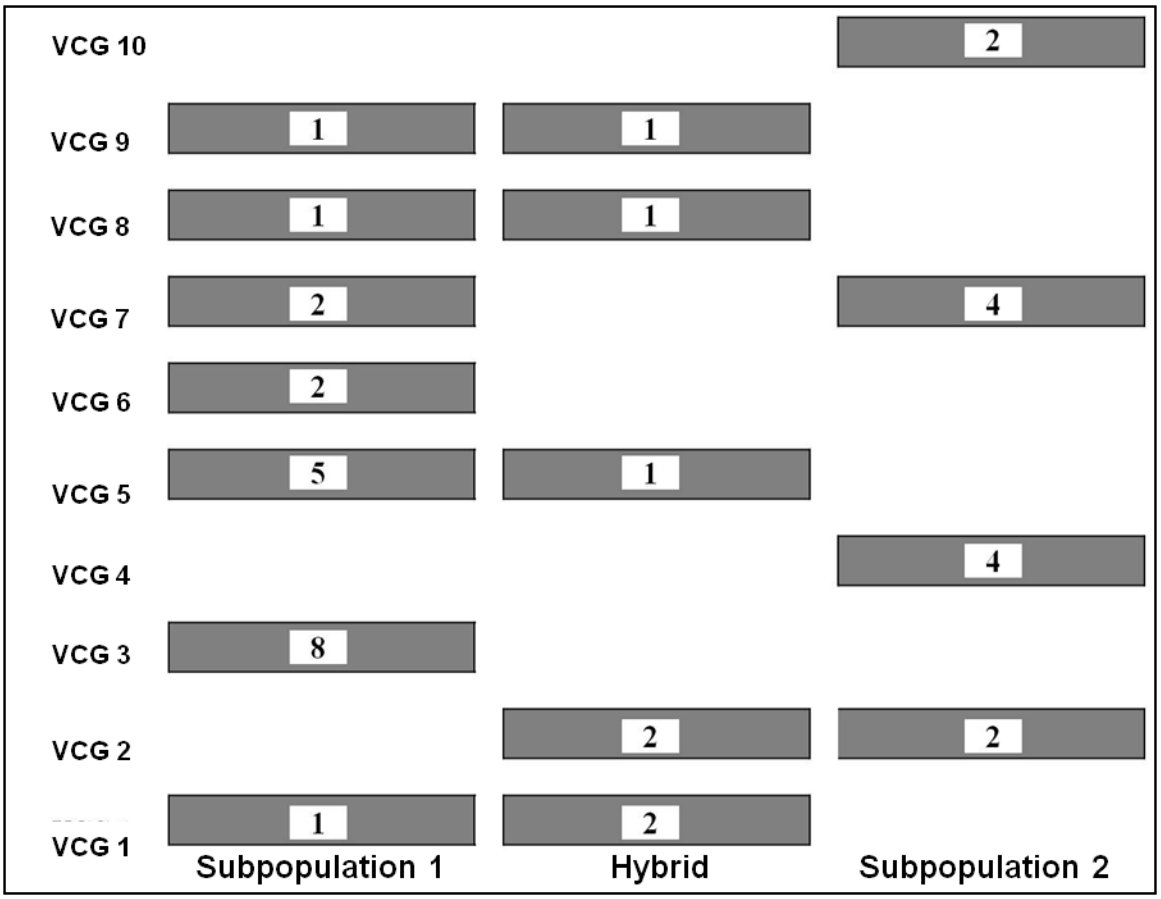

Figure 2.2. Population membership of isolates belonging to the 10 multi-member VCGs identified in this study. The number of isolates in each VCG is indicated in the boxes within the gray-shaded rectangles.

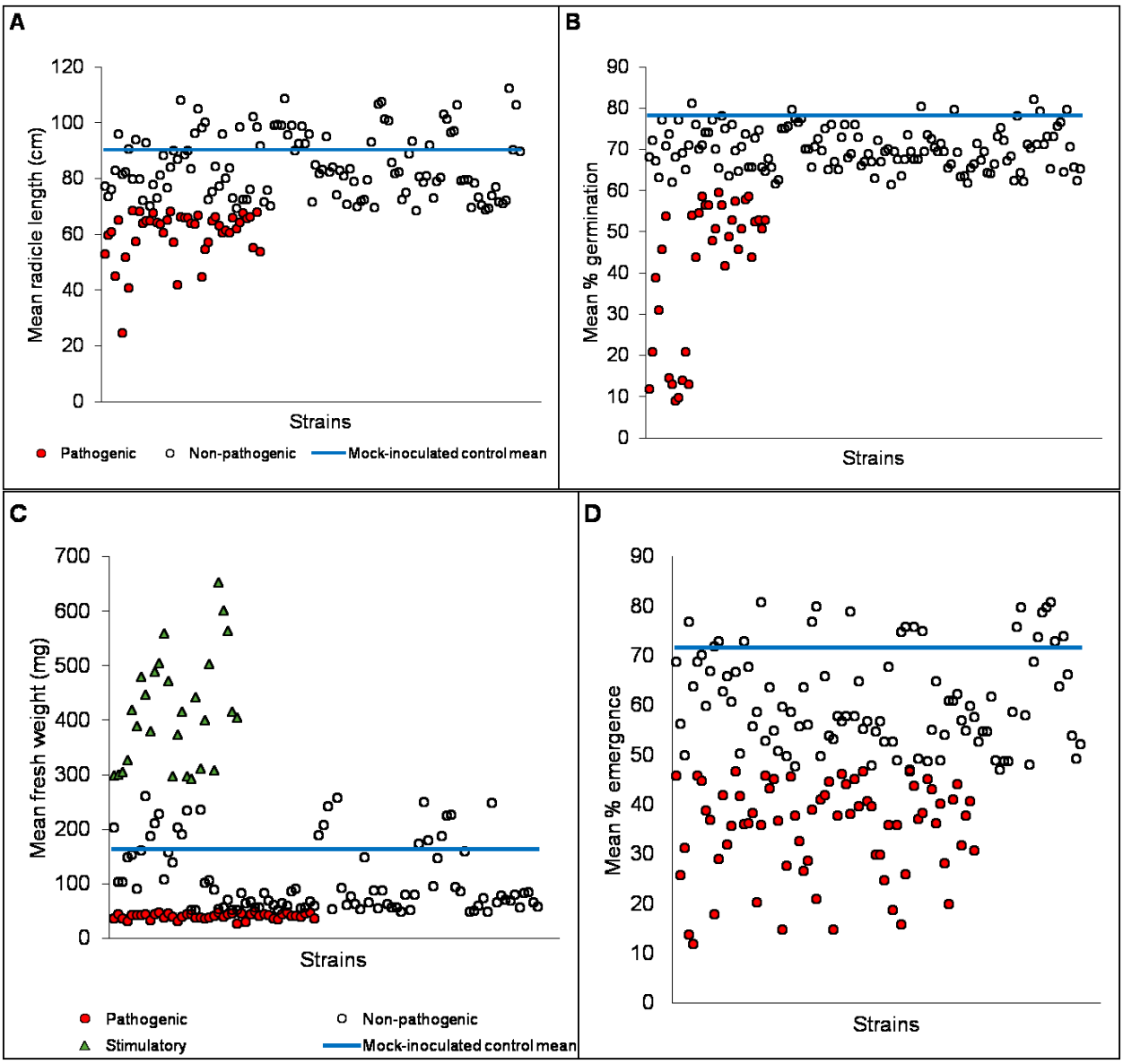

Figure 2.3. Pathogenicity of 167 strains of *F. thapsinum* on grain sorghum seedlings. Mean radicle length (cm) and mean % germination were evaluated in the rolled-towel assay (**A** and **B**). Mean fresh weight and mean % emergence were evaluated in a pot-based greenhouse assay (**C** and **D**). Means of pathogenic strain values were significantly less ($P < 0.05$) than the mock-inoculated control, non-pathogenic strain values were not significantly different, and stimulatory strain values were significantly larger.

Table 2.4. Seedling pathogenicity means of subpopulation 1, subpopulation 2, and the hybrid group.

Parameter	Number of strains	Rolled-towel assay		Pot-based greenhouse assay	
		Radicle length (cm)	% germination	Fresh weight (mg)	% emergence
Subpopulation 1	78	79 bc	64 b	118 b	50 b
Hybrid group	34	83 b	66 b	140 ab	53 b
Subpopulation 2	55	76 c	64 b	179 a	50 b
Mock-inoculated	-	90 a	78 a	163 ab	72 a

Means with the same letter are not significantly different at $P < 0.05$.

Chapter 3 - Relatedness of *Fusarium thapsinum* populations from Kansas, Africa, Thailand, and Australia

Abstract

Grain sorghum (*Sorghum bicolor*) originated in Africa and is an introduced crop in the USA, Thailand, and Australia, although Australia, like Africa, has endemic sorghum species. *Fusarium thapsinum*, a causative agent of both grain mold and stalk rot of commercial sorghum is found everywhere sorghum is cultivated. A Kansas population of *F. thapsinum* showed evidence of introduction from two locations. Using amplified fragment length polymorphism (AFLP) markers and sexual crosses, we evaluated the genetic relationship between *F. thapsinum* populations from sorghum in Kansas (167 isolates), three African countries: Cameroon, Mali, and Uganda (81 isolates), Thailand (20 isolates), and Australia (18 isolates). Genotypic diversity for the *F. thapsinum* populations ranged from 72% for Australia to 94% for Kansas. Putative clones ranged from 7% of the Kansas population to 33% of the Australian population. These results are evidence that both the sexual and asexual modes of reproduction are important components of the life cycle of *F. thapsinum*. The effective population number was greatly reduced by low female fertility. Two genetically distinct populations were inferred by STRUCTURE. One population consisted of strains from Kansas and the second of strains from Africa. The genotypes of the two smaller populations (Australia and Thailand) were similar to those from Kansas and these two populations were phylogenetically more closely related to the Kansas population than they were to the African population. Within the total global population, 77% of the genetic variation was within the populations and 23% was between the populations. These results suggest that gene flow occurs through movement of strains between locations. The presence of private alleles at relatively

high frequency, however, suggests more historical than contemporary gene flow is occurring. The high genotypic diversity of strains from Kansas, Australia, and Thailand suggests that there may be multiple source populations contributing to the populations evaluated from each of these three locations. Additional work is needed to better define potential centers of diversity of *F. thapsinum*, e.g. India, and eastern, western and southern Africa, where sorghum has been domesticated for a long time and where commercial sorghum breeding programs have a long history.

Introduction

Grain sorghum (*Sorghum bicolor*) originated in Africa and is the fifth most important grain crop globally after wheat, rice, maize, and barley (Maunder, 2002). Sorghum serves as a staple food for millions living in drought prone and food-insecure regions in Africa and southern Asia. In numerous African countries, including Mali, Cameroon, and Uganda, sorghum is a subsistence crop used for numerous foods including leavened-breads, couscous, fermented and unfermented-porridges, and traditionally brewed African beers (Rooney and Waniska, 2000). In Southeast Asian countries, such as Thailand, sorghum is an important cereal crop ranking third after rice and maize. In Thailand sorghum is used mainly as an animal feed (Jaisil, 2003). Commercial grain sorghum was first imported to the United States and Australia in the 1850s (Maunder, 2002). In Australia, grain sorghum is grown under rain fed conditions, and is used primarily as animal feed, with a limited amount going into human food as breakfast cereals and snacks (Ryley et al., 2002). Australia also has native sorghum grass species such as *S. laxiflorum* and *S. macrospermum* that are closely related to the cultivated sorghum, *S. bicolor* (Dillon et al., 2004). Grain sorghum grown in the United States either is exported, or used domestically as animal feed or as a feedstock for the biofuel industry.

Diseases on sorghum caused by diverse microorganisms reduce yield and grain quality. Grain mold and stalk rot of sorghums are both yield-limiting diseases that reduce seed quality and vigor (Little and Magill, 2003). Members of the genus *Fusarium*, such as *F. thapsinum*, *F. andiyazi*, *F. verticillioides*, *F. proliferatum*, *F. napiforme*, *F. nygamai*, *F. pseudonygamai*, and additional undescribed species are commonly part of a consortium of fungal species that cause diseases on sorghum (Leslie and Summerell, 2006). *F. thapsinum* is a major causative agent of both grain mold and stalk rot of sorghum, and is found everywhere sorghum is grown (Klittich et

al., 1997; Leslie and Summerell, 2006). Spores of *F. thapsinum* can be recovered from the air and soil associated with sorghum fields (Funnell-Harris and Pedersen, 2011). *F. thapsinum* is not limited to sorghum and also has been recovered from the native tallgrass prairie plants (*Andropogon gerardii*) in Kansas (Leslie et al., 2004), from Australian indigenous grasses (*Austrostipa aristiglumis*) (Bentley et al., 2007), bananas, and maize (Leslie et al., 2005; Leslie and Summerell, 2006).

There is evidence of genetic structure in a population of *F. thapsinum* from sorghum in Kansas with at least two populations and an inferred hybrid group detected (Chapter 2). These populations are presumed to reflect multiple introductions of *F. thapsinum* to Kansas. Our objective in this study was to determine whether genetic substructure exists in other *F. thapsinum* populations, whether this substructure is similar to that observed in Kansas, and whether potential centers of divergence for the species can be identified.

Materials and methods

Fungal isolates

Strains used in this study (Appendices A and C) were obtained from the culture collection in the Department of Plant Pathology, Kansas State University, Manhattan. One-hundred-and-sixty-seven *F. thapsinum* strains used in our previous study (Chapter 2) were from sorghum and constituted the Kansas population. Eighty-one strains were from sorghum seed collected in one of three African countries: Mali (71 strains), Cameroon (6 strains), and Uganda (4 strains) and constituted the African population. Twenty strains were initially recovered from sorghum grain and stalks in Thailand. These strains were partially characterized previously by Nor (2014) and constituted the Thai population. Eighteen strains from Australia were from sorghum field soils (3 strains), diseased sorghum seeds and stalks (12 strains), and a native grass, *A. aristiglumis* (6

strains) (Bentley et al., 2007). All cultures were purified by subculturing a single conidium with a micromanipulator. Pure culture spore suspensions were stored in 15% glycerol at -70°C (Leslie and Summerell, 2006).

Culture preparation and DNA extractions

Cultures were grown on complete medium (CM) slants for 7 days (Correll et al., 1987). A spore suspension was made by flooding the culture with 1 ml of an aqueous 2.5% Tween 60 (Sigma-Aldrich Corp., St Louis, MO) solution and scraping the agar surface with the tip of a Pasteur pipette. The spore suspension, ~ 2 ml of 1×10^6 conidia/ml, was used to inoculate a 125-ml Erlenmeyer flask containing 40 ml of CM broth. Flasks were incubated on an orbital shaker (135 rpm) for 2 d at room temperature (22-25°C). Approximately 500 mg of fungal mycelia was recovered per ml of culture broth following filtration through a milk filter disc (Ken AG, Ashland, OH). Mycelia were blotted dry with paper towels, wrapped in aluminum foil, and stored at -70°C until DNA was extracted. DNA was isolated by following the cetyltrimethylammonium bromide (CTAB) protocol (Murray and Thompson, 1980) as modified by Leslie and Summerell (2006). DNA pellets were washed twice with 70% ethanol, air-dried for 5 min in an oven at 60°C, and dissolved in 50 µl of 1× TE buffer (10 mM Tris base, 1 mM EDTA, pH 8.0). DNA concentrations were determined with an ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) and the extracts stored at -20°C until used.

Amplified fragment length polymorphisms (AFLPs) reaction and analysis

Three primer-pairs (*EcoRI*+TT/*MseI*+AC, *EcoRI*+GG/*MseI*+CT, and *EcoRI*+AA/*MseI*+TT) were used to generate AFLP profiles (Vos et al., 1995) as described by Leslie and Summerell

(2006). *F. thapsinum* mating type tester strains obtained from the Fungal Genetics Stock Center, Manhattan, KS, KSU4093 (FGSC 7056) and KSU4094 (FGSC 7057) were included as reference strains in the AFLP analysis. A binary matrix data was created by scoring bands between 200 and 500 base pairs in length for their presence ("1") or absence ("0"). Bands from different individuals of the same molecular size were assumed to be identical.

MAT-1 and MAT-2

MAT-1 and *MAT*-2 PCR reactions used the primers and amplification conditions of Steenkamp et al. (2000). Amplified DNA fragments were separated in a 2% agarose gel stained with 10 mg/ml of ethidium bromide. *MAT* alleles were diagnosed based on the size of the fragment amplified (~200 bp for *MAT-1* and ~800 bp for *MAT-2*).

Sexual crosses and female fertility

Sexual crosses were made on carrot agar as previously described (Klittich and Leslie, 1988). In tests of male fertility, one of the *F. thapsinum* tester-strains FGSC 7056 (*MAT-2*) or FGSC 7057 (*MAT-1*) served as the female parent and a field strain served as the male parent. Crosses were made between strains of different mating type. Crosses in which the field strains were tested for female-fertility had the field strain as the female parent and the tester strain as the male parent. All crosses were repeated at least three times.

Genotypic diversity

Based on the AFLP data, allele frequencies of polymorphic loci, the number of loci in linkage disequilibrium (LD) in clone-censored populations, and genetic identity among populations, as

described by Nei (1972), were determined with the shareware program POPGENE version 1.32 (available at http://www.ualberta.ca/~fyeh , Yeh et al., 1999). Genotypic diversity ($\hat{G}$) for each population was estimated as described by Milgroom (1996), the value obtained was normalized by dividing by the number of unique haplotypes identified in the population. Analysis of molecular variance (AMOVA) (Excoffier et al., 1992) and the presence of private alleles in populations were determined in GenAlex, version 6.5 (Peakall and Smouse, 2006).

Effective population number (N_e)

A chi-square (χ^2) test was used to test the goodness of fit to a 1:1 segregation ratio for mating types in each population. N_e based on mating type [$N_{e(mt)}$] and female fertility [$N_{e(f)}$] were calculated by using the equations of Leslie and Klein (1996).

Population structure

Population structure was determined by using the Bayesian clustering method in STRUCTURE version 2.2 (Pritchard et al., 2000). STRUCTURE was run with four different sets of AFLP loci: (i) 52 loci polymorphic in at least one of the four populations, (ii) 21 loci polymorphic in all four populations, (iii) the 40 polymorphic loci identified in the Kansas population, and (iv) the 39 polymorphic loci identified in the African population (Appendix D).

Individuals were assigned to putative ancestral groups based on an admixture model with sampling locations using the LOCPRIOR option of STRUCTURE. The number of groups, K, was set from 1 to 5. Ten independent runs were made with "burn-in" replicates set at 40,000 and a run length of 80,000 steps. The most likely number of clusters (K) was determined by using the method of Evanno et al. (2005).

Phylogenetic analysis

The phylogenetic relationship among strains was determined from a binary matrix generated from AFLP markers by Unweighted Pair Group by Mathematical Average (UPGMA) in PAUP* 4.0 beta 10 (Swofford, 1998). The support for any resulting subgroups in the UPGMA tree was assessed by bootstrap analysis with 1,000 iterations. The relationship among populations was determined in a UPGMA-based analysis of allele frequencies of the 21 common polymorphic AFLP loci by using POPTREE2 version 2 software (Takezaki et al., 2010).

Results

Genotypic diversity

Fifty-two polymorphic AFLP loci (Appendix D) were used to analyze genotypic diversity in *F. thapsinum* populations. Genotypic diversity was highest (94%) in the Kansas population and lowest (72%) in the Australia population (Table 3.1). Putative clones ranged from 7% (12/167) of the Kansas population to 33% (6/18) of the Australian population. Within each population there were putative clonal strains that were recovered from the same host plant and others that came from different geographic regions.

Linkage disequilibrium was detected within the population as a whole and in the subpopulations. The highest LD (25%) at $P < 0.05$ was observed in the population as a whole. Amongst subpopulations, LD was highest in Kansas (23%) followed by Australia (14%), and then both the Thai and African populations at 11% (Table 3.1). The pattern followed by the relative LD frequencies was the same at $P < 0.01$ as it was at $P < 0.05$ (Table 3.1).

The population from Africa had eight private alleles, Australia had two, and Kansas and Thailand had one each (Table 3.2). With two exceptions, one from Africa and the other from Kansas, the frequency of the private alleles all were ≤ 15% (Table 3.2).

Effective population number (N_e)

N_e in *Fusarium* can be estimated from the proportion of female-fertile strains or from the relative frequency of the mating type alleles. Mating type did not segregate 1:1 in any of the subpopulations. The *MAT*-1 allele was the most frequent in all of the populations except for the Australian population, where only the *MAT*-2 allele was recovered (Table 3.5). In the three populations where both *MAT* alleles were identified, $N_{e(mt)}$ ranged from 75% to 96% of the count, suggesting small to moderate reductions in N_e due to a skewed ratio of the *MAT* alleles. $N_{e(mt)}$ for the Australian population was 0, as only a single *MAT* allele was detected in our survey (Table 3.5).

Hermaphroditic (female fertile) strains were identified in Kansas (4), Africa (8), and Thailand (3), but not in Australia. $N_{e(f)}$ for the Australian population was 0, as only male-fertile strains were recovered. $N_{e(f)}$ for the other three populations ranged from 9 % to 45% of the count and were smaller than the $N_{e(mt)}$ values for the corresponding populations (Table 3.5).

Population structure

Two populations (K = 2), were inferred by STRUCTURE when strain genotypes for the 52 AFLP loci that were polymorphic in any of the *F. thapsinum* populations were analyzed (Figure 3.1). Strains from Kansas were members of one population and strains from Africa were members of a second population (Figure 3.1). Strains from Australia and Thailand grouped with the strains from Kansas. Similar results were observed when STRUCTURE was run with the 21 loci that were polymorphic in all of the populations, the 40 loci that were polymorphic only in Kan-

sas, and the 39 loci that were polymorphic only in Africa (Figure 3.1). Most of the genetic variation (77%) was found within the populations, while 23% was among the populations (Table 3.4).

Phylogenetic analyses

A phylogenetic tree containing all 286 strains was constructed by using the UPGMA algorithms and the genotypes of the 52 polymorphic AFLP loci (Appendix E). Strains from the same geographic region usually, but not always, belonged to the same cluster (Appendix E). As expected if genetic recombination is occurring, the branching structure of this tree was not strongly supported statistically.

In UPGMA population trees (Figure 3.2), the Kansas and Thai populations always were more closely related to each other than they were to either the African or the Australian populations. In the population tree based on 21 AFLP loci polymorphic in all populations, the Australia, Thai, and Kansas populations were more closely related to one another than they were to the African population (Figure 3.2 B). The branching pattern of this tree was statistically significant, which is consistent with the differentiation of these populations in other analyses. The lack of polymorphisms for all of the loci in the other trees evaluated could explain the differences in branching patterns observed.

Discussion

The relatively larger number of strains from Kansas (167) and Africa (81) means that these populations carry more weight in the analyses than do the smaller number of strains available from Thailand (20) and Australia (18). When we first designed this study, we planned to include the 203 strains recovered by Petrovic (2007) from Australia in our study. However, none of these

strains are available and the data on mating type, female fertility, and N_e are the only direct comparisons possible between our study and this earlier one.

Relatively high genotypic diversity indices (Table 3.1) were observed in the four *F. thapsinum* populations. Even so, putative clones were identified in all four populations. The Australian population in our study had a modest genotypic diversity of 72%. This result can be attributed to the relative small sample size and the presence of putative clones in this population. Six of the 18 strains of *F. thapsinum* from Australia were recovered from an uncultivated native grass (*A. aristiglumis*) growing on private farmland adjacent to a sorghum field (Bentley et al., 2007), and four of these six strains from *A. aristiglumis* were putative clones. A previous study (Leslie et al., 2004) reported recovering a single *F. thapsinum* strain in the prairie grass in Kansas. These data indicate that strains of *F. thapsinum* can move from cultivated sorghum to native grasslands or vice versa in multiple locations globally. We interpret these results to mean that asexual reproduction leading to clonality, and sexual reproduction leading to high genotypic diversity are both part of the life cycle in these populations.

N_e can be affected by the number of female fertile strains in the population and the ratio of the *MAT* alleles present. The presence of only one mating type (*MAT*-2) and the absence of female-fertile strains in the Australian population affected both $N_{e(mt)}$ and $N_{e(f)}$ parameters in the current study (Table 3.2). Normally this result means that sexual recombination never or rarely occurs in the population. However, the sample size in the current study was small, and results from a more extensive study of *F. thapsinum* from Australia are available (Petrovic, 2007). In the previous study mating type frequency ratio was not significantly different from 1:1 (113:90 *MAT-1*:*MAT-2*), and female fertility was 192:11 (male:hermaphrodite), suggesting that the potential for sexual reproduction component had not been completely lost in the three populations

evaluated. $N_{e(mt)}$ was 99% of the count and $N_{e(f)}$ was 20% of the count in these previously described Australian populations suggesting that N_e was reduced much more by the lack of female-fertile strains than it was by the skewing of the *MAT* allele ratios. Results similar to those of Petrovic (2007) have been reported for the composite global population of Leslie and Klein (1996), and *F. thapsinum* populations from sorghum in Kansas (Chapter 2) and in Tanzania (Mansuetus et al., 1997).

For the other three populations in the current study, $N_{e(mt)}$ was highest in Kansas (96% of the count), lower in the African population (81% of the count) and lowest in the Thai population (75% of the count) (Table 3.2). There was more variation in $N_{e(f)}$. The Thai population had the highest $N_{e(f)}$ value (45% of the count) of any population reported to date. The African population in this study also had a relatively high $N_{e(f)}$ of 33% of the count, while the Kansas population has the lowest value $N_{e(f)}$ (9% of the count) reported thus far for *F. thapsinum*. As previously reported for *F. thapsinum* and other species of *Fusarium*, the presence of female fertile (hermaphrodite) strains usually is much more important for limiting N_e and sexual reproduction than are imbalances in *MAT* allele ratios (Leslie and Klein, 1996; Mansuetus et al., 1997; Petrovic, 2007). More data from a larger set of strains is needed to confirm the differences observed in the current study. Variation in sexuality especially in introduced populations, e.g. Australia, Kansas and Thailand, is expected depending upon the immigrants that founded these populations.

Kansas and African *F. thapsinum* populations, regardless of the polymorphic AFLP locus set used, provided most of the genetic structure of the four populations (Figure 3.1). *F. thapsinum* populations from Australia and Thailand had the same pattern of genotypic diversity as that observed for the Kansas population. These results suggest that Africa is not the sole source for the populations of *F. thapsinum* in Kansas, Thailand or Australia.

Based on the AMOVA (Table 3.3), the high within population genotypic variation suggests that significant gene flow and mixing occurs through sexual recombination and/or movement of isolates between these populations (Table 3.4). However, the presence of high frequency putative private alleles in Kansas (~34%) and Africa (~38%), suggests more historical gene flow occurred than has happened recently. Phylogenetic analysis of *F. thapsinum* populations are consistent with the population relationships inferred from the STRUCTURE and AMOVA analyses (Figure 3.2) and that the populations from Kansas, Australia, and Thailand are more closely related to one another than they are to the African population.

In general, there is evidence of high genotypic diversity in populations of *F. thapsinum* from Australia, Thailand, Kansas, and Africa. Based on the present study and previous reports, female fertility in *F. thapsinum* populations is very low at both country and continent levels (Leslie and Klein, 1996; Mansuetus et al., 1997; Petrovic, 2007; Chapter 2). These results support the assertion that both the sexual and asexual modes of reproduction are important components of the life cycle of *F. thapsinum* populations. Populations from outside Africa, contain genotypic variation that is significantly different from that found in African populations of *F. thapsinum*, suggesting that Africa is not the only source population for Kansas, Thailand, and Australia. The two smaller populations, Australia and Thailand, also are more closely related to the Kansas population than they are to the African population. Other centers of origin for *F. thapsinum* might occur at locations where there are extensive domestication and breeding programs for commercial sorghum, e.g. India, and eastern, western and southern Africa. To the extent that host germplasm from all of these regions is introduced as commercial germplasm, *F. thapsinum* gene and genotype diversity could be introduced as well. For the Australian population the native sorghum grasses are potentially an alternate source population, but there is little evidence to sup-

port this hypothesis since the detected genotypic diversity is similar to that in Thailand and Kansas. Thus, we think that the *F. thapsinum* populations in Australia were brought in with cultivated sorghum rather than evolving in concert with the native Australian sorghum species. Future work, should focus on identifying additional center(s) of origin for *F. thapsinum* by obtaining strains from potential center(s) of origin and evaluating their genetic relationships with those from the populations evaluated to date.

Table 3.1. Estimates of genotypic diversity ($\hat{G}$) for populations of *F. thapsinum* from Australia, Kansas, Thailand, and Africa based on 52 polymorphic AFLP loci.

Population	No. of Isolates	Unique haplo-types	No. of polymor-phic loci	$\hat{G}$[a]	$\hat{G}$/n[b]	Number of locus pairs in linkage disequilibrium (%)	
						At $P < 0.05$	At $P < 0.01$
Whole population	286	255	52	222	0.87	338/1326 (25)	222/1326 (17)
Australia	18	14	32	10.1	0.72	68/496 (14)	23/496 (5)
Thailand	20	17	32	15.4	0.91	56/496 (11)	23/496 (5)
Kansas	167	159	40	149	0.94	178/780 (23)	115/780 (15)
Africa	81	65	39	53.3	0.82	80/741 (11)	40/741 (5)

[a] $\hat{G}$ was calculated as described in Milgroom (1996) from comparisons of AFLP allelic data at 52 AFLP loci. $\hat{G} = 1/\sum p_i^2$ where p_i = the observed frequency of the i^{th} multilocus genotype in a population.

[b] $\hat{G}/n$ was calculated by dividing $\hat{G}$ by the number of unique haplotypes observed in each population.

Table 3.2. Goodness of fit test (χ^2) for mating type and the effective population size parameters for *Fusarium thapsinum* populations from Australia, Thailand, Kansas, and Africa.

Population	Mating type			N_{fs}:N_h[b]	$N_{e(mt)}$[c]	$N_{e(f)}$[d]
	MAT-1:MAT-2	χ^2[a]	*P*			
All populations	174:112	13.4	0.00025	271:15	95	19
Australia[e]	0:18	18.0	< 0.0001	18:0	0	ND
Thailand	15:5	5.0	0.025	17:3	75	45
Kansas	101:66	7.3	0.007	163:4	96	9
Africa	58:23	15.1	<0.0001	73:8	81	33

[a] All tests have one degree of freedom

[b] N_{fs} – number of female-sterile male fertile strains; N_h – number of hermaphroditic strains.

[c] Effective population size based on mating type expressed as a percent of the count based on Equation 4 of Leslie and Klein (1996).

[d] Effective population size based on numbers of male and hermaphrodites expressed as a percentage of the actual count based on Equation 6 of Leslie and Klein (1996).

[e] $N_{e(f)}$ was not determined (ND) since no hermaphroditic strains were present.

Table 3.3. Analysis of molecular variance (AMOVA) based on 52 polymorphic AFLP loci for populations of *F. thapsinum* from Australia, Kansas, Thailand, and Africa.

Source of variation	Degrees of freedom	Variance	% of total variance
Among populations	3	1.638	23
Within populations	282	5.442	77
Total	285	7.080	100

Table 3.4. Summary of private alleles in populations of *F. thapsinum* from Australia, Kansas, Thailand, and Africa.

Population	Number of isolates	No. of private alleles	Locus	Allele*	No. of isolates with private allele	Frequency of private allele
Australia	18	2	ETT17	0	2	0.111
			ETT20	0	1	0.056
Thailand	20	1	UEaa7	1	3	0.150
Kansas	167	1	EAA20	1	56	0.335
Africa	81	8	UEaa3	1	7	0.086
			EAA4	0	5	0.062
			UEaa18	1	9	0.111
			UEaa19	1	12	0.148
			UEgg15	1	5	0.062
			UEgg16	1	7	0.086
			UEgg20	1	31	0.383
			UEtt14	1	6	0.074

* 0 = no band, 1 = band present.

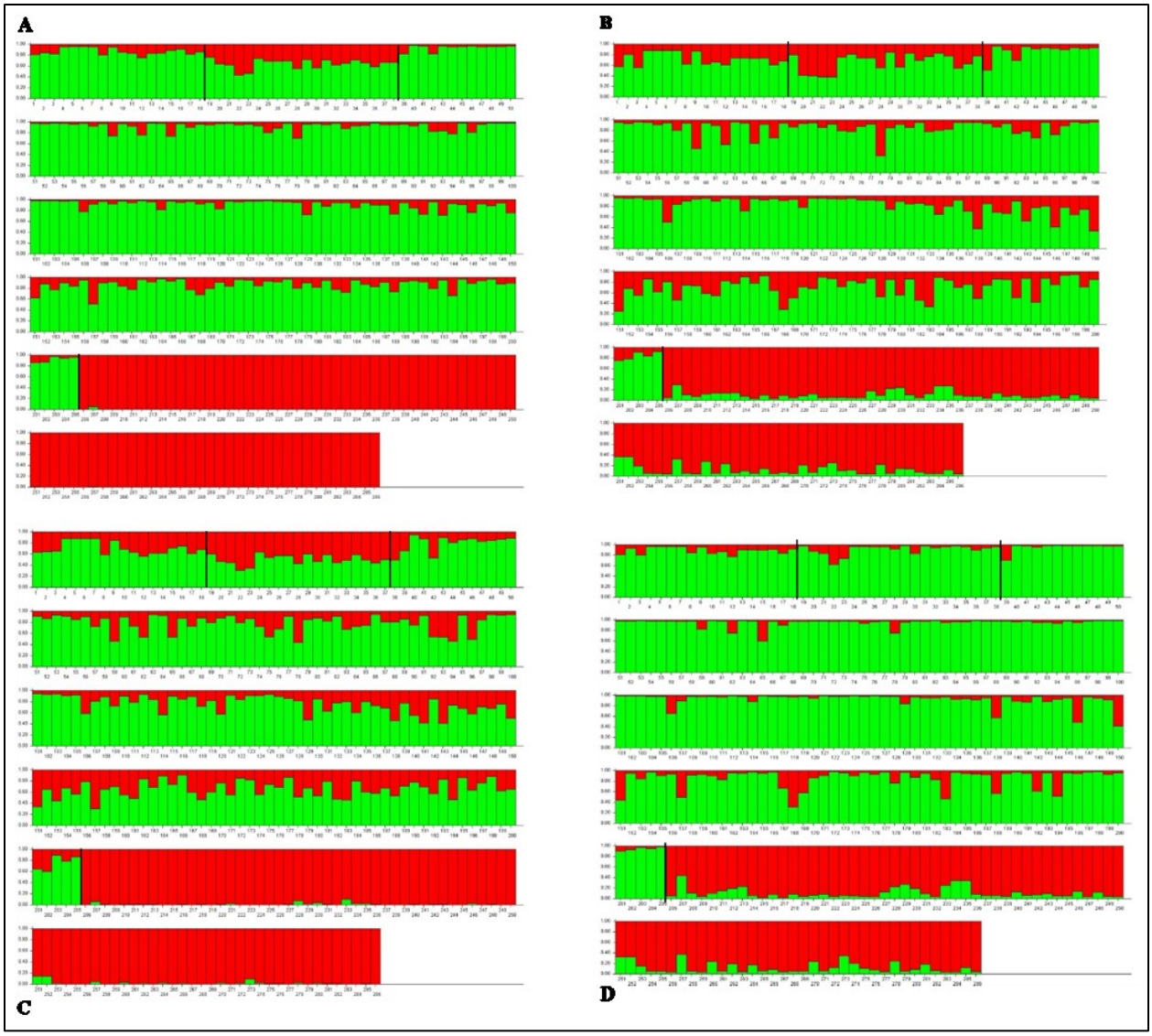

Figure 3.1. STRUCTURE analyses of *F. thapsinum* populations from Australia, Thailand, Kansas, and Africa. Columns represent individual strains. Strains 1-18 are from Australia, 19-38 are from Thailand, 39-205 are from Kansas, and 206-286 are from Africa. STRUCTURE based on (A) all 52 AFLP loci polymorphic in any of the populations; (B) 21 AFLP loci polymorphic in all populations; (C) 40 AFLP loci polymorphic in Kansas; and (D) 39 AFLP loci polymorphic in Africa.

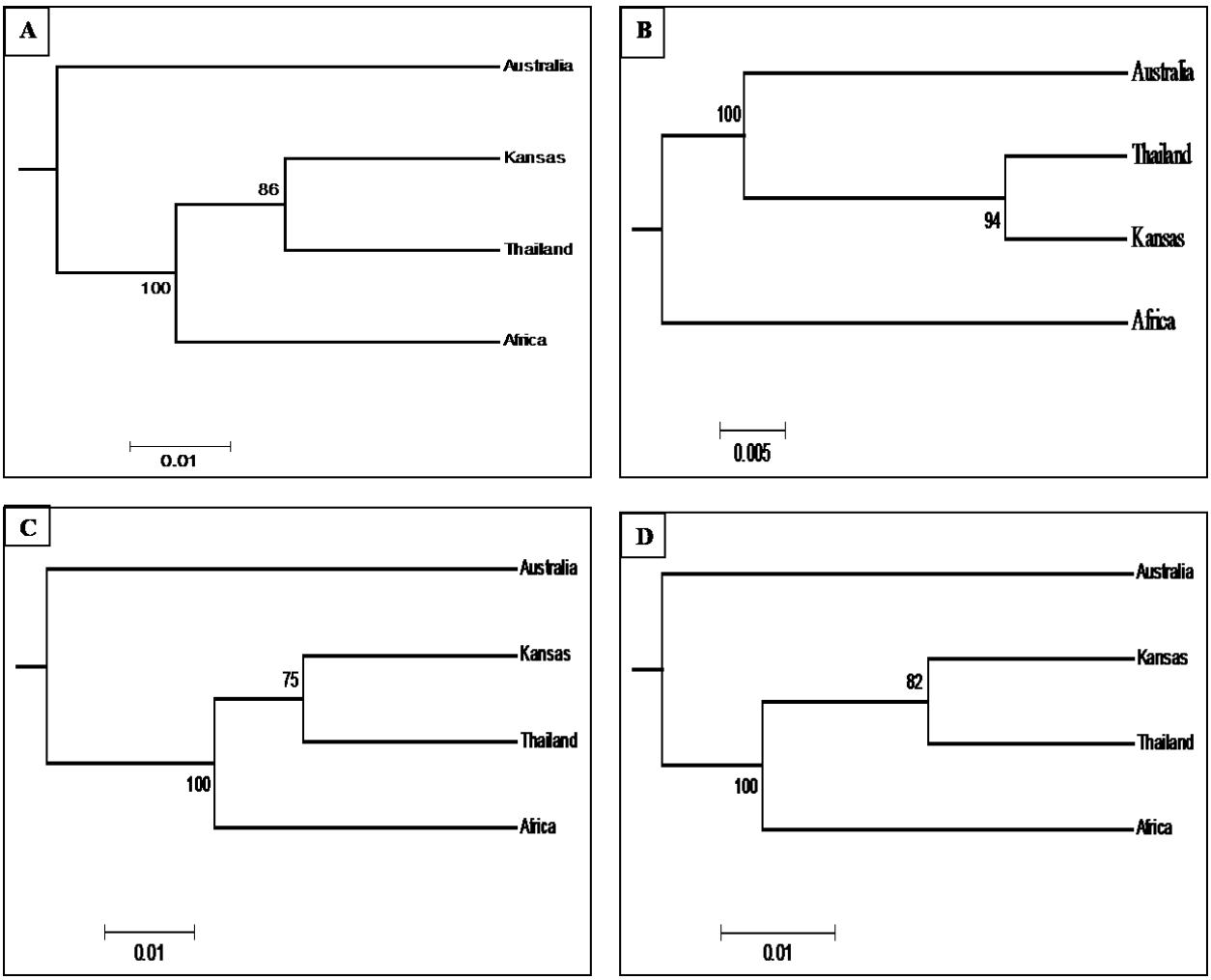

Figure 3.2. Population tree representing clustering of the four populations based on allele frequencies inferred by a UPGMA method. UPGMA trees based on (A) all 52 AFLP loci polymorphic in any of the populations; (B) 21 AFLP loci polymorphic in all populations; (C) 40 AFLP loci polymorphic in Kansas; and (D) 39 AFLP loci polymorphic in Africa. Numbers within the trees represent the bootstrap values (values above 50% are indicated; 1000 replications).

Chapter 4 - Stalk rot of sorghum caused by genetically diverse *Fusarium thapsinum* strains

Abstract

Stalk rot of grain sorghum is a yield-limiting disease that reduces seed quality and plant vigor. Grain harvest is more difficult due to plant lodging. *Fusarium thapsinum* is the primary causal agent of sorghum stalk rot. The *F. thapsinum* population from sorghum in Kansas is genetically diverse. Within-species genetic variation in pathogen populations may indicate variation in pathogenicity. The objective of this study was to evaluate the severity of sorghum stalk rot caused by genetically diverse *F. thapsinum* strains under field and greenhouse conditions. Experiments were conducted in the summers of 2014 and 2015. Six *F. thapsinum* strains from Kansas were evaluated for aggressiveness towards a stalk-rot susceptible line (Tx7000), a moderately resistant line (BTx399), and a resistant line (SC599) in the field. Tx7000 and SC599 also were evaluated in the greenhouse. Disease severity of pathogens was measured by major lesion length (MLL) (cm) and the number of nodes crossed (NC) by the lesion. *F. thapsinum* strains varied in stalk rot aggressiveness under both field and greenhouse conditions. Differences in aggressiveness were not attributable to population differentiation for the *F. thapsinum* strains. Generally, there was a highly significant positive correlation of disease scores in both field and greenhouse experiments for three strains (FGSC 26427, FGSC 26428, and FGSC 26431). Strain FGSC 26428, originally from an asymptomatic sorghum stalk was highly aggressive on Tx7000 under both field and greenhouse conditions. This strain is an ideal choice for use in screening sorghum germplasm. This work provides evidence of differences in stalk rot disease scores by *F. thapsinum* strains and the potential of these strains to be inconsistent in their aggressiveness under field and greenhouse

conditions. Thus, selection of a pathogen strain for studies of sorghum resistance or susceptibility to stalk rot is a crucial decision that can significantly alter the result of the germplasm screening effort.

Introduction

Sorghum bicolor originated in Africa and is the fifth most important grain crop worldwide after wheat, rice, maize, and barley (Maunder, 2002). In Sub-Saharan Africa and southern Asia, grain sorghum is an important subsistence crop and is used for numerous human foods including leavened-breads, couscous, fermented and unfermented-porridges, and traditionally brewed beers (Rooney and Waniska, 2000; Taylor and Dewar, 2000). In countries such as the United States, Australia, China, Japan, Mexico, Brazil, and Argentina, grain sorghum is used as livestock feed and for bio-ethanol production (Maunder, 2002). Diseases on sorghum caused by diverse microorganisms reduce yield and grain quality. Disease severity depends upon the pathogen species present and the environmental conditions (Bramel-Cox et al., 1988)

Stalk rot of grain sorghum is a yield-limiting disease that causes lodging and reduces seed quality, plant vigor, and harvestability (Little and Magill, 2003, 2009; Jardine, 2006). Fusarium stalk rot can be caused by several *Fusarium* spp., including *F. thapsinum*, *F. andiyazi*, *F. proliferatum*, and *F. verticillioides* (Leslie and Summerell, 2006; Tesso et al., 2012). *F. thapsinum* is the primary causal agent of stalk rot and is associated with grain sorghum wherever it is grown (Tesso et al., 2005, 2010; Petrovic et al., 2009). In Kansas, stalk rot is the most common disease of sorghum, with average annual losses of 4%, but that can range up to 50% or even more in some years (Jardine, 2006; Jardine and Leslie, 1992). Dry conditions early in the season favor the initiation of stalk rot in commercial fields. Subsequent warm, wet conditions favor increased disease incidence and severity (Jardine, 2006).

Breeding efforts for resistance to Fusarium stalk rot have been slow as the causal agent associated with this disease for many years was *F. moniliforme*. *F. moniliforme* has since been subdivided into more than 40 species (Leslie, 1995; O'Donnell et al., 1998) and the name for-

mally retired (Seifert et al., 2003). Only some of the species split from the former *F. moniliforme* are sorghum pathogens (Leslie et al., 2005; Leslie and Summerell, 2006).

There are genetic and phenotypic (pigmentation and pathogenicity) differences amongst strains of *F. thapsinum* collected from sorghum in Kansas. Strains that produce the characteristic yellow pigment and those that are non-pigmented were found in an approximately 2:1 ratio. In seedling pathogenicity tests, there were significant differences between two genotypically distinct, but inter-breeding subpopulations of *F. thapsinum* (Chapter 2). Studies evaluating sorghum germplasm for resistance to Fusarium stalk rot commonly use a single strain of the pathogen (Bramel-Cox et al., 1988; Tesso et al., 2005, 2010; Adeyanju, 2014; Bandara et al., 2015), and the ability of multiple, genetically distinct and identifiable strains of *F. thapsinum* to cause stalk rot is unknown at either the field or greenhouse level.

The objective of this study was to determine if multiple strains of *F. thapsinum* had similar abilities to cause stalk rot under field and greenhouse conditions. The working hypothesis, was that all strains were equally capable of causing disease on susceptible and resistant sorghum germplasm in both the field and the greenhouse. If there is a wide spread in pathogenic capability due to genetic background or environmental conditions, then selection of strains for use in breeding programs becomes an important variable, and studies of the variation in host-pathogen interactions a key to understanding observed variation in stalk rot as it occurs under both field and greenhouse conditions.

Materials and methods

Inoculum preparation and inoculation

Six *F. thapsinum* strains from Kansas (Table 4.1) were obtained from the Fungal Genetics Stock Center (FGSC) in the Department of Plant Pathology, Kansas State University, Manhattan. Population designation of the *F. thapsinum* strains was based on genetic fingerprinting with amplified fragment length polymorphism (AFLP) markers (Chapter 2).

Cultures were grown on complete medium (CM) slants for 7 days (Correll et al., 1987). A spore suspension was made by flooding the culture with 1 ml of an aqueous 2.5% Tween 60 (Sigma-Aldrich Corp., St Louis, MO) solution and scraping the agar surface with the tip of a Pasteur pipette. The spore suspension, approximately 2 ml of 1×10^6 conidia mL^{-1}, was used to inoculate a 125-ml Erlenmeyer flask containing 40 ml CM broth. Flasks were incubated on an orbital shaker (135 rpm) for 2 d at room temperature (22-25°C). Cultures were filtered through four layers of cheesecloth to remove mycelial fragments. Conidia remaining in the filtrate were counted with a hemacytometer and spore density adjusted to 5×10^6 conidia mL^{-1} with 10 mM phosphate-buffered saline (PBS; pH 7.2). The inoculum was stored in the refrigerator (4°C) and used within two days of preparation.

Field experiment

The field experiment was conducted in the summer of 2014 at the Ashland Bottoms Research Farm of Kansas State University, Department of Agronomy, near Manhattan (39° 6′ 51.92″ N, 96° 38′ 10.30″ W). Three sorghum lines were evaluated: Tx7000, a stalk rot susceptible line; BTx399, a stay-green line with moderate resistance to stalk rot; and SC599, a stalk rot resistant line (Tesso et al., 2010). The experiment was laid out as a complete randomized design. Each

sorghum line was planted in a 5-row plot that was 5 m long with rows spaced 0.7 m apart. At flowering, 84 uniform plants (7 × 12) were tagged for each sorghum line with 12 tagged plants per strain and control and used for pathogen or mock inoculation.

At 14 d post-flowering, a cordless compact drill (Performax®, Menards®, Eau Claire, WI) was used to make a 1.0 cm deep injection site at the first internode. A sterile surgical syringe fitted with a 20 gauge × 1 inch (0.902 mm × 2.5 cm) needle was used to inject 0.2 ml of a conidial suspension into the stalk. Twelve plants were inoculated for each of the six tested strains. Twelve control plants were mock inoculated with 0.2 ml of 10 mM PBS. Plants were assessed for stalk rot 28 d post-inoculation. This assessment was made by longitudinally splitting the stalks and measuring the major lesion length (MLL) (cm), i.e, the uninterrupted necrotic lesion with red-purple discoloration extending on either side of the inoculation point (Figure 4.1), and by counting the number of nodes crossed (NC) by the lesion.

Greenhouse experiment

The greenhouse experiment was conducted in the summer of 2015. Tx7000 (stalk rot susceptible) and SC599 (stalk rot resistant) sorghum lines were evaluated. Fifty-six 19-L Poly-Tainer pots (Nursery Supplies Inc., Fairless Hill, PA) were filled with a 1:1 mixture of soil and Metro-mix 360 growing medium (Sun Gro, Bellevue, WA). The experiment was arranged in a complete randomized design with four replicates (three plants per replicate). Prior to planting, seeds were surface-sterilized with a mixture of Maxim 4FS, Apron XL, Concept III, and a colorant (Maulana and Tesso, 2013).

For each sorghum line, 28 pots were planted with ten seeds each. At 10 d post emergence, seedlings were thinned to three plants per pot. Pots were supplied with a fast-release nu-

trient solution (Scotts Miracle-Gro, Marysville, OH) in the first week after planting and then a controlled release fertilizer, Osmocote® (Scotts Miracle-Gro) post-emergence. Pots were watered every third day. Insect pests were controlled by spraying the plants with a Floramite SC (Chemtura Corporation, Middleburg, CT) insecticide, as necessary.

The greenhouse inoculation procedure differed from that in the field. Holes were not drilled in the stalks of plants grown in the greenhouse since these stalks were readily punctured with a needle. At 14 d post-flowering, stalks were inoculated with 0.2 ml of a 5×10^6 conidia mL^{-1} suspension prepared in PBS by using a sterile surgical syringe and needle (as described above). There were four pots each with three inoculated plants for each strain. Plants mock-inoculated with PBS served as the controls. Plants were assessed for stalk rot at 28 d post-inoculation by longitudinally splitting the stalks and measuring the major lesion length (MLL) (cm), i.e., the uninterrupted necrotic lesion with red-purple discoloration extending on either side of the inoculation point and counting the number of nodes crossed (NC) by the lesion.

Data collection and statistical analysis

Data from the field and greenhouse experiments were analyzed independently with the generalized linear mixed model (GLIMMIX) procedure as implemented in SAS (v.9.4., SAS Institute, Cary, North Carolina). To evaluate the consistency of stalk rot disease scores under both environments, a Pearson correlation was analyzed between MLL and NC with the CORR procedure in SAS.

Results

Stalk rot aggressiveness of *F. thapsinum* strains in the field

The strains used in this study belong to two subpopulations (1 and 2) of *F. thapsinum* from sorghum in Kansas. Strains FGSC 26428, 26431, and 26426 are from subpopulation 1 and FGSC 26427, 26430, and 26429 are from subpopulation 2 (Table 4.1; Chapter 2).

In the field experiment, all of the strains were noticeably more aggressive (Figures 4.2A and B) on the stalk rot susceptible line (Tx7000). MLL and NC ranged from 3.2 cm and 1.0 nodes crossed for FGSC 26430 (subpopulation 2) to 19.1 cm and 3.2 nodes crossed for FGSC 26428 (subpopulation 1). The mock-inoculated control had lower disease scores than any of the plants inoculated with a strain of *F. thapsinum*.

On the stalk rot resistant line (SC599), MLL ranged from 1.6 cm for FGSC 26426 (subpopulation 1) to 2.7 cm for FGSC 26431 (subpopulation 1). The trend was different for NC, and ranged from 0.0 nodes crossed for FGSC 26427 (subpopulation 2) to 1.3 nodes crossed for FGSC 26429 (subpopulation 2). The mock-inoculated control had the lowest MLL score (1.5 cm) but not the lowest NC score (0.5 nodes crossed) (Figures 4.2C and D).

For the moderately resistant line (BTx399) with the stay-green trait, MLL and NC ranged from 1.7 cm and 0.0 nodes crossed for FGSC 26428 (subpopulation 1) to 2.4 cm and 0.9 nodes crossed for FGSC 26426 (subpopulation 1). The mock-inoculated control had lowest MLL (0.4 cm) but not the lowest NC score (0.2 nodes crossed) (Figures 4.2 E and F).

Overall, neither MLL nor NC could be used to differentiate fungal strains in subpopulation 1 from those in subpopulation 2. Disease severity scores, as expected, were lower on both the Fusarium stalk rot moderately resistant (BTx399) and resistant (SC599) lines than they were on the stalk rot susceptible (Tx7000) line.

Stalk rot aggressiveness of *F. thapsinum* strains in the greenhouse

For Tx7000 in the greenhouse, MLL ranged from 3.6 cm for strain FGSC 26429 to 10.5 cm for strain FGSC 26428 (Figure 4.3A). Diseased nodes crossed (NC) followed the same trend; lesions covered by strains FGSC 26429 and FGSC 26428 crossed 1.1 and 2.7 nodes, respectively (Figure 4.3B).

For SC599, MLL ranged from 2.2 cm for strain FGSC 26428 and 4.1 cm for strain FGSC 26429 (Figure 4.3C), while the NC was 1.0 for both the strains (Figure 4.3D).

There were no significant differences ($P = 0.693$) in MLL and NC ($P = 0.726$) between strains from subpopulation 1 and subpopulation 2. Similar to the field data, disease severity scores were numerically higher in Tx7000 than in SC599 (data not shown).

Comparison of aggressiveness of *F. thapsinum* strains under field and greenhouse conditions

To evaluate the consistency of stalk rot disease scores under both environments, MLL and NC results in the field were correlated with those obtained in the greenhouse. There were significant positive Pearson correlations: $r = 0.539$ ($P = 0.047$) and $r = 0.575$ ($P = 0.031$) for MLL and NC, respectively (Figures 4.4A and B). Three strains, FGSC 26430 (subpopulation 2), FGSC 26429 (subpopulation 2), and FGSC 26426 (subpopulation 1) were noticeably inconsistent in their MLL and NC on Tx7000 under field and greenhouse conditions. For example, strain FGSC 26430 had MLL = 3.2 cm and NC = 1.0 in the field (Figures 4.2A and B), and MLL = 10.0 cm and NC = 2.4 in the greenhouse (Figures 4.3A and B). The other two strains, FGSC 26429 and FGSC 26426, had disease scores that were high in the field (Figures 4.2A and B) and low in the greenhouse (Figures 4.3A and B). When these three strains were excluded from the correlation analy-

sis, the Pearson correlation was positive and highly significant (r = 0.977, $P < 0.0001$), for MLL (Figure 4.4C) but low and non-significant for NC (r = 0.345, $P = 0.402$) (Figure 4.4D).

Discussion

The main objective of this study was to determine if strains of *F. thapsinum* had similar abilities to cause sorghum stalk rot under field and greenhouse conditions. Our current data showed that strain aggressiveness varied and depended upon experimental conditions. There were numerical differences in MLL and NC between the six *F. thapsinum* strains. These differences were more pronounced on the stalk rot susceptible line, Tx7000 (Figures 4.2A and B, 4.3A and B).

When Kansas subpopulations were compared, there were no significant differences in aggressiveness between the subpopulations under either field or greenhouse conditions. Increasing the number of strains tested to more than three per population, however, could alter this conclusion. Differences in the stalk rot aggressiveness for individual *F. thapsinum* strains can be attributed to factors other than subpopulation differentiation, and might be more readily identified in larger experiments evaluating more fungal strains and/or host lines. Our results are consistent with those of Jardine and Leslie (1992) who found no significant differences in aggressiveness of 11 strains each representing the common vegetative compatibility groups (VCGs) of *F. thapsinum* from grain sorghum in Kansas.

For the stalk rot susceptible line (Tx7000) under both field and greenhouse conditions, FGSC 26428 (subpopulation 1) was the most aggressive and had the highest disease scores. FGSC 26428 was originally recovered from an asymptomatic sorghum stalk (Table 4.1) and was non-pathogenic in seedling tests (Chapter 2). This strain may be an endophyte or a latent patho-

gen that can cause disease when the host plant is under sufficient environmental stress (Photita et al., 2004).

In the current study, plants in the field were considered water-stressed compared to the greenhouse where plants were watered consistently throughout the experiment. The highest MLL and NC values for this strain were in the field (Figure 4.2A and B). This result is consistent with a previous study evaluating the effect of water stress on charcoal rot development in sorghum. More disease was observed in water-stressed plants (Diourte et al., 1995).

When host genotypes were compared, patterns of stalk rot susceptibility or resistance were discernable. Major lesion length and NC for all six strains were higher for the susceptible line (Tx7000) than for BTx399 and SC599 in the field, and for Tx7000 in the greenhouse. These results are consistent with previous stalk rot studies in which these three genotypes were used (Bandara et al., 2015; Tesso et al., 2010).

Consistent disease ratings across different environments is a requirement for a breeding program to successfully develop disease resistant germplasm (Bramel-Cox et al., 1988; Tesso et al., 2010). Essentially, the strains evaluated in this study can be grouped as highly aggressive in the field and the greenhouse (FGSC 26428); least aggressive in the field and greenhouse (FGSC 26427 and FGSC 26431); highly aggressive in field and least aggressive in the greenhouse (FGSC 26429 and FGSC 26426); and least aggressive in the field and highly aggressive in the greenhouse (FGSC 26430). FGSC 26427, FGSC 26428, and FGSC 26431 had consistent disease scores and a highly significant, positive Pearson correlation for MLL under both field and greenhouse experiments. Correlation of NC was considered less informative than MLL for these strains because the distance between the nodes can vary. Strain FGSC 26428 was the most ag-

gressive on Tx7000 under both environmental conditions and is a good choice for use in screening sorghum germplasm.

In conclusion, it is common practice to use a single strain of *F. thapsinum* or of other *Fusarium* species when evaluating sorghum germplasm for resistance to Fusarium stalk rot (Bramel-Cox et al., 1988; Tesso et al., 2005, 2010; Kapanigowda et al., 2013; Adeyanju, 2014; Bandara et al., 2015) without considering that strains can vary in stalk rot aggressiveness. Thus, the selection of a pathogen strain for studies of sorghum resistance and susceptibility to stalk rot can be a crucial decision that can significantly alter the result of the germplasm screening effort. Our null hypothesis was that all strains of *F. thapsinum* would be equally capable of causing stalk rot on susceptible and resistant sorghum germplasm in both the field and the greenhouse.

Based on the current results there were large numerical differences in MLL and NC amongst the strains, which leads to the rejection of this hypothesis. The critical need now is to identify the fungal genetic background and environmental conditions responsible for the variation observed in disease severity. Clearly our understanding of the critical parameters in the disease triangle – host, pathogen and environment – remain incomplete for sorghum stalk rot.

Table 4.1 Strains of *Fusarium thapsinum* evaluated for stalk rot disease of grain sorghum grown under field and greenhouse conditions.

KSU number[a]	FGSC number[b]	Subpopulation[c]	Host material	Geographic origin
F-00915	26427	2	Asymptomatic sorghum stalk	Gove county, Kansas
F-00916	26428	1	Asymptomatic sorghum plant	Gove county, Kansas
F-01153	26431	1	Diseased sorghum stalk	Cowley county, Kansas
F-01163	26430	2	Diseased sorghum stalk	Morris county, Kansas
F-02576	26429	2	Diseased sorghum stalk	Dickinson county, Kansas
F-02718	26426	1	Diseased sorghum stalk	Pottawatomie county, Kansas

[a] KSU number = accession number of strain from the culture collection in the Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506.

[b] FGSC = Fungal Genetics Stock Center, Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506.

[c] Subpopulation inferred in STRUCTURE v2.2 (Pritchard et al., 2000) using 34 polymorphic AFLP markers (Chapter 2).

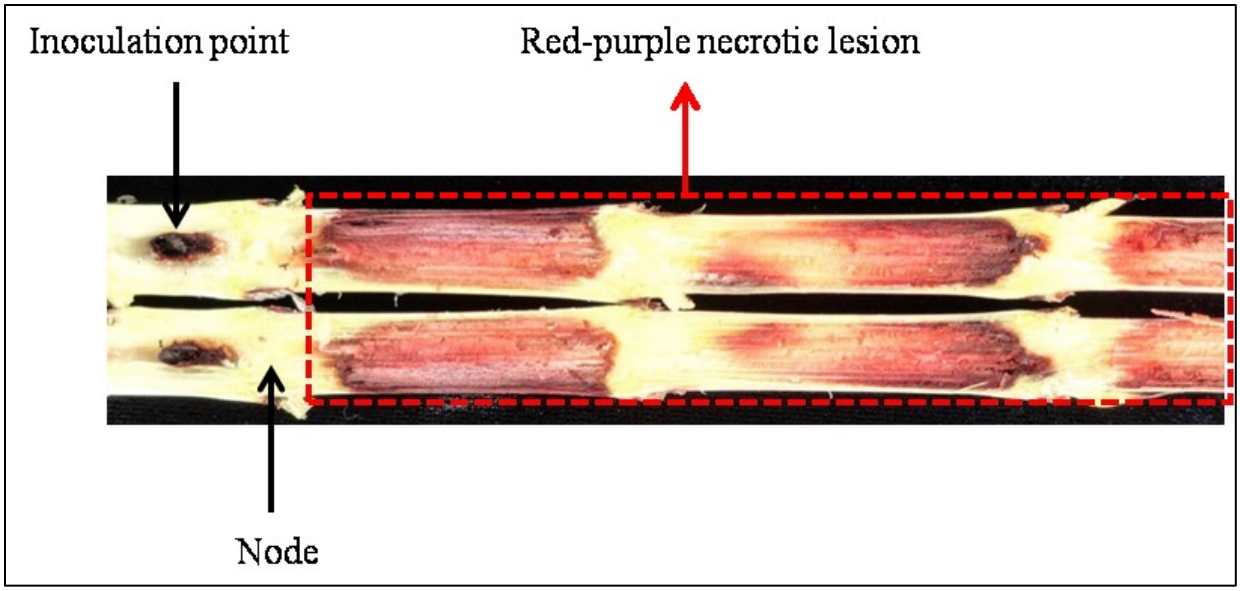

Figure 4.1. Stalk rot phenotypes. Stalk rot assessment was made by longitudinally splitting the stalk and measuring (cm) the major lesion length (MLL), i.e., the uninterrupted necrotic lesion with red-purple discoloration extending out on either side of the inoculation point, and by counting the number of nodes crossed (NC) by the lesion.

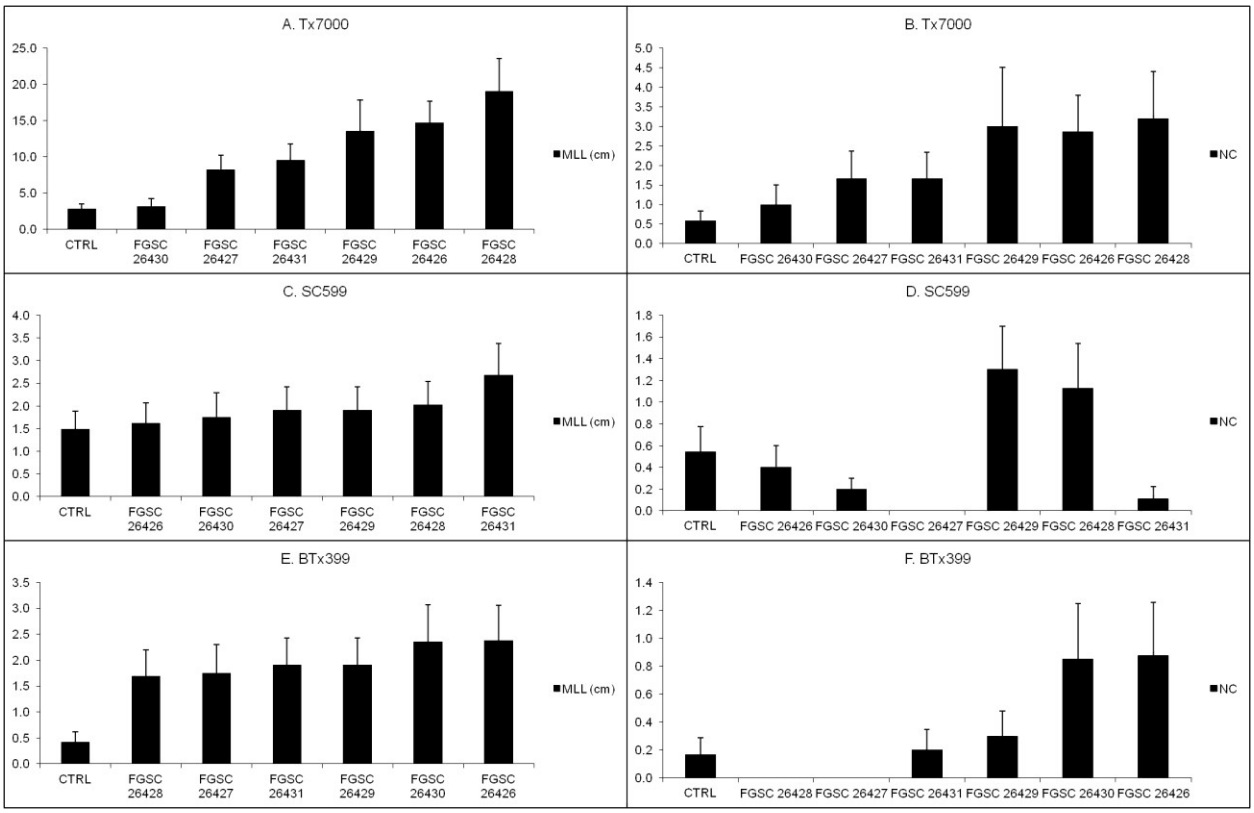

Figure 4.2. Aggressiveness of six genetically diverse *F. thapsinum* strains towards sorghum under field conditions. Mean (± SE) of major lesion length (MLL) and number of nodes crossed (NC) by the lesion on (A and B) susceptible Tx7000, (C and D) resistant SC599, and (E and F) moderately resistant BTx399 lines. Strain numbers from the Fungal Genetics Stock Center, Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506. CTRL = mock-inoculated with phosphate-buffered saline (pH 7.2).

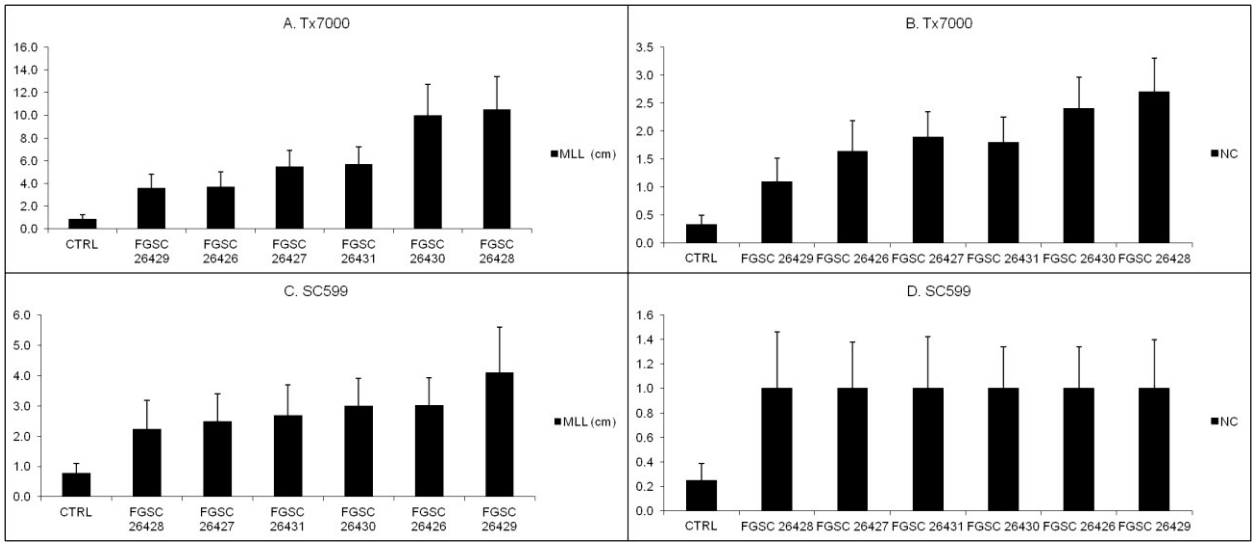

Figure 4.3. Aggressiveness of six genetically diverse *F. thapsinum* strains towards sorghum under greenhouse conditions. Mean (± SE) of major lesion length (MLL) and nodes crossed (NC) by the lesion (A and B) on susceptible Tx7000 and (C and D) resistant SC599 lines. Strain numbers from the Fungal Genetics Stock Center, Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506.

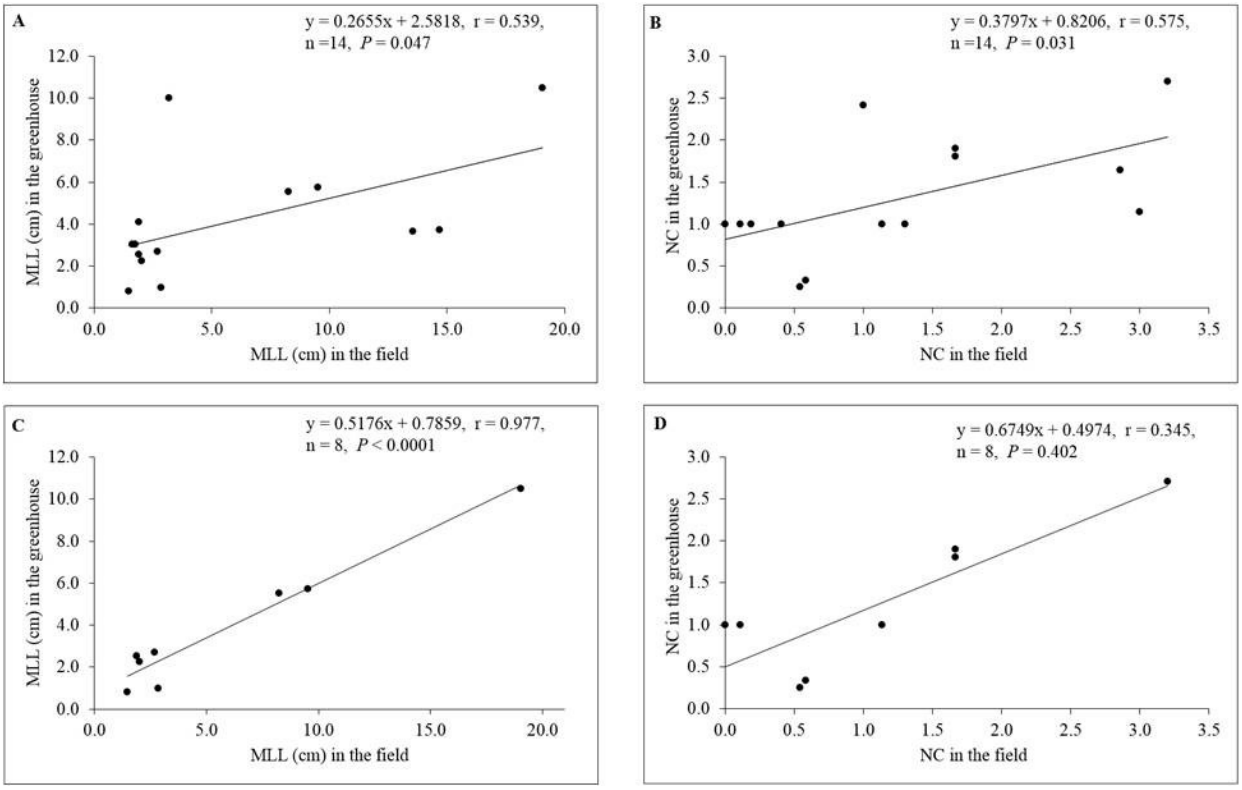

Figure 4.4. Pearson correlations between (A) mean lesion length (MLL) and (B) nodes crossed (NC) for six *F. thapsinum* strains and the mock-inoculated control under field and greenhouse conditions. Correlations between (C) MLL and (D) NC when strains FGSC 26430, FGSC 26429, and FGSC 26426 were excluded from the analysis.

Chapter 5 - Genetic diversity of *Fusarium andiyazi* and *F. andiyazi*-like populations from sorghum

Abstract

Fusarium andiyazi, is a member of the *F. fujikuroi* species complex and is an important pathogen of grain sorghum (*Sorghum bicolor*) causing grain mold and stalk rot. There have been growing numbers of reports of "*F. andiyazi*-like" strains recovered from sorghum around the world; however, very little is understood about the genetic variation in *F. andiyazi* and these *F. andiyazi*-like populations. The objective of this study was to evaluate genetic diversity in *F. andiyazi* and closely related species. Eighty-six isolates recovered from sorghum in Kansas (6), Uganda (30), and Cameroon (50) were analyzed by using amplified fragment length polymorphism (AFLP) markers. An unweighted pair group method with arithmetic mean analysis (UPGMA) based on 56 AFLP loci separated the strains into three distinct clusters. All clusters contained strains from multiple geographic origins. The first cluster had 59 strains that grouped with *F. andiyazi* reference strains. The second and third clusters contained 13 and 14 strains, respectively. Further, STRUCTURE differentiated two populations when the 86 strains were analyzed. Population 1 consisted of the 59 *F. andiyazi* strains, while a second population contained 27 strains in two more clusters. These results indicate the presence of at least one and perhaps two sister-species of *F. andiyazi* are present but have not yet been formally recognized or described. More work is needed to characterize these sister-species of *F. andiyazi* and to understand their role in sorghum pathogenicity.

Introduction

Fusarium andiyazi is an important pathogen of sorghum causing both grain mold and stalk rot (Marasas et al., 2001; Leslie et al., 2005; Tesso et al., 2005; Leslie and Summerell, 2006). This fungal species is a member of the *F. fujikuroi* species complex (FFSC) and is closely related to *F. thapsinum* and *F. verticillioides* (Marasas et al., 2001). Members of the FFSC share similar morphological characteristics and can be difficult to distinguishing based on morphology alone. Molecular markers such as amplified fragment length polymorphisms (AFLPs), partial sequences of genes such as translocation elongation factor 1α, β-tubulin, mitochondrial small subunit (mtSSU) rDNA, and nuclear 28S rDNA (O'Donnell et al., 1998; Marasas et al., 2001; Leslie and Summerell, 2006).

F. andiyazi has been recovered from many sorghum-growing regions of the world including the United States, South Africa, Australia, Ethiopia and Nigeria (Leslie and Summerell, 2006). In previous studies, *F. andiyazi* was the dominant species on sorghum grown in several agro-climatic regions of Australia (Petrovic et al., 2009). *F. andiyazi* also has been recovered from presumed non-hosts such as maize, sugarcane, and rice, and has been reported as an opportunistic human pathogen (Leslie and Summerell, 2006; Kebabci et al., 2014).

There have been growing numbers of reports of "*F. andiyazi*-like" strains from sorghum and other substrates around the world (Madania et al., 2013). However, little is understood about the genetic diversity within and between *F. andiyazi* and the *F. andiyazi*-like populations. The objective of this study was to evaluate genetic diversity in *F. andiyazi* and closely related species.

Materials and methods

Strains used in this study (Table 5.1) were obtained from the culture collection in the Department of Plant Pathology, Kansas State University, Manhattan. Strains were initially recovered from asymptomatic and diseased sorghum seed in Cameroon (50), Uganda (30), and Kansas (6). All cultures were purified by subculturing a single microconidium with a micromanipulator. Pure culture spore suspensions were stored in 15% glycerol at -70°C (Leslie and Summerell, 2006).

Culture preparation and DNA extractions

Cultures were grown on complete medium (CM) slants for 7 days (Correll et al., 1987). A spore suspension was made by flooding the culture with 1 ml of an aqueous 2.5% Tween 60 (Sigma-Aldrich Corp., St Louis, MO) solution and scraping the agar surface with the tip of a Pasteur pipette. The spore suspension, ~ 2 ml of 1×10^6 conidia/ml, was used to inoculate a 125-ml Erlenmeyer flask containing 40 ml CM broth. Flasks were incubated on an orbital shaker (135 rpm) for 2 d at room temperature (22-25°C). Approximately 500 mg of fungal mycelia was recovered per ml of culture broth following filtration through a milk filter disc (Ken AG, Ashland, OH). Mycelia were blotted dry with paper towels, wrapped in aluminum foil, and stored at -70°C until DNA was extracted. DNA was isolated by following a cetyltrimethylammonium bromide (CTAB) protocol (Murray and Thompson, 1980) as modified by Leslie and Summerell (2006). DNA pellets were washed twice with 70% ethanol, air-dried for 5 min in an oven at 60°C, and dissolved in 50 µl of 1 × TE buffer (10 mM Tris base, 1 mM EDTA, pH 8.0). DNA concentrations were determined with an ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) and the extracts were stored at -20°C until used.

Amplified fragment length polymorphisms (AFLPs) reaction and analysis

AFLP profiles (Vos et al., 1995) were generated by using three primer-pairs (*EcoRI*+TT/*MseI*+AC and *EcoRI*+AA/*MseI*+TT) as described by Leslie and Summerell (2006). *Fusarium* reference strains included as references in the AFLP analysis were: *F. thapsinum* (FGSC 7057 and FGSC 7056); *F. verticillioides* (FGSC 7415 and FGSC 7416); *F. napiforme* (KSU 5015 and KSU 5016); *F. proliferatum* (FGSC 7421 and FGSC 7422); *F. pseudonygamai* (KSU 10762 and KSU 10770); *F. andiyazi* (KSU 4647 and KSU 4804); and "*F. andiyazi*-like" (KSU 3089 and KSU 4846). Bands between 200 and 500 base pairs in length were scored as present "1" or absent "0" and a binary matrix data was generated. Bands from different individuals of the same molecular size were assumed to be identical.

Phylogenetic analysis

The phylogenetic relationship among strains was determined based on the binary matrix generated from AFLP markers by Unweighted Pair Grouping by Mathematical Average (UPGMA) in PAUP* 4.0 beta 10 (Swofford, 1998). The support for any resulting subgroups in the UPGMA tree was assessed by bootstrap analysis with 1,000 iterations.

Population structure

Population structure was determined by using the Bayesian clustering method in STRUCTURE version 2.2 (Pritchard et al., 2000) based on 56 polymorphic AFLP loci. Individuals were assigned to putative ancestral groups based on an admixture model. The number of groups, *K*, was set from 1 to 4. Ten independent runs were made with "burn-in" replicates set at 50,000 and a

run length of 100,000 steps. The most likely number of clusters (*K*) was determined by using the method of Evanno et al. (2005).

Results and discussion

A UPGMA tree based on 56 AFLP loci separated the 86 strains into three clusters. Cluster 1 contained 59 strains that grouped with the *F. andiyazi* reference strains (Figure 5.1). Cluster 2 contained 13 strains that grouped together with several reference strains for a fungal species not yet formerly described (Saleh et al., *unpublished*). Cluster 3 contained 14 strains that belong to an unknown, or potentially second new, species (Figure 5.1). These results are consistent with other reports of strains that are closely related to, but not in the same species as, *F. andiyazi* (Leslie et al., 2005; Madania et al., 2013).

Strain clustering was not based on a strain's geographic origin. The branching pattern of the UPGMA tree was statistically significant, and clearly clustered *F. andiyazi* and *F. andiyazi*-like strains separately from other closely related *Fusarium* species such as *F. thapsinum*, *F. verticillioides*, *F. proliferatum*, *F. napiforme*, and *F. pseudonygamai* (Figure 5.1). Thus, strains from *F. andiyazi* and the *F. andiyazi*-like populations can be clearly distinguished from each other and from other closely related species based on AFLP markers.

Six strains from Kansas were identified as *F. andiyazi* (Figure 5.1). This report is the first of *F. andiyazi* from sorghum in Kansas. This result is consistent with those of Marasas et al. (2001) who recovered *F. andiyazi* from sorghum growing in Colorado near the Kansas/Colorado state line.

In the current study, reference strains of a new species in the process of being identified were included (Saleh et al., *unpublished*), which enabled a subset (13) of the *F. andiyazi*-like

strains to be identified as members of this as yet undescribed species. The 13 strains in question were recovered from sorghum in Uganda and Cameroon. The undescribed species is widely distributed across Africa and is a potential pathogen of a number of important cereals including maize, sorghum, and millets (Leslie, *personal communication*).

STRUCTURE analysis differentiated the 86 strains into two subpopulations. Subpopulation 1 contained 59 field strains plus the two *F. andiyazi* reference strains, while subpopulation 2 contained the 27 strains in clusters 2 and 3 plus the reference strains for the undescribed species (Figure 5.2). These results indicate the presence of at least two groups of strains from clades that appear to be sister-species of *F. andiyazi*. The results from the current study emphasize the need to continue re-evaluating *Fusarium* species associated with sorghum from all growing regions of the world (Leslie et al., 2005; Marasas et al., 2001). Further work is needed to better characterize these sister-species of *F. andiyazi* and to understand their role in sorghum pathogenicity.

Table 5.1 *F. andiyazi* and *F. andiyazi*-like strains used in this study.

Cluster	KSU strain number	Geographic origin	Host substrate
1 *(F. andiyazi)*			
	15115	Cameroon	Sorghum seed
	15137	Cameroon	Sorghum seed
	16093	Cameroon	Sorghum seed
	16095	Cameroon	Sorghum seed
	16097	Cameroon	Sorghum seed
	16098	Cameroon	Sorghum seed
	16100	Cameroon	Sorghum seed
	16111	Cameroon	Sorghum seed
	16115	Cameroon	Sorghum seed
	16118	Cameroon	Sorghum seed
	16122	Cameroon	Sorghum seed
	16123	Cameroon	Sorghum seed
	16127	Cameroon	Sorghum seed
	16130	Cameroon	Sorghum seed
	16134	Cameroon	Sorghum seed
	16139	Cameroon	Sorghum seed
	16147	Cameroon	Sorghum seed
	16151	Cameroon	Sorghum seed
	16154	Cameroon	Sorghum seed
	16155	Cameroon	Sorghum seed
	16164	Cameroon	Sorghum seed
	16171	Cameroon	Sorghum seed
	16172	Cameroon	Sorghum seed
	16173	Cameroon	Sorghum seed
	16174	Cameroon	Sorghum seed
	16175	Cameroon	Sorghum seed
	16178	Cameroon	Sorghum seed
	16179	Cameroon	Sorghum seed
	16183	Cameroon	Sorghum seed
	16184	Cameroon	Sorghum seed
	16185	Cameroon	Sorghum seed
	16186	Cameroon	Sorghum seed
	16188	Cameroon	Sorghum seed
	17822	Cameroon	Sorghum seed
	2791	Kansas	-
	2804	Kansas	-
	903	Kansas	-

Cluster	KSU strain number	Geographic origin	Host substrate
1 *F. andiyazi*	904	Kansas	-
	908	Kansas	-
	911	Kansas	-
	9015	Uganda	Sorghum seed
	9016	Uganda	Sorghum seed
	9019	Uganda	Sorghum seed
	9022	Uganda	Sorghum seed
	9030	Uganda	Sorghum seed
	9032	Uganda	Sorghum seed
	9035	Uganda	Sorghum seed
	9037	Uganda	Sorghum seed
	9039	Uganda	Sorghum seed
	9198	Uganda	Sorghum seed
	9201	Uganda	Sorghum seed
	9463	Uganda	Sorghum seed
	9497	Uganda	Sorghum seed
	9499	Uganda	Sorghum seed
	9500	Uganda	Sorghum seed
	9502	Uganda	Sorghum seed
	9503	Uganda	Sorghum seed
	9504	Uganda	Sorghum seed
	9507	Uganda	Sorghum seed
	4804 (*F. andiyazi*)	Reference strain	
	4647 (*F. andiyazi*)	Reference strain	
2			
	15118	Cameroon	Sorghum seed
	15131	Cameroon	Sorghum seed
	9040	Uganda	Sorghum seed
	9052	Uganda	Sorghum seed
	9066	Uganda	Sorghum seed
	9091	Uganda	Sorghum seed
	9217	Uganda	Sorghum seed
	9270	Uganda	Sorghum seed
	9478	Uganda	Sorghum seed
	9501	Uganda	Sorghum seed
	9505	Uganda	Sorghum seed
	9511	Uganda	Sorghum seed
	9527	Uganda	Sorghum seed

Cluster	KSU strain number	Geographic origin	Host substrate
2	4846	Reference strain	
	3089	Reference strain	
3			
	15071	Cameroon	Sorghum seed
	15074	Cameroon	Sorghum seed
	15077	Cameroon	Sorghum seed
	15082	Cameroon	Sorghum seed
	15086	Cameroon	Sorghum seed
	15095	Cameroon	Sorghum seed
	15100	Cameroon	Sorghum seed
	15128	Cameroon	Sorghum seed
	15135	Cameroon	Sorghum seed
	16107	Cameroon	Sorghum seed
	16108	Cameroon	Sorghum seed
	16109	Cameroon	Sorghum seed
	16148	Cameroon	Sorghum seed
	16166	Cameroon	Sorghum seed

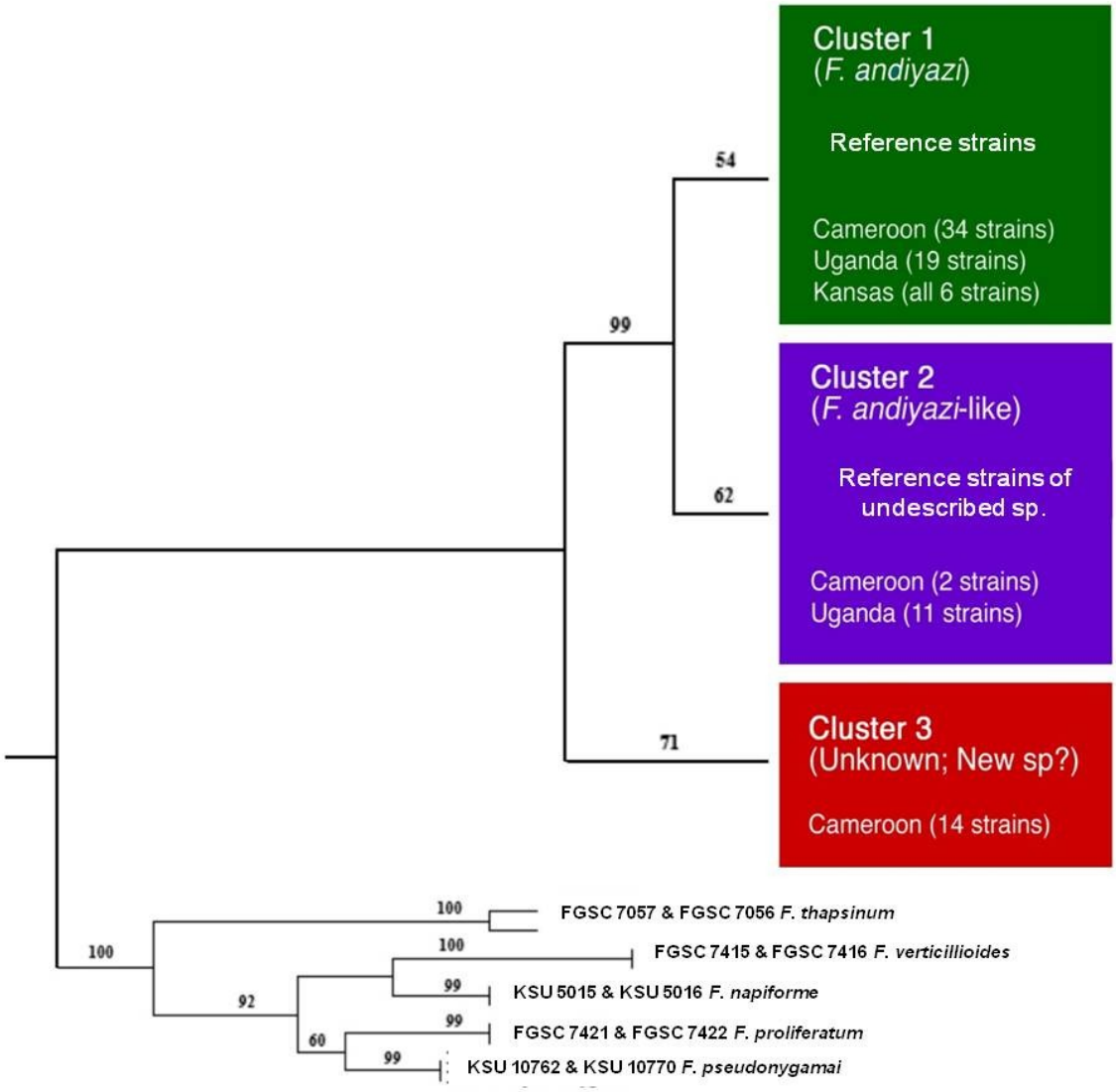

Figure 5.1. UPGMA tree of *F. andiyazi* and *F. andiyazi*-like isolates based on 56 AFLP bands. Numbers within the tree represent the bootstrap values (values above 50% are indicated; 1000 replications).

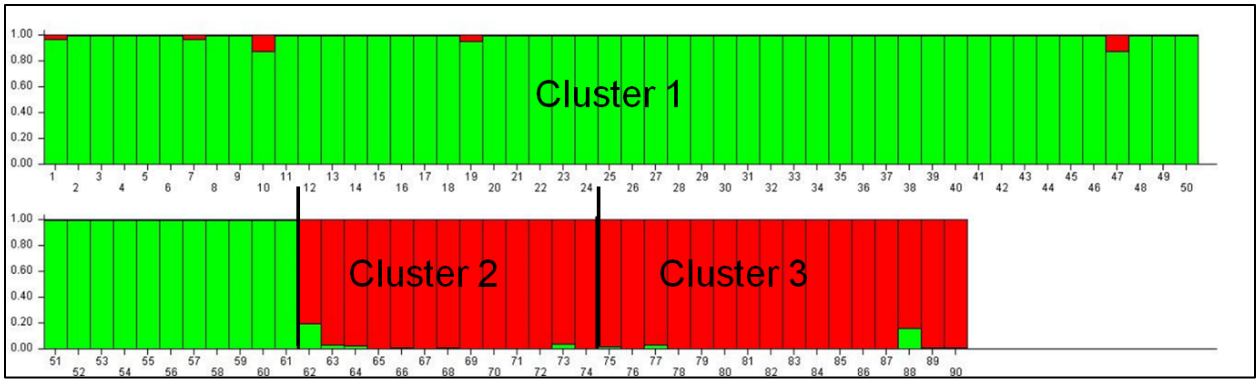

Figure 5.2. STRUCTURE analysis of *F. andiyazi* and *F. andiyazi*-like isolates based on 56 AFLP bands. Columns represent individual strains. Strains 1-61 (Cluster 1) are *F. andiyazi* (includes two *F. andiyazi* tester strains); 62-90 (Clusters 2 and 3) are *F. andiyazi*-like strains with Cluster 2 also including two tester strains of an as yet undescribed species.

Chapter 6 - Conclusions

Prior to the current work, *F. thapsinum* and *F. andiyazi* were thought of as highly clonal species (Leslie and Klein, 1996; Marasas et al., 2001) with very little known about the population structure of these important sorghum pathogens. Also, comparable seedling and adult plant pathogenicity evaluations of multiple strains of these two species were lacking. The current work addressed these issues in *F. thapsinum* by evaluating genetic variation in field isolates from multiple continents and by conducting both seedling and adult plant pathogenicity tests of *F. thapsinum*.

The study of population genetic structure of *F. thapsinum* from sorghum in Kansas identified two subpopulations and a genetically intermediate group. High genotypic diversity, but relatively low female fertility, suggests that both sexual and asexual reproduction are important parts of the *F. thapsinum* life cycle. These results were consistent with our working hypothesis that *F. thapsinum* populations were similar to other *Fusarium* populations with a known sexual stage. The presence of the hybrid group suggests that at least two previously separated populations of *F. thapsinum* are coming into regular contact in the same niche on sorghum in Kansas and that hybridization between them is occurring. The number of strains in the "hybrid" group is small relative to those in the groups representing the two subpopulations. This small number might mean that the hybrids are not as competitive as members of the parental subpopulations, or that the hybrids primarily are mating with members of one of the two subpopulations and that their progeny appear to be part of a subpopulation rather than a part of the hybrid group. Since the work on population structure of *F. thapsinum* from Kansas is based on isolates that were recovered in the 1980s, a contemporary study is now needed to determine how the partition of ge-

netic variation might have changed in this fungus over time. The current study can serve as the baseline for such comparisons.

The work on population structure of *F. thapsinum* from Kansas, Africa, Thailand, and Australia, found that migration with seeds brought from different parts of the world and random mating *in situ* probably are important in determining the population structure of this fungal pathogen. In an extensive study of *F. thapsinum* from Australia similar conclusions were reached, *i.e.*, that the Australian population of *F. thapsinum* is part of an international panmictic population of this species (Petrovic, 2007). Large collections of *F. thapsinum* from, *e.g.*, India and eastern, western and southern Africa, where sorghum has been domesticated and commercial sorghum breeding programs have a long history, are needed to determine that additional center(s) of diversity exist and to evaluate the genetic relationships observed within and between the populations evaluated to date.

In seedling pathogenicity tests, *F. thapsinum* strains from Kansas ranged from non-pathogenic to pathogenic. Surprisingly, <5% of the tested strains were pathogenic in both the rolled towel and greenhouse seedling assays. These results are consistent with the majority of the *F. thapsinum* strains being endophytes or latent pathogens. There were significant differences in radicle length and fresh weight between the two Kansas subpopulations and the hybrid group. This result suggested that variation in seedling pathogenicity may be partially attributable to genetic variability in the *F. thapsinum* population. However, more in depth studies are needed to confirm and to establish the molecular genetic basis of this phenotype.

For adult plant pathogenicity, stalk rot of sorghum caused by six genetically diverse *F. thapsinum* strains from Kansas was evaluated. Our working hypothesis was that strains would be equally capable of causing disease on susceptible and resistant sorghum germplasm in both the

field and the greenhouse. Based on stalk rot aggressiveness, there are differences in pathogenicity amongst the strains and the working hypothesis was rejected. The results of this study challenge the common practice of using a single, arbitrarily selected strain of *F. thapsinum* for evaluating sorghum germplasm for resistance to *Fusarium* stalk rot without considering the known aggressiveness of the strain used. These results highlight the point that strain selection is an important consideration when screening sorghum germplasm for disease resistance and that all strains of the species should not be considered equivalent to one another.

The study of genetic diversity of *F. andiyazi* and *F. andiyazi*-like populations identified at least two groups that represent sister-species of *F. andiyazi* that have not yet been formally described. More work is needed to characterize these putative sister-species and to understand their role, if any, in sorghum pathogenicity.

Overall, the current study found substantial genetic variation in *F. thapsinum* populations, and that this variation may contribute to observed variation in pathogenicity, at least in seedling pathogenicity (in the field seedling pathogenicity would be manifested as poor stand establishment). There was no correlation between differences in the stalk rot aggressiveness for individual *F. thapsinum* strains and subpopulation differentiation. A more extensive study evaluating more fungal strains and/or host lines could alter this conclusion. The results of both seedling and adult plant pathogenicity tests of *F. thapsinum* provide a textbook example of the need for optimized pathogenicity assays that include adequate characterization of the strain(s) used to screen germplasm for seedling blight, stalk rot, or any other disease prior to widespread use. Such efforts will assist in identifying contributions from both the pathogen's genetic background and the environmental conditions to the variation observed in disease severity in both the seedling and adult portions of the host plant's life cycle. Clearly, an understanding of the genetic variation

within populations of *F. thapsinum* is important in understanding where the fungus originated, how it continues to evolve, and how results of tests of it as a pathogen should be evaluated and interpreted. This thesis has touched all of these issues and should make it easier for scientists interested in either the practical or applied study of this fungus in the future to ask new questions about this fungus and to limit the damage that it can cause to cultivated sorghum.

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Appendix A - List of strains of *Fusarium thapsinum* from sorghum in Kansas.

Population[a]	KSU strain number[b]	Year isolated	Geographic origin[c]	Pigmentation[d]	Mating type *MAT-1*/*MAT-2*	VCG[e]
1	F-00496	1986	Pottawatomie	Y	1	–
	F-00754	1986	Brown	Y	1	10
	F-00916	1986	Gove	Y	2	U
	F-00917	1986	Lane	Y	2	U
	F-00918	1986	Finney	Y	2	U
	F-00919	1986	Hodgeman	Y	2	U
	F-00920	1986	Hodgeman	Y	2	U
	F-00921	1986	Rice	Y	2	6
	F-00922	1986	Rice	Y	2	6
	F-00923	1986	Rice	Y	2	U
	F-00965	1987	Riley	Y	1	5
	F-00966	1987	Riley	Y	2	U
	F-01049	1987	Wabaunsee	Y	1	U
	F-01053	1987	Osage	Y	2	U
	F-01056	1987	Osage	Y	1	7
	F-01064	1987	Douglas	Y	1	7
	F-01087	1987	Linn	Y	2	8
	F-01088	1987	Linn	Y	1	5
	F-01096	1987	Crawford	Y	1	5
	F-01139	1987	Cowley	NP	1	–
	F-01151	1987	Cowley	Y	1	U
	F-01153	1987	Cowley	Y	2	U
	F-01155	1987	Cowley	Y	1	9

Population[a]	KSU strain number[b]	Year isolated	Geographic origin[c]	Pigmentation[d]	Mating type *MAT-1*/*MAT-2*	VCG[e]
1	F-01294	1987	Washington	Y	1	U
	F-01320	1987	Washington	Y	1	1
	F-01321	1987	Washington	Y	1	3
	F-01338	1987	Republic	Y	1	U
	F-01352	1987	Smith	Y	2	U
	F-01353	1987	Smith	Y	1	–
	F-01372	1987	Trego	Y	1	3
	F-01378	1987	Trego	Y	1	3
	F-01383*	1987	Trego	Y	1	3
	F-01390	1987	Trego	Y	1	3
	F-01391	1987	Trego	Y	1	3
	F-01392	1987	Trego	Y	1	3
	F-01394	1987	Trego	Y	1	U
	F-01395	1987	Trego	Y	1	3
	F-01427	1987	Trego	Y	1	5
	F-01521	1988	Marion	Y	1	U
	F-01524	1988	Marion	Y	2	U
	F-01526	1988	Marion	Y	1	U
	F-01544	1988	Harvey	Y	1	U
	F-01545	1988	Harvey	Y	1	U
	F-01546	1988	Harvey	Y	1	U
	F-01672	1988	Harvey	NP	1	–
	F-01684	1988	Harper	Y	1	U
	F-02392	1989	Dickinson	Y	1	–
	F-02405	1989	Dickinson	NP	1	–
	F-02579	1989	Dickinson	Y	1	–

Population[a]	KSU strain number[b]	Year isolated	Geographic origin[c]	Pigmentation[d]	Mating type *MAT-1*/*MAT-2*	VCG[e]
1	F-02645	1989	Pottawatomie	Y	1	–
	F-02673	1989	Pottawatomie	Y	1	–
	F-02686	1989	Pottawatomie	Y	2	–
	F-02687	1989	Pottawatomie	Y	2	–
	F-02689	1989	Pottawatomie	Y	2	–
	F-02701	1989	Pottawatomie	Y	1	–
	F-02704	1989	Pottawatomie	Y	2	–
	F-02705	1989	Pottawatomie	Y	1	–
	F-02718	1989	Pottawatomie	Y	1	–
	F-02719	1989	Pottawatomie	Y	1	–
	F-02720	1989	Pottawatomie	Y	2	–
	F-02723	1989	Pottawatomie	Y	1	–
	F-02724	1989	Pottawatomie	Y	1	–
	F-02727	1989	Pottawatomie	Y	2	–
	F-02728	1989	Pottawatomie	Y	1	–
	F-02729	1989	Pottawatomie	Y	2	–
	F-02735	1989	Pottawatomie	Y	1	–
	F-02739	1989	Pottawatomie	Y	1	–
	F-02744	1989	Pottawatomie	Y	2	–
	F-02746	1989	Pottawatomie	Y	2	–
	F-02760	1989	Dickinson	NP	1	–
	F-02779	1989	Pottawatomie	Y	1	–
	F-02788	1989	Riley	Y	2	–
	F-02793	1989	Riley	Y	2	–
	F-02796	1989	Riley	Y	2	–
	F-02798	1989	Cloud	Y	1	–

Population[a]	KSU strain number[b]	Year isolated	Geographic origin[c]	Pigmentation[d]	Mating type *MAT-1*/*MAT-2*	VCG[e]
1	F-02805	1989	Cloud	Y	1	–
	F-02810	1989	Dickinson	Y	1	–
	F-02815	1989	Dickinson	Y	2	–
Hybrid						
	F-01083	1987	Anderson	Y	1	U
	F-01106	1987	Cherokee	Y	2	1
	F-01127	1987	Chautauqua	NP	1	–
	F-01137	1987	Elk	Y	1	9
	F-01154	1987	Cowley	Y	2	8
	F-01183	1987	McPherson	Y	1	2
	F-01184	1987	McPherson	Y	1	2
	F-01292	1987	Washington	Y	2	1
	F-01388	1987	Trego	Y	1	5
	F-01428	1987	Trego	Y	1	U
	F-01531	1988	Marion	Y	2	U
	F-01671	1988	Harvey	NP	2	–
	F-02390	1989	Dickinson	Y	2	–
	F-02396	1989	Dickinson	Y	2	–
	F-02401	1989	Dickinson	Y	1	–
	F-02404	1989	Dickinson	Y	1	–
	F-02432	1989	Dickinson	Y	1	–
	F-02439	1989	Dickinson	NP	1	–
	F-02483	1989	Dickinson	NP	1	–
	F-02582	1989	Dickinson	NP	2	–
	F-02584	1989	Dickinson	Y	1	–
	F-02664	1989	Pottawatomie	NP	2	–

Population[a]	KSU strain number[b]	Year isolated	Geographic origin[c]	Pigmentation[d]	Mating type *MAT-1*/*MAT-2*	VCG[e]
Hybrid	F-02667	1989	Riley	Y	1	–
	F-02698	1989	Pottawatomie	NP	2	–
	F-02716	1989	Pottawatomie	Y	1	–
	F-02717	1989	Dickinson	Y	1	–
	F-02725	1989	Pottawatomie	Y	2	–
	F-02726	1989	Pottawatomie	Y	2	–
	F-02745	1989	Pottawatomie	Y	1	–
	F-02758	1989	Dickinson	NP	2	–
	F-02775	1989	Pottawatomie	NP	2	–
	F-02802	1989	Cloud	NP	2	–
	F-02803	1989	Cloud	Y	2	–
	F-02813	1989	Dickinson	NP	2	–
2						
	F-00494	1986	Pottawatomie	NP	1	–
	F-00719	1986	Brown	NP	2	–
	F-00728	1986	Brown	Y	1	11
	F-00915	1986	Gove	Y	1	U
	F-01054	1987	Osage	Y	2	7
	F-01061	1987	Douglas	NP	1	–
	F-01103	1987	Crawford	Y	2	–
	F-01163	1987	Morris	Y	2	7
	F-01187	1987	Clay	NP	1	–
	F-01283	1987	Washington	NP	2	–
	F-01324	1987	Washington	NP	1	–
	F-01335	1987	Washington	Y	2	7
	F-01337	1987	Washington	Y	2	7

Population[a]	KSU strain number[b]	Year isolated	Geographic origin[c]	Pigmentation[d]	Mating type *MAT-1*/*MAT-2*	VCG[e]
2	F-01339	1987	Republic	Y	1	2
	F-01340	1987	Republic	Y	1	2
	F-01375*	1987	Trego	Y	1	4
	F-01376	1987	Trego	Y	1	11
	F-01377*	1987	Trego	Y	1	4
	F-01379	1987	Trego	Y	1	4
	F-01381	1987	Trego	Y	1	4
	F-01429	1987	Trego	Y	1	U
	F-01455	1987	Geary	NP	1	–
	F-01525	1988	Marion	NP	2	–
	F-01530	1988	Marion	NP	2	–
	F-01674	1988	Reno	Y	1	U
	F-02421	1989	Dickinson	NP	2	–
	F-02426	1989	Dickinson	NP	1	–
	F-02434	1989	Dickinson	NP	2	–
	F-02435	1989	Dickinson	NP	1	–
	F-02472	1989	Dickinson	NP	1	–
	F-02477	1989	Dickinson	NP	2	–
	F-02484	1989	Dickinson	Y	1	–
	F-02487	1989	Dickinson	NP	1	–
	F-02576	1989	Dickinson	Y	1	–
	F-02586*	1989	Dickinson	NP	1	–
	F-02648	1989	Pottawatomie	Y	2	–
	F-02650	1989	Pottawatomie	NP	1	–
	F-02652	1989	Pottawatomie	NP	2	–
	F-02655	1989	Pottawatomie	NP	2	–

Population[a]	KSU strain number[b]	Year isolated	Geographic origin[c]	Pigmentation[d]	Mating type *MAT-1*/*MAT-2*	VCG[e]
	F-02658	1989	Pottawatomie	NP	2	–
	F-02659	1989	Pottawatomie	NP	1	–
	F-02662	1989	Pottawatomie	NP	2	–
	F-02670	1989	Pottawatomie	NP	2	–
	F-02671	1989	Pottawatomie	NP	1	–
	F-02682	1989	Pottawatomie	NP	2	–
	F-02696	1989	Pottawatomie	NP	1	–
	F-02699	1989	Pottawatomie	NP	1	–
	F-02707	1989	Pottawatomie	NP	2	–
	F-02708	1989	Pottawatomie	NP	1	–
	F-02711	1989	Pottawatomie	NP	1	–
	F-02715	1989	Pottawatomie	NP	1	–
	F-02731	1989	Pottawatomie	NP	1	–
	F-02742	1989	Pottawatomie	Y	2	–
	F-02761	1989	Dickinson	Y	1	–
	F-02778	1989	Pottawatomie	NP	2	–

[a] Population inferred in STRUCTURE v2.2 software (Pritchard et al., 2000) using 34 polymorphic AFLP markers.

[b] KSU = accession number of strain from the culture collection in the Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66502.

[c] Kansas county where strains were recovered.

[d] Pigmentation of cultures on full strength PDA, Y = yellow-pigmented, NP = non-pigmented.

[e] Vegetative compatibility group (VCG), U = unique, Number = VCG group, (–) = not determined.

*Indicates the strain was both male and female fertile; all other strains were fertile only as males.

Appendix B - List of *Fusarium thapsinum* strains and their pathogenicity based on the rolled-towel and pot-based greenhouse assays.

Population[a]	KSU strain number[b]	Pathogenicity[c]			
		Mean radicle length (mm)	Mean % germination	Mean fresh weight (mg)	Mean % emergence
1					
	F-00496	74	72	204	**46**
	F-00754	76	63	104	69
	F-00916	83	71	105	56
	F-00917	**53**	**21**	150	**26**
	F-00918	96	74	155	**31**
	F-00919	**60**	**39**	92	**14**
	F-00920	**61**	62	162	50
	F-00921	82	68	262	77
	F-00922	82	77	189	64
	F-00923	91	69	213	69
	F-00965	**45**	**31**	228	70
	F-00966	**65**	65	109	60
	F-01049	80	71	159	67
	F-01053	94	81	139	72
	F-01056	80	70	204	73
	F-01064	72	74	191	63
	F-01087	93	77	236	66
	F-01088	70	70	*299*	61
	F-01096	78	66	*301*	67

Population[a]	KSU strain number[b]	Pathogenicity[c]			
		Mean radicle length (mm)	Mean % germination	Mean fresh weight (mg)	Mean % emergence
1	F-01139	73	**54**	**37**	50
	F-01151	81	76	53	73
	F-01153	**25**	**15**	52	**12**
	F-01155	**52**	**8.9**	238	**46**
	F-01294	88	66	102	68
	F-01320	77	73	107	56
	F-01321	84	75	90	59
	F-01338	90	66	*305*	81
	F-01352	87	75	54	**45**
	F-01353	**41**	**14**	*328*	53
	F-01372	108	75	57	**39**
	F-01378	89	80	**45**	**37**
	F-01383	90	78	**37**	**18**
	F-01390	84	70	72	64
	F-01391	96	66	**33**	**29**
	F-01392	105	71	54	**42**
	F-01394	98	73	52	**32**
	F-01395	100	70	**43**	55
	F-01427	72	75	84	51
	F-01521	75	65	65	**36**
	F-01524	**68**	**54**	69	**47**
	F-01526	84	76	57	60
	F-01544	77	76	58	50
	F-01545	96	73	85	59
	F-01546	80	66	69	48
	F-01672	84	69	62	56

Population[a]	KSU strain number[b]	Pathogenicity[c]			
		Mean radicle length (mm)	Mean % germination	Mean fresh weight (mg)	Mean % emergence
1	F-01684	73	63	53	**42**
	F-02392	77	67	66	64
	F-02405	**57**	**55**	61	56
	F-02579	**68**	68	87	**36**
	F-02645	**64**	**49**	**44**	**36**
	F-02673	**65**	**58**	92	**38**
	F-02686	**65**	70	*420*	77
	F-02687	**68**	66	*390*	80
	F-02689	69	**59**	56	**20**
	F-02701	99	80	**44**	**36**
	F-02704	73	69	**45**	**46**
	F-02705	72	**53**	58	**43**
	F-02718	**64**	72	69	**46**
	F-02719	**64**	67	**35**	50
	F-02720	77	70	*480*	66
	F-02723	**61**	64	**45**	54
	F-02724	**65**	**53**	**48**	53
	F-02727	102	73	**40**	**37**
	F-02728	99	75	**46**	**15**
	F-02729	92	72	**40**	**28**
	F-02735	72	68	**32**	58
	F-02739	76	62	**40**	57
	F-02744	70	64	**45**	58
	F-02746	99	71	**45**	**46**
	F-02760	99	82	**39**	**38**
	F-02779	99	73	**39**	**33**

Population[a]	KSU strain number[b]	Pathogenicity[c]			
		Mean radicle length (mm)	Mean % germination	Mean fresh weight (mg)	Mean % emergence
1	F-02788	110	65	**38**	**27**
	F-02793	96	73	**39**	**29**
	F-02796	99	76	**42**	**39**
	F-02798	90	77	60	**21**
	F-02805	93	71	**49**	**41**
	F-02810	99	66	**41**	**42**
	F-02815	93	65	**45**	**45**
Hybrid					
	F-01083	96	74	190	79
	F-01106	72	75	210	58
	F-01127	**68**	64	240	65
	F-01137	**57**	**46**	55	55
	F-01154	**42**	**13**	*450*	57
	F-01183	85	70	260	**15**
	F-01184	82	65	*380*	48
	F-01292	83	66	93	55
	F-01388	95	70	62	57
	F-01428	82	65	78	53
	F-01531	84	69	64	68
	F-01671	74	67	55	53
	F-02390	83	72	150	49
	F-02396	**66**	70	*490*	75
	F-02401	81	70	**46**	**38**
	F-02404	71	**44**	**28**	**46**
	F-02432	84	70	66	**44**
	F-02439	**66**	64	89	**38**

Population[a]	KSU strain number[b]	Pathogenicity[c]			
		Mean radicle length (mm)	Mean % germination	Mean fresh weight (mg)	Mean % emergence
Hybrid	F-02483	**66**	**48**	56	**45**
	F-02582	**64**	**60**	*510*	76
	F-02584	79	**57**	88	47
	F-02664	70	**58**	*560*	76
	F-02667	**64**	**46**	**47**	49
	F-02698	72	67	*470*	75
	F-02716	72	65	**31**	49
	F-02717	80	67	63	55
	F-02725	93	64	**45**	**40**
	F-02726	70	66	57	65
	F-02745	107	62	**49**	**47**
	F-02758	108	70	57	**41**
	F-02775	101	79	50	**40**
	F-02802	101	65	82	**30**
	F-02803	86	80	53	49
	F-02813	**67**	63	81	54
2					
	F-00494	82	68	170	61
	F-00719	**45**	**12**	250	**30**
	F-00728	83	67	180	61
	F-00915	72	77	96	62
	F-01054	75	76	150	57
	F-01061	89	71	190	55
	F-01103	94	78	230	60
	F-01163	**68**	**9.7**	230	**25**
	F-01187	81	70	*300*	**36**

Population[a]	KSU strain number[b]	Pathogenicity[c]			
		Mean radicle length (mm)	**Mean % germination**	**Mean fresh weight (mg)**	**Mean % emergence**
2	F-01283	79	74	*370*	**19**
	F-01324	81	66	95	58
	F-01335	92	65	87	53
	F-01337	73	68	*420*	55
	F-01339	79	62	*300*	**36**
	F-01340	80	63	160	**16**
	F-01375	**55**	**21**	*290*	55
	F-01376	**57**	**13**	*440*	62
	F-01377	103	76	**42**	**26**
	F-01379	101	78	49	49
	F-01381	97	77	52	47
	F-01429	97	76	60	49
	F-01455	106	67	75	49
	F-01525	79	73	**46**	**47**
	F-01530	80	68	**44**	**44**
	F-01674	80	67	50	59
	F-02421	**65**	62	250	76
	F-02426	70	**59**	68	**37**
	F-02434	**66**	68	*310*	80
	F-02435	**63**	**57**	80	58
	F-02472	**61**	**57**	72	48
	F-02477	79	68	*400*	69
	F-02484	**62**	**51**	**38**	**38**
	F-02487	73	74	69	**45**
	F-02576	70	70	81	**43**
	F-02586	**61**	**42**	58	**36**

Population[a]	KSU strain number[b]	Pathogenicity[c]			
		Mean radicle length (mm)	Mean % germination	Mean fresh weight (mg)	Mean % emergence
2	F-02648	**66**	68	*500*	74
	F-02650	69	81	84	**40**
	F-02652	69	70	*310*	79
	F-02655	74	74	*650*	80
	F-02658	**62**	73	*600*	81
	F-02659	**64**	**53**	**36**	**28**
	F-02662	77	71	*560*	73
	F-02670	**68**	70	*420*	64
	F-02671	**66**	**51**	**45**	**20**
	F-02682	**66**	72	*410*	74
	F-02696	**55**	**44**	85	66
	F-02699	**68**	**53**	**48**	**41**
	F-02707	72	63	**42**	54
	F-02708	71	64	67	**44**
	F-02711	**54**	**51**	60	49
	F-02715	72	62	**42**	52
	F-02731	112	67	**41**	**32**
	F-02742	90	78	**46**	**38**
	F-02761	106	71	**49**	**41**
	F-02778	90	71	**38**	**31**

[a] Population inferred in STRUCTURE v2.2 software (Pritchard et al., 2000) using 34 polymorphic AFLP markers.

[b] KSU = accession number of strain from the culture collection in the Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66502.

[c] Value in regular font indicates that a strain was non-pathogenic; value in bold font indicates that a strain was pathogenic; value in italics indicates that a strain had a stimulatory effect.

Appendix C - List of *Fusarium thapsinum* strains from sorghum in Australia, Africa (Mali, Cameroon, Uganda), and Thailand.

Country	KSU strain number	Host	Mating type (*MAT*-1/*MAT*-2)
Australia			
	F-24060	*Austrostipa aristiglumis*	2
	F-24061	*A. aristiglumis*	2
	F-24062	Soil	2
	F-24063	*A. aristiglumis*	2
	F-24064	*A. aristiglumis*	2
	F-24065	*A. aristiglumis*	2
	F-24066	*A. aristiglumis*	2
	F-24067	Grain sorghum	2
	F-24068	Grain sorghum	2
	F-24069	Grain sorghum	2
	F-24070	Grain sorghum	2
	F-24072	Grain sorghum	2
	F-24073	Unknown	2
	F-24078	Soil	2
	F-24081	Grain sorghum	2
	F-24082	Grain sorghum	2
	F-24083	Soil	2
	F-24084	Soil	2
Thailand			
	F-3051	Grain sorghum	2
	F-3133	Grain sorghum	1
	F-3140	Grain sorghum	1
	F-3148	Grain sorghum	1
	F-3149	Grain sorghum	1
	F-3150	Grain sorghum	2
	F-3154	Grain sorghum	1
	F-3156	Grain sorghum	2
	F-3157	Grain sorghum	2
	F-3158	Grain sorghum	1
	F-3159	Grain sorghum	1
	F-3160	Grain sorghum	1
	F-3161	Grain sorghum	1

Country	KSU strain number	Host	Mating type (*MAT*-1/*MAT*-2)
Mali	F-3162	Grain sorghum	1
	F-3163*	Grain sorghum	1
	F-3164	Grain sorghum	1
	F-3165*	Grain sorghum	1
	F-3168	Grain sorghum	2
	F-3169	Grain sorghum	1
	F-3172*	Grain sorghum	1
	F-12023	Grain sorghum	1
	F-12034	Grain sorghum	2
	F-12040	Grain sorghum	2
	F-12056	Grain sorghum	1
	F-12065	Grain sorghum	1
	F-12066	Grain sorghum	2
	F-12084*	Grain sorghum	2
	F-12215	Grain sorghum	1
	F-12222	Grain sorghum	2
	F-12226	Grain sorghum	2
	F-12228	Grain sorghum	1
	F-12243	Grain sorghum	1
	F-12244	Grain sorghum	1
	F-12441	Grain sorghum	2
	F-12458	Grain sorghum	1
	F-12459	Grain sorghum	1
	F-12471	Grain sorghum	1
	F-12510*	Grain sorghum	1
	F-12691	Grain sorghum	2
	F-12698	Grain sorghum	1
	F-12702	Grain sorghum	1
	F-12705	Grain sorghum	1
	F-12706	Grain sorghum	1
	F-12710	Grain sorghum	2
	F-12713	Grain sorghum	1
	F-12720	Grain sorghum	1
	F-12723	Grain sorghum	1
	F-12724*	Grain sorghum	2
	F-12725	Grain sorghum	2
	F-12727	Grain sorghum	1

Country	KSU strain number	Host	Mating type (*MAT*-1/*MAT*-2)
	F-12728	Grain sorghum	2
	F-12821	Grain sorghum	1
	F-12827	Grain sorghum	1
	F-12836	Grain sorghum	1
	F-12842	Grain sorghum	1
	F-12847	Grain sorghum	1
	F-12848	Grain sorghum	2
	F-12849	Grain sorghum	2
	F-12869	Grain sorghum	2
	F-12871	Grain sorghum	1
	F-12716	Grain sorghum	1
	F-12829*	Grain sorghum	1
	F-12834	Grain sorghum	2
	F-12262	Grain sorghum	2
	F-12428	Grain sorghum	1
	F-12433	Grain sorghum	1
	F-12446	Grain sorghum	1
	F-12453	Grain sorghum	2
	F-12457	Grain sorghum	1
	F-12461	Grain sorghum	1
	F-12470*	Grain sorghum	2
	F-12472	Grain sorghum	1
	F-12476	Grain sorghum	1
	F-12689	Grain sorghum	2
	F-12838	Grain sorghum	1
	F-12843	Grain sorghum	1
	F-12845	Grain sorghum	1
	F-12852	Grain sorghum	1
	F-12857	Grain sorghum	1
	F-12862	Grain sorghum	1
	F-12227	Grain sorghum	1
	F-12230	Grain sorghum	1
	F-12242	Grain sorghum	2
	F-12039	Grain sorghum	1
	F-12060	Grain sorghum	1
	F-12068	Grain sorghum	1
	F-12245*	Grain sorghum	1
	F-12686	Grain sorghum	1

Country	KSU strain number	Host	Mating type (*MAT*-1/*MAT*-2)
Cameroon	F-12693	Grain sorghum	2
	F-12828	Grain sorghum	1
	F-12870	Grain sorghum	1
	F-15141	Grain sorghum	1
	F-16129	Grain sorghum	1
	F-15094	Grain sorghum	1
	F-15148*	Grain sorghum	1
	F-15149	Grain sorghum	1
	F-17751	Grain sorghum	1
Uganda	F-9191*	Grain sorghum	1
	F-9197	Grain sorghum	2
	F-9199	Grain sorghum	1
	F-9207	Grain sorghum	1

*Indicates strains that were both male and female fertile; all other strains were fertile only as males.

Appendix D - Fifty-two AFLP loci polymorphic in populations of *F. thapsinum* from Australia, Kansas, Thailand, and Africa.

Locus[a]	Australia	Kansas	Thailand	Africa
EAA2	P	P	P	P
UEAA3	M (allele 0)	M (allele 0)	M (allele 0)	P
EAA4	M (allele 1)	M (allele 1)	M (allele 1)	P
UEAA7	M (allele 0)	M (allele 0)	P	M (allele 0)
EAA7	P	P	P	P
UEAA8	M (allele 0)	M (allele 0)	P	P
EAA8	P	P	P	P
EAA9	M (allele 0)	P	P	M (allele 0)
EAA11	P	P	P	P
EAA12	P	P	P	P
EAA13	P	P	P	P
EAA14	M (allele 0)	P	P	M (allele 0)
EAA15	M (allele 1)	P	P	P
EAA16	M (allele 0)	P	P	M (allele 0)
EAA17	M (allele 0)	P	P	P
UEAA18	M (allele 0)	M (allele 0)	M (allele 0)	P
UEAA19	M (allele 0)	M (allele 0)	M (allele 0)	P
EAA18	P	P	M (allele 0)	M (allele 0)
EAA19	P	P	M (allele 0)	M (allele 0)
EAA20	M (allele 0)	P	M (allele 0)	M (allele 0)
EGG2	M (allele 1)	P	P	P
EGG3	P	P	P	P
EGG4	P	P	P	P
EGG6	P	P	P	P
EGG7	P	P	P	P
EGG8	P	P	P	P
EGG9	P	P	P	P
EGG11	P	P	P	P
EGG12	P	P	P	P
EGG13	P	P	M (allele 0)	M (allele 0)
EGG14	P	P	M (allele 1)	M (allele 1)
UEGG15	M (allele 0)	M (allele 0)	M (allele 0)	P
UEGG16	M (allele 0)	M (allele 0)	M (allele 0)	P
EGG18	P	P	P	M (allele 1)
UEGG20	M (allele 0)	M (allele 0)	M (allele 0)	P
ETT2	P	P	P	P

Locus[a]	Australia	Kansas	Thailand	Africa
ETT3	P	P	P	P
ETT4	M (allele 0)	P	M (allele 0)	P
ETT6	P	P	P	P
ETT7	P	P	P	P
ETT8	P	P	M (allele 0)	P
ETT9	P	P	M (allele 0)	P
ETT10	P	P	P	M (allele 0)
ETT11	M (allele 0)	P	P	P
ETT12	P	P	P	P
UETT14	M (allele 0)	M (allele 0)	M (allele 0)	P
ETT14	M (allele 1)	P	M (allele 1)	P
ETT15	P	P	M (allele 0)	P
ETT16	P	P	P	P
ETT17	P	M (allele 1)	M (allele 1)	M (allele 1)
ETT19	P	P	P	P
ETT20	P	M (allele 1)	M (allele 1)	M (allele 1)

[a] Loci for which at least one population is polymorphic. P = polymorphic locus, M = monomorphic locus with fixed allele in parentheses.

Appendix E - UPGMA tree of strains from Australia, Thailand, Kansas, and Africa based on 52 polymorphic AFLP markers.