POPULATION DYNAMICS OF *PUCCINIA EMACULATA* IN NATURAL AND EXPERIMENTAL ENVIRONMENTS

By

JESSICA CARRIE PAVLU

Bachelor of Science in Soil and Crop Sciences and

Horticulture Food Crop Production

Colorado State University

Fort Collins, Colorado

2013

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 2015

POPULATION DYNAMICS OF *PUCCINIA EMACULATA* IN NATURAL AND EXPERIMENTAL ENVIRONMENTS

Thesis Approved:

Dr. Carla Garzon

Thesis Adviser

Dr. Jaqueline Fletcher

Dr. Stephen Marek

ACKNOWLEDGEMENTS

I would like to express gratitude to all my lab members that have helped me get to this point in academic career. A very special thank you goes to Gabriela Orquera Tornakian, without your training, advice, and patience I couldn't become the scientist I am today. I would also like to mention in gratitude Omar Arias, Alexandra Moya, Maddi Shires, Yisel Carrilo, Kihyuck Choi, and Patricia Garrido for all their contributions to this study.

Finally I would like to thank my fiancé Brad Lindenmayer and my family for all their love and support during my degree program.

Acknowledgements reflect the views of the author and are not endorsed by committee members or Oklahoma State University.

Name: JESSICA PAVLU

Date of Degree: DECEMBER, 2015

Title of Study: POPULATION DYNAMICS OF *PUCCINIA EMACULATA* IN NATURAL AND EXPERIMENTAL ENVIRONMENTS

Major Field: ENTOMOLOGY AND PLANT PATHOLOGY

Abstract:

Panicum virgutum L., commonly referred to as switchgrass, is a C4 grass native to most of North America. With its deep rooting system allowing for low nutrient and water requirements and high biomass production, switchgrass was selected by the U.S. Department of Energy (DOE) as a good crop for biofuel production. However, switchgrass has been reported to have substantial losses in biomass due to the fungal plant pathogen *Puccinia emaculata* also known as switchgrass rust. Thought to be a macrocyclic rust, switchgrass serves as *P. emaculata*'s uredinial and telial host. In this study the population dynamics *of P. emaculata* was analyzed for the purpose of both epidemiological discovery and as a model for scientific attribution methods for rust. The first study used single spores across two states (Oklahoma and Virginia) and four years (2011, 2012, 2013, and 2014) to evaluate four previously described polymorphic microsatellite loci. The purpose of this study was to generate a conceptual analysis of population dynamics in a field setting over a series of years in two geographically distant states. Use of fixation indices showed low to moderate differentiation between states and moderate differentiation between years. All populations were discrete, with minute amounts of genotype migration between years. This result points to a similar inoculums source. The second study compared the field samples from the first study to growth chamber populations generated at Oklahoma State University over the same years to evaluate if the analysis method could provide scientific attribution. Similar origin populations with an unknown environment and to a completely unknown population were used to test the analysis method. The results showed the four microsatellite loci could be used to correlate populations of the same identity or reject the attribution of a population to the known population, but not to provide scientific attribution with the statistical support needed for a microbial forensic investigation. This study was unique in the use of single spores and a rust model for microbial forensic applications. With more microsatellite loci this method could be statistically sound enough for scientific attribution for a microbial forensic investigation.

TABLE OF CONTENTS

Chapter Page

I. INTRODUCTION 1

II. REVIEW OF LITERATURE..3

Biofuel Importance in the United States 3
Switchgrass (*Panicum virgatum L.)*..4
Puccinia emaculata..5
Agricultural Biosecurity and Microbial Forensics 7
Model System for Rust Pathogen Microbial Forensics: Study Justification 9
Methods Overview & Simple Sequence Repeat Analysis 10
Microsatellite Based Population Genetics in related *Puccina* species..................11
Simple Sequence Repeat Based DNA Profiling in Forensic Sciences 12
Experimental Approach 14
Literature Cited 16

III. OBJECTIVE ONE: 22
"A Population Diversity Analysis of *Puccina emaculata* Single Spores in OK and VA"

Abstract 22
Introduction..23
Methodology & Single Spore Collection...26
Microsatellite Analysis 28
Results..30
Statistical Analysis...31
Discussion 35
Literature Cited 39

Chapter Page

IV. OBJECTIVE TWO 42
"The Rust *Puccinia Emaculata* as a Model Rust for Microbial Forensic Investigation"

Abstract 42
Introduction 43
Experimental Design 44
Methodology & Potential Source Identification 45
Validation using Unknown Population for Attribution 47
Statistical Analysis 47
Results & Comparison of Known Field and Chamber Populations (Obj2.1) 48
Unknown Environment Analysis (Obj2.2) 52
Statistical Analysis 52
Blind Sample Analysis (Obj2.3) 56
Discussion & OK Unknown Environment Populations Identity 61
Unknown Population Identity 62
Conclusions 63
Literature Cited 67

LIST OF TABLES

Table Page

1. Eight Potentially Polymorphic Primers (Obj1) 29
2. Allelic Diversity Patterns (Obj1) 31
3. Pair-wise Population F_{ST} Values (Obj1) 32
4. Allelic Values Across All Populations (Obj1) 35
5. Pair-wise Population F_{ST} Values (Obj2.1) 48
6. Average Allele Values Across All Populations (Obj2.1) 51
7. Pair-wise Population F_{ST} Values (Obj2.2) 53
8. Pair-wise Population F_{ST} Values (Obj2.3) 58

LIST OF FIGURES

Figure Page

1. Native Distribution of Switchgrass across North America (Obj1) 24
2. Upland and Lowland Variety Distribution across the United States 24
and Canada (Obj1)
3. Principal Coordinate Analysis by Sample (Obj1) 33
4. STRUCTURE Bar Plot (Obj1) 33
5. Allelic Patterns across All Populations (Obj1) 34
6. STRUCTURE Bar Plot (Obj2.1) 49
7. Principal Coordinate Analysis by Sample (Obj2.1) 50
8. Allelic Patterns across all Populations (Obj2.1) 51
9. Principal Coordinate Analysis by Sample (Obj2.2) 55
10. Principal Coordinate Analysis by Population (Obj2.2) 55
11. Principal Coordinate Analysis by Sample (Obj2.3) 59
12. Principal Coordinate Analysis by Population (Obj2.3) 60

CHAPTER I

INTRODUCTION

In this project the utility of a microsatellite based DNA profiling analysis for a fungal plant pathogen to discriminate potential sources of inoculum for a confidence level suitable for forensic investigation was assessed. In two studies, microsatellite DNA fingerprinting was used to examine the spatial and temporal population dynamics of the rust fungus *Puccinia emaculata* in natural and experimental environments. Single urediniospores were used to genotype individuals within populations, trends in allele frequencies were evaluated leading to better understanding of spatial and temporal changes in population allele diversity of the pathogen. The results generated in the first study provided background information about allele frequency and diversity differences among natural colonizing field populations, and founded the assessment of whether the DNA profiling protocol would be suitable for use in a forensic investigation to identify the environmental origin, field or growth chamber, of inocula used in a hypothetical agricultural intentional or inadvertent release.

This research project had two main objectives, addressed by three studies. The first study examined the allelic diversity and population structures of *P. emaculata* populations in two states (Oklahoma and Virginia) over a period of four years (2011, 2012) to determine if the populations of those states differ in genetic composition and

structure over space and time. The information generated provided background knowledge about the allelic diversity of field populations of local origin (OK) compared to a geographically distant population (VA). The information generated was used in the second study by providing basic information to understand the epidemiology of switchgrass rust.

The first assay of the second study assessed whether a previously standardized microsatellite based DNA profiling protocol can be used in forensic applications to discriminate between naturally occurring and artificially propagated rust inoculum. The populations used in this objective included field collected samples from Oklahoma in the years 2011, 2012, 2013 and 2014. Additionally, samples collected originally in 2009 from the same Oklahoma field and continuously propagated on growth chamber grown plants were sampled periodically throughout 2012, 2013 and 2014 and compared to the field collected samples. The goal of this assay was to understand the genetic differences between populations in the two environments in order to determine if a sample of unknown origin could be attributed to one population or the other.

The background information generated in the first study (in which naturally colonizing and chamber populations were compared) was used to attempt attribution of origin with similar origin populations with unrevealed environment (field or chamber) origins. The final assay of the second study analyzed an entirely unknown population for attribution of origin. There is a need for scientific attribution methods for plant pathogens in the discipline of microbial forensics because of the severe implications of crop or natural habitat loss due to bioterrorism, biocrime, or inadvertent release of a pathogen.

Fungal rust plant pathogens are of high consequence; this study is justified as a step toward the development of microbial forensic methods for rust fungi.

CHAPTER II

LITERATURE REVIEW

Biofuel importance to the United States

Biofuel has become one of the most important energy sources in the United States because it is renewable energy source and has a small environmental footprint. Ethanol's popularity has risen, not only because of its previously mentioned attributes, but also because ethanol has been mandated for use in commercial and non-commercial vehicles since the passage of the Energy Independence and Security Act (EISA) of 2007 [1]. This law states that 36% of fuel should be renewable and that 16% of that fuel must be cellulosic. Cellulosic fuel is described as an "energy crop" that is not used primarily for livestock feed or direct consumption [1]. Energy crops have had an increasing importance due to concern about global food shortages, food price increases, and water demand increases for corn grown for ethanol production [2]. Currently, the United States farms more than 84 million acres of corn, grossing 63.9 billion dollars in income for the Nation. Of this corn, 80% is used for domestic and international livestock feed [3], leaving the remaining 20% for health products, bio-degradable products, direct consumption, and, most importantly for the purpose of this study, biofuel in the form of ethanol [3].

Because of EISA 2007 and the potential shortages of food production, alternative crops for cellulosic biofuel production have been a priority for research. One of the most promising cellulosic biofuel crops being researched today is *Panicum virgatum* or switchgrass.

Switchgrass (*Panicum virgatum L.*)

Switchgrass (*Panicum virgatum L.*) is a perennial C4 grass native to the North American prairies growing east of the Rocky Mountains, across the Great Plains, and over much of the Northeast and Canada [4]. Belonging to the family *Poaceae* in the section Virgata, switchgrass's closest relatives are *P. tricholaenoides, P. amarum,* and *P. amarulum,* which are also warm season native forage grasses [5-7]. Like its United States native grass relatives, switchgrass has been studied for its deep roots and carbon sequestration potential along with applications in soil erosion control, forage, and ornamental use [8]. In conjunction with its soil restoration and conservational qualities, switchgrass can sustain productivity across an array of environmental conditions, including what has been determined "marginal land". Marginal land, described as undesired land or unfit for row crop production, can also be described by its unsuitability for economic production, not fit for development. It has been suggested that land be used for forage and grazing [9]. Switchgrass has low water and nutrient requirements, and requires no specialized farming equipment or practices [10 , 11]. When compared directly, switchgrass's ethanol production, biomass and cost of production were always competitive, if not better, than those of corn [12]. Switchgrass has continued to be studied and has potential to be a widely cultivated crop. In addition to its use as feed and in

biofuel production, several switchgrass cultivars have been developed as ornamental plants and are broadly used in landscaping [13].

As with all other crops, many pathogens can cause damage on switchgrass. Common fungal pathogens include *Bipolaris oryzae* Link, *Fusarium graminearum* Schwabe, *Uromyces graminicola* Burril, *Puccinia graminis* Pers.: Pers., *Puccinia emaculata* Schwein and *Alternaria alternate* Nees. Of these fungal pathogens the fungal rust pathogen caused by *Puccinia emaculata* is the most damaging, making this rust the most important disease of switchgrass and the focus of this study [14-18].

Puccinia emaculata

The switchgrass rust pathogen *P. emaculata* was first described by Schweinitz in 1900 on the host *Panicum capillare* L. [19]. Believed to be a heteroecious rust pathogen, having at least two hosts to complete its life cycle, *P. emaculata* is most likely a macrocyclic rust as well. This rust cannot be confirmed a macrocyclic rust until the alternate host is confirmed. Switchgrass is the telial and uredinial host and flowering spurge (*Euphorbia corollata* L.) was reported to be the basidial and aecial host [16, 20].

Characterized by its large numbers of globose shaped, cinnamon brown urediniospores and oblong, dark brown to black teliospores, *P. emaculata* is highly pathogenic on a variety of upland and lowland switchgrass cultivars and a few of its closest relatives including the previously mentioned *P. amarum* [21]. Found on both ornamental and cultivated varieties, *P. emaculata* can have up to 100% leaf coverage and infect up to 100% of the leaves on a single plant, depending on variety [22]. Though the rust diminishes the aesthetics of the ornamental varieties, the damage on cultivated

switchgrass is both economical and physical. Along with up to 50% biomass reduction, *P. emaculata* can lower the amount of ethanol produced by 60% in the popular varieties Alamo and Kanlow [23]. Though switchgrass had been studied for many years, it was not until 2007 that *P. emaculata,* was described in Virginia [24] as an economically relevant pathogen. Most of the current rust management practices revolve around seasonal fungicide treatments. Some of the most effective fungicides for ornamental use are azoxystrobin, myclobutanil, and propiconazole. However, their efficacy for biomass promotion of infected plants vary greatly year to year depending on environmental conditions [25]. The inconsistency in efficacy, potential environmental risks, and risk of pathogen resistance when using multiple fungicide applications has supported cultivar resistance in switchgrass as the best means for management. Upland variety "Cave-in-Rock" and lowland varieties "Kanlow" and "Alamo" have shown moderate resistance, whereas other varieties, such as the upland variety "Sunburst", were susceptible based on ecotype variation and reaction to infection. The variation in incidence and damage to switchgrass by *P. emaculata* suggests that improved rust resistant varieties can be developed [14]. To deploy improved resistance in switchgrass varieties it is crucial to first understand the pathogen diversity and as well as disease epidemiology.

Reported frequently in states such as Oklahoma, Tennessee, Virginia, Arkansas, Mississippi, Kansas, Nebraska, South Dakota, Iowa, Louisiana, and multiple regions of Canada, *P. emaculata* is widespread has a distribution most likely matching that of the host [16, 26]. Due to its wide distribution and host range, *P. emaculata* is likely to have high genetic diversity. Most likely a heteroecious rust, there would be a high expected rate of sexual recombination leading to genetically diverse populations throughout its

expansive distribution[16]. Additionally, though not reported in *P. emaculata*, somatic recombination within the dikaryotic urediniospores of *P. striiformis* has been observed [27]. Having heterokaryotic nuclei within the urediniospores of *P. emaculata* suggests this may be true for this rust as well leading to higher genetic variability within the genome of the rust [16].

However, without the confirmation of the aecial host, it is difficult to speculate the source of diversity within switchgrass rust populations. Additionally, without the aecial host it is very difficult to determine the origin of inoculum to better infer movement and evolution of the pathogen. Preliminary genetic analysis [16] suggested that the spores are not localized populations bases on the lack of geographic differentiation. This finding may suggest thar the *P. emaculata* urediniospores may migrate in a fashion similar to that of its relatives along the *Puccinia* pathway (South to Northward moving wind patterns that carry rusts from Mexico to a majority of the United States, then on North to Canada) [28]. This theory cannot be proven until the aecial host is found in or close to Mexico. It is with population analyses that the genetic diversity of the pathogen can begin to reveal details about the epidemiology of such a complex organism.

Agricultural biosecurity and microbial forensics

Though the production of biological weapons had predominantly ceased by the end of WWII, the anthrax attacks of 2001 revealed how unprepared the United States was for bioterror acts [29, 30]. It was after these attacks that a coalition of many different scientific fields and government organizations created an initiative to prevent, detect and

attribute biocrimes; this area of study is termed microbial forensics. A widely accepted definition of microbial forensics is "a scientific discipline dedicated to analyzing evidence from a bioterrorism act, biocrime or inadvertent microorganism/toxin release for attribution purposes" [30]. In the same context scientific attribution for this study is defined as "the assignment of a sample of questioned origin to a source, or sources, of known origin, to the highest possible degree of scientific certainty—while excluding origination from other sources" [30]. Microbial forensics allows for multi-disiplinary research to help protect both people and agriculture.

Agricultural microbial forensics encompasses all aspects of forensics and microbiology as they pertain to agriculture. This may include animal pathogens, food contaminants, water contamination, and plant pathogens [29, 31]. Though there is no concrete historical evidence of plant pathogens being used for bioweapons, there are many examples of inadvertent or unintentional releases that have caused substantial economic damage. Some examples of these types of epidemics are Asiatic citrus canker, bacterial wilt, sudden oak death, Pierce's disease of grape vine, karnal bunt of wheat, and Asian soybean rust [29]. Agricultural microbial forensic methods were used in all these cases to identify the microbe and attribute origin. Though never released, the United States did develop a method of releasing wheat stem rust on an opponent [30]. This is an example of an intentional release that prompted this study.

One of the most important aspects of microbial forensics, or even forensics in general, is the attribution of the origin of a sample. In microbial forensics for plant pathogens this concept of attribution is often addresses by the application of molecular epidemiology [30], especially in the use of genotyping or fingerprinting of pathogen

samples for comparative typing and source tracking. Genotyping samples found in a particular location and comparing them to previously analyzed populations of the same organism to determine similarities in allelic diversity, using genetic markers to attribute origin, track movement, and predict evolutionary modifications, is most commonly referred to as population genetics [30, 32, 33]. In this way population genetics becomes especially important in microbial forensics when analyzing plant pathogen outbreaks.

Model system for rust pathogen microbial forensics: Study justification

As world population continues to increase so does the demand for grains such as corn, wheat, rice, oats, barley, and milo. It is projected that by 2030 the world will be consuming nearly 300 million tons of wheat, and, though not as great, all other small grain consumption is projected to follow in suit [34]. Rust pathogens cause severe damage to all small crops, leading, in some cases, to major food crises [35]. With the development of resistant varieties large scale endemics have been greatly reduced. However, in the right environmental conditions rust pathogens can still cause substantial epidemics, causing up to 70% yield loss , as seen in a stem rust epidemic in 2000 in the United States [28]. When resistant crop varieties cannot be implemented fast enough to withstand evolving rust fungi [35]. Recently, *Puccina* spp. has become recognized for its biosafety threat to United States agriculture. For example, it is one of the targets of the a Department of Homeland Security effort sequence select agents and other high-threat pathogens that pose a threat to US human, livestock, or crop health [36]. By adapting microbial forensic practices to plant pathogens the US can be prepared for intentional or inadvertent inoculations of potentially devastating pathogens like those in the *Puccina* genus.

Methods overview

Simple Sequence Repeat Analysis

Repetitive nucleotide sequences occur throughout the entire genome of all prokaryotic and eukaryotic organisms [37,38], often in non-coding regions. Tandem repeats are caused by slippage during recombination and contribute to chromatin organization, regulation of DNA metabolic processes, and regulation of gene activity [38,39]. An important genetic marker based on repetitive nucleotide sequences and slippage for inferring conclusions on evolution of a population are called simple sequence repeats (SSRs), also referred to as short tandem repeats (STRs), multilocus variable number tandem repeat analysis (MLVA), or variable number of tandem repeats (VNTRs). The name assigned to tandem repeat genetic markers varies depending of the discipline. For example the names SSR and VNTR are commonly used in disciplines associated with environmental studies, plant breeding, and non-human based microbiology [33, 37]. In disciplines pertaining to human microbiology, criminal forensics, and animal genetic analysis the terms STR and MLVA are more recognized [30, 31]. Due to the large variability in genome location, and being highly polymorphic, SSRs have also been used widely for analysis of pedigrees, population genetics, and genetic fingerprinting and forensic profiling [31, 37].

Currently for rusts, primarily wheat rusts, population analyses SSRs or single nucleotide polymorphisms (SNPs) are being used for population characterization [39]. SNPs are used primarily in conjunction with genome sequencing data. Currently, a full

genome has not been made public for *P. emaculata*, but *Puccinia graminis* and *Puccinia pachyrhizi* have been widely sequenced [40].

Microsatellite based population genetics in related *Puccinia* species

Microsatellite loci, widely used for studies of population genetics of many organisms, are usually found in unique gene regions of the genome but are conserved within the population and are easily reproducible [41]. Microsatellites are the markers most commonly used for rust population analysis because they are co-dominant, making them ideal for use with dikaryotic organisms that are treated as diploid [42]. Microsatellites in rust pathogens have been used to determine origin, map movement, predict evolutionary mutations and support other population studies [43-45].

Since rust fungi cannot be grown on artificial media to obtain pure cultures, the experimental units used in rust population studies are often uredinia, or urediniospore bearing pustules. In Moscow, Russia, a *P. triticina* study evaluated multiple geographically differentiated populations across the entire country. For this study an isolate was defined by uredinia generated on different varieties of wheat seedling containing multiple combinations of resistance genes. The seedlings were infected with urediniospores collected directly from the field, then one or two isolates were collected from each group of wheat seedlings of varieties that differed in susceptibility. DNA was extracted and 23 SSR loci were analyzed. The study demonstrated a high probability that the inoculum for all *P. triticina* populations in Russia were from a common source in Europe. The Russian populations showed less genetic similarity to populations from other former Soviet Union countries [45].

In Africa, SSRs were used to determine inocula origin of different races of *P. triticina*. The study defined isolates as single pustules collected from five geographically distant populations. Twelve SSR loci were used to define populations using Bayesian STRUCTURE analysis and genetic distance analyses. This study found migration of alleles from South Africa to North and West Africa suggesting that new susceptibility to this rust in South Africa will be seen in West and North Africa as well [44] .

In 2011, 20 microsatellites were designed from 20 labeled and transformed single *P. emaculata* pustules [20], and tested on the 20 single pustules. The conclusions led to the discovery of 2-5 alleles per locus and evidence that supports that genotypes at one locus are independent from those at another. Furthermore, because of the significant deviation from Hardy Weinberg Equilibrium that was seen in the asexual pustules, sexual recombination maybe rare in *P. emaculata* [20].

Simple Sequence Repeat based DNA profiling in Forensic Sciences

The main objective of this project was to examine whether a rust pathogen (*P. emaculata*) can be genetically profiled and analyzed in a fashion similar to the way humans are genetically profiled in the criminal justice system. The most used genetic profiling methods in human forensic investigations in the United States are short tandem repeats (STRs) [31], also referred to as Simple Sequence Repeats (SSR) or microsatellite markers [37,31]. STRs can be found in multiple sites in the human genome. The location of the STR in the genome allows for differentiation of different characteristics or function. For example, sex identification involves using the presence or absence of Y-STR's located on the Y chromosome of males. DNA can be extracted from a forensic

sample using an organic or commercial kit extraction such as the QIAamp DNA Investigator kit by QIAGEN [46]. Following extraction, the DNA is quantified and cleaned in preparation for amplification and analysis using STRs. The analysis can be performed in a multitude of ways including but not limited to; evaluation of presence or absence of individual bands in standard agarose gel electrophoresis, multiplex PCR followed by agarose gel electrophoresis, or multiplexing followed by capillary electrophoresis. Real-time PCR has also been used widely, eliminating the need for electrophoresis gels [30].

The most common method of genetic profiling is multiplex PCR and capillary electrophoresis. Commercial kits like the "STR Blue Kit" by Applied Biosystems can extract DNA while simultaneously amplifying multiple STR loci. Though the Blue Kit amplifies only three STRs, other kits can amplify up to fifteen STRs [31]. Depending on the depth of genetic profiling necessary for criminal investigation, forensic scientists can utilize as many previously standardized STRs as necessary. Typically, more than twenty STRs are evaluated for population studies, while fewer are necessary for criminal investigations [47]. Forensic analysis used for conviction, designated the Combined DNA Index System (CODIS), recognizes thirteen standardized STRs for genetic profiling [48]. The system works by comparing the probability that two genetic profiles were identical within a particular population to help identify individuals using their genetic fingerprints [31]. Overall, the methods used in forensic laboratory genetic analysis are very similar to those used for population analysis of plant pathogens, differing mostly on the questions asked and the interpretation of the data.

Experimental approach

In this study simple sequence repeat (SSR) microsatellite loci were used to compare the genetic profiles of *P. emaculata* from different populations originating from multiple environments. SSR microsatellites are the most common marker used for rust population analysis because they are co-dominant heritable markers found throughout the eukaryotic genome, making them ideal for dikaryotic organisms [42]. However, because most population software doesn't account for dikariotic heterokaryons, the rusts are scored as diploid to account for both nuclei. Population allele frequencies were analyzed spatially and temporally to provide background information on population genetic fluctuations over space and time in the natural environment. To assess the applicability of the standardized microsatellite based DNA profiling for biosecurity and forensic applications, the genetic profiles of naturally colonizing collected samples and growth chamber/lab produced samples were compared over time.

This study was analyzed using GenAlEx statistical software, used for the analysis of SSRs and other markers for a variety of organisms. Through this software the allele frequencies of the SSRs were analyzed using Fixation statistics (F_{ST}). F_{ST}s were first developed by Wright (1921) to be used in conjunction with Hardy-Weinberg principals to define the reduction in heterozygosity (based on allelic frequencies) in a random breeding population, also known as a population in Hardy-Weinberg equilibrium (HWE) [49]. Since *P. emaculata* and many other rust pathogens are heteroecious, sexually recombining organisms, they are considered to be in HWE. For this reason F_{ST} analysis fits the biological rust system. F_{ST} values are calculated by subtracting the heterozygosity in the total population from the heterozygosity in the subpopulation, divided by the total.

The product is a percentage of heterozygosity loss [33]. The loss in heterozygosity dictated by alleles common and novel in the population is what causes divergence between populations indicating evolutionary trend. Another analysis used often in rust population genetics studies for differentiation is the principal coordinate analysis (PCoA), which uses allele distance mapping to produce a generic pattern of distribution and to quickly identify key trends in populations [50, 51, 52]. However, PCoAs may not be as accurate as F_{ST}s for *P. emaculata* populations because the PCoA doesn't assume HWE [50]. By using these widely standardized statistical analyses, this study allowed concise conclusions about the population trends of *P. emaculata*.

Though this study is very similar to most previous rust population studies regarding the methods used, aspects that differentiate this study from previous work includes how the SSRs were designed and what we considered a sample to be. This study uses four polymorphic SSRs designed using *de novo* sequences and ESTs designed previously [16].

This study is one of very few in which single spores were used for genotype characterization rather than bulk uredinospores from pustules. This method ensures that every sample is an individual genotype that can be used to properly evaluate the genetic variation within populations of *P. emaculata* [32]. Most commonly, for rust population studies, single pustules (containing thousands of individuals) or even plant based isolates (containing multiple pustules) are used [43-45].

.

Literature Cited

1. Energy, U.D.O. *The Energy Independence and Security Act of 2007 (EISA 2007)*. 2007; Available from: http://energy.gov/about-us/web-policies.
2. Gu, Y.X., *Projecting future grassland productivity to assess the sustainability of potential biofuel feedstock areas in the Greater Platte River Basin.* Global Change Biology Bioenergy, 2014. 6(1): p. 35-43.
3. Environmental Protection Agency. *Renewable Fuel Standard (RFS)* Fuels and Fuel Additives 2013 12/10/2013; Available from: http://www.epa.gov/otaq/fuels/renewablefuels/.
4. Sokhansanj, S., et al., *Large-scale production, harvest and logistics of switchgrass (Panicum virgatum L.) - current technology and envisioning a mature technology.* Biofuels Bioproducts & Biorefining-Biofpr, 2009. **3**(2): p. 124-141.
5. Palmer, P.G., *A Biosystematic Study of the Panicum amarum-P. amarulum Complex (Gramineae).* Brittonia, 1975. 27(2): p. 142-150.
6. Wood, J.M. and M.S. Evans, *Natal plants*. Vol. Second. 1899-1904.
7. Triplett, J.K., et al., *Five nuclear loci resolve the polyploid history of switchgrass (Panicum virgatum L.) and relatives.* PLoS One, 2012. **7**(6): p. e38702.
8. Navreet Deo *Switchgrass carbon sequestration*. Smart solutions for climate change, 2013.
9. (CGIAR), C.G.o.I.A.R., *CGIAR Research Priorities for Marginal Lands*, in *SDR/TAC:IAR/99/12*, F. Food and Agriculture Organization, Editor. 1999, CGIAR Secretariat: Washington, D.C. .

10. Perrin, R., et al., *Farm-Scale Production Cost of Switchgrass for Biomass.* Bioenergy Research, 2008. 1(1): p. 91-97.

11. Sanderson, M.A., et al., *Switchgrass as a biofuels feedstock in the USA.* Canadian Journal of Plant Science, 2006. 86(5): p. 1315-1325.

12. Varvel, G.E., et al., *Comparison of corn and switchgrass on marginal soils for bioenergy.* Biomass & Bioenergy, 2008. 32(1): p. 18-21.

13. Jimmy Carter Plant Materials Center *Plant fact sheet for switchgrass (Panicum virgatum L.)*. 2011.

14. Uppalapati, S.R., et al., *Characterization of the Rust Fungus, Puccinia emaculata, and Evaluation of Genetic Variability for Rust Resistance in Switchgrass Populations.* Bioenergy Research, 2013. 6(2): p. 458-468.

15. Linh Vu, A., *Identifying pathogens of switchgrass and investigating antimicrobial activity of switchgrass-derived extractives*, in *Tennessee Research Creative Exchange*. 2011, University of Tennessee: TN.

16. Orquera, G., *Population biology of Switchgrass rust (Puccinia emaculata Schw.)*, in *Entomology and Plant Pathology*. 2014, Oklahoma State University: Stillwater Oklahoma. p. 94.

17. Woudenberg, J.H.C., et al., *Alternaria redefined.* Studies in Mycology, 2013. **75**(1): p. 171-212.

18. Manamgoda, D.S., et al., *The genus Bipolaris.* Studies in Mycology, 2014. 79: p. 221-288.

19. Loring, C., J. N., and A.E. Menke, *Proceedings of the American Academy of Arts and sciences*. 1883, Oxford, MA: University Press: John Wilson and Son. 2.

20. Wadl, P.A., et al., *Development and characterization of microsatellites for switchgrass rust fungus (Puccinia emaculata).* Conservation Genetics Resources, 2011. 3(1): p. 185-188.

21. Black, J., A. Windham, and M. Windham, *Switchgrass rust epidemics (Puccinia emaculata) in agronomic fields in Tennessee.* Phytopathology, 2011. 101(6): p. S16-S16.

22. Hirsch, R.L., et al., *First Report of Rust Caused by Puccinia emaculata on Switchgrass in Arkansas.* Plant Disease, 2010. 94(3): p. 381-381.

23. Sykes, V.R., et al., *Effect of Puccinia emaculata infection on ethanol production potential of Panicum virgatum*, in *Phytopathology*, A.P. Society, Editor. 2011, University of Tennessee: Knoxville Tennessee.

24. Zale, J., et al., *First Report of Rust on Switchgrass (Panicum virgatum) Caused by Puccinia emaculata in Tennessee.* Plant Disease, 2008. 92(12): p. 1710-1710.

25. Hagan, A.K. and J. Akridge, *Impact of Fungicide Selection and Application Interval on the Control of Rust and Biomass Yield of an Ornamental Switchgrass*, in *Pathology and Nematology*. 2013, SNA Research Conference. p. 196-203.

26. Black, J., *The epidemiology of Puccinia emaculata (rust) in switchgrass and evaluation of the mycoparasite Sphaerellopsis filum as a potential biological control organism for switchgrass rust.*, in *Entomology and Plant Pathology*. 2012, University of Tennessee: Knoxville Tennessee. p. 73.

27. Little, R. and J.G. Manners, *Somatic recombination in yellow rust of wheat (Puccinia striiformis): I. The production and possible origin of two new*

physiologic races. Transactions of the British Mycological Society, 1969. **53**(2): p. 251-258.

28. Schumann, G.L. and K.J. Leonard, *Stem rust of wheat.* The plant health instuctor, 2011. PHI-I-2000-0721-01.
29. Fletcher, J., et al., *Plant Pathogen Forensics: Capabilities, Needs, and Recommendations.* Microbiology and Molecular Biology Reviews, 2006. 70(2): p. 450-471.
30. Budowle, B., et al., *Microbial Forensics* Second ed. 2011, Burlington MA: Acedemic Press/Elsevier.
31. Saferstein, R., *Forensic science: from the crime scene to the crime lab*. Second ed, ed. V.R. Anthony. 2013, New Jersey Pearson
32. Milgroom, M.G., *Population biology of plant pathogens: genetics, ecology, and evolution*. 2015: APS Press.
33. Hartl, D.L. and A.G. Clark, *Principals of population genetics*. Fourth ed. 2007, Sunderland MA: Sinauer Associates Inc.
34. United Nations, *World agricultture: towards 2015/2030*, F. Food and Agriculture Organization, Editor. 2015, FAO: Rome.
35. Chaves, M.S., et al., *The importance for food security of maintaining rust resistance in wheat.* Springer Science+Business Media Dordrecht and International Society for Plant Pathology 2013, 2013. Food Security (5): p. 157-176.
36. Blagden, T., *Microbial forensics and ag biosecurity – closing the gaps*, D.O.E.A.P. Pathology, Editor. 2015: Oklahoma State University.

37. Acquaah, G., *Principals of plant genetics and breeding*, ed. J.W.S. Ltd. 2012, Maryland: Wiley-Blackwell. 740.

38. Li, Y.C., et al., *Microsatellites: genomic distribution, putative functions and mutational mechanisms: a review.* Molecular Ecology, 2002. 11(12): p. 2453-2465.

39. Rajender, S., et al., *Analysis of simple sequence repeats (SSRs) dynamics in fungus Fusarium graminearum.* Bioinformation, 2011. 5(10): p. 402-404.

40. Naik, B.K., et al., *Molecular mapping and validation of the microsatellite markers linked to the Secale cereale-derived leaf rust resistance gene Lr45 in wheat.* Molecular Breeding, 2015. 35(2).

41. Peixoto, R.F., et al., *Genetic diversity among Puccinia melanocephala isolates from Brazil assessed using simple sequence repeat markers.* Genetics and Molecular Research, 2014. 13(3): p. 7852-7863.

42. Luo, H.Y., et al., *Genome-Wide Analysis of Simple Sequence Repeats and Efficient Development of Polymorphic SSR Markers Based on Whole Genome Re-Sequencing of Multiple Isolates of the Wheat Stripe Rust Fungus.* PLOS One, 2015. 10(6).

43. Zhan, G.M., et al., *Screening for simple sequence repeat markers in Puccinia striiformis tritici based on genomic sequence.* Journal of Zhejiang University-Science B, 2015. 16(8): p. 727-732.

44. Pretorius, Z.A., et al., *Races of Puccinia triticina detected on wheat in Zimbabwe, Zambia and Malawi and regional germplasm responses.* Australasian Plant Pathology, 2015. 44(2): p. 217-224.

45. Kolmer, J.A., et al., *Russian populations of Puccinia triticina in distant regions are not differentiated for virulence and molecular genotype.* Plant Pathology, 2015. 64(2): p. 328-336.

46. Hebda, L.M., A.E. Doran, and D.R. Foran, *Collecting and Analyzing DNA Evidence from Fingernails: A Comparative Study.* Journal of Forensic Sciences, 2014. 59(5): p. 1343-1350.

47. Hanson, E.K. and J. Ballantyne, *A Y-short tandem repeat specific DNA enhancement strategy to aid the analysis of late reported (>= 6 days) sexual assault cases.* Medicine Science and the Law, 2014. 54(4): p. 209-218.

48. Kline, M.C., J.W. Redman, and J.M. Butler, *Training on STR typing using commercial kits and ABI 310/3100*. 2001,ational Institute of Standards and Technology:ational Institute of Standards and Technology.

49. Schweighardt, A.J., et al., *Evaluation of Commercial Kits for Dual Extraction of DNA and RNA from Human Body Fluids.* Journal of Forensic Sciences, 2015. 60(1): p. 157-165.

50. Holtz, M.D., et al., *Genetic diversity of Puccinia striiformis from cereals in Alberta, Canada.* Plant Pathology, 2014. 63(2): p. 415-424.

51. Rajender, S., et al., *Analysis of simple sequence repeats dynamics in the genic regions of wheat rust fungi (Puccinia sp.).* Research Journal of Biotechnology, 2014. 9(4): p. 69-74.

52. Ordonez, M.E. and J.A. Kolmer, *Simple sequence repeat diversity of a worldwide collection of Puccinia triticina from durum wheat.* Phytopathology, 2007. 97(5): p. 574-583.

CHAPTER III

OBJECTIVE ONE: A POPULATION DIVERSITY ANALYSIS OF *PUCCINIA EMACULATA* SINGLE SPORES IN OKLAHOMA AND VIRGINIA

ABSTRACT

Native to the North American prairies, *Panicum virgatum,* most commonly known as switchgrass, has been chosen for biofuel production due to its low input cost of production and high feed stock quality. However, a fungal rust pathogen (*Puccinia emaculata*) has become a major threat to switchgrass, causing significant widespread biomass reductions. The objective of this study was to spatially and temporally analyze the population dynamics of switchgrass rust over a period of two years (2011 - 2014) in two different states, Virginia and Oklahoma. Individual *P. emaculata* urediniospores were collected and whole genome amplified. Following confirmation of the rust genus and species, 20 previously designed microsatellite loci were screened. Four microsatellites consistently produced polymorphic amplicons. A STRUCTURE analysis indicated that all years and states had few migrant genotypes making the six populations very discrete. These findings suggest a possible movement of alleles, year by year, from a common inoculum source rather than localized populations. This movement could be due to migration along the *Puccinia* pathway. The results also pointed to high allele diversity per locus, and non significant Chi^2 values for expected and observed heterozygosity, pointing to a population in Hardy Weinberg Equilibrium. Population analysis of representative population samples over space and time will allow a better understanding of the natural epidemiology and movement of the rust pathogen, which will lead to the development of best management practices.

Introduction

The Energy Independence and Security Act (EISA) of 2007 mandated that 36% of all fuel should be renewable, and of that 16% must come from cellulosic extraction [4]. Currently, most crops used for ethanol production are also grain crops, primarily corn. Corn is a direct consumption crop, so its division for ethanol as well may lead to food shortage challenges in the future [6]. For this reason the Department of Energy [7] has supported research towards finding high biomass producing plants that are not used for direct consumption by humans or livestock. These are referred to as "energy crops", and switchgrass is among these crops [8].

Panicum virgatum L., commonly known as switchgrass, is a C4 grass native to most of North America (Figure 1). With deep rooting systems, switchgrass has low water and nutrient requirements making it an excellent candidate for soil conservation on marginal land [9]. Switchgrass is also known for its very high biomass, with two variety classifications defined by their growth habit. With upland varieties growing up to ten feet tall and lowland varieties (Figure 2) growing as high as fifteen feet, the crop is ideal for lignocellulosic biofuel extraction [10]. However, many plant pathogens infect and cause damage to switchgrass, reducing its biomass production. The most damaging of these is the basidiomycete *Puccinia emaculata* [11, 12], causal agent of switchgrass rust.

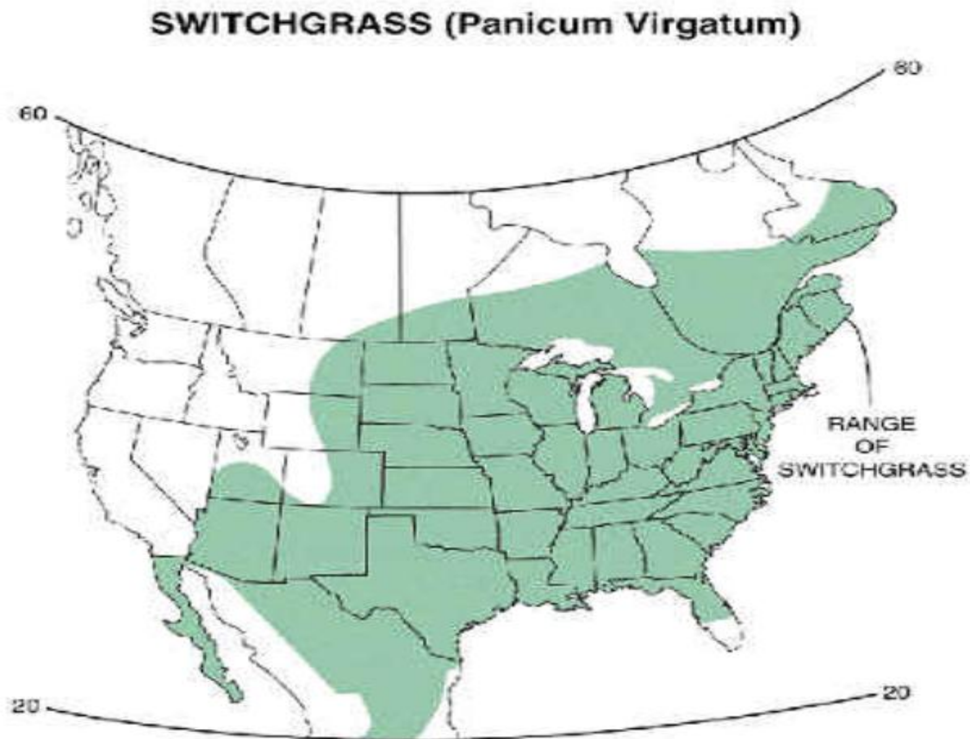

Figure 1: Native distribution of switchgrass across North America (Obj1) [2].

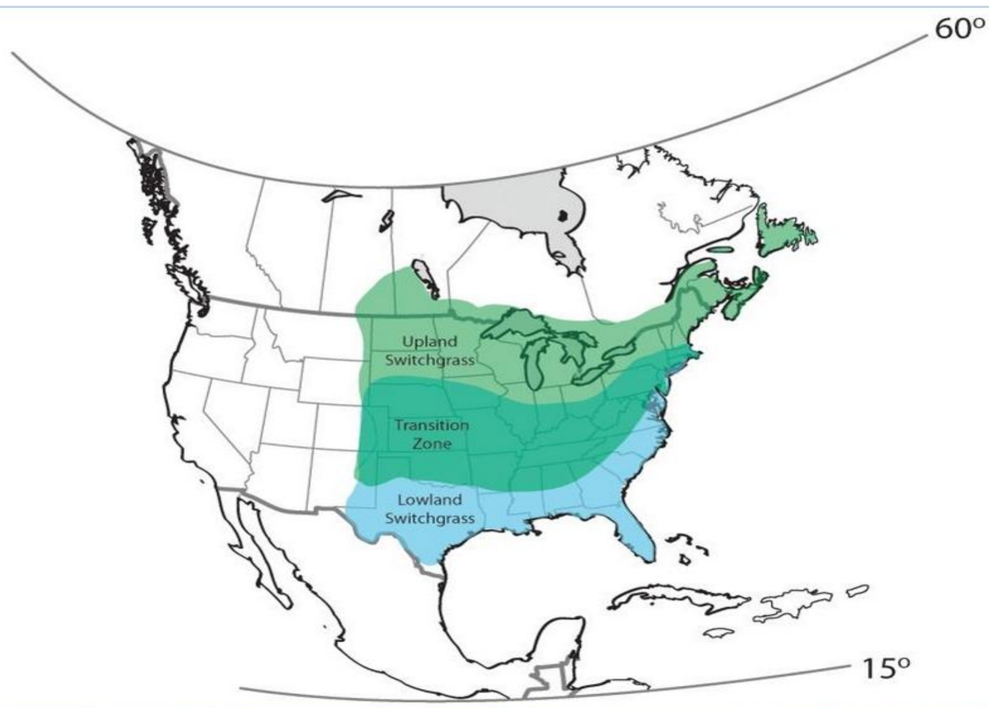

Figure 2: Upland and lowland variety distribution across the United States and Canada (Obj1) [1].

Believed to be a heteroecious rust, meaning at least two hosts to complete its life cycle, *P. emaculata* is most likely a macrocyclic rust as well. Having a narrow host range, switchgrass is the main telial and uredinial host, however *Puccina emaculata* has been confirmed to produce urediniospores also on two different accessions of *Panicum amorum* (bitter panicgrass), a native prairie grass [13]. Though not yet confirmed in growth chamber tests, flowering spurge (*Euphorbia corollata* L.) was reported to be the potential basidial and aecial host [5, 13, 14]. Since the entire disease cycle has not been confirmed, these assumptions are based on the knowledge of the disease cycle to date.

First described by Schweinitz in 1900 on the host *Panicum capillare* L., *P. emaculata* has become more economically important in recent years [15] the switchgrass rust fungus is extremely prolific in the uredial stage, and has been reported to cause up to 100% leaf coverage, causing photosynthetic losses leading to severe damage [16]. Severe infections can cause up to 50% biomass reduction resulting in a 60% reduction in ethanol production [17]. Several research initiatives are currently focused on breeding rust resistant switchgrass cultivars.

A better understanding of the biology and epidemiology of *P. emaculata* may enhance the ability of breeding programs to develop broadly deployable cultivars with durable resistance. Population genetics analyses of this rust may provide information about the movement and allelic diversity of pathogen populations across its geographic distribution over several years. Understanding this rust's genetic diversity in its natural setting can provide insights into its reproductive biology, generating hypothesis that could be tested with further research. Additionally, genetic characterization of *P.*

emalulata populations may lead to identification of pathogenicity factors and races, which could facilitate epidemic predictions.

The objective of this study was to examine and compare the allelic diversity and population structures of *P. emaculata* populations in Oklahoma over the course of four years (2011- 2014) and in Virginia over a period of two years (2011-2012). These comparisons will show whether the populations in those states differ in genetic composition and structure over space and time. The information generated will provide background knowledge about the allelic diversity of field populations of local origin (OK) compared to a geographically distant population (VA). This information will also serve as background for to understand the epidemiology of switchgrass rust in field vs. experimental environments.

Methodology:

Single Spore Collection:

P. emaculata urediniospores (asexual propagative spore stage) were maintained and collected as described by Orquera (2014) [5]. The spores were collected from the surfaces of individual switchgrass leaves, collected from the field, using custom designed micro-vacuums that deposit the spores into 2mL collection tubes, and then placed in desiccators with silicon beads for 24 hours prior to being placed at -80° C for long term storage [5]. One spore population was collected per location and year, and each population was stored in a separate collection tube.

Field *P. emaculata* urediniospore populations collected every summer from one field in Oklahoma (OSU) in 2011 (OSU11F, n=9), 2012 (OSU12F, n=16), 2013

(OSU13F, n=13) and 2014 (OSU14F, n=12), and from one field in Virginia (VT11, n=10) in 2011 and 2012 (VT12, n=18) were studied. The source plants from included upland and lowland switchgrass cultivars including predominately Alamo, Blackwell, Kanlow, Dacotah, and Cave-In-Rock. Individual spores were used as experimental units. To recover spores from collection tubes for single-spore collection, the frozen tubes were placed in a freezer block to prevent thawing. The spores were collected from the rim and wall of the tubes using a sterile 20µL disposable pipette tip, spread evenly onto the surface of water agar, incubated at 37°C for one to two hours, then placed at room temperature for two to four additional hours to induce urediniospores germination.

Single spore collection was performed with the aid of a VistaVision compound microscope (VWR, Radnor, PA). The germinated spores were collected with a micro-dissecting needle holder with a 0.125mm dissecting needle at 40x magnification. Once the germ tube had attached to the needle the spore was placed in an individual 0.2mL PCR tube containing 9µL of Illustra GenomiPhi V2 DNA Amplification Kit (GE Healthcare, Marlborough, MA) sample buffer and incubated for ten minutes at 95°C, before proceeding with whole genome amplification (WGA). In accordance with the Illustra GenomiPhi V2 DNA Amplification Kit protocol, the sample was maintained at 4°C while 1µL of enzyme and 9µL of reaction buffer were added to the spore tube. The sample was then incubated in the thermocycler at 30°C for two hours, followed by ten minutes at 65°C to stop ligation.

Following amplification, 5µL of each sample were aliquoted into 2mL PCR tube strips with 45µ of 1% TE buffer (a tenfold dilution). The diluted samples were quantified using Nanodrop 1000 (Thermofisher Scientific, Wilmington, DE) with TE buffer as a

blank. After quantification the samples were diluted to 25ng/µl and used as template for PCR amplification with *Puccinia* spp. and *P. emaculata* specific primers [12] to confirm that the WGA products correspond to *P. emaculata*. Samples confirmed to be *P. emaculata* were used later for SSR fingerprinting analysis.

Microsatellite Analysis

Template DNA (2µL per reaction) was amplified in 20µl PCR reactions that included GoTaq green master mix (Promega, Madison, WI) and 5mM each forward and reverse primers (Promega, Madison, WI). PCR cycling followed the conditions standardized by Orquera (2014) [5]. The 20 EST and *de novo* microsatellite loci were screened first on a subsample of 52 individual urediniospore WGA products representing Virginia populations from 2011-2012 and Oklahoma populations from 2011-2012. Following PCR amplification, 18µl of product were loaded into a 2% TAE agarose gel and electrophoresed at 98V for two to two and a half hours. Microsatellite loci having amplicons resolved by agar electrophoresis in over 95% of the samples across all populations analyzed were used in subsequent assays. Fluorescent primers specific for the selected loci, obtained from IDT (Coralville, IA), were used to generate amplicons that were resolved by capillary electrophoresis at Oklahoma State University's Biochemistry and Molecular Biology Recombinant DNA and Protein Core Facility using an ABI 3730 DNA Analyzer (Thermofisher Scientific, Wilmington, DE). Eight loci that were polymorphic in the preliminary assessment (Table 1) were selected for fingerprinting four populations (Oklahoma 2011 and 2012, and Virginia 2011 and 2012). Capillary electrophoresis identified polymorphisms and consistent amplification of one or two alleles per sample in four of the eight SSRs. Allelic information generated for the four

SSR loci were used for statistical analyses. Data of SSR markers having large numbers of missing data or multiple bands per sample were not included in the statistical analysis. After completing the characterization of the four initial populations, two additional Oklahoma populations (2013 and 2014) were fingerprinted for the four selected SSR loci OEPE 68, OPE5, OPE32, and OEPE6a.

Table 1 (Obj1): Eight potentially polymorphic primers. Bold indicates four loci that were informative and used for statistical analysis [5].

Locus	Primer sequences (5' to 3')
OPE5F	**AAAGGTTGAGTGGTAGTGGT**
OPE5R	**GCTAATGATGACGAAGTTGT**
OPE21F	TGGCTGATTACAAGACAGAGTT
OPE21R	GGTTGAGTGGTAGTGGTAGAGA
OPE28F	CCCGAAATGACACATCAAAA
OPE28R	ACACACACACACACACAGCAC
OPE32F	**ACAAGCCATCCAAGGGAGT**
OPE32R	**CAAGTTCATCGGCATCGTT**
OEPE6aF	**GTAATAAGAGCCAACACGGAGG**
OEPE6aR	**TAGGATTAGGCATGGCGTACTT**
OEPE28F	AACGTCTCCAAAAGCTGATCTC
OEPE28R	GATTGCAGTAAGACAAGGGGAC
OEPE68F	**TGGAGAGATAGACCCAATAGCC**
OEPE68R	**CAACTCATCAACACACAACCCT**
OEPE71F	AGCGTGACAAGTGAACAAGAGA
OEPE71R	TACAACCCGAAACTCCTCAACT

Fluorescent forward primers were paired with non-tagged reverse primers in 25µl reactions using GoTaq Colorless master mix (VWR). PCR conditions and thermal cycling were as previously described. Following amplification, 8µl of each sample was mixed with 2µl of loading dye and electrophoresed as previously described. After confirmation, by visual inspection under UV light, of amplicons of the expected size on agarose gels, PCR products were resolved by capillary electrophoresis, and amplicon

sizes were evaluated using PeakScanner version 1.0 software (Applied Biosystems, Foster City, CA). The band sizes were recorded into an allelic database, which was analyzed using GenAlEx v6.501 (Rutgers University, New Brunswick NJ). Statistical analyses performed included estimation of F_{ST} values, Pairwise Population F_{ST} Comparisons, Principal Coordinates Analysis (PCoA), expected and observed heterozygosities, and Hardy-Weinberg Equalibrium (HWE). STRUCTURE version 2.3.4. (Pritchard, Wen, and Falush, 2010) was used to conduct a Bayesian analysis to identify genetic lineages and potential migration and admixture patterns. The parameters for STRUCTURE analyses included a 10,000 burn-in length and 50,000 MCMC repetitions. The samples were tested for immigration and all frequencies were correlated among populations. The number of populations assumed or K was found by using the "STRUCTURE simulation function" and testing hypotheses between two and ten. Following the simulation the results for ΔK that gave a statistical best match value for each hypothesis was analyzed in a line graph. The highest peak was selected as the optimum K value for the analysis.

Results:

The 77 individual urediniospore genotypes were analyzed, corresponding to 6 subpopulations.

Statistical Analysis:

F_{ST} analyses identified great genetic population structure throughout (F_{ST}= 0.220). Inbreeding analysis revealed the populations were in Hardy-Weinberg Equilibrium (Table 2). Pair-wise comparisons revealed significant differentiation among most of the populations compared with the exception of OSU12, VT12 and OSU11, which were not significantly different from each other (Table 3). Differentiation among Oklahoma populations ranged from low to great genetic differentiation, with OSU14 having great genetic differentiation from all other populations. While VT11 had moderate to great genetic differentiation when compared with other Oklahoma and Virginia populations, VT12 had low genetic differentiation compared to OSU11 and OSU12, and moderate genetic differentiation from OSU13 and OSU14. The Oklahoma population that shared the most alleles with a Virginia population was OSU12 with VT12, with a fixation index of 0.033, comparable to the F_{ST} of 0.032 found between OSU11F and OSUF12.

Table 2 (Obj1): Allelic diversity patterns. The average expected heterozygosity, observed heterozygosity, Chi^2, number of observed alleles, and number of effective alleles for all populations and all loci in this study.

Expected Heterozygosity	**Observed Heterozygosity**	**Chi^2**	**Number of Observed Alleles**	**Number of Effective Alleles**
0.699	0.528	4.19^{ns}	8.417	5.224

Table 3 (Obj1): **Pair-wise Population F_{ST} Values**: F_{ST} 00-0.05: Little genetic differentiation; F_{ST} 0.05-0.15: Moderate genetic differentiation* ; F_{ST} 0.15-0.25: Great genetic differentiation** ; F_{ST} 0.25<:Very great genetic differentiation*** [3]

	OSU11	**OSU12**	**OSU13**	**OSU14**	**VT12**	**VT11**	
OSU11							**OSU11**
OSU12	0.032						**OSU12**
OSU13	0.095*	0.095*					**OSU13**
OSU14	0.183**	0.174**	0.151**				**OSU14**
VT12	0.045	0.033	0.074*	0.149**			**VT12**
VT11	0.083*	0.063*	0.136*	0.220**	0.062*		**VT11**
	OSU11F	**OSU12F**	**OSU13F**	**OSU14F**	**VT12**	**VT11**	

PCoA analysis illustrates population diversity patterns in agreement with the F_{ST} results (Figure 3). Variance of the samples was evenly distributed between the first and second coordinate. Samples from Oklahoma and Virginia grouped predominately by year rather than by state, with few genotypes being admixed among the other populations. VT12 genotypes clustered closely with Oklahoma genotypes from the years 2011 and 2012 and had two genotypes that clustered with VT11. In the left coordinate there are five VT12 genotypes that are closer related to the OSU13 and OSU14, with one completely overlapped with a tight cluster of OSU13 and OSU14. Overall OSU13 and OSU14 genotypes seem to cluster together furthest away from 2011 genotypes from both states. OSU13 had two genotypes on the right coordinate making that population closer related to 2011 and 2012 genotypes than OSU14, which, was most differentiated in both the PCoA and F_{ST} analysis.

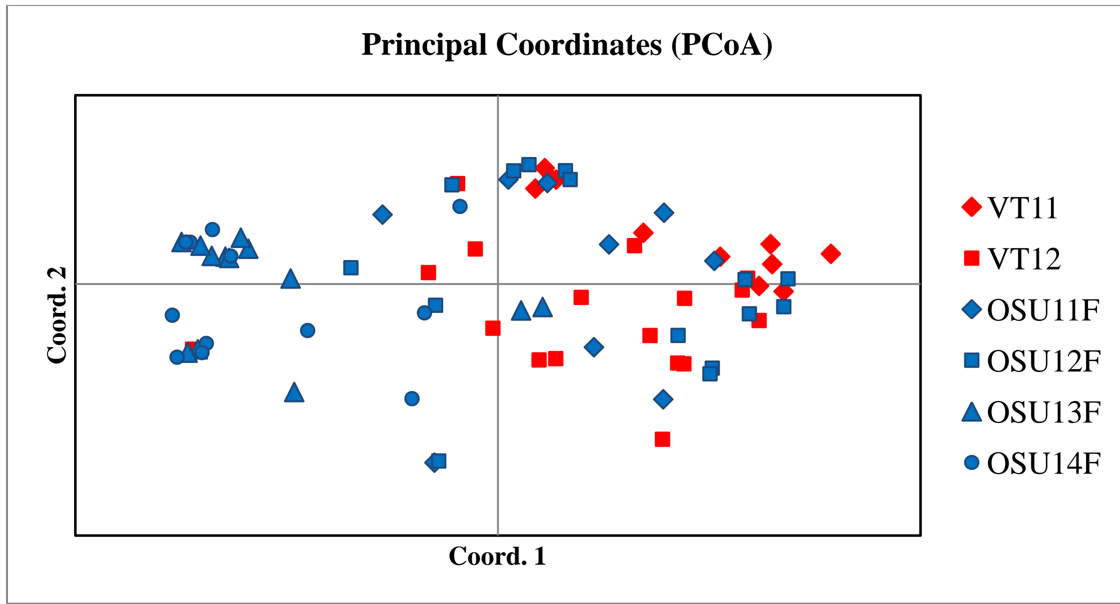

Figure 3 (Obj1): Principal coordinate analysis of four Oklahoma (OSU 2011, OSU 2012, OSU 2013, OSU 2014) and two Virginia (VT 2011, and VT 2012) analyzed using four polymorphic SSR markers.

A *K* value of 6 was determined by a simulation summary based on data correlation to ΔK. A bar plot illustrating the genotypes of these populations is shown in Figure 4. Six mostly discrete populations corresponding to the populations defined by state and year were found. Mixed genotypes were found in VT11, OSU13F and OSU14F.

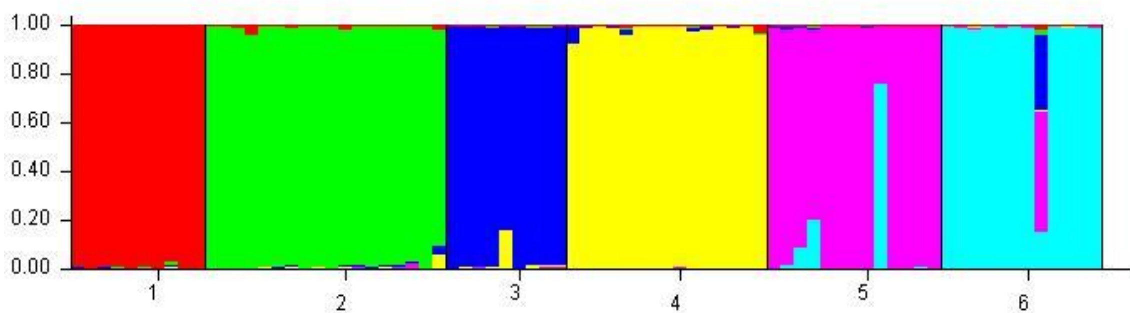

Figure 4(Obj1): Bar plot graphic built by STRUCTURE based on Baysiean analysis to predict probability of each sample genotype belonging to a particular population. 1= VT11, 2= VT12, 3= OSU11F, 4= OSU12F, 5= OSU13, and 6= OSU14F.

Allelic patterns across populations are shown in Figure 5 and Table 4. OSU14 had the lowest average allele diversity (4.8 alleles), number of effective alleles (3 alleles), and number of private alleles (0.5 alleles). All other populations were similar in these categories. VT12 had the highest allele diversity with an average of 12.25 per locus. The average allele diversity in OSU11, OSU12, OSU13 and VT12 ranged between 6.5 and 10. Besides OSU14, the number of effective alleles in all populations averaged of four to six. Finally, the number of private alleles in VT12 was the greatest with average of 6.25 private alleles across all four loci. The other populations averaged two to four private alleles per locus.

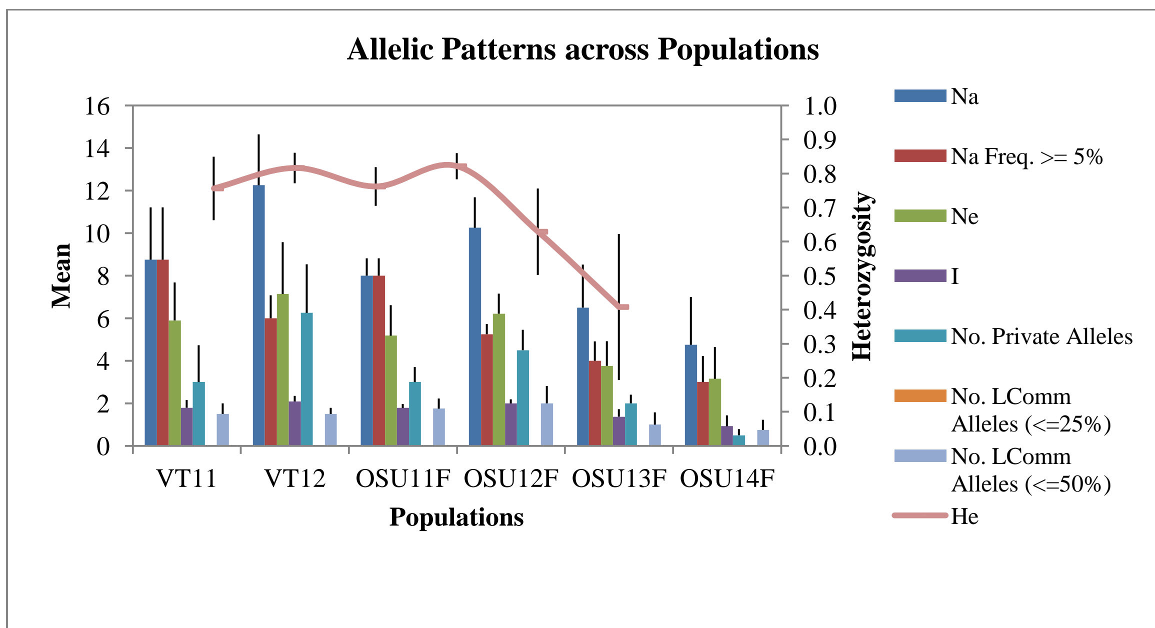

Figure 5 (Obj1): Allelic Patterns across all populations with all six populations represented below the graph. Bare codes are described as follows: Na = No. of Different Alleles, Na (Freq >= 5%) = No. of Different Alleles with a Frequency >= 5%, Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), No. Private Alleles = No. of Alleles Unique to a Single, No. LComm Alleles (<=25%) = No. of Locally Common Alleles (Freq. >= 5%) Found in 25% or Fewer Populations, He = Expected Heterozygosity = 1 - Sum pi^2

Table 4 (Obj1): Allelic values across all populations with all six populations represented below the graph.

Population Means	VT11	VT12	OSU11F	OSU12F	OSU13F	OSU14F
Na	8.750	12.250	8.000	10.250	6.500	4.750
Na Freq. >= 5%	8.750	6.000	8.000	5.250	4.000	3.000
Ne	5.899	7.138	5.178	6.212	3.763	3.153
I	1.791	2.090	1.780	1.997	1.373	0.922
No. Private Alleles	3.000	6.250	3.000	4.500	2.000	0.500
No. LComm Alleles (<=25%)	0.000	0.000	0.000	0.000	0.000	0.000
No. LComm Alleles (<=50%)	1.500	1.500	1.750	2.000	1.000	0.750
uHe	0.796	0.842	0.811	0.852	0.702	0.426

Codes are described as follows: Na = No. of Different Alleles, Na (Freq >= 5%) = No. of Different Alleles with a Frequency >= 5%, Ne = No. of Effective Alleles= 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), No. Private Alleles = No. of Alleles Unique to a Single, No. LComm Alleles (<=25%) = No. of Locally Common Alleles (Freq. >= 5%) Found in 25% or Fewer Populations, He = Expected Heterozygosity = 1 - Sum pi^2

Discussion

The objective of this study was to evaluate population structure of *P. emaculata* in Oklahoma over a period of four years in comparison to the geographically distant populations in Virginia over a period of two years, in order to identify population dynamics within and among these six populations. Results show moderate to great genetic differentiation between *P. emaculata* populations of the two states. The F_{ST} values, Bayesian analysis, and PCoA provide statistical support for significant differentiation among and between populations. The differentiation found is consistent with variation among relatively discrete state populations, with some differentiation also observed temporally. This variation could be caused by introduction of new alleles every year to the local allelic pool. Although Virginia samples in 2011 shared only some alleles with Virginia samples in 2012, one mixed genotype in the Virginia 2012 population

shared alleles with Oklahoma populations in 2012, 2013, and 2014. This phenomenon would suggest migration of alleles found in the Oklahoma populations to the Virginia populations between the growing seasons in 2011 to 2012, followed by the resurgence in the Oklahoma 2014 population of some alleles present in Virginia in 2011. Because of the repression and resurgence of alleles in later years in the Oklahoma populations future studies should include samples from Virginia 2013 and 2014, and from other geographical locations from 2011 to 2014, to evaluate if similar patterns occur among those populations in order to better support the one inoculums source hypothesis.

According to the Bayesian analysis bar plot there is good probability that some of the alleles found in Virginia in 2012 arrived by immigration from the 2011 Oklahoma population. This hypothesis is suggested by the reduction in differentiation caused by the introduction of identical alleles between Oklahoma 2012 and Virginia 2012. However, these introduced similar alleles could be from another geographical location not in this study that shares alleles with both Oklahoma and Virginia. Some of these alleles were observed in Virginia in 2012 and again in Oklahoma 2012 and 2014 populations. These common alleles dwindling and reappearing after a year may suggest common inoculum that survives locally, with local allele frequency fluctuations, becomes locally predominant again due to random genetic drift, and is dispersed by air currents. This scenario would not be uncommon since many rust pathogens, like the wheat pathogens *P. graminis*, *P. striiformis*, and *P. tritici,* migrate in a similar fashion [18]. Annual wind patterns, such as the *Puccinia* pathway in the United States, or storms and other natural disasters can move rust pathogens to new locations [19].

The *Puccinia* pathway hypothesis, previously suggested by Orquera (2014) after analysis of nuclear and mitochondrial haplotypes, is further supported by the results of the present study. The bar plot generated by STRUCTURE clearly illustrates that the genotypes in these population have the highest probability of belonging to their own population than to other populations [20]. These findings, along with the numbers of private alleles observed, indicates the occurrence of alleles unique to each population. The origin of these alleles is unknown, but they may represent rare alleles arriving by migration that are eliminated from the local genetic pool every year.

Another objective of this study was to observe the allelic diversity within naturally occurring populations of *P. emaculata*. With the exception of the Virginia population in 2011, there is a high allelic diversity within every population [21]. Across all individuals there were up to 42 different alleles in one locus, OPE5. All individuals were different genotypes. Because of this high genetic diversity, small sample size may result in a poor representation of the allelic variation present in *P. emaculata* populations and underestimation of the true diversity within this species. Yet the challenges of obtaining single spore genotypes for this study made it difficult to reach the sample numbers needed for very strong statistical support [20]. Additionally, analyses of more genetic markers may generate more reliable statistical inferences. With higher population numbers the confidence in overarching population trends would be considerably greater. These factors must be considered in future studies of *P. emaculata* populations.

Overall this study gave evidence of the substantial population information that can be obtained from a single spore. This study promotes further exploration of switchgrass rust populations across the United States and provides insight into the high

genetic diversity that could be found in a native grass rust pathogen at a genotypic level. The study provided evidence that new alleles are introduced into Oklahoma and Virginia populations every year, and create unique population patterns. With the completion of this work there is a stronger understanding of the composition of *P. emaculata* field populations in Oklahoma.

Literature Cited

1. Casler, M.D., et al., *The Switchgrass Genome: Tools and Strategies*. 2011: American Society of Agronomy.
2. Moser and J.P. Vogel, *Switchgrass (Panicum virgatum)*, V.I.A.I.T.G. agriculture, Editor. 1995: Ames, IA.
3. Hartl, D.L. and A.G. Clark, *Principals of population genetics*. Fourth ed. 2007, Sunderland MA: Sinauer Associates Inc.
4. Energy, U.D.o. *The Energy Independence and Security Act of 2007 (EISA 2007)*. 2007; Available from: http://energy.gov/about-us/web-policies.
5. Orquera, G., *Population biology of Switchgrass rust (Puccinia emaculata Schw.)*, in *Entomology and Plant Pathology*. 2014, Oklahoma State University: Stillwater Oklahoma. p. 94.
6. Gu, Y.X., et al., *Projecting future grassland productivity to assess the sustainability of potential biofuel feedstock areas in the Greater Platte River Basin.* Global Change Biology Bioenergy, 2014. **6**(1): p. 35-43.
7. Doerrer, N., et al., *Evaluating biological variation in non-transgenic crops: Executive summary from the ILSI Health and Environmental Sciences Institute workshop, November 16-17, 2009, Paris, France.* Regulatory Toxicology and Pharmacology, 2010. **58**(3): p. S2-S7.
8. Tobias, C.M. and G. Sarath, *Why sequence switchgrass?*, in *CSP Plans-FY 2007*, J.G. Institute, Editor. 2007, UC Berkeley US Department of Energy: California.

9. (CGIAR), C.G.O.I.A.R., *CGIAR Research Priorities for Marginal Lands*, in *SDR/TAC:IAR/99/12*, F. Food and Agriculture Organization, Editor. 1999, CGIAR Secretariat: Washington, D.C. .

10. Perrin, R., et al., *Farm-Scale Production Cost of Switchgrass for Biomass.* Bioenergy Research, 2008. **1**(1): p. 91-97.

11. Linh Vu, A., *Identifying pathogens of switchgrass and investigating antimicrobial activity of switchgrass-derived extractives*, in *Tenneessee Research Creative Exchange*. 2011, University of Tenneessee: TN.

12. Uppalapati, S.R., et al., *Characterization of the Rust Fungus, Puccinia emaculata, and Evaluation of Genetic Variability for Rust Resistance in Switchgrass Populations.* Bioenergy Research, 2013. 6(2): p. 458-468.

13. Johnson, B., et al., *Defining the host range of the switchgrass rust fungus, Puccinia emaculata.*, in *Biology and Disease Management - Mycology*, A.P. Society, Editor. 2015: Pasadena, CA.

14. Wadl, P.A., et al., *Development and characterization of microsatellites for switchgrass rust fungus (Puccinia emaculata).* Conservation Genetics Resources, 2011. 3(1): p. 185-188.

15. Loring, C., J. N., and A.E. Menke, *Proceedings of the american academy of arts and sciences*. 1883, Oxford, MA: University Press: John Wilson and Son. 2.

16. Hirsch, R.L., et al., *First Report of Rust Caused by Puccinia emaculata on Switchgrass in Arkansas.* Plant Disease, 2010. 94(3): p. 381-381.

17. Sykes, V.R., et al., *Effect of Puccinia emaculata infection on ethanol production potential of Panicum virgatum*, in *Phytopathology*, A.P. Society, Editor. 2011, University of Tennessee: Knoxville Tennessee

18. Ali, S., et al., *Origin, migration routes and worldwide population genetic structure of the wheat yellow rust pathogen Puccinia striiformis f.sp. tritici.* PLOS, 2014(1003903).

19. Schumann, G.L. and K.J. Leonard, *Stem rust of wheat.* The plant health instuctor, 2011. PHI-I-2000-0721-01.

20. Milgroom, M.G., *Population biology of plant pathogens: genetics, ecology, and evolution*. 2015: APS Press.

21. Wright, S., *Evolution and the genetics of populations*. Vol. 2. 1969, Chicago: The University of Chicago Press.

CHAPTER IV

OBJECTIVE TWO: THE RUST *PUCCINIA EMACULATA* AS A MODEL RUST FOR MICROBIAL FORENSICS INVESTIGATIONS

ABSTRACT

For this study the background information generated by the analysis of SSR fingerprints from single spores collected from the field populations in objective one were used in comparison to growth chamber populations generated at Oklahoma State University collected over the same years 2012, 2013, and 2014. This study had two hypotheses. The first was that natural field populations and growth chamber populations of *Puccinia emaculata* uredinospores would be significantly different due to the potential genetic bottlenecking that could occur in an artificial environment where only asexual propagation occurred on a limited selection of switchgrass cultivars. The results of this study showed moderate to great genetic differentiation between field and chamber populations using F_{ST} analysis. STRUCTURE analysis showed admixed genotypes within the growth chamber populations, resulting in substantial differentiation from the very discrete field populations. The second hypothesis of was that, using the background information previously generated, the SSR fingerprinting protocol could be used to attribute or reject the origin of an unknown population when compared to the previously analyzed populations. These two hypotheses resulted in the four microsatellite loci used were useful to correlate populations of the same identity or reject the attribution of origin of a population to the known population, but were unable to provide scientific attribution with the statistical support needed for a microbial forensic application. This study was unique in the use of single spores and in the use of a rust model for microbial forensic applications. There is high probability that with more microsatellite loci, this method would be sufficiently sound, statistically, for attribution of origin for a microbial forensic investigation.

Introduction:

The world population continues to rise, and as a result, so does the demand for grains such as corn, wheat, rice, oats, barley, and milo. It is projected that by 2030 the world will be consuming nearly 300 million tons of wheat. Though not as substantial, all other small grain consumption is projected to follow in suit [2]. Rust pathogens cause severe damage to all small grains, leading in some cases to major food crises [3]. Though less damage occurs since the adaptation of resistant varieties, in ideal environmental conditions rust pathogens have been known to cause substantial epidemics that result in up to 70% yield loss [4].

The devastating wheat stem rust pathogen *Puccinia graminis* f. sp. *tritici* lineage Ug 99 (Race TTKSK) is capable of overcoming many wheat resistance genes, causing severe losses and spreading rapidly over extensive areas. This disease demonstrates what can happen when resistance genes cannot be implemented fast enough to avert the damage from an ever evolving organism [3]. The challenges to identify new wheat genes for resistance to stem rust have brought renewed attention to rust pathogens. *Puccina* spp. have been recognized for their biosafety threat to United States agriculture. For example *Puccina* spp. are some of the targets of a Department of Homeland Security effort to sequence select agents and pathogens that pose a threat to US human, livestock, or crop health [5].

Microbial forensics is "a scientific discipline dedicated to analyzing evidence from a bioterrorism act, biocrime or inadvertent microorganism/toxin release for attribution purposes" [6]. Scientific attribution is defined as "the assignment of a sample of questioned origin to a source, or sources, of known origin, to the highest possible degree of scientific certainty – while excluding origination from other sources" [7]. These two definitions were used as guidelines to assess the potential of the DNA fingerprinting protocol and statistical analyses used in objective

one for microbial forensic purposes. Validation protocols for scientific attribution methods are focused on replication for standardization [8]. In this study, switchgrass rust was used as a model to validate a statistical analysis method to determine if it could be used for scientific attribution of a rust pathogen [9-12]. Being able to profile rust pathogens genetically in the same way that humans and microbial organisms are profiled for criminal investigations could lead to attribution in cases of biological attacks or, most likely, an inadvertent inoculation in small grain cropping systems [5, 8].

Experimental design

Two hypotheses were tested. The first was that natural field populations and growth chamber populations of *P. emaculata* uredinospores are significantly differentiated due to the potential genetic bottle necking that could occur in an artificial environment such as a growth chamber, where only asexual propagation occurred on a limited selection of switchgrass cultivars [13]. To evaluate the validity of this hypothesis, two assays were conducted. In the first experiment urediniospore field collections from Virginia in 2011 (VT11) and 2012 (VT12) and from Oklahoma in the years 2011 (OSU11F), 2012 (OSU12F), 2013 (OSU13F) and 2014 (OSU14F), and urediniospore populations propagated and periodically sampled from growth chambers at the Oklahoma State University, Stillwater campus, were compared. The Oklahoma growth chamber population was collected originally in 2009 from the same fields as the Oklahoma field collected samples, and continuously propagated under controlled environmental conditions, in growth chambers, on plants of a selection of switchgrass cultivars, and sampled in 2012, 2013 and 2014. The first experiment was used to compare general population trends between the growth chamber populations and the field populations in populations of similar year and origin.

The second assay compared blind (researcher was not aware of the identity of the samples) field and chamber population samples collected in 2015 from Oklahoma. The aim of this assay was to assess the validity of the SSR fingerprinting analysis protocol to determine the potential source (field or growth chamber) of urediniospores of related origin (same field).

The second hypothesis of was that by using the background information previously generated, SSR fingerprinting could be used to accept or reject attribution of origin of an unknown population when compared to the previously analyzed populations. To test this hypothesis, SSR fingerprints of urediniospore samples of origin unknown to the researcher were statistically analyzed against the background information previously generated to assess the potential of the fingerprinting analysis.

Methodology:

Potential source identification

The sampling protocol described in objective one was used. *P. emaculata* urediniospores (asexual propagative spore stage) were collected and stored as described by Orquera (2014) [11]. Field samples were obtained annually from 2011 to 2015 by members of Dr. Stephen Marek's and Dr. Carla Garzon's research groups. The author of this research participated in sampling, collection, and storage of 2013, 2014, and 2015 urediniospore field samples. Sampling was conducted once a year during summer months (July and August) when spore production is at its peak in the field. Growth chamber populations were collected in 2009 from a switchgrass breeding plot located close to Cow Creek, within the limits of the Oklahoma Agricultural Experiment Station in Stillwater, Oklahoma, and continuously propagated under controlled environmental conditions (27°C and have a 12 hour photoperiod) in growth chambers at the Noble Research Center, Oklahoma State University. A selection of low-land and high-land

switchgrass varieties that included Kanlow, Dacotah, Blackwell, and Cave-in-Rock were used for urediniospore propagation. Plant maintenance and urediniospore propagation were conducted by Gabriela Orquera. Switchgrass plants were allowed to grow for approximately six months before being cut back and replaced with new switchgrass plants. New plants were inoculated by shaking infected leaves of old plants to spread urediniospores over the tillers of young plants. This process was repeated every six months. There were no deliberate introductions of new field collected spores into the growth chamber since 2009. The samples used in this study, referred to as growth chamber populations, were generated as described above and collected annually as described in objective one for field and chamber samples.

The protocol for single uriediniospore SSR fingerprinting described in objective one was followed to produce DNA fingerprints for all known growth chamber and field populations for the first assay as well as for four 2015 urediniospore population samples provided to the researcher without disclosure of their origin. These four 2015 populations were used for the second assay of hypothesis one in objective two. The four SSRs markers selected for DNA fingerprinting in objective one, OEPE68, OPE5, OPE32, and OEPE6a, were used in objective two following the protocol described by Orquera (2014).

At the end of the study the identities of the samples were disclosed to the researcher as follows: Group 1 and Group 4 contained urediniospores collected in 2014 from the Cow Creek breeding plot, while Group 2 and Group 3 contained urediniospores collected in 2014 from the growth chamber. The total number of single urediniospore genotypes analyzed was 164, corresponding to populations as follows: OSU11F= 9, OSU12F =15, OSU13F = 13, OSU14F = 12, OSU12C = 14, OSU13C =15, and OSU14C =15 samples, VT11 = 10, VT12= 18 samples, and the Group 1-4 samples.

Validation using unknown population for attribution:

The validation of an unknown population study was used to evaluate the ability of the statistical analyses to accept or decline attribution of origin to an unknown population. Nine SSR fingerprints of a "blinded" urediniospore population sample were generated by an undisclosed researcher using the four SSR markers selected by the author. Fingerprinting data was provided to the author to be compared with the combined database of Oklahoma (OSU11-14F) and Virginia field (VT11-12), and Oklahoma growth chamber urediniospore collections (OSU12-14C), as well as the Group 1-4 data. In total this analysis consisted of 173 samples.

Statistical analysis

Fixation index analysis (F_{ST}), principal coordinate analysis, and allelic patterns across population were assessed using GenAlEX v6.501 (Rutgers University, New Brunswick NJ) and STRUCTURE version 2.3.4. (Pritchard, Wen, and Falush, 2010). STRUCTURE analysis parameters included a 10,000 burn in length and 50,000 MCMC repetitions. The samples were tested for immigration and all frequencies were correlated among populations. The optimum K was found by using the "STRUCTURE simulation function" and testing hypotheses between two and fifteen. Following the simulation the results for ΔK that gave a statistical best match value for each hypothesis was analyzed in a line graph. The highest peak was selected as the K for the analysis.

Results:

Comparison of known field and chamber populations (Obj 2.1):

Fixation index analyses (F_{ST}) values for pair-wise comparisons of the population are summarized in Table 5. The results for this study show little genetic differentiation between VT12F and OSU11F and OSU12F populations, in congruence with the findings in objective one. There was moderate genetic differentiation between the OSU12-14C populations and each of all other populations except OSU14F population. OSU14F had moderate genetic differentiation from OSU13C and OSU14C.

Table 5 (Obj2.1):: Pair-wise Population F_{ST} Values F_{ST} 00-0.05: Little genetic differentiation; F_{ST} 0.05-0.15: Moderate genetic differentiation* ; F_{ST} 0.15-0.25: Great genetic differentiation** ; F_{ST} 0.25<:Very great genetic differentiation*** [1]

	VT 11	**VT 12**	**OSU11F**	**OSU12F**	**OSU13F**	**OSU14F**	**OSU12C**	**OSU13C**	**OSU14C**
VT11									
VT12	0.062*								
OSU11F	0.083*	0.045							
OSU12F	0.063*	0.033	0.032						
OSU13F	0.136*	0.074*	0.095*	0.095*					
OSU14F	0.220**	0.149**	0.183**	0.174**	0.151**				
OSU12C	0.117*	0.058*	0.074*	0.067*	0.078*	0.140*			
OSU13C	0.113*	0.064*	0.077*	0.071*	0.128*	0.192**	0.087*		
OSU14C	0.142*	0.096*	0.109*	0.107*	0.081*	0.152**	0.055*	0.126*	
	VT 11	**VT 12**	**OSU11F**	**OSU12F**	**OSU13F**	**OSU14F**	**OSU12C**	**OSU13C**	**OSU14C**

The PCoA (Figure 6) revealed an overall trend of Virginia field populations (red) overlapping with Oklahoma field populations (blue). OSU12C and OSU13C were broadly dispersed, while OSU14C were more closely clustered. Most VT11F genotypes clustered closely. These results are congruent with F_{ST} results. As F_{ST} suggested, the chamber populations show trends of separation in clustering from the Oklahoma and Virginia field populations. Yet there is overlap in clusters containing Oklahoma chamber populations and OSU13F and OSU14F.

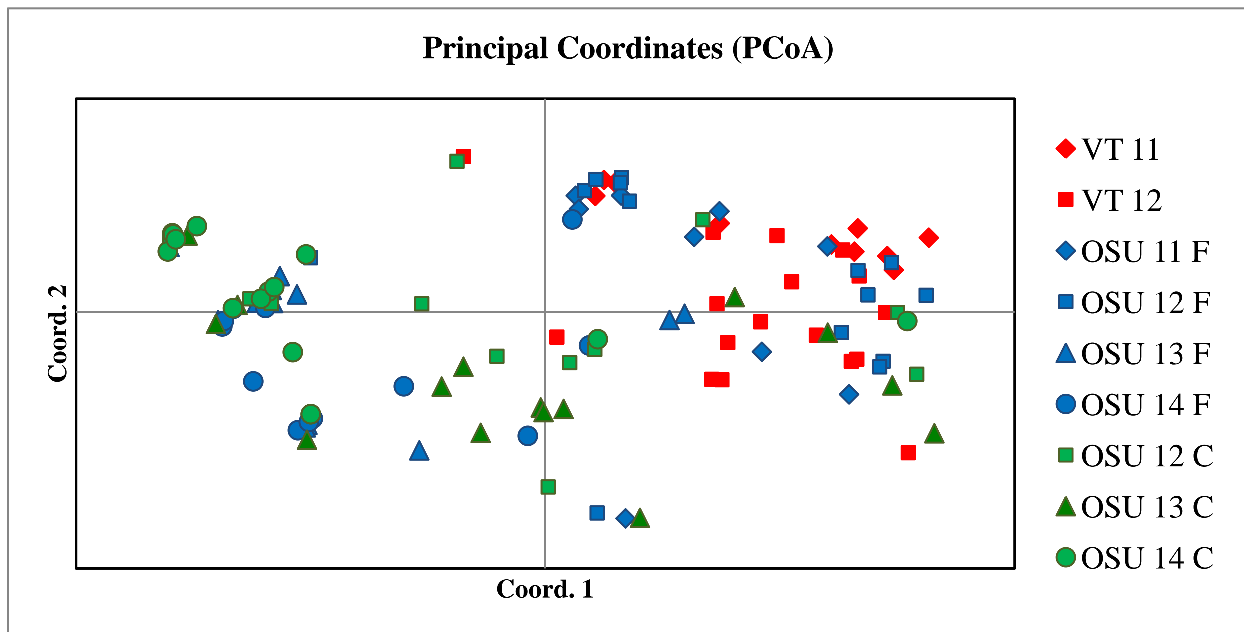

Figure 6 (Obj2.1): PCoA of microsatellite multi-locus genotypes (*n=93*) of nine populations of *Puccinia emaculata* in Oklahoma and Virginia, from switchgrass plants grown under growth chamber (OSU12-14C) and field (OSU11-14F, VT11-12) conditions.

Bayesian analysis was conducted in STRUCTURE 2.3.4 to further examine population differentiation. The *K* value (*K6*) was determined by a simulation summary based on data correlation to ΔK. The bar plot illustrating the genotypes of these populations is shown in Figure 7. The Bayesian analysis shows the first six discrete populations from the field collections. Within the six field populations some migrant genotypes (Virginia 2012, Oklahoma 2011, Oklahoma 2013, and Oklahoma 2014 field populations). Yet, the slight mixing of genotypes in

the field populations is minimal in comparison to the highly mixed genotypes that make up the growth chamber produced populations in this study.

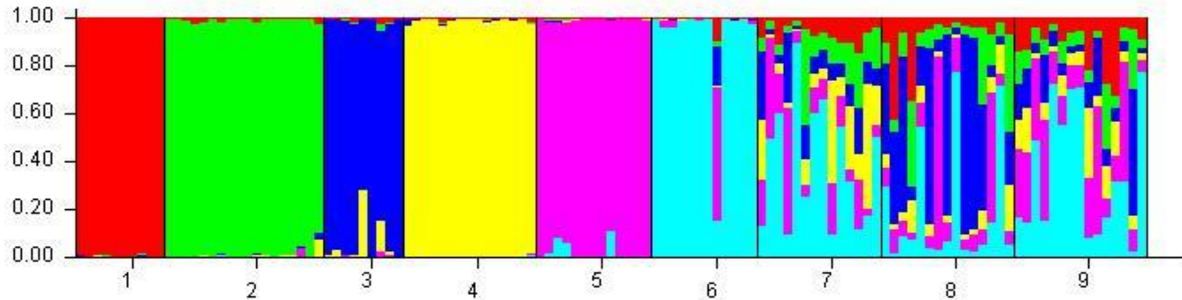

Figure 7 (Obj2.1): STRUCTURE bar plot of 121 single urediniospore genotypes of 9 populations of *Puccinia emaculata* in Oklahoma and Virginia, from switchgrass plants grown under growth chamber (OSU12-14C) and field (OSU11-14F, VT11-12) conditions, inferred using Bayesian analysis using STRUCTURE v. Columns represent individual genotypes, with partitions that correspond to the genotype's estimated mean membership coefficient to one of 6 genetic clusters (K=6) which predict probability of each sample genotype belonging to a particular population: Population 1= VT11, population 2= VT12, population 3= OSU11F, population 4= OSU12F, population 5= OSU13F, population 6= OSU14F, population 7= OSU12C, population 8= OSU13C, and population 9= OSU14C.

The Bayesian analysis bar graph revealed putative admixed (hybrid) genotypes in the growth chamber populations, which is congruent with PCoA and F_{ST} results. In the allelic patterns in Figure 8 and Table 6, growth chamber populations show, on average, lower numbers of private alleles and lower allele diversity with frequencies higher than five percent in comparison to field populations.

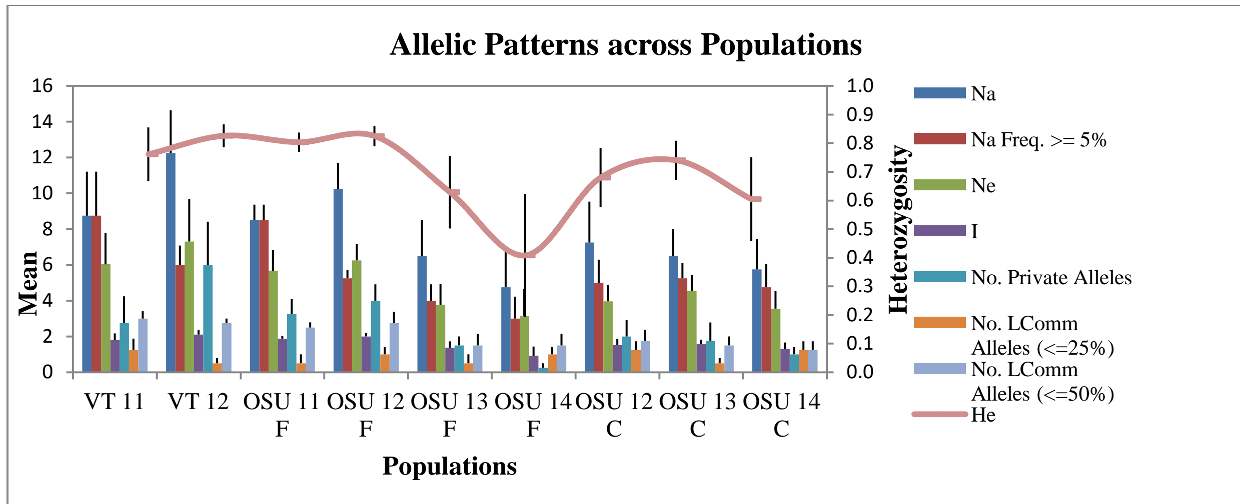

Figure 8 (Obj2.1): Allelic Patterns across all populations with all nine populations represented below the graph. Bare codes are described as follows: Na = No. of Different Alleles, Na (Freq >= 5%) = No. of Different Alleles with a Frequency >= 5%, Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), No. Private Alleles = No. of Alleles Unique to a Single, No. LComm Alleles (<=25%) = No. of Locally Common Alleles (Freq. >= 5%) Found in 25% or Fewer Populations, He = Expected Heterozygosity = 1 -

Table 6 (Obj2.1**):** Average allele values across all populations with all nine populations represented below the graph. Bare codes are described as follows: Na = No. of Different Alleles, Na (Freq >= 5%) = No. of Different Alleles with a Frequency >= 5%, Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), No. Private Alleles = No. of Alleles Unique to a Single, No. LComm Alleles (<=25%) = No. of Locally Common Alleles (Freq. >= 5%) Found in 25% or Fewer Populations, He = Expected Heterozygosity = 1 - Sum pi^2

Population	VT11	VT12	OSU11 F	OSU12 F	OSU13 F	OSU14 F	OSU12 C	OSU13 C	OSU14 C
Na	8.750	12.250	8.500	10.250	6.500	4.750	7.250	6.500	5.750
Na Freq. >= 5%	8.750	6.000	8.500	5.250	4.000	3.000	5.000	5.250	4.750
Ne	6.031	7.308	5.671	6.253	3.763	3.153	3.964	4.539	3.554
I	1.801	2.107	1.879	2.002	1.373	0.922	1.516	1.571	1.296
No. Private Alleles	2.750	6.000	3.250	4.000	1.500	0.250	2.000	1.750	1.000
No. LComm Alleles (<=25%)	1.250	0.500	0.500	1.000	0.500	1.000	1.250	0.500	1.250
No. LComm Alleles (<=50%)	3.000	2.750	2.500	2.750	1.500	1.500	1.750	1.500	1.250
He	0.761	0.826	0.804	0.825	0.629	0.408	0.680	0.741	0.604
uHe	0.801	0.852	0.852	0.856	0.702	0.426	0.722	0.788	0.677

Unknown environment analysis:

Unknown Environment Analysis (Obj2.2):

The unknown environment analysis consisted of a comparison between the known populations from field and chamber in Oklahoma and the field population of Virginia versus the Oklahoma 2015 unknown environment samples (Group 1-4). Group 1 consisted of ten individuals, whereas group 2-4 consisted of 11 individuals each, making a total of 164 total individuals in this analysis.

Statistical analysis:

A fixation index statistical analysis (F_{ST}) (Table 7), showed that groups one through four all showed moderate genetic differentiation from Virginia 2012, Oklahoma 2013 field populations and Oklahoma 2013, and Oklahoma 2014 chamber populations. Groups one, three, and four had great genetic differentiation from the Virginia field population in 2011. Group one also had great genetic differentiation from Oklahoma 2011, Oklahoma 2012, and Oklahoma 2014 field populations. Groups two and three showed very low genetic differentiation suggesting that they may be of the same origin. Groups two and three also show low genetic differentiation from the Oklahoma 2012 chamber population. This suggests that both groups two and three were from the growth chamber and of the same population origin. Groups two and three also show low genetic differentiation from group four, but have moderate genetic differentiation from group one. Group four showed moderate genetic differentiation from all other populations with the exception of Virginia 2011. This analysis shows that groups two and three are of very similar origin and both may have come from the growth chamber. Groups one and four could not be attributed to a known environment.

Table 7 (Obj2.2): Pair-wise Population F_{ST} Values: F_{ST} 00-0.05: Little genetic differentiation; F_{ST} 0.05-0.15: Moderate genetic differentiation* ; F_{ST} 0.15-0.25: Great genetic differentiation** ; F_{ST} 0.25<:Very great genetic differentiation*** [1]

	VT 11	**VT 12**	**OSU11F**	**OSU12F**	**OSU13F**	**OSU14F**	**OSU12C**	**OSU13C**	**OSU14C**	**Group1**	**Group 2**	**Group 3**	**Group 4**	
VT11														**VT11**
VT12	0.062*													**VT12**
OSU11F	0.083*	0.045												**OSU11F**
OSU12F	0.063*	0.033	0.032											**OSU12F**
OSU13F	0.136*	0.074*	0.095*	0.095*										**OSU13F**
OSU14F	0.220**	0.149**	0.183**	0.174**	0.151**									**OSU14F**
OSU12C	0.117*	0.058*	0.074*	0.067*	0.078*	0.140*								**OSU12C**
OSU13 C	0.113*	0.064*	0.077*	0.071*	0.128*	0.192**	0.087*							**OSU13C**
OSU 14 C	0.142*	0.096*	0.109*	0.107*	0.081*	0.152**	0.055*	0.126*						**OSU14C**
Group 1	0.207*	0.147*	0.158**	0.154**	0.103*	0.171**	0.113*	0.147*	0.106*					**Group 1**
Group 2	0.141*	0.076*	0.088*	0.086*	0.068*	0.105*	**0.040**	0.062*	0.061*	0.059*				**Group 2**
Group 3	0.154**	0.075*	0.093*	0.093*	0.076*	0.127*	**0.045**	0.090*	0.065*	0.088*	**0.024**			**Group 3**
Group 4	0.159**	0.107*	0.119*	0.119*	0.078*	0.119*	0.071*	0.097*	0.065*	0.074*	**0.032**	**0.041**		**Group 4**
	VT 11	**VT 12**	**OSU11F**	**OSU12F**	**OSU13F**	**OSU14F**	**OSU12C**	**OSU13C**	**OSU14C**	**Group 1**	**Group 2**	**Group 3**	**Group 4**	

PCoA using a standardized covariance by sample model (Figure 9) grouped Group 1 with Oklahoma 2014 field genotypes, but Group 1 can also be found in several mixed clusters of field and chamber populations. Group two is found primarily clumping with Oklahoma 2012 chamber populations, as the F_{ST} analysis suggested. Group three, like group two, is found primarily with clumps including Oklahoma 2012 chamber populations and group two. Group four is well integrated in all samples, but can be found in clumps predominantly with field samples. The sample based PCoA shows a high amount of integration with all populations and therefore doesn't seem to point to an attribution of environmental origin (field or chamber) for the group samples.

A principal coordinate analysis (PCoA), using a standardized covariance model by population instead of by sample (Figure 10), yielded better visualization of general relationship trends of each population and its closest genetic relative. This figure shows the four group populations most closely related to themselves. In this analysis groups three and four are closest in genetic distance, and groups one and two are the furthest apart in genetic distance. There is a short distance between group one and the Oklahoma 2014 field population.

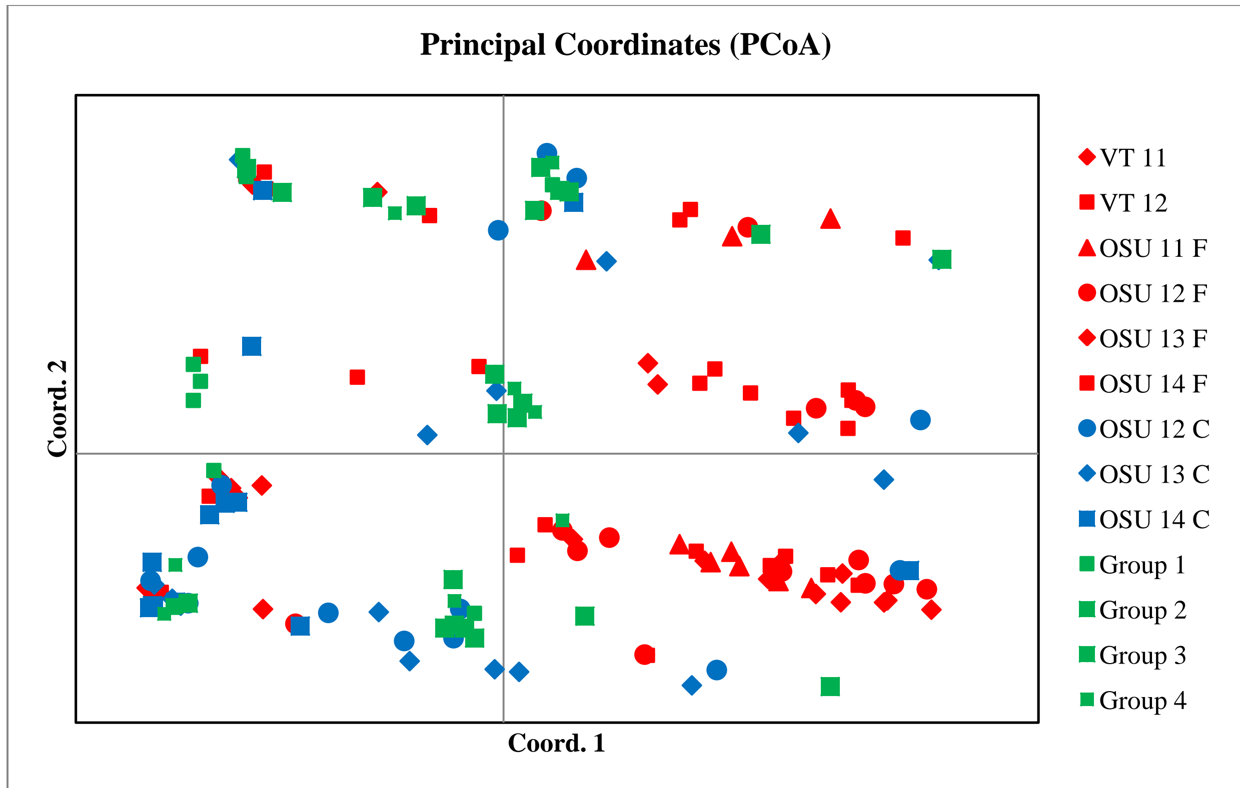

Figure 9 (Obj2.2)**:** Principal coordinate analysis using standardized covariance model by sample. The figure illustrates a spatial comparison of the samples using a coordinate graph. The 13 populations (OSUF 2011, OSUF 2012, OSUF 2013, OSUF 2014, OSUC 12, OSUC 13, OSUC14 VT 2011, VT 2012, and Group 1-4) are displayed with corresponding shapes and colors for differentiation.

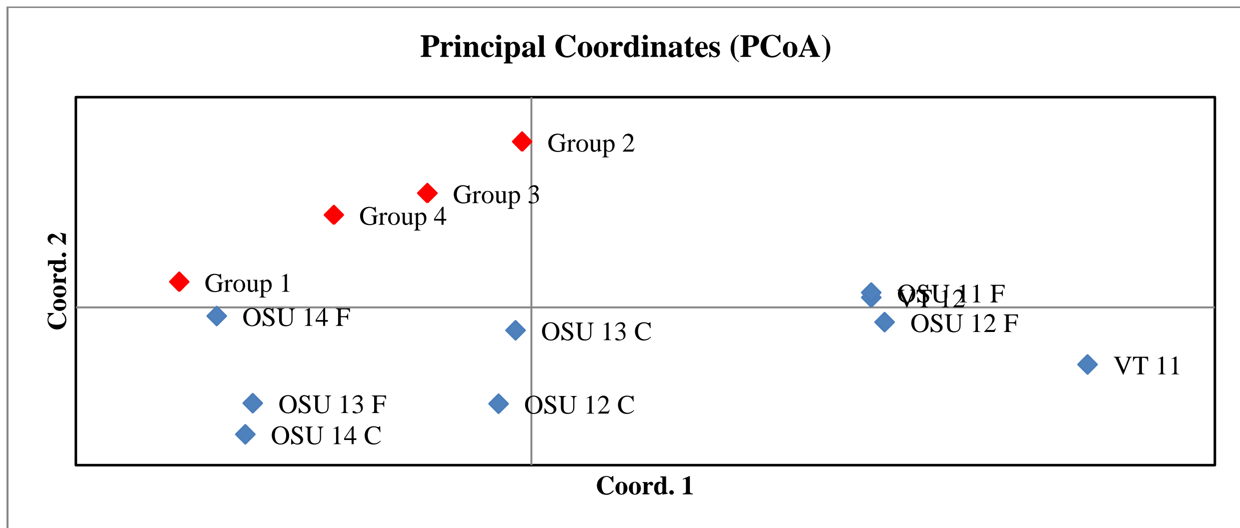

Figure 10 (Obj 2.2): Principal coordinate analysis (PCoA) using a standardized covariance model by population instead of by sample

From this analysis it could be it could be inferred that group one is a field population based on its relationship to the Oklahoma 2014 field population. Groups two, three, and four cannot be attributed to either field or growth chamber.

In summation the F_{ST} analysis suggests that the groups two and three are chamber populations based on their low genetic differentiation from Oklahoma 2012 field population and from one another. The PCoA by population suggested that group one could be attributed to a field population based on its relationship to the Oklahoma 2014 field population. None of the analyses allowed determination of which environment was the source of group four

Blind sample analysis (Obj 2.3):

In the pair-wise population comparison F_{ST} index featured in Table 8, the unknown population had moderate to very great genetic differentiation when compared to the other 13 populations, giving support not only to what population the unknown samples belong to, but also providing evidence as to where the population does not belong. The unknown population had very great genetic differentiation from Group 1 of the 2015 samples, which excludes this population from originating with the unknown population. The unknown sample has great genetic differentiation from both Oklahoma and Virginia in 2011, yet has only moderate genetic differentiation from those states both in the field and in the chamber in 2012. This suggests that the genetic differentiation for *P. emaculata* in these populations diversifies more by year than by the geographic location of the population. The unknown sample also has great genetic differentiation from both field and growth chamber populations of Oklahoma 2013. Interestingly, Oklahoma year 2014 doesn't follow the pattern of previous years with great genetic differentiation from the growth chamber population, and the lowest F_{ST} score for the unknown

population when compared to 2014 field population. From the F_{ST} evaluation the unknown population seems most closely related to the Oklahoma 2014 field population, followed by Virginia 2012, Oklahoma 2012 chamber population, and finally Oklahoma 2012 field population. Excluding the group populations, Oklahoma 2014 field population is only moderately differentiated from Oklahoma 2012 chamber population. Oklahoma 2014 field population has great genetic differentiation from all other 2012 populations that the unknown population is least differentiated from. This could suggest the unknown population is of Oklahoma 2012 chamber descent or Oklahoma 2014 field descent due to the low moderate genetic differentiation between the two populations and the unknown population.

Table 8 (Obj2.3): Pair-wise Population F_{ST} Values: F_{ST} 00-0.05: Little genetic differentiation; F_{ST} 0.05-0.15: Moderate genetic differentiation* ; F_{ST} 0.15-0.25: Great genetic differentiation** ; F_{ST} 0.25<:Very great genetic differentiation*** [1]

	VT 11	**VT 12**	**OSU11 F**	**OSU12 F**	**OSU13 F**	**OSU14 F**	**OSU12 C**	**OSU13 C**	**OSU14 C**	**Unknown**	**Group 1**	**Group 2**	**Group 3**	**Group 4**	
VT11															**VT11**
VT12	0.062*														**VT12**
OSU11F	0.083*	0.045													**OSU11F**
OSU12F	0.063*	0.033	0.032												**OSU12F**
OSU13F	0.136*	0.074*	0.095*	0.095*											**OSU13F**
OSU14F	0.220**	0.149**	0.183**	0.174**	0.151**										**OSU14F**
OSU12C	0.117*	0.058*	0.074*	0.067*	0.078*	0.140*									**OSU12C**
OSU13C	0.113*	0.064*	0.077*	0.071*	0.128*	0.192**	0.087*								**OSU13C**
OSU14C	0.142*	0.096*	0.109*	0.107*	0.081*	0.152**	0.055	0.126*							**OSU14C**
Unknown	0.190**	0.125*	0.162**	0.149*	0.203**	**0.117***	0.145*	0.174**	0.201**						**Unknown**
Group 1	0.207**	0.147*	0.158**	0.154**	0.103*	0.171**	0.113*	0.147*	0.106*	**0.269*****					**Group 1**
Group 2	0.141*	0.076*	0.088*	0.086*	0.068*	0.105*	**0.04**	0.062*	0.061*	0.155**	0.059				**Group 2**
Group 3	0.154**	0.075*	0.093*	0.093*	0.076*	0.127*	**0.045**	0.090*	0.065*	0.167**	0.088*	0.024			**Group 3**
Group 4	0.159**	0.107*	0.119*	0.119*	0.078*	0.119*	0.071*	0.097*	0.065*	0.191**	0.074*	0.032	0.041		**Group 4**
	VT11	**VT12**	**OSU11 F**	**OSU12 F**	**OSU13 F**	**OSU14 F**	**OSU12 C**	**OSU13 C**	**OSU14 C**	**Unknown**	**Group 1**	**Group 2**	**Group 3**	**Group 4**	

The principal coordinate analysis (PCoA) (Figure 11), using the standardized covariance model by sample, gives evidence that the unknown population groups most closely with Virginia 2012 field populations and, as the F_{ST}s suggested, Oklahoma 2014 field population and Oklahoma 2012 field population. The PCoA does not show a connection between the unknown samples and Oklahoma 2012 chamber populations. After evaluating the PCoA the unknown population would appear to most likely belong to field populations from Virginia 2012, Oklahoma 2013, or Oklahoma 2014.

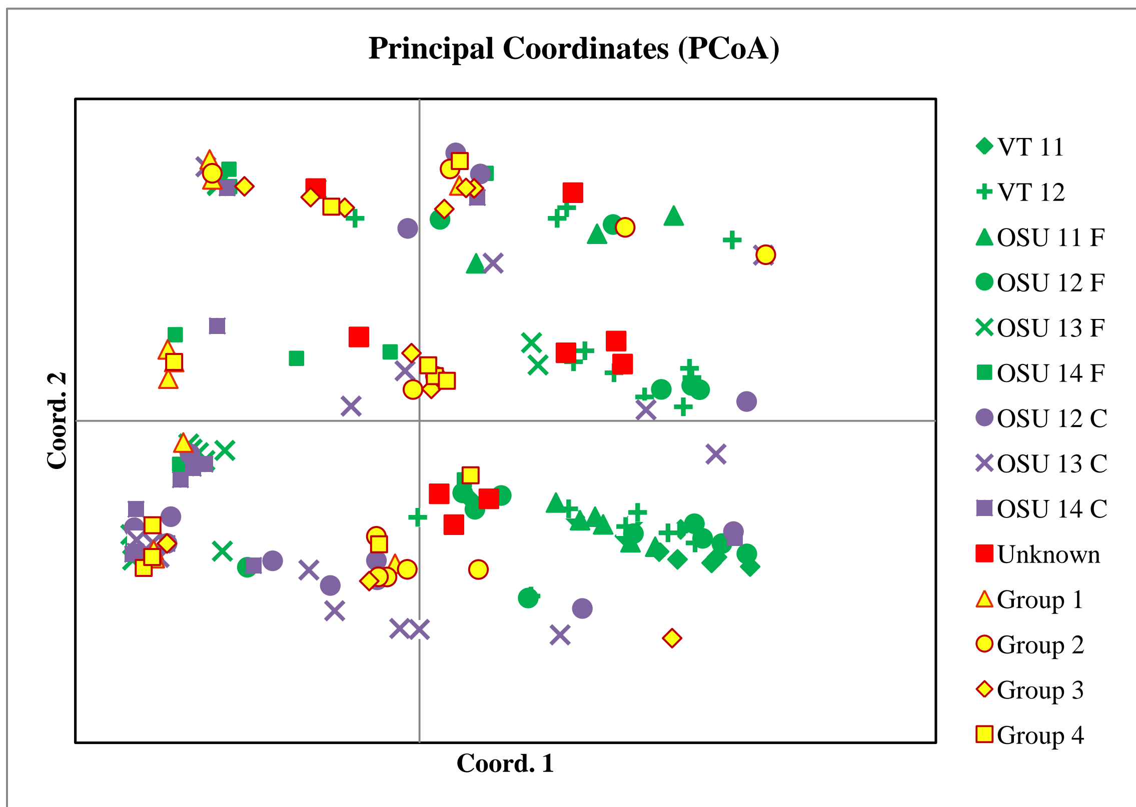

Figure 11 (Obj2.3)**:** Principal coordinate analysis using standardized covariance model by sample. The figure illustrates a spatial comparison of the samples using a coordinate graph. The 14 populations (OSUF 2011, OSUF 2012, OSUF 2013, OSUF 2014, OSUC 12, OSUC 13, OSUC14 VT 2011, VT 2012, Group 1-4, and Unknown population) are displayed with corresponding shapes and colors for differentiation.

To further illustrate population relatedness, a principal coordinate analysis (PCoA) using the standardized distance matrix model was done by population as opposed to sampling by (Figure 12). These data place the unknown sample in the very center of the matrix along with field populations from Virginia and Oklahoma in 2011 and 2012 to the right and all other populations to the left. This placement could reflect the unknown population's intermixed allele commonality with multiple other populations, hampering the ability to group it with any specific population.

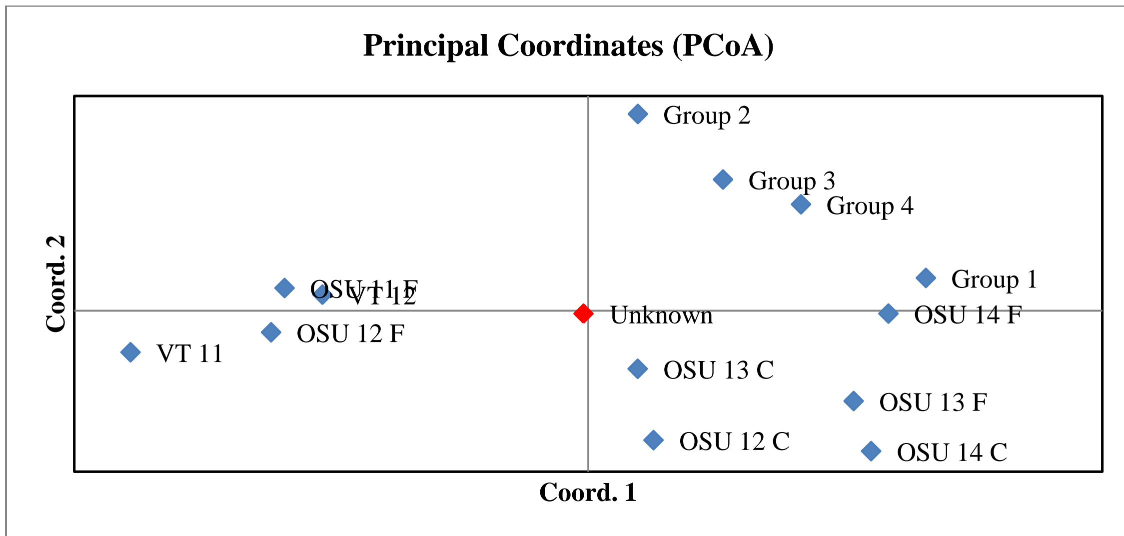

Figure 12 (Obj2.3): Principal coordinate analysis (PCoA) using a standardized distance matrix model by population instead of by sample

To better understand the populations relatedness based on the allelic patterns the private alleles and common alleles were analyzed. The unknown population and Oklahoma 2014 field both have only one private allele, from locus OPE5. This evidence suggests that the unknown population is most closely related to Oklahoma 2014 field population. The common alleles for all the populations were counted and compared to these of the unknown population. The two populations that had the highest number of common alleles with the unknown population were Oklahoma 2012 and 2013 chamber populations. Neither the FST nor PCoA analysis indicated a relationship between these populations and the unknown population.

After analyses were conducted and reported, the blind sample was disclosed as a population of spores from a switchgrass field in Mississippi, collected in 2011.

In summation, all analysis methods showed the unknown population to be a different population from the others analyzed, with the exception of the slight overlap of the F_{ST} analysis and the PCoA showing origin to Oklahoma 2014 field population. The low statistical support in the F_{ST} analysis, in addition to multiple analyses giving contradictory answers suggests the hypothesis that these analyses with these four SSR loci can be used for forensic attribution of the unknown population should be rejected.

Discussion:

Oklahoma 2015 unknown environment populations identity:

In the unknown environment study, the identity of groups one and four was Oklahoma 2015 chamber populations and that of groups two and three were identified as Oklahoma 2015 field collected populations. The evidence suggested multiple environmental origins for all four populations. Though there was some evidence as to the origin for three of the four groups, there is no support that this statistical analysis, given these SSR loci, could be used to attribute these populations to a field or chamber environment with enough confidence to be suitable for a microbial forensic purpose. The clumping of the group populations in the population PCoA suggests that, with more samples per population, there could be stronger affiliation to the field or chamber populations. Yet, the F_{ST} analysis, even with very few SSR loci, gave evidence that group two and three were of a similar or even identical origin. Similarly, the F_{ST} analysis was able to differentiate group one from two and three. These findings suggest that, with one

or two additional polymorphic SSRs, there is a greater possibility of environment attribution for *P. emaculata* using F_{ST} statistics.

Unlike the F_{ST}, the PCoA by sample neither associated the group populations to field or growth chamber environments, nor drew clear differentiation between the group populations. However the PCoA by population, much like the F_{ST} analysis, though unable to provide origin of environment, supported to the relationship between groups one and four and groups two and three. The confidence of the PCoA analysis could be increased by the addition of more individuals per population.

Unknown population identity:

The unknown population was revealed to be a Mississippi 2011 field population. When evaluating the results of the unknown population validation experiment, it is clear why the unknown sample didn't show less than moderate genetic differentiation from any other population in the F_{ST} analysis. The identity of Mississippi 2011 population validates the very great genetic differentiation from the Oklahoma 2015 chamber population previously mentioned in the F_{ST} analysis.

The PCoA by sample provided insufficient evidence for attribution of the Mississippi population due to the inconsistent clumping of individual samples. The PCoA by population, however, did illustrate the separation of the Mississippi samples from all other populations, demonstrating the ability to differentiate such genetically distant populations. The validity of this attribution study would be enhanced significantly by having previously analyzed Mississippi populations in the database.

From an epidemiological perspective the population PCoA, displaying Mississippi 2011 intermediately between other 2011 field populations and 2013 and 2014 Oklahoma field populations, gives further support to the hypothesis that *P. emaculata* is moved by south to northward moving winds. This conclusion is further supported by the F_{ST} analysis, which showed great genetic differentiation between Mississippi 2011 and Oklahoma and Virginia 2011 field populations, but only moderate genetic differentiation from Oklahoma field populations in 2012 and 2014. A pattern seen in the F_{ST} analysis was that in odd years (2011, 2013, 2015) the Oklahoma field populations had great genetic differentiation from Mississippi 2011, but in even years (2012 and 2014) it had only moderate genetic differentiation. A possible explanation is that though new alleles are dispersed by winds from the south, they may not have migrated to Oklahoma [14]. There is a chance that the alleles found in Mississippi in 2011 migrated to Virginia and Oklahoma in 2012 and became the dominate genotype, and in the following year, perhaps due to environmental selection, new alleles were dispersed into Oklahoma and became the dominate genotype of that year. If those alleles did not contribute to fitness they may not have become established, allowing the migrants from 2012 to re-emerge as the dominate genotype in 2014. Unfortunately, without the addition of Mississippi populations from 2012, 2013, and 2014 no statistically supported inferences can be made.

Conclusions:

The results of this studyshow no evidence that the four microsatellite loci chosen can differentiate, with a high degree of statistical confidence, Oklahoma growth chamber populations of *P. emaculata* from field populations collected in Oklahoma and Virginia. For this reason, the hypothesis predicting that this analysis can be used to attribute a population to a natural or artificial environment must be rejected. However, the study did support conclusions on allele diversity in an artificial environment.

The fact that the growth chamber population genotypes were present in one population suggests that in 2009 all genotypes were present and the growth chambers artificial and extreme selection pressure contributes to the mixed population structure. The fact that, every six months the plants in the growth chamber were cut back and replaced with new plants, represents a catastrophic event that may account for the disappearance and reemergence of certain alleles generating these mixed genotypes [14]. This phenomenon may also have led to the moderate genetic differentiation between populations within the growth chamber.

The mixed genotypes of the chamber populations could be explained by the small population size compared to that in the natural environment. Objective one evidence pointed to a dominate genotype being present each year. The growth chamber, not receiving a new dominate genotype each year, maintained the different alleles that were present in 2009. Because of the size of the populations in nature, the study may require much larger sample sizes. Picking roughly the same number of individuals to represent

both populations, and comparing the field to the exponentially smaller growth chamber population, biases the genotypes analyzed.

As for hypothesis two, one cannot reject or deny the attribution ability of this analysis method to reveal origin without a validated known population to which the unknown sample can be compared. Like human forensic analysis, with the intention of scientific attribution, without a previous genotyping of the individual, SSR analysis cannot identify an unknown suspect [8]. Yet, like human SSR analysis, using the database generated by previous experiments, this analysis provided evidence of which origins could be excluded. With the addition of more SSR loci, it is highly likely that the F_{ST} analysis and PCoA, used in conjunction, is a robust approach for scientific attribution. Though this study did not confirm the ability to attribute natural or artificial environment, or specific origin, with sufficient statistical support to be used as a microbial forensic analysis, this study was successful in assessing single spore diversity per loci.

Other important fungi that have been studied for microbial forensic purposes are *Fusarium spp*., *Aspergillus fumigatus*, *Pneumocystis jiovecii*, and *Coccidioides pasadasii* and *immitis* [9]. Unlike *P. emaculata* these fungi are studied primarily because of their direct harm to people; however, the methods used for scientific attribution are very much the same. All of these systems have used microsatellite loci for genotyping and population analysis [9].   One case that had methods very similar to this study was done in Texas on *Coccidioides immitis.* The study's goal was to define the origin of an outbreak of *C. immitis*. Using 164 isolates and nine SSRs this study used F_{ST} and PCoA to attribute the outbreak to South America [15]. The attribution was done by comparing

previously evaluated samples from South America to the clinical samples obtained in Texas. The previously mentioned statistical analysis gave evidence to origin.

Objective two followed methods that have been used frequently. However it is important to state that all the fungi listed above that are commonly studied in microbial forensics are haploid and clonal when infecting their host [6, 15, 16]. *Puccina spp*'s are mostly heterozygous and are dikaryons when infecting their host of economic importance [17-19]. This complexity in the biological system adds for complexity in the sampling and genetic analysis of these organisms. Still, the importance of rust will continue to grow as the world population does. The use of *P. emaculata* as a model has opened opportunities for future studies to contribute to the microbial forensic.

Literature Cited

1. Hartl, D.L. and A.G. Clark, *Principals of population genetics*. Fourth ed. 2007, Sunderland MA: Sinauer Associates Inc.
2. United Nations, *World agricultture: towards 2015/2030*, F. Food and Agriculture Organization, Editor. 2015, FAO: Rome.
3. Chaves, M.S., et al., *The importance for food security of maintaining rust resistance in wheat.* Springer Science+Business Media Dordrecht and International Society for Plant Pathology 2013, 2013. Food Security (5): p. 157-176.
4. Schumann, G.L. and K.J. Leonard, *Stem rust of wheat.* The plant health instuctor, 2011. PHI-I-2000-0721-01.
5. Blagden, T., *Microbial forensics and ag biosecurity – closing the gaps*, D.O.E.A.P. Pathology, Editor. 2015: Oklahoma State University.
6. Budowle, B., et al., *Microbial Forensics* Second ed. 2011, Burlington MA: Acedemic Press/Elsevier.
7. Murch, R. *Exploring an International Microbial Forensics Capability Microbial Forensics Capability to Support Attribution and Advance Global Biosecurity*. in *Trends in Science and Technology Relevant to the BWC*. 2010. Beijing, China: The National Academics.
8. Saferstein, R., *Forensic science: from the crime scene to the crime lab*. Second ed, ed. V.R. Anthony. 2013, New Jersey.
9. Budowle, B., et al., *Role of law enforcement response and microbial forensics in investigation of bioterrorism.* Croatian Medical Journal, 2007. 48(4): p. 437-449.

10. Linh Vu, A., *Identifying pathogens of switchgrass and investigating antimicrobial activity of switchgrass-derived extractives*, in *Tenneessee Research Creative Exchange*. 2011, University of Tenneessee: TN.

11. Orquera, G., *Population biology of Switchgrass rust (Puccinia emaculata Schw.)*, in *Entomology and Plant Pathology*. 2014, Oklahoma State University: Stillwater Oklahoma. p. 94.

12. Wadl, P.A., et al., *Development and characterization of microsatellites for switchgrass rust fungus (Puccinia emaculata).* Conservation Genetics Resources, 2011. 3(1): p. 185-188.

13. Acquaah, G., *Principals of plant genetics and breeding*, ed. J.W.S. Ltd. 2012, Maryland: Wiley-Blackwell. 740.

14. Milgroom, M.G., *Population biology of plant pathogens: genetics, ecology, and evolution*. 2015: APS Press.

15. Fisher, M.C., et al., *Biogeographic range expansion into South America by Coccidioides immitis mirrors New World patterns of human migration.* Proc Natl Acad Sci U S A, 2001. 98(8): p. 4558-62.

16. Woudenberg, J.H.C., et al., *Alternaria redefined.* Studies in Mycology, 2013. 75(1): p. 171-212.

17. Niu, Z.X., et al., *Genetic analysis and molecular mapping of crown rust resistance in common wheat.* Theoretical and Applied Genetics, 2014. 127(3): p. 609-619.

18. Uppalapati, S.R., et al., *Characterization of the Rust Fungus, Puccinia emaculata, and Evaluation of Genetic Variability for Rust Resistance in Switchgrass Populations.* Bioenergy Research, 2013. 6(2): p. 458-468.

19. Suenaga, K., et al., *Microsatellite markers for genes Lr34/Yr18 and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat.* Phytopathology, 2003. 93(7): p. 881-890.

VITA

Jessica Carrie Pavlu
Candidate for the Degree of

Master of Science

Thesis: POPULATION DYNAMICS OF PUCCINIA EMACULATA IN NATURAL AND EXPERIMENTAL ENVIRONMENTS

Major Field: Entomology and Plant Pathology

Biographical:

Jessica C. Pavlu grew up in the rural town of Hudson, Colorado. Throughout high school, she raised livestock and participated heavily in Future Farmers of America. After high school graduation in 2009, she attended Colorado State University and, while working toward her degree requirements, she was also a College of Agricultural Sciences Ag. Ambassador as well as worked in the Wheat Breeding Lab. In 2012 Jessica participated in a semester abroad program at Lincoln University in Lincoln, New Zealand. There, she studied plant pathology and biological control of insect pests. Following her undergraduate studies she joined the Department of Entomology and Plant Pathology at Oklahoma State University in 2014. During her Master's program she gave multiple presentations all over the nation including National American Phytopathological Society meeting in Pasadena, CA, and the Bio-Threat Advisory Group meeting in Miami, FL. In the fall of 2015, she was chosen to be an Honorary Graduate Marshal for the Fall 2015 graduation ceremony. After obtaining her Master's Degree, Jessica intends to continue her education by perusing a PhD in Entomology at Oklahoma State University.

Education:

Completed the requirements for the Bachelor of Science in Soil and Crop Sciences and Horticulture Food Crop Production at Colorado State University, Ft. Collins, Colorado, USA, in December 2013.

Experience:

- USDA APHIS CPHST Intern, Miami Lab, Miami Florida, May-July, 2015
- USDA National Needs Fellow, January 2014-December 2015
- Entomology & Plant Pathology Graduate Student Assoc. Secretary, 2015
- Oklahoma State University, Stillwater, January 2014-December 2015
- Wheat Breeding Lab Technician, July 2010-September 2013

Professional Memberships:

American Phytopathological Society