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EVALUATION OF SWICTHGRASS (*Panicum virgatum* L.) AS A BIOENERGY
FEEDSTOCK FOR THE NORTHEASTERN AND MID-ATLANTIC USA

by Laura Mary Cortese

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ABSTRACT OF THE DISSERTATION

EVALUATION OF SWITCHGRASS (*Panicum virgatum* L.) AS A BIOENERGY FEEDSTOCK FOR THE NORTHEASTERN AND MID-ATLANTIC USA

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Stacy A. Bonos

Switchgrass (*Panicum virgatum* L.) is a warm season, C4 perennial grass native to most of North America with numerous applications, including use as a bioenergy feedstock. Although switchgrass has emerged as a bioenergy crop throughout the midwestern and southern US, little information is available on the performance of switchgrass in the Northeast/Mid-Atlantic. In the first genetic diversity study of switchgrass populations to utilize both morphological and molecular markers, it was found that the combination of morphological and molecular markers differentiated populations best, and should be useful in future applications such as genetic diversity studies, plant variety protection, and cultivar identification. In a study that evaluated several bioenergy traits of 10 switchgrass cultivars in NJ, populations with improved agronomic characteristics were identified. Cultivar Timber exhibited the best combination of characteristics and has promise for biomass production in the Northeast/Mid-Atlantic US. In a third study, the effects of cultivar, location, and harvest date on biomass yield, dry matter, ash, and combustion energy content in three

switchgrass cultivars were investigated. Results indicated that a January harvest allowed for optimal feedstock quality and that cultivars Alamo, Carthage, and Timber produced high yielding, high quality biomass. In an effort to improve the establishment capacity of switchgrass, a fourth study was conducted examining the effects of divergent selection for seed weight on germination and emergence in three switchgrass populations over two cycles of selection, and cold stratification on germination in the derived populations. Selection for seed weight alone was not sufficient to improve germination and germination rate in populations tested, while cold stratification improved germination. Therefore, breeding efforts should be directed towards reducing dormancy in order to improve switchgrass germination and establishment. The final two studies examined genotype x environment effects, estimated broad-sense heritability, and stability analysis on lignocellulosic and agronomic traits in switchgrass clones grown on marginal and prime soils in NJ. Results support the existence of both specifically and broadly adapted switchgrass germplasm, and demonstrate the need for evaluation of germplasm across multiple years and environments (including prime and marginal sites) in order to develop cultivars with optimal lignocellulosic and agronomic characteristics.

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"We do not know one millionth of one percent about anything." Thomas Alva Edison

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1. INTRODUCTION

As the world's population increases, so does the demand for food, water, and energy. According to the Food and Agricultural Organization of the United Nations (FAO, 2005), fossil fuels are the primary energy source worldwide, with petroleum, coal, and natural gas accounting for approximately 80% of the developed world's energy consumption. The world's major energy sources are non-renewable, found in limited quantity, and have serious environmental impacts such as greenhouse gas emission. In addition, the few countries that produce oil control the pricing of this commodity, causing instability in the market. During the 1970's, the United States became more dependent on foreign oil while the global oil market remained unstable. In response, the US initiated the Bioenergy Feedstock Development Program (BFDP) in 1978 under the sponsorship of the US Department of Energy (USDOE) to evaluate potential feedstocks to be used as biofuels. In the early 1990's, the USDOE named switchgrass (*Panicum virgatum* L.) as a model species for the development of herbaceous energy crops after 10 years of research (McLaughlin and Kszos, 2005; Sanderson et al., 1996). In 1991, research into yield improvement of switchgrass began with a single breeding project at Oklahoma State University. In 1996 a second major breeding project at the University of Georgia was designed to address the soils and climate of the Southeastern US. Additional, smaller-scale breeding projects were carried out in the Northern Great Plains, coordinated through the USDA, Agricultural Research Service, Lincoln, Nebraska, which pioneered breeding research on switchgrass and other forage grasses, and at the University of Tennessee, where applications of clonal propagation and crossing of elite parents were

evaluated. The initial breeding strategy for switchgrass improvement based on fast-track recurrent restricted phenotypic selection (RRPS) was modified to recurrent genotypic selection (RGS) (McLaughlin and Kszos, 2005).

Although the USDOE eliminated the BFDP in 2002, interest was renewed again in 2005 with the United States Department of Agriculture (USDA) and USDOE survey highlighting the need for development of herbaceous energy crops like switchgrass. Increased investment into the genetic improvement of switchgrass as a bioenergy feedstock followed, and a second wave of investigation into switchgrass was born (Bouton, 2007). In 2007 alone, the USDOE dedicated over \$1 billion towards multiyear biofuels research and development (Ebert, 2007). Ken Vogel (2004, p. 582), a supervisory research geneticist with the USDA Agricultural Research Service stated, “If economical conversion technologies can be developed, [switchgrass] will likely be planted on millions of hectares as a biomass energy crop”.

As mentioned above, most switchgrass breeding efforts have been limited to a few programs funded through the DOE or USDA-ARS concentrated in the Midwest despite the fact that differential response of ecotypes in biomass production across latitudes has been shown. Researchers have identified the importance of breeding for improved biomass production for specific sites (Fike et al., 2006; Lewandowski et al., 2003; Vogel and Jung, 2001) and suggest that no single switchgrass cultivar is likely to have yield advantages across all locations (Casler et al., 2004; Hopkins et al., 1995). The Northeast/ Mid-Atlantic region is the most populated and urbanized region of the United States and responsible for a large amount of the fossil fuels consumed in this country. It is unlikely that it will be economically feasible to transport biomass from far distances to

utilize as renewable energy in the Mid-Atlantic and Northeast. Therefore it will be important to identify and breed germplasm specifically for this environment if biomass is going to be a significant source of renewable energy for this region. New Jersey and other states throughout the region are interested in utilizing renewable energy sources (New Jersey Clean Energy Program – Board of Public Utilities, Maryland Public Interest Research Group). Even though NJ is a small state, there are more than 150,000 acres of land enrolled in the farmland preserve program that are not suitable or are not currently being used for typical agricultural crop production. In addition, the most consistent success in establishing switchgrass has developed in the Mid-Atlantic region where recommendations for breaking seed dormancy, planting depth by no-till techniques, planting dates, herbicide types and levels have been established and validated in on farm trials (McLaughlin and Kszos, 2005). Therefore, a switchgrass breeding program was started by Dr. Stacy Bonos at Rutgers University in 2005 to develop germplasm for use in the northeastern and Mid-Atlantic regions of the US.

The following work describes studies conducted at Rutgers University investigating switchgrass as a bioenergy feedstock for the northeastern and Mid-Atlantic USA. The dissertation is divided into seven sections: (1) Literature review; (2) Genetic diversity of twelve switchgrass populations using molecular and morphological markers; (3) Bioenergy traits of ten switchgrass populations grown in the northeastern/Mid-Atlantic US; (4) Biomass energy characteristics of switchgrass cultivars grown in New Jersey; (5) Germination in three populations of switchgrass divergently selected for seed weight; (6) Genotype x environment, broad-sense heritability, and stability analysis of lignocellulosic characteristics of switchgrass clones grown on marginal and prime soils in

New Jersey; and (7) Genotype x environment, broad-sense heritability, and stability analysis of agronomic characteristics of switchgrass clones grown on marginal and prime soils in New Jersey.

2. LITERATURE REVIEW

2.1. TAXONOMIC DESCRIPTION

Panicum virgatum (L.), commonly referred to as switchgrass, is a warm-season perennial grass, native to North America, and belonging to the Paniceae tribe in the subfamily Panicoideae of the Poaceae (Missaoui et al., 2006). Switchgrass is adapted to a wide range of environmental conditions, occurs primarily east of the Rocky Mountains, from southern Canada to Mexico, but is also found in Bermuda, Cuba, and Costa Rica, as well as Argentina (as a possible introduction), and is adapted to a wide range of habitats (Barkworth et al., 2007). Switchgrass was historically a dominant species of the true tallgrass prairie of North America, as well as of floodplain grasslands (Porter, 1966). Today, switchgrass is found in prairies, meadows, open woods, brackish marshes, bottomlands, and riverbanks, often inhabiting sandy or rocky soils (Quinn, 1969).

Switchgrass is a coarse grass that grows from 0.5 to 3.0 m tall, with rhizomatous roots that reach depths of 3 m. The leaves are erectophile and can have stomata on both sides, with rounded sheaths and firm, flat blades, ranging in length from 10 to 60 cm (Vogel, 2004; Parrish and Fike, 2005). The ligule is fringed and membranous with hairs, 1.5 to 3.5 mm long. The switchgrass inflorescence is a diffuse panicle, ranging from 15 to 55 cm in length, with spikelets found at the end of the long branches. The spikelets contain two flowers, the first floret being sterile or staminate and the second floret being fertile. Spikelets are 3-5 mm long and florets are awnless and glabrous (Vogel, 2004). Switchgrass flowers basipetally, with an individual panicle at anthesis for up to 12 days

(Martinez-Reyna and Vogel, 1998). Switchgrass reproduces both vegetatively via rhizome and by seed, and with its perennial life cycle, an established stand can last indefinitely (McLaughlin et al., 1999). Switchgrass seed consists of the palea and lemma, which are tightly attached to the caryopsis, with the lemma margins enrolled over the margins of the palea. Glumes can be nearly entirely removed by combining and cleaning (Vogel, 2004). All switchgrass shoots are able to produce flowers and seedheads, with vegetative buds found at or below the soil surface.

Switchgrass has been grouped into upland and lowland types, with distinctly different growth habits and habitat adaption. Porter (1966) provided one of the earliest descriptions of two “types” of switchgrass, in an investigation of morphological differences between the two types in Oklahoma. Lowland types tend to be taller, with thicker stems, longer, wider, blue-green leaves, and a more coarse growth habit than Upland types. Lowland populations also have longer ligules, larger panicles, and more resistance to the rust fungus *Puccinia* sp. than upland types (Parrish and Fike, 2005; Quinn, 1969). Upland ecotypes have semi-decumbent stems, pubescence on the upper surface of the leaf blades, short rhizomes, and they grow up to 1.5 m high (Barnett and Carver, 1967; Porter, 1966). Upland types are adapted to northern latitudes, while lowland types are adapted to southern latitudes (Casler, 2005).

2.2. SWITCHGRASS – A MODEL BIOENERGY CROP

Switchgrass has several attributes that led to its designation of a model bioenergy species. First, it is native to most of the US. This has several implications, one of which is that it is not likely to become invasive, as it has existed here for the last 12,000 years without being cited as an invasive species (Vogel and Burson, 2004; Vogel and Jung, 2001). Switchgrass is also productive over a wide geographic range and grows in marginal land not suitable for traditional agricultural crops. As a perennial crop, switchgrass is advantageous over annual crops in that it does not need to be replanted every year, which translates to less fossil fuel inputs. The perennial nature of the plant also helps to reduce soil erosion and runoff, while increasing soil carbon and improving soil texture and chemistry (Sanderson et al., 1996). In addition, Gebhart et al. (1994) and McLaughlin et al. (1994) showed that switchgrass has a near zero net carbon exchange resulting from burning biomass fuels instead of fossil fuels and net CO₂ reduction associated with replacement of soil C. In a field-scale study (plots ranging in size from 3-9 ha) of cellulosic ethanol produced from switchgrass grown on marginal land in several locations in the Midwestern US, Schmer et al. (2008) reported a net energy yield of 60 GJ Mg ha⁻¹ yr⁻¹, and that switchgrass produced 540% more renewable energy than nonrenewable energy consumed.

Another advantage is that switchgrass is a low input crop that has low water, fertility, and pesticide requirements, while the farming community is familiar with grass production, harvesting and equipment needed to produce the crop (McLaughlin and Kszos, 2005). McLaughlin and Walsh (1998) reported average yields of 16 Mg ha⁻¹ yr⁻¹

in unirrigated research plots, with a one-year single plot maximum yield of 37 Mg ha⁻¹ yr⁻¹. Switchgrass is a C₄ grass species with the anatomical and physiological characteristics of C₄ grasses, including four-carbon acids as the first products of carbon fixation, phosphoenolpyruvate carboxylase (PEP) as the first carboxylating enzyme, and Kranz leaf anatomy. C₄ grasses generally grow well at high temperatures, which can induce photorespiration, a wasteful process whereby Glyceraldehyde 3-phosphate (G3P) is formed at a reduced rate and higher metabolic cost. Because PEP has no affinity for O₂, C₄ grasses overcome photorespiration. C₄ grasses have high water use efficiencies and require about one-third to one-half as much water to produce a unit of dry matter as C₃ grasses. C₄ grasses also have high N-use efficiencies than C₃ grasses due to the more efficient photosynthetic process in C₄ plants that compartmentalizes rubisco in the bundle sheath cells. In addition, C₄ grasses respond to high light flux. At full sunlight (light flux), photosynthetic rates per leaf are still increasing in C₄ grasses, while in C₃ grasses photosynthetic rates peak at one-third full sunlight (Moser, Burson, and Sollenberger, 2004). Currently, there are few reports of switchgrass insect pests. Grasshoppers (*Othoptera*) can cause some damage by feeding on leaves and the yellow sugarcane aphid may feed on switchgrass, but not as a preferred host (Parrish and Fike, 2005). More recently, the stem-boring caterpillar *Blastobasis repartella* Dietz., commonly referred to as the switchgrass moth, was observed in both cultivated and natural stands of switchgrass (Prasifka et al., 2010) in the north central US (Illinois, Iowa, Michigan, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin). In addition, a new species of gall midge, *Chilophaga virgate* Gagné was observed in both spaced plant nurseries and seeded plots of switchgrass in South Dakota (Boe and Gagne, 2011). There

is currently little information available investigating the impacts of the switchgrass moth and gall midge on switchgrass as a bioenergy feedstock. Diseases that have the potential to cause losses in switchgrass include rust caused by *Puccinia* sp., Helminthosporium spot blotch (causal agent *Helminthosporium sativum* Pam., King, & Brakke), smuts caused by *Tilletia maclaganii* (Berk.) G.P. Clinton, anthracnose (*Colletotricum navitas*), and panicum mosaic virus. Vogel (2004) reported that smuts reduce seed yields and biomass production in Iowa, while Helminthosporium spot blotch can be problematic in Pennsylvania and other eastern states with high humidity. Dr. Stacy Bonos observed a significant amount of rust on switchgrass grown in New Jersey (personal communication, 2008). Switchgrass has a large degree of genetic variability for disease resistance ranging from resistance to tolerance. If switchgrass is grown on a large scale, it is likely that insect and disease pressures will increase. Therefore, it is important that we gain a better understanding of the mechanisms behind disease and insect resistance in switchgrass (Parrish and Fike, 2005; Vogel, 2004).

As a bioenergy feedstock, switchgrass is amenable to several different conversion processes. Cellulose and hemicellulose fractions of total plant biomass can undergo biochemical conversion to produce alcohols including methane and ethanol. All biomass can undergo thermochemical processes such as pyrolysis, gasification, and direct liquification to produce methanol, syngas, and pyrolysis oils. Finally, all biomass can also undergo direct combustion, either alone or with coal to produce heat, steam and electricity (Vogel, 2004). Clearly the above processes differ and have variable ideal feedstock qualities. Therefore, care must be taken to optimize switchgrass varieties so they are best suited for particular conversion processes. Unfortunately, scientists are still

developing and optimizing these conversion processes (Vogel, 2004). Another problem that needs to be addressed is the designation of accurate, rapid, reproducible, and high throughput methods for quantifying cell wall components of bioenergy feedstocks such as switchgrass. Much of the literature on cell wall concentration and composition for agriculturally important grasses is not accurate due to the analysis methods employed. Researchers commonly rely on the detergent system on analysis, whereby samples are sequentially solubilized to provide measures of neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and ash. However, these methods can be inaccurate because of incomplete solubilization of protein and hemicellulose in the NDF and ADF steps, respectively (Boudet et al., 2003; Hatfield and Fukushima, 2005; Vogel, 2004; Vogel and Jung, 2001; Bonos and Casler, personal communication, 2008). Currently there are no accepted industry standards for accurately determining cellulose, hemicellulose, and lignin content in switchgrass. This represents one of many gaps that exist between the different areas involved in producing energy from switchgrass, whereby in this example, breeders are limited by both lack of knowledge of ideal feedstock characteristics and no accurate way to measure them (Sanderson, et al., 2004; Bonos and Casler, personal communication, 2008).

2.3. BREEDING AND GENETICS

Despite the knowledge gap described above, breeders have been able to improve switchgrass for use as a bioenergy feedstock. A brief description of some significant findings in switchgrass breeding and genetics follows. The initial step in grass breeding is to gather basic information about the reproductive biology of the species of interest (Vogel, 2004). Switchgrass yields little to no seed when self-pollinated, but the mechanism behind this proposed self-incompatibility was not known (Taliaferro and Hopkins, 1996). Martinez-Reyna and Vogel (2002) conducted a study to determine the features of the incompatibility systems that exist in switchgrass. They suggest both a prefertilization incompatibility system resembling the gametophytic S-Z system found in other grasses, as well as a postfertilization incompatibility system that does not allow plants with differing ploidy levels to intermate. The authors state a need for further research on incompatibility mechanisms in switchgrass in order to produce hybrid cultivars. Martinez-Reyna and Vogel (1998) performed the first successful controlled cross between upland and lowland switchgrass plants. While the two varieties used in the crosses differed in ecotype, they were both tetraploids. In this study, Martinez-Reyna and Vogel (1998) reported a new technique for emasculating and hybridizing switchgrass. Emasculations proved to be successful when the top of the stigmas could be seen through the transparent tips of the palea and lemma. Emasculations and hybridizations were made before natural pollen shed.

Despite being a primarily outcrossing wind-pollinated species, little is known about switchgrass pollen biology. Ge et al. (2011) investigated pollen size, viability,

longevity, and the effects of several environmental factors on pollen in Alamo, Cave-in-Rock, and transgenic Alamo switchgrass. Cave-in-Rock pollen was found to be larger than that of Alamo, and no difference in size was observed between transgenic and control Alamo pollen. Staining with aniline blue and Lugol solution was unable to detect a difference between fresh and dead pollen. Pollen viability and longevity decreased with increasing temperatures, with Cave-in-Rock demonstrating a longer half-life than that of Alamo. Relative humidity levels below 40% reduced pollen longevity in Alamo, and sunny atmospheric conditions greatly reduced viability in both cultivars. Ecker et al. (2013) investigated pollen longevity and dispersion in switchgrass cultivars Blackwell, Kanlow, and Alamo. Predicted half-life of released pollen was reported as 17.3 minutes. The simulation used in this study showed that pollen could travel 3.5 km from the source under mild wind conditions, and up to 6.5 km from the source under turbulent wind conditions, with the majority of pollen being deposited closer to the source.

Martinez-Reyna, et al. (2001) studied chloroplast DNA (cpDNA) polymorphisms, morphological traits, and meiotic stability in upland x lowland hybrids of switchgrass. They crossed 'Kanlow', a lowland tetraploid, with 'Summer', an upland tetraploid using Martinez-Reyna and Vogel's (1998) emasculation and hybridization technique. All morphological traits evaluated (pubescence, foliage color, and seed size) were intermediate in hybrids except for seed width, and all hybrids produced viable seed. The authors reported predominant maternal inheritance of cpDNA (but could not rule out some paternal inheritance), and bivalent chromosome pairing between hybrids, indicating a high degree of homology between chromosomes of the two genomes. This suggests

that the transfer of genes between ecotypes is possible by meiotic homologous recombination, and that the two ecotypes are closely related.

Knowledge of the ploidy level of germplasm is important because a trait that may be simply inherited in a diploid may appear to be inherited in a quantitative manner in a polyploid because of the number of genes segregating. In addition, switchgrass plants of varying ploidy levels do not readily hybridize, so a breeder must know the ploidy level of their germplasm in order to cross plants successfully (Vogel and Burson, 2004). Switchgrass is high in genetic diversity, with variation in its basic chromosome number ($x = 9$), typically ranging from tetraploid to octaploid (McLaughlin, 1999). Switchgrass has small chromosomes, making them difficult to count using traditional cytological tools. Church (1940) provided the earliest account of multiple ploidy levels within switchgrass, and Neilson (1944) reported that switchgrass ranges in ploidy from $2n = 2x = 18$ to $2n = 12x = 118$. As switchgrass breeding progressed, various ploidy levels were reported for the same switchgrass ecotype or population. Much of this conflict was resolved when Hopkins et al. (1996) used both light microscopy and flow cytometry to determine that most upland cultivars are octaploid, with the exception of one upland cultivar, 'Summer' being tetraploid. All lowland types whose genomes have been examined to date have been tetraploid, while upland types range from tetraploid to hexaploid to octaploid. Thus, ploidy level is not strictly correlated to type status in switchgrass, as 'Dakotah' and 'Summer' are examples of upland tetraploid switchgrass cultivars (Hultquist et al. 1996). Flow cytometry was useful in discriminating between tetraploid and octaploid plants, but it was suggested that other methods should be utilized to verify the ploidy of plants thought to be hexaploid. Hexaploid switchgrass plants

rarely occur in nature today, and controlled crosses made between tetraploids and octaploids have produced very few progeny (Martinez-Reyna and Vogel, 2002; Taliaferro and Hopkins, 1996). Intermating between octaploids and tetraploids is believed to be prevented by post-fertilization processes that inhibit normal seed development, similar to endosperm imbalance found in other species (Missaoui et al., 2005). It is therefore possible that reports of hexaploid switchgrass (Barnett and Carver, 1967; Burton, 1942; Church, 1940; Nielson, 1944; Porter, 1966; Wullschlegel et al., 1996) were made in error, or that hexaploid switchgrass plants rarely occur in nature today. Both explanations are plausible, as microscopy and cytogenetic tools have improved over the last 70 years, and Vogel and Jung (2001) report a rapid decline of the Tallgrass Prairie that began in the 1930's with only 4% of it remaining today. The most commonly utilized methods for determining ploidy levels in plants include cytology and flow cytometry, both of which have been performed extensively in switchgrass. Therefore, an opportunity for the application of novel methods of ploidy level detection exists in switchgrass.

Switchgrass has historically been used in the US as a forage crop, and most of the early publications on switchgrass included botanical descriptions or evaluation of phenotypic variation among ecotypes (Barnett and Carver, 1967; McMillan, 1959; McMillan, 1965; McMillan and Weiler, 1959; Porter, 1966; Quinn, 1969). In the 1970s, scientists began to study switchgrass as a monoculture, with a focus on forage value and yield (Balasko and Smith, 1971; Berg, 1971). Research on switchgrass as a forage crop has continued through the present, but the species has also become interesting for other purposes, such as wind barriers and erosion control (Bilbro and Fryrear, 1997; Kemper et

al., 1992; Retta et al., 2000), streamside buffers and vegetative filter strips (Dillaha et al., 1989; Lee et al., 1999; Mersie et al., 1999), as an ornamental grass (Davidson and Gobin, 1998), a wildlife habitat (Clark et al., 1998; Clark et al., 1989; Gottwald and Adam, 1998; Schwartz and Whitson, 1987), a component of substrate used in commercial mushroom production (Royse et al., 2004), and most recently as a biofuel crop (Bouton, 2007; McLaughlin and Kszoz, 2005; McLaughlin and Walsh, 1998; Sanderson et al. 1996; Sanderson et al., 2004; Vogel, 2004; Vogel and Burson, 2004).

Breeding switchgrass for use as a biofuel in the US began in the early 1990s when the USDOE designated switchgrass as a model bioenergy species. Much of the initial breeding work involved selection for improved biomass production and feedstock quality, via recurrent restricted phenotypic selection (RRPS) (Parrish and Fike, 2005). Switchgrass breeders then modified their strategy to recurrent genotypic selection (RGS), which provided increased gains in yield (McLaughlin and Kszos, 2005). Godshalk et al. (1988) reported significant genetic variation for arabinose, galactose, glucose, xylose, hexose, pentose, and total sugars expressed as g kg⁻¹ NDF and for in vitro dry matter digestibility (IVDMD) (a laboratory test used as a plant quality index for animal feed), NDF, and ADF in switchgrass, and heritability estimates of greater than 0.5 for all traits. Vogel and Jung (2001) noted that breeding for cell wall traits such as IVDMD and lignin concentration alters these traits over breeding cycles, thereby suggesting that future breeding efforts to optimize both switchgrass yield and cell wall components for improved feedstock quality will be successful (Bouton, 2007; McLaughlin and Kszoz, 2005; Sanderson et al., 2004; Vogel and Jung, 2001).

Hopkins and Taliaferro (1997) conducted a study that investigated genetic variation for and level of acid soil tolerance at the seedling stage of 2 populations ('Kanlow' and 'Blackwell') of switchgrass. They found little or no genetic variation for acid soil tolerance in the 2 populations under controlled conditions, as both were well adapted to acidic soil conditions at the seedling stage. Due to their findings, the authors suggest that selection for greater acid soil tolerance is not reasonable, and that similar studies in the field should be conducted to further enhance understanding.

Hopkins et al. (1995a) performance of 20 elite switchgrass populations for agronomic and forage quality traits as well as genotype x environment effects on these characteristics at three locations for two years. Significant genotype by environment interactions were seen for biomass yield at heading, and differences in yield between populations was not detected. Disease ratings for stem rust and anthracnose did not differ among populations, but a significant population by location by year effect was observed. Significant population by year and population by location effects were observed for holocellulose yield and IVDMD. Multi-year, multi-location analysis of switchgrass germplasm was recommended for developing cultivars adapted to the Midwest. Similarly, Hopkins et al. (1995b) investigated genotypic variability and the impact of genotype x environment interaction for agronomic and forage quality traits in random accessions of switchgrass collected from remnant prairie sites in MN, SD, NE, IA, MO, and IL. Genotypic variation was found for yield at heading and in vitro dry matter digestibility at a vegetative growth stage. Genotype x environment interactions were significant, but accessions IA34, IL62, and NE3 performed well across locations for forage yield at heading. It is suggested that switchgrass accessions with high forage yield

can be selected for from native prairie sites and utilized in future development of improved cultivars.

Das et al. (2004) investigated selection methods that would increase biomass yield in *Panicum virgatum* to establish breeding improvement. One experiment showed significant genetic variability among 3 half-sib families evaluated for biomass yield. A second experiment included in this study showed significant variation over 2 locations (OK) for biomass yield per plant, tiller length, leaf blade length, and leaf blade width. The authors found genetic variation within switchgrass populations significant enough to warrant breeding improvement of biomass yield. It was suggested that selection for increased number of tillers per plant would prove most valuable for increasing biomass yield. This report refutes the work of Muir, Sanderson, Ocumpaugh, Jones, and Reed (2001), who stated that tiller mass was more indicative of biomass production than tiller density.

Casler et al. (2002) researched the possibility of a genetic relationship between in vitro dry matter digestibility (IVDMD) and agricultural fitness of 4 herbaceous perennial grass species selected for IVDMD. *Dactylis glomerata*, *Bromus inermis*, *Panicum virgatum* 'Pathfinder', and *Medicago sativa* seedlings were established in a greenhouse and then transplanted to the field in IA, NE, and WI. In switchgrass it was reported that selection for increased IVDMD had negative effects on cold tolerance and growth rate. The authors suggest that genes controlling lignin synthesis and cold hardiness are linked and/or that pleiotropic effects of individual genes are responsible for their observations.

Vogel et al. (2002) studied genetic variability for winter survival in *Panicum virgatum* selected for high in vitro dry matter digestibility (IVDMD). It was shown that

switchgrass selected for high IVDMD, either by transformation or traditional breeding techniques, had reduced levels of agricultural fitness. These findings support the work of Casler et al. (2002) with regards to diminished agricultural fitness. Vogel et al. suggest that switchgrass selected for increased IVDMD should be tested for fitness throughout the selection process.

Based on the slow progress for gain in yield for switchgrass, Casler and Brummer (2008) conducted a study investigating theoretical gains for among-and-within family selection methods and comparing these methods to standard family and progeny-test selection methods. Among-and-within family selection was shown to be as effective or better than family selection when single-plant heritability is high, within-family selection intensity is greater than among-family selection intensity, and cycle time can be decreased.

Casler (2010) demonstrated significant gains in biomass yield in an upland tetraploid switchgrass population after two cycles of within-family selection for increased biomass yield. A mean increase of 0.36 Mg ha^{-1} per cycle of selection was reported, and while selection occurred at one location, increase were consistent across two locations with differing soil types and hardiness zones.

Casler and Smart (2013) investigated the effectiveness of using natural selection for survivorship in switchgrass swards in existing breeding methods in six switchgrass populations evaluated at five locations. While some cultivar by environment effects were seen, no negative responses were reported, and natural selection tended to increase biomass yield by 6.4% after one generation.

The use of hybrids has been utilized in maize to produce progeny with superior traits (heterosis). Developing hybrid switchgrass has the potential to greatly increase biomass yields per acre, thereby increasing the amount of ethanol per acre produced from a switchgrass field. For example, one hybrid switchgrass cultivar could contain ideal traits such as high yield, disease resistance, and drought tolerance currently found in different switchgrass ecotypes (Bouton, 2007). In order to use heterosis in a breeding program, one must first identify heterotic groups. Martinez-Reyna and Vogel (2008) evaluated potential heterotic groups of switchgrass for their potential to produce progeny with hybrid vigor. They showed that population hybrids can be used to identify heterotic groups. Within these heterotic groups, test crosses would need to be performed to identify genotypes that would yield heterotic progeny. Martinez-Reyna and Vogel (2008) suggest that in order to develop a hybrid switchgrass breeding program, one would need to classify ploidy level, ecotypes, and plant adaptation region of germplasm. Although this would require a significant amount of time and resource allocation initially, it may be worthwhile to invest in such a breeding strategy if we are to reach the goal set by The Energy Policy Act of 2005 (EPACT 2005), which issued a mandate for the use of up to 7.5 billion gallons of renewable fuel in gasoline by 2012 (Farrell et al., 2006).

Vogel (2013) compared two breeding systems using two switchgrass populations to determine which was most effective in increasing biomass yield and improving IVDMD. Between-and-within-family selection, where half-sib families are generated and the best plants within families are selected as breeding material, was more effective at improving biomass yield and IVDMD than multistep family selection, which was not as effective and was more resource intensive.

2.4. GENETIC DIVERSITY

Past studies on diversity in switchgrass utilized either molecular or morphological markers. Quinn (1969) studied the nature of ecotypic variation in upland switchgrass and its adaptive significance. He found that differences in phenology suggest ecological differentiation rather than discrete ecotypes. This study also showed intraspecific morphologic variation in plants collected from various sites. No adaptive significance to these differences was able to be determined.

Gunter et al. (1996) used random amplified polymorphic DNA (RAPD) markers to analyze genetic relatedness in switchgrass and found them helpful in separating populations into upland and lowland ecotypes. Hultquist et al. (1996) found one chloroplast DNA (cpDNA) RFLP associated with the upland-lowland ecotype classification, and designated two cytotypes, U (upland) and L (lowland). Both studies found differences between upland and lowland populations, but no variation associated with ploidy level or individual ecotypes.

Quinn (2002) studied amounts of genetic variability and phenotypic plasticity in phenology in *Panicum virgatum* and *Sporobolus cryptandrus*. He found significant variation in phenology among populations and among clones within populations. Switchgrass showed more genetic variation among clones in a population as well as greater phenotypic plasticity than sand dropseed. No consistent relationship between amounts of clonal genetic variations and plasticity was found, and Quinn stated a need for further experimentation on species diversity.

Casler et al. (2004) evaluated 20 switchgrass populations that originated from TX to SD at five locations to investigate population x location interaction and existence of differential latitudinal adaptation over two years for several traits including yield, survival, plant height, maturity, lodging, dry matter, holocellulose, lignin, and IVDMD. Significant population x location interaction attributed mainly to latitude and germplasm origin was seen for survival, yield, and plant height. However, a significant amount of variation within populations was also observed. It was suggested that lowland populations should not be moved north of their origin by more than one hardiness zone due to risk of winter injury, and upland populations should not be moved south of their origin by more than one hardiness zone due to risk of loss of biomass yield.

Casler (2005) utilized measurements of morphological variables to characterize phenotypic variability among switchgrass collected from prairie remnant sites in the Northern US as well as switchgrass cultivars. He also found a distinction between upland and lowland ecotypes, and that a high degree of phenotypic variability was present between populations collected from sites within close proximity. Missaoui et al. (2006) utilized RFLPs to assess genetic diversity between three switchgrass populations (two lowland tetraploids and one upland tetraploid) as well as sequencing of the chloroplast trnL intron in 34 switchgrass accessions and again found a distinction between upland and lowland ecotypes. Casler et al. (2007) utilized RAPDs to identify structural patterns and spatial variation of switchgrass populations from the northern and central US. No distinction could be made between switchgrass cultivars and prairie remnant populations, and little to no marker variation was associated with geographic zones. Narasimhamoorthy et al. (2008) utilized expressed sequence tag-simple sequence repeats

(EST-SSR) to determine genetic variability within and among 31 switchgrass populations as well as flow cytometry to determine any relationship between ploidy level and genetic variation. They reported the distinction between upland and lowland ecotypes and no variation associated with ploidy level as in previous studies, yet found some evidence of clustering of populations based on geographic location.

2.5. BIOTECHNOLOGY

While traditional breeding techniques show promise in improving switchgrass as a bioenergy crop, biotechnology may also play an important role in increasing efficiency of switchgrass breeding through the use of marker assisted selection or plant transformation (Bouton, 2007; McLaughlin and Kszos, 2005; Sanderson et al., 2004; Vogel, 2004; Vogel and Burson, 2004; Vogel and Jung, 2001). There are few reports of the use of genomic and transgenic technologies in switchgrass (Bouton, 2007; Missaoui et al., 2005; Tobias et al., 2005). Hultquist et al. (1996) conducted a study investigating whether cpDNA restriction fragment length polymorphisms (RFLP) occur in switchgrass cultivars that differ in ploidy level or ecotype. They surveyed eighteen (18) cultivars of switchgrass for cpDNA polymorphisms. One polymorphism was detected, and was associated with the upland and lowland ecotype designation for switchgrass. The authors designated two cytoplasm types (cytotypes), 'L' and 'U', that correspond to lowland and upland switchgrass ecotypes respectively, and demonstrated the probe/enzyme combination of BamHI/pLD 5 can be used to distinguish between upland and lowland ecotypes (Vogel, 2004). Martinez-Reyna et al. (2001) confirmed findings of Hultquist et al. (1996) of a BamHI site polymorphism between upland and lowland ecotypes and showed that it occurred outside the chloroplast *rbcl* gene region. Missaoui et al. (2006) reported a polymorphism in the non-coding chloroplast *trnL* (UAA) intron, where all twelve (12) accessions classified as lowland (out of 34) contained a 49 bp deletion. However, only three (3) of these were of known classification, while the other nine (9) were classified based on morphology and chloroplast *trnL* intron deletion. To date there

is no published information on variation within the nuclear genome that further differentiates the two cytotypes (Bouton, 2007; Tobias et al., 2005).

Huang et al. (2003) studied genetic variation in the nuclear gene encoding plastid acetyl-CoA carboxylase from 6 cultivars of *Panicum virgatum* in an attempt to define evolutionary relationships between these cultivars. This was the first reported view of genetic variation at the gene sequence level. The authors stated that tetraploid and octaploid switchgrass ecotypes had a tendency to form 2 separate groups of populations (upland and lowland), which supports findings of Gunter et al. (1996), and Hultquist et al. (1996). In addition, the close relationship between Blackwell and Caddo suggested by Gunter et al. (1996) was reflected in some Acetyl-CoA carboxylase gene sequences.

Missaoui et al. (2005) constructed the first linkage map in switchgrass based on a mapping population of 85 individuals derived from a cross between an upland tetraploid, Summer, and a lowland tetraploid Alamo (Bouton, 2007). The authors utilized 102 restriction fragment length polymorphic (RFPL) markers and identified eight (8) homology groups of the nine (9) expected in switchgrass, and inferred that switchgrass is an autotetraploid with a high degree of preferential pairing. If breeders plan to use marker assisted selection switchgrass, useable genetic maps must be constructed and made available (Bouton, 2007). More recently, Serba et al. (2013) developed switchgrass linkage maps using a full-sib pseudo-testcross mapping population that was derived from a cross between a tetraploid lowland Alamo genotype and a tetraploid upland Summer genotype, the first that includes both upland and lowland ecotypes. Maps were highly collinear and showed the two parents have similar recombination rates, and that both maps were similar to previously published maps. The authors highlight the potential use

of the maps to identify quantitative trait loci associated with traits of interest, especially biomass yield and biomass quality.

With the advent of transformation technology, breeders are now able to move genes from almost any organism into their organism of interest. Richards et al. (2001) reported the first transformation in switchgrass using particle bombardment. Richards et al. (2001) constructed a dual marker plasmid (GRP-BAR) for use in *Panicum virgatum* transformation. The technique had been used previously in *Festuca rubra* and *F. arundinacea*. A particle inflow gun was used to bombard embryonic callus tissue with the constructed plasmid. GFP was observed in leaf tissue and pollen of transgenic plants, and the bar transgene was inherited in T₁ progeny.

In 2002, Somleva et al. gave the first published report of Agrobacterium-mediated transformation in switchgrass. Somleva et al. (2002) evaluated the efficiency of Agrobacterium-mediated transmission of transgenes into *Panicum virgatum*. The GFP-BAR plasmid used by Richards et al. (2001) was also utilized in this study. Switchgrass was the second forage or turf grass (*Agrostis palustris* utilized it first) to use Agrobacterium-mediated transformation. Transgenes were sexually transmitted through both male and female gametes and expressed in T₁ progeny. The greatest efficiency was found when embryogenic calluses and somatic embryos were used as targets for infection, with somatic embryos showing superior performance. While this technology is available for use in switchgrass, caution must be exercised before transformed switchgrass plants are released. As a native species that occurs in most of the eastern two-thirds of the US, the likelihood that transgenes will be transferred to existing switchgrass populations via pollen is great. In fact, Casler (2012) stated "...deregulation

of any switchgrass transgene is guaranteed to result in its dissemination and introduction into natural populations”. It is also possible that transgenes could be transferred to wild relatives (Bouton, 2007; Vogel and Burson, 2004). No one has explored the implications of such events in switchgrass scientifically, but Vogel and Burson (2004) report the possibility of the occurrence of problems such as loss of genetic diversity in native genotypes, loss of population sizes and structure via the introduction of deleterious or lethal alleles, and unforeseen changes in plant-animal interactions. It is clear that a better understanding of the switchgrass genome is needed (Bouton, 2007; Huang et al., 2003; Martinez-Reyna and Vogel, 2008) if swift progress is to be made in utilizing switchgrass as a bioenergy crop. Information is lacking on switchgrass genomics, genetic variation, taxonomy, genetic mapping, and transgenic technologies. However, progress has been made in improving switchgrass for use as a forage and bioenergy crop, despite these large gaps in knowledge (Parrish and Fike, 2005).

2.6. SEED

One of the advantages switchgrass has over other bioenergy feedstocks like *Miscanthus x giganteus* is that it is established by seed, which is typically less expensive and labor-intensive than clonal establishment. However, switchgrass establishment is often slow and inconsistent, limiting its adaptation and use (Haynes et al, 1997). Switchgrass seeds have varying degrees of dormancy; however this dormancy is not well understood (Zegada-Lizarazu et al., 2012). Several studies have investigated methods of reducing dormancy in switchgrass. In a 2001 study, Shen et al. investigated optimal methods of overcoming seed dormancy in Cave-in-Rock switchgrass. Effects of prolonged stratification, post-stratification drying, restratification, and afterripening were tested. It was found that germination was increased to about 80% with 14 days of stratification at 5°C. It was also determined that if seeds are re-dried and then hydrated following the 14 days of stratification, germination decreases by half. The reoccurrence of dormancy that accompanies post-stratification drying is termed reversion. Reversion was prevented with stratification periods of 42 days and afterripening.

Beckman et al. (1993) examined switchgrass seedling emergence as affected by cold stratification, and in the greenhouse, the moistened and wetchill treatments increased seedling emergency of switchgrass 35 and 150%, respectively. In the greenhouse, solid matrix priming treatments (SMP) slightly increased the percentage of switchgrass plants with adventitious root development 5 weeks after planting but not in the field studies. Number of adventitious roots per plant were unaffected by treatment. In the field, the SMP-treated seed produced the highest seedling emergence for switchgrass under moist

planting conditions and had the potential to improve stands when seed was planted without drying. However, final seedling emergence from dry untreated seed was greater than that for SMP-treated seed under dry soil conditions.

Several studies have demonstrated the effects of seed treatments on switchgrass germination. Jensen and Boe (1991) showed that mechanical and chemical scarification increased germination in upland populations Sunburst and a North Dakota ecotype of neoteric switchgrass seed switchgrass. Scarification treatments of 15 and 30s both increased germination of seed that was one to five months old in five upland switchgrass populations. However, the effects of scarification should be evaluated under field conditions to determine effectiveness on a large scale. Similarly, Haynes et al. (1997) demonstrated that a sodium hypochlorite treatment (5.25% NaOCl for 15 min) and acid scarification (8 M H₂SO₄ for 5 min) treatment followed by a cold stratification treatment (moist storage at 5°C for 2 weeks) with resulted in an increase in final germination percentage in a northern upland switchgrass population. Additionally, several studies demonstrated the effectiveness of hydrogen peroxide, sodium nitroprusside, potassium ferrocyanide (Sarath et al., 2006; Sarath et al., 2007), and gibberellic acid (Madakadze et al., 2000) applications on improving germination in switchgrass.

Outside of seed treatments, few studies have investigated breeding efforts directed towards improving switchgrass germination and establishment (Casler, 2012). Smart et al. (2003) studied seedling growth rate, morphological development, and establishment of *Andropogon gerardii* and *Panicum virgatum* populations developed by divergent selection for tiller number while selecting for increased shoot weight. In the NE field grown plants, no major population differences in shoot weight, root weight, and

morphological root stage were detected. It was found that populations divergently selected for seedling tiller number showed no difference in their ability to become established. The authors suggested that selection for high shoot weight had no beneficial effects on seedling vigor in the field. Similarly, a study that investigated the effects of selection for crown node placement on switchgrass establishment demonstrated no consistent improvement in establishment (Elbersen et al., 1999). Finally, selection for reduced post-harvest seed dormancy resulted in the release of switchgrass germplasm line TEM-LoDorm, which showed improved germination (Burson et al., 2009).

Seed size has been shown to play a role in germination and establishment in several grass species (Kneebone and Cremer, 1955; Glewen and Vogel, 1984; Aiken and Springer, 1995). Aiken and Springer (1995) showed that germination and emergence increased with larger seed in switchgrass cultivars Alamo, Blackwell, Cave-in-Rock, Kanlow, Pathfinder, and Trailblazer. Smart and Moser (1999) found similar results, where germination was greater in heavy seed compared to light seed lots of switchgrass cultivars Blackwell and Trailblazer. Boe and Johnson (1987) suggested seed cleaning and breeding for large seeded cultivars as two possible methods for improving switchgrass establishment. They demonstrated that selection for high seed weight was effective in increasing seed weight of progeny from a bulk seed lot in an open pollinated field.

Boe (2003) investigated the effects of genetic and environmental influences on seed weight and seed yield for two northern switchgrass cultivars (Sunburst and Summer) grown in 2 locations in South Dakota. All plants grew taller in noncompetitive environments and with increased moisture in the competitive environment (in *Poa*

pratensis sod). Boe found that selecting for large seed is likely to increase environmental sensitivity for seed weight in both cultivars of switchgrass tested. It was also noted that a high degree of variation existed between and among cultivars of switchgrass for seed yield at one of the locations in the study.

Madakadze et al. (2003) conducted a study to estimate the appropriate base temperatures for optimal seedling growth in *Panicum virgatum*, *Andropogon gerardii*, *Sorghastrum nutans*, and *Calamovilfa longifolia*. Seedlings were grown at 4, 8, 12, 16, and 24°C in growth chambers for 4 weeks and were harvested each week. Relative growth rates were calculated at each temperature, were correlated with chilling sensitivity of the plants, and were used to determine base temperatures for growth. The four switchgrass cultivars utilized include: Cave-in-Rock, Dakota, Pathfinder, and New Jersey 50. Estimated base temperatures for the above cultivars were reported as follows: 7.26, 2.79, 5.80, and 4.49°C. Madakadze et al. (2003) suggest the variation in base temperatures is due possibly to the differing origins of cultivars or cultivar adaptation to environmental factors.

Evers and Parsons (2003) studied the interaction of soil type and moisture on emergence and seedling growth of Alamo switchgrass. In this greenhouse study, soil textures included: very fine sandy loam, loamy fine sand, silt loam, and clay, and were watered at intervals of 3 to 4, 7, 10 to 11, or 14 days. The authors found that watering frequency had a greater impact on seedling survival and growth than soil texture. The results showed that growth and development of seedlings decreased with an increase in watering intervals, with at least weekly watering necessary for more than 90% seedling survival in all soil textures.

A study by Byrd and May (2000) examined transpiration efficiency in 9 cultivars of *Panicum virgatum*. Cultivars in this study included: Alamo, Blackwell, Carthage, Cave-in-Rock, Forestburg, Greenville, Kanlow, Pathfinder, and Shelter. Transpiration efficiency differed in all cultivars, but it did not differ between water-stressed plants and well-watered plants. The authors concluded that specific leaf weight is an indicator of transpiration efficiency.

Van Esbroek et al. (2003) studied the effects of photoperiod on leaf and floral development in one northern (Cave-in-Rock) and one southern (Alamo) cultivar of *Panicum virgatum*. Plants were harvested from the field and grown in the greenhouse under natural (11.5-13 hours) and extended (16 hours, 12 hours natural + 4 hours light extension) photoperiods. For Cave-in-Rock, panicle emergence was delayed by 18 days and duration of panicle exertion was extended by 17 days under the extended photoperiod. For 'Alamo', panicle emergence was not altered with the extended photoperiod, but the duration of panicle exertion was extended by 15 days. The authors concluded that photoperiod influenced duration of panicle growth and panicle size, and noted that further studies were necessary to determine whether their findings resulted from photoperiodic responses or from an indirect effect on vegetative morphology.

In another study by Van Esbroek et al. (2004), effects of photoperiod extension were examined to determine their influence on the dry matter yield of *Panicum virgatum* cultivars that originated from varying latitudes. Five cultivars of switchgrass and their latitudes used in this study include: Shelter 40°N, Cave-in-Rock 40°N, Caddo 36°N, Kanlow 36°N, and Alamo 29°N. Cave-in-Rock showed the greatest response to the extended photoperiod, while Caddo and Kanlow were variable for photoperiodic

responses. Shelter had limited growth and was not included in the analysis. Alamo was unaffected by photoperiod. Cave-in-Rock and Caddo displayed increased dry matter yields with extended photoperiods. Van Esbroek et al. (2004) suggest that dormancy for some *P. virgatum* cultivars can be overcome by a photoperiodic signal. The authors also suggest that this work and the related study they conducted mentioned above (2003) show that sensitivity to photoperiod varies among cultivars and with developmental phase in switchgrass.

2.7. MANAGEMENT

Sanderson and Reed (2000) investigated the effects of water, nitrogen, and planting density on dry weight, tiller number, leaf area, and morphological development stage of Alamo switchgrass in the field. They found that dry weight, tiller number, leaf area, and morphological development stage increased as plant spacing increased. It was reported that nitrogen input in the establishment year did not affect dry weight and tiller number, but established plants at low densities showed an increase in dry weight and leaf area with high nitrogen input. Sanderson and Reed (2000) suggest that response to competition in *Panicum virgatum* is controlled by aboveground resources.

Muir et al. (2001) conducted a study on determining optimal levels of nitrogen and phosphorous fertilizers and row spacing for Alamo switchgrass production. Five varying rates of both nitrogen and phosphorous were applied to field plots in two Texas locations over three years, with tiller mass and density measurements serving as indicators of biomass production. It was determined that tiller mass was more indicative of biomass production than tiller density. Biomass production was not influenced by phosphorous addition, but addition of nitrogen (at least 168 Kg ha⁻¹ yr⁻¹) and increasing row width increased biomass production response.

Suplick et al. (2002) conducted a field and greenhouse study on how fertilizer nitrogen affects leaf appearance and laminar extension rates in *Panicum virgatum*. Five different levels of nitrogen were applied to Alamo switchgrass in the field and under a controlled environment. They found that rates of leaf appearance and extension are

affected by nitrogen application. Lamina extension rate showed a strong correlation to yield, suggesting its value as a potential indicator of yield.

Reed et al. (2002) conducted a study to determine the interaction of soil pH and cadmium application on the accumulation and allocation of cadmium in switchgrass. Cadmium is toxic to humans and many animals and can enter the food chain via grazing. It was found that switchgrass tolerates moderate soil levels of cadmium, but that it becomes an unsuitable pasture grass at these levels. In addition, the optimal pH to minimize cadmium accumulation in switchgrass was found to be near neutral.

Stroup et al. (2003) studied the effects of nitrogen and water stress on biomass production of two lowland (Alamo and Kanlow) and two upland (Blackwell and Caddo) cultivars of *Panicum virgatum* in Texas. This study found that nitrogen availability had a greater affect on growth potential than water availability. In general, the lowland cultivars produced greater biomass yields than the upland cultivars, but upland cultivars showed less sensitivity to water stress. Alamo had the greatest biomass production of all cultivars tested, and the authors suggest its use for forage and biomass production in central Texas.

Vogel et al. (2002) investigated optimum harvest periods and nitrogen application rates for biomass production of switchgrass in the Midwest. Established stands of Cave-in-Rock switchgrass were evaluated in 2 locations, IA and NE. The authors found that optimum yields were obtained when switchgrass was harvested at maturity stages ranging from full emergence of panicle from the boot to post-anthesis and fertilized with a nitrogen rate of 120 Kg ha⁻¹.

In another evaluation, Thomason et al. (2004) studied the effect of nitrogen application rate and harvest time on switchgrass biomass at two locations in OK. They reported that harvest frequency and time had the greatest impact on biomass production, with three harvests per year giving the best results. In contrast to work done by Vogel et al. (2002), Thomason et al. showed greatest yield with a nitrogen application rate of 488 Kg ha⁻¹.

3. GENETIC DIVERSITY OF TWELVE SWITCHGRASS POPULATIONS USING MOLECULAR AND MORPHOLOGICAL MARKERS

3.1. INTRODUCTION

Switchgrass (*Panicum virgatum* L.) is a warm season, C₄ perennial grass native to most of North America. It has numerous applications including hay, forage, habitat restoration and erosion control as well as a component in seeded native grass mixtures and buffer strips (Missaoui et al., 2005). Switchgrass has also been selected by the U.S. Department of Energy (US-DOE) as a model bioenergy feedstock species. Switchgrass is an outcrossing polyploid that has been classified into upland and lowland ecotypes based on morphology and habitat preference (Das et al., 2004). Upland ecotypes are commonly octaploids ($2n=8x=72$) and occasionally hexaploids ($2n=6x=54$) or tetraploids ($2n=4x=36$) and are fine-stemmed with various amounts of pubescence on the leaf blades, semi-decumbent, and broad based, with heights of 92 to 152 cm and adapted to drier habitats. The lowland ecotypes are typically tetraploid ($2n=4x=36$) and are coarse stemmed, erect, glabrous, more robust, and found in bunches in wetter sites with heights of 61 to 305 cm (Hultquist et al., 1996; Lewandowski et al., 2003).

The US-DOE launched a research effort beginning in the early 1990's on breeding, culture, and physiology of switchgrass for use as a biofuel because it demonstrates high productivity across a wide geographic range, suitability for marginal land, low water and nutrient requirements as well as positive environmental benefits (Sanderson et al., 1996). Breeding programs are dependent on genetic variation for the

development of improved cultivars. Therefore the knowledge of genetic diversity is pertinent to improving overall plant characteristics which will allow for a systematic sampling of germplasm for breeding and conservation purposes (Kumar, 1999; Rajasekar et al., 2006). Significant genetic diversity has been observed in switchgrass (Hultquist et al., 1996; Casler, 2005; Missaoui et al., 2006; Casler et al., 2007a; Narasimhamoorthy et al., 2008).

Previous genetic diversity studies in switchgrass utilized either molecular (Gunter et al., 1996; Hultquist et al., 1996; Missaoui et al., 2006; Casler et al., 2007a; Narasimhamoorthy et al., 2008) or morphological markers (Casler, 2005; Casler et al., 2007b). Random amplified polymorphic DNA (RAPD) markers were used to analyze genetic relatedness in switchgrass and separate populations into upland and lowland ecotypes (Gunter et al., 1996). Additionally, Hultquist et al. (1996) identified a chloroplast DNA (cpDNA) restriction fragment length polymorphism (RFLP) associated with the upland-lowland ecotype classification, and was able to designate two cytotypes, U (upland) and L (lowland), in switchgrass. Both studies found differences between upland and lowland populations, but no variation associated with ploidy level or individual ecotypes. RFLPs as well as sequencing of the chloroplast *trnL* intron were also shown to be useful in distinguishing between upland and lowland populations in switchgrass (Missaoui et al., 2006). RAPDs were also used to identify structural patterns and spatial variation of switchgrass populations from the northern and central US. However, switchgrass cultivars could not be distinguished from prairie remnant populations, and little to no marker variation was associated with geographic zones (Casler et al., 2007). Expressed sequence tag-simple sequence repeats (EST-SSR) and

flow cytometry were also used to determine genetic variability within and among 31 switchgrass populations to determine relationships between ploidy level and genetic variation (Narasimhamoorthy et al., 2008). Upland and lowland ecotypes were differentiated and there was some evidence of clustering of populations based on geographic location but no association with ploidy level was identified (Narasimhamoorthy et al., 2008). Morphological variables have also been utilized to characterize phenotypic variability among switchgrass ecotypes. In a study comparing prairie remnant collections in the northern US with switchgrass cultivars, morphological markers were useful in distinguishing between upland and lowland ecotypes and identified a high degree of phenotypic variability between populations collected from sites within close proximity (Casler, 2005). No studies to date on genetic diversity in switchgrass have been conducted that use both molecular and morphological markers. The utility of using both molecular and morphological markers has been demonstrated in other species, including cotton (*Gossypium hirsutum* L.) (Tatineni et al., 1996), common bean (*Phaseolus vulgaris* L.) (Gomez et al., 2004; Duran et al., 2005), peanut (*Arachis hypogea* L.) (Ferguson et al., 2004), and *Cucurbita pepo* L. (Franco et al., 2001; Ferriol et al., 2003). For example, in *C. pepo*, many landraces cannot be assigned to a given known morphotype; therefore, characterization based on the use of both molecular and morphological markers is essential for elucidating the genetic relationships of ecotypes within this species (Ferriol et al., 2003). In general, the use of both molecular and morphological markers is recommended because each data set provides complementary information with greater power of resolution in genetic diversity analyses (Marvaldi et al., 2002; Gomez et al., 2004). The use of both morphological and molecular markers

classifies genotypes better than employing only continuous phenotypic variables or only discrete phenotypic variables when assessing genetic diversity (Franco et al., 2001) and phylogenetic relationships (Marvaldi et al., 2002). Both molecular and morphological markers are also valuable for the identification of distinct populations or genotypes for conservation, optimum sites for germplasm collection, and ongoing changes in the pattern of diversity over time. Additionally, morphological and molecular markers are useful for the evaluation and utilization of genetic resources, the study of diversity of pre-breeding and breeding germplasm, and for the protection of breeder's intellectual property rights (Newbury and Ford-Lloyd, 1997; Franco et al., 2001).

Furthermore, although a significant amount of genetic diversity exists within switchgrass, little research has been conducted on the level of genetic diversity and local adaptation among different ecotypes of switchgrass currently recommended for habitat restoration and biofuel production in the northeast region of the US. The objectives of this study were to assess genetic diversity and determine molecular and morphological differences within and between 12 different switchgrass populations grown in New Jersey by examining both morphological and molecular characteristics, and to determine whether morphological, molecular, and/or combined data sets can detect ecotype and/or geographical differences between these populations.

3.2. MATERIALS AND METHODS

3.2.1. Plant Material

Switchgrass seed from 12 populations was obtained from various sources. Brooklyn, 'Carthage' (Miller et al., 2006), 'High Tide' (Miller et al., 2007; <http://plant-materials.nrcs.usda.gov/NJPMC/releases.html>), 'Shelter', and Timber germplasm sources were obtained from the Natural Resources Conservation Service – United States Department of Agriculture Plant Materials Center in Cape May NJ and represented eastern ecotypes. All of the additional germplasm sources [Argentina, 'Caddo' (Hein, 1958), 'Kanlow', 'Pathfinder' (Newell, 1968), 'Shawnee' (Vogel et al., 1996), 'Sunburst' (Boe and Ross, 1998), and Turkey] were obtained from the Plant Introduction (PI) collection curated by the Germplasm Resources Information Network (GRIN) and included standard cultivars developed in the Midwest and other germplasm sources from other countries (Table 1). Kanlow represented a lowland ecotype, while Brooklyn, Caddo, Carthage, Pathfinder, Shawnee, Shelter, and Sunburst, represented upland ecotypes (Hultquist et al., 1996; Hopkins et al., 1996; Hultquist et al., 1997; Lu et al., 1998). Carthage, High Tide, Argentina, and Turkey have not yet been classified as upland or lowland ecotypes but have morphologies consistent with the upland designation, while Timber has morphology consistent with the lowland designation (Table 1). Seed of each population was germinated in Pro-Mix HP (K.C. Shafer, York, PA) in 30.5 x 38.1 cm flats. Individual plants were transplanted to 48-celled flats and held under greenhouse conditions for approximately 8 weeks. Plants were transplanted to

a spaced-plant nursery in the spring of 2005 at the Rutgers University Plant Biology Research and Extension Farm at Adelphia, NJ. Individual plants were spaced 0.9 M apart with 12 plants per row. Four rows (or 48 genotypes) of each population were planted together and were spaced 0.9 M apart. Populations were not replicated in space; therefore, valid hypothesis tests regarding cultivar differences for phenotypic traits cannot be made. Morphological measurements were taken on 12 random individuals from each of the 12 different switchgrass populations in 2005 and 2006. Only a total of 139 individual plants were included in the morphological analysis due to death of some individual plants between 2005 and 2006. Measurements included plant height, panicle length, flag leaf height, length and width, heading date (when panicles first became visible), and anthesis date (50% flowering). Plant height was measured from the soil surface to the average height of the majority of the panicles. Panicle length was measured from the bottom node to the tip of the panicle. Flag leaf height was measured as the distance between the soil surface and the collar of the flag leaf. Flag leaf width was measured at the widest point of the flag leaf. Flag leaf length was measured from the collar to the tip of the leaf blade. Plant height, heading date, and anthesis date were measured on an individual plant basis to obtain one measurement per plant. The remaining measurements were taken from 3 panicles from each of the 12 plants per population and averaged to obtain one measurement per plant. All measurements were averaged over both years, and averages for each plant were used in subsequent analyses.

Table 1. Twelve switchgrass populations evaluated in NJ for morphological and molecular markers and their ecotype designation and origin

Population	Ecotype	GRIN Accession ID	Origin
Argentina	Upland ^a	PI 337553	Rafaela Experiment Station, Santa Fe, Argentina
Brooklyn	Upland		Brooklyn, NY
Caddo	Upland	PI 476297	Stillwater, OK
Carthage	Upland ^a	PI 421138	Carthage, NC
High Tide	Upland ^a		Chesapeake Bay area, Perryville, MD
Kanlow	Lowland	PI 421521	Wetumka, OK
Pathfinder	Upland	PI 642192	Domestic collections from NE and KS
Shawnee	Upland	PI 591824	Shawnee National Forest, IL; from 1 cycle of selection of Cave-in-Rock, selected in Nebraska
Shelter	Upland	PI 430240	Saint Mary's, WV
Sunburst	Upland	PI 598136	Near Yankton, SD
Timber	Lowland ^a		NC
Turkey	Upland ^a	PI 204907	Ankara, Turkey

^a These populations have not been characterized but exhibit characteristics very similar to the ecotype designation written

3.2.2. DNA Extraction

Leaf tissue was also collected from 12 individuals from each population for molecular marker analysis. Leaf tissue was ground in liquid nitrogen, and DNA was isolated using the Sigma® GenElute™ Plant Genomic DNA Miniprep kit (Sigma-Aldrich Co., St. Louis, MO) with the following modifications. Microcentrifuge tubes containing ground plant samples were kept on ice prior to incubation at 65° C. Five µL RNaseA (Qiagen Inc., Valencia, CA) was added to microcentrifuge tubes prior to the addition of Lysis Solution [Part A and Part B]. Binding Solution was added to flow-through liquid and was mixed by pipetting. In addition, pre-warmed Elution Solution was incubated for 5 minutes at room temperature (15-25° C) before centrifuging at maximum speed for one minute.

3.2.3. PCR Reaction and Genotyping

Publicly available microsatellite (SSR) markers derived from expressed sequence tag (EST) sequences from switchgrass were utilized for the molecular marker analysis (Tobias et al., 2006). Thirty-two SSR primer pairs (Integrated DNA Technologies, Coralville, IA) were tested for polymorphism on the 12 individuals from each population totaling 144 individual plant samples. Each polymerase chain reaction contained 10x Ramp-Taq Buffer (160 mM (NH₄)₂SO₄, 670 mM Tris-HCl pH 8.3, 0.1% Tween-20), 2 mM MgCl₂ (Denville Scientific Inc., Metuchen, NJ), 0.25 mM each dNTP, 2 µL of 500 pM/µL reverse primer, 1.5 µL of 500 pM/µL forward primer (the 5' end of the forward

primer was fluorescently labeled with one of four possible dyes - 6-FAM™, NED™, PET™, or VIC®) (Applied Biosystems, Foster City, CA), 0.5 units of Ramp-Taq™ DNA Polymerase, and 25 ng of template DNA in a total volume of 12.5 uL. PCR was performed using Applied Biosystems GeneAmp® PCR System 9700 thermocyclers with the following profile: initial denaturation at 94° C for 7 min; 30 cycles of 94° C for 30 s, 56° C for 45 s, and 72° C for 45 s; 8 cycles of 94° C for 30 s, 53° C for 45 s, and 72° C for 45 s; final extension at 72° C for 10 min. Four polymerase chain reaction products labeled with different fluorescent dyes were pooled (1µL of each reaction) and the volume was brought to 10 µL with sterile water. One µL of the pooled mixture was combined with 9 µL Hi-Di Formamide (Applied Biosystems, Foster City, CA) and 1 µL of the Genescan – 500 ROX Size Standard (Applied Biosystems Foster City, CA). The samples were heated at 95° C for 5 min and quenched on ice. Raw data was generated on the ABI 3130 genetic analyzer and genotypes were scored using GeneMapper Version 3.7 software (Applied Biosystems, Foster City, CA).

3.2.4. Morphological Data Analysis

Population means of plant measurements over both years including plant height, panicle height, flag leaf height, length, and width, heading date, and anthesis date from 139 individuals as well as phenotypic standard deviations of each mean value were determined using the Proc Means procedure in SAS Version 9.1 (SAS Institute, 2004). Given the experimental design utilized in this experiment, no statistical hypothesis tests regarding population differences for phenotypic traits, actual or implied, were used. Population means were used only as a descriptive tool without any statistical inferences. The 12 population means were then subjected to principal component analysis (PCA) using the Proc Princomp procedure in SAS (SAS Institute, 2004). Principal components were used as input variables for a cluster analysis using the unweighted pair-group method of averages (UPGMA) to generate a dendrogram using the Proc Cluster procedure in SAS Version 9.1 (SAS Institute, 2004).

3.2.5. Molecular Data Analysis

Statistical procedures for genetic analysis were developed for diploid organisms and are not sufficient for analysis of organisms with higher ploidy levels that contain more than two alleles at a given locus. Switchgrass is primarily tetraploid and octaploid and can have four or eight possible alleles at a given locus. Therefore, the polymorphic SSR bands for each individual were scored individually for presence or absence (Gunter et al., 1996; Missaoui et al., 2006; Kubik et al., 2009). This resulted in a data set of 1's

and 0's for 103 alleles (141 individuals from 12 populations). Only 141 individuals were included in the molecular analysis due to poor amplification of three individual genotypes. Nei's genetic distance matrix was calculated from presence/absence data from 141 individuals according to Nei (1972) using GenAlEx Version 6.2 (Peakall and Smouse, 2006). The genetic distance matrix was used as input for a cluster analysis using the unweighted pair-group method of averages (UPGMA) to generate a dendrogram using the Proc Cluster procedure using Ward's minimum-variance criteria in SAS Version 9.1 (SAS Institute, 2004). Ward's method utilizes an analysis of variance approach for evaluation of distances between clusters and attempts to minimize the sum of squares (SS) of any two clusters that could be formed (Ward, 1963). Genetic distances were subjected to an analysis of molecular variance (AMOVA) using GenAlEx 6.2 based on 999 permutations (Peakall and Smouse, 2006). AMOVA allows for a partitioning of molecular variance within and among populations and tests the significance of partitioned variance components using permutational testing procedures (Excoffier et al., 1992). Φ_{PT} values, analogous to F_{ST} when data are haploid or binary, are calculated in an AMOVA and represent the proportion of the total variance that is partitioned between populations (Excoffier et al., 1992; Peakall and Smouse, 2006).

3.2.6. Combined Data Analysis

Plants that had both morphological and molecular marker data were included in a third data set. This combined data set contained 110 characters, the 7 morphological measurements from the morphological marker data set and the 103 SSR alleles from the molecular marker data set, from 119 total individuals from 12 populations, with 8 to 12 individuals per population. Only 119 individuals had both morphological and molecular data due to plant death and poor amplification and were thus included in the combined analysis. Means of morphological measurements for each population, used only as a descriptive tool without any statistical inferences, were computed using the Proc Means procedure in SAS, and were subjected to a PCA using the Proc Princomp analysis in SAS to generate seven principal components. Nei's genetic distance matrix (Table 2) was calculated from the molecular presence/absence data according to Nei (1972) using GenAlEx Version 6.2 (Peakall and Smouse, 2006). The genetic distance matrix was then subjected to PCA to generate seven principal components. The seven principal components from the morphological data and the seven from the molecular marker data were combined into one data set of 14 principal components, comprising the third combined data set. This data set was then used as input for a cluster analysis using the unweighted pair-group method of averages (UPGMA) to generate a dendrogram using the Proc Cluster procedure in SAS Version 9.1 (SAS Institute, 2004).

Table 2. Pairwise population matrix of Nei's genetic distance for 12 switchgrass populations

	CAD	SHA	AR	TU	SU	KA	SHE	HT	PA	BR	TI
Shawnee (SHA)	0.028										
Argentina (AR)	0.035	0.042									
Turkey (TU)	0.072	0.064	0.083								
Sunburst (SU)	0.062	0.050	0.067	0.078							
Kanlow (KA)	0.197	0.225	0.233	0.218	0.236						
Shelter (SHE)	0.101	0.105	0.118	0.071	0.112	0.213					
High Tide (HT)	0.123	0.129	0.150	0.147	0.163	0.170	0.135				
Pathfinder (PA)	0.047	0.052	0.069	0.103	0.083	0.209	0.117	0.129			
Brooklyn (BR)	0.128	0.158	0.168	0.162	0.169	0.151	0.142	0.065	0.134		
Timber (TI)	0.169	0.193	0.192	0.189	0.204	0.073	0.188	0.113	0.180	0.110	
Carthage (CAR)	0.073	0.091	0.104	0.097	0.117	0.193	0.104	0.110	0.062	0.106	0.173

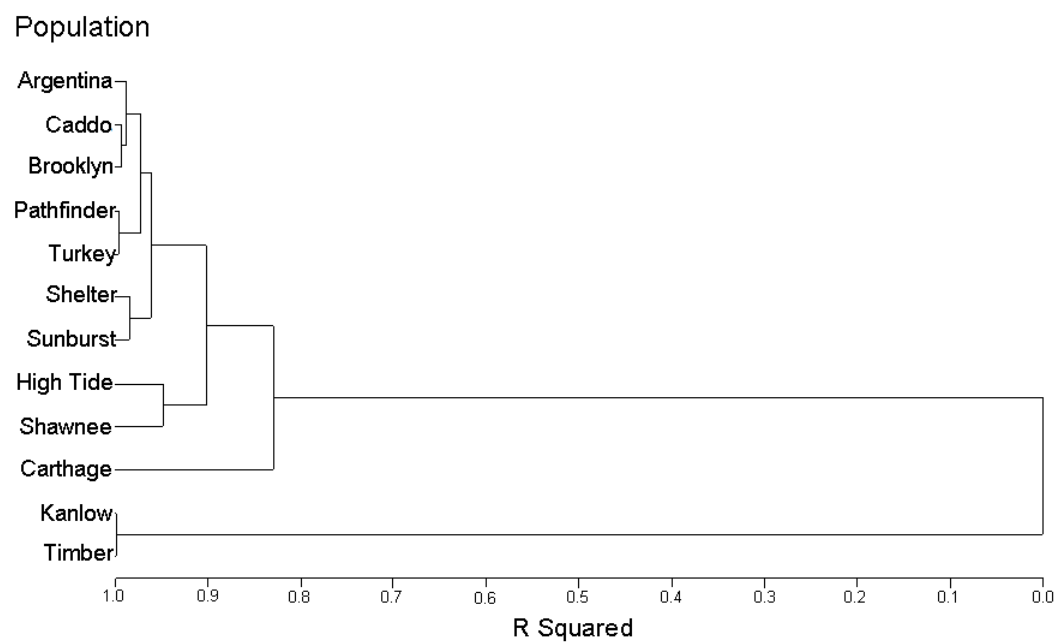
Individuals from each population were evaluated for presence or absence of 103 alleles amplified by 16 EST-SSR primer pairs. The distance values were generated based on the similarity index between populations.

3.3. RESULTS AND DISCUSSION

3.3.1. Morphological Characterization

The UPGMA analysis of morphological measurements (Figure 1) corresponded well to the ecotype origins (Table 1) for the populations evaluated with a few exceptions. The UPGMA analysis resulted in three distinct clades with the lowland types (Kanlow and Timber) forming a separate clade from the upland types (Figure 1). Within the upland group, Carthage formed a separate clade distinct from the other upland ecotypes. This is not surprising since Carthage is the only upland ecotype collected from North Carolina and is visibly distinct from other upland ecotypes evaluated (Table 3). The remaining upland populations consisting of Argentina, Caddo, Brooklyn, Pathfinder, Turkey, Shelter, Sunburst, High Tide, and Shawnee formed the third clade. These populations also grouped together in a Principal Component Analysis (data not shown). Of interest was the inclusion of the ecotypes from Argentina and Turkey in the upland clade. Similar results were observed for the Argentina and Turkey germplasm sources by Narasimhamoorthy et al. (2008). It is possible that these were introductions from North America (Freckmann and Lelongford, 1993). These results indicate that the morphological measurements utilized in the cluster analysis were effective in distinguishing between upland and lowland switchgrass ecotypes which is similar to previous studies of morphological characterization of switchgrass ecotypes (Casler, 2005).

Figure 1. Cluster analysis using UPGMA of morphological measurements of seven characters averaged over two years in 12 switchgrass populations.



Distances between clusters are expressed in R^2 values, where high R^2 values indicate more similarity.

Table 3. Means of morphological measurements of 12 switchgrass populations grown in NJ in 2005 and 2006*

Population	Ecotype	Plant Height (cm)	Panicle Length (cm)	Flag Leaf Height (cm)	Flag Leaf Length (cm)	Flag Leaf Width (cm)	Heading Date ^a (Julian)	Anthesis Date ^b (Julian)
Argentina	Upland ^c	129 ± 10 ^d	44 ± 4	78 ± 11	39 ± 4	1.0 ± 0.1	194 ± 2	235 ± 4
Brooklyn	Upland ^c	126 ± 8	44 ± 8	71 ± 8	43 ± 6	1.0 ± 0.2	186 ± 6	232 ± 5
Caddo	Upland	129 ± 15	46 ± 7	79 ± 13	41 ± 4	0.9 ± 0.2	185 ± 5	230 ± 3
Carthage	Upland ^c	156 ± 14	67 ± 8	92 ± 15	50 ± 3	1.1 ± 0.1	195 ± 8	234 ± 3
High Tide	Upland ^c	149 ± 8	48 ± 10	95 ± 6	47 ± 8	1.1 ± 0.2	197 ± 6	238 ± 6
Kanlow	Lowland	198 ± 11	58 ± 6	140 ± 9	52 ± 4	1.3 ± 0.2	208 ± 6	243 ± 5
Pathfinder	Upland	137 ± 11	49 ± 8	86 ± 12	44 ± 4	1.0 ± 0.1	190 ± 4	234 ± 5
Shawnee	Upland	133 ± 8	45 ± 10	98 ± 21	40 ± 4	0.9 ± 0.1	190 ± 5	235 ± 6
Shelter	Upland	129 ± 11	37 ± 4	79 ± 9	40 ± 5	1.0 ± 0.1	180 ± 6	233 ± 2
Sunburst	Upland	137 ± 10	37 ± 7	84 ± 13	36 ± 7	0.9 ± 0.1	184 ± 7	237 ± 6
Timber	Lowland ^c	198 ± 11	56 ± 8	138 ± 12	54 ± 4	1.2 ± 0.1	203 ± 4	243 ± 6
Turkey	Upland ^c	137 ± 10	44 ± 6	82 ± 6	41 ± 7	0.9 ± 0.1	186 ± 2	232 ± 8

*Measurements were averaged over both years

^aHeading date was recorded when panicles were first visible.

^bAnthesis date was recorded when about 50% of flowers were open.

^cThese populations have not been characterized but exhibit characteristics very similar to the ecotype designation written.

^dPhenotypic standard deviation of each mean value. Statistical comparisons between populations are not valid due to lack of spatial replication.

3.3.2. Molecular Characterization

The 32 EST-derived SSR primer pairs obtained from Tobias et al. (2006) were tested on the 12 individual plants from each population. Sixteen of the 32 primer pairs amplified a polymorphic SSR locus and were highly informative among the switchgrass populations evaluated. These 16 were chosen for genetic analysis. The 16 polymorphic loci identified between two and 12 alleles with an average of 6.5 alleles per locus in 12 populations of switchgrass. The 16 SSR loci amplified a total of 103 alleles that were used for genetic analysis.

3.3.3. Genetic diversity among switchgrass populations

Similarity coefficients of Nei's genetic distance between switchgrass populations ranged from 0.028 to 0.236 (Table 2). The highest degree of similarity was observed between Caddo and Shawnee, two upland populations, while the lowest degree of similarity (most diversity) was observed between Sunburst and Kanlow, an upland and a lowland population, respectively. Some ecotypes evaluated in this study were shown to be very closely related and shared a high degree of genetic similarity, while other ecotypes were quite diverse. Pairwise Jaccard genetic distances based on a dissimilarity index among three switchgrass populations evaluated with RFLP markers ranged from 0.70 to 0.82 between genotypes (Missaoui et al., 2006). These findings were similar to those reported in this paper, which when converted to a dissimilarity matrix (1-similarity coefficient) (data not shown) range from 0.76 to 0.97. It is possible that the values

reported here indicated a greater degree of similarity between populations due to the fact that more ecotypes were analyzed and a different marker system was used. Similarity coefficients among 14 switchgrass populations evaluated with RAPD markers ranged from 0.53 to 0.78 (Gunter et al., 1996), and indicated a greater degree of diversity between populations and differed considerably from what was shown here or by Missaoui et al. (2006).

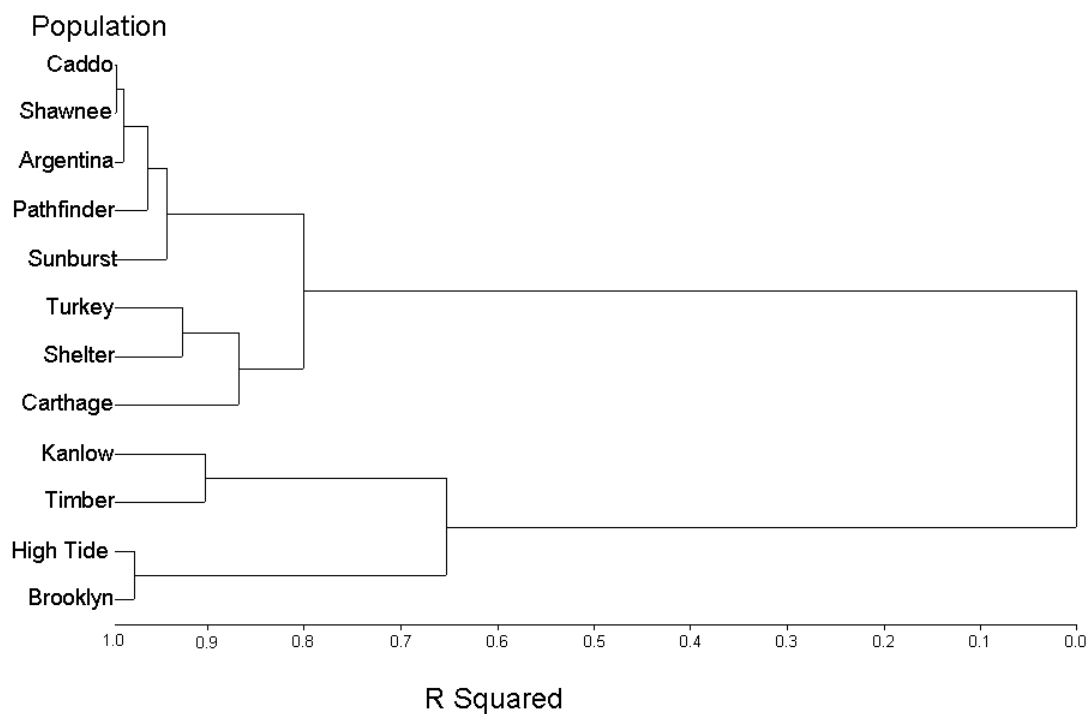
The difference in similarity coefficients between this study and Missaoui et al. (2006) and that of Gunter et al. (1996) could be due to the fact that different populations were evaluated in each study and different marker systems were utilized. In a comparison of RAPD, RFLP, AFLP, and SSR markers for utility in diversity studies in maize, it was shown that AFLPs and RFLPs were the most highly correlated marker systems for genetic distances, closely followed by SSRs and RFLPs. SSRs and RAPDs were the least highly correlated of the markers (Garcia et al., 2004). The study indicated that AFLPs, RFLPs, and SSRs all showed similar degrees of genetic diversity, while RAPDs produced results inconsistent with those of the aforementioned markers. Additionally, EST-SSRs and RFLPs are both highly conserved and therefore may result in the generation of smaller genetic distances than a marker system such as RAPDs which are not well conserved.

UPGMA analysis of EST-SSR marker variation corresponded well to collection sites (Table 1) and to the morphological analysis (Figure 1) with a few exceptions. The UPGMA analysis of the marker data resulted in four distinct clades (Figure 2). In this analysis, Kanlow and Timber, the two lowland ecotypes, formed their own group. High Tide and Brooklyn, both collected from eastern US (Table 1) formed a distinct group.

The other two clades made up the rest of the upland ecotypes and were more similar to each other than to the two previously mentioned clades (Kanlow and Timber and High Tide and Brooklyn). The smaller of the two clades contained Turkey, Shelter and Carthage while the large clade contained Caddo, Shawnee, Argentina, Pathfinder, and Sunburst. These two clades are similar to the results of the morphological analysis for the upland ecotypes, except that Carthage is included with the other upland ecotypes. It is interesting to note that the UPGMA analysis grouped some of the upland ecotypes by geographic region. High Tide was collected from MD and Brooklyn was collected from NY. Additionally, Shelter was collected from WV while Carthage from NC. This is an indication that ecotypes collected from close geographic regions may share some common alleles.

These results indicate that EST-SSR markers were effective in distinguishing between switchgrass populations. The similarity between morphological and molecular marker analysis indicates the genic SSR markers may be highlighting expressed traits with adaptive significance. EST-SSRs are derived from transcribed genes and often characterize functionally relevant polymorphisms. They have been shown to have roles in gene expression, regulation of DNA recombination, transcription and translation, as well as putative roles in providing an adaptive advantage (Li et al., 2004; Varshney et al., 2005; Chistiakov et al., 2006). The results shown here reveal that EST-SSR markers were useful in differentiating between closely related germplasm sources and could be used to supplement morphological and agronomic data used for plant variety protection and/or cultivar identification.

Figure 2. An UPGMA dendrogram analysis of 12 switchgrass populations using 103 alleles from 16 EST-SSR primer pairs.



Distances between clusters are expressed in R^2 values, where high R^2 values indicate more similarity.

3.3.4. AMOVA

Results of the Analysis of Molecular Variance (AMOVA) (Table 4) indicated that most (64%) of the molecular variation in switchgrass populations exists among individuals within populations, with lesser amounts among populations (36%). Permutation tests (based on 999 permutations) suggest that the overall Φ_{PT} was significant ($\Phi_{PT} = 0.37$, $P = 0.001$) (Table 4), which indicates the differences among ecotypes are significant. Similar results were observed in switchgrass (Gunter et al., 1996; Casler, 2005; Missaoui et al., 2006; Casler et al., 2007a; Narasimhamoorthy et al., 2008) as well as other outcrossing species including buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.] (Huff et al., 1993; Peakall et al., 1995), *Physaria bellii* G. A. Mulligan (Kothera et al., 2007), perennial ryegrass (*Lolium perenne* L.) (Kubik et al., 2001), and creeping bentgrass (*Agrostis stolonifera* L.) (Kubik et al., 2009).

Table 4. Analysis of molecular variance (AMOVA) for 141 individuals from 12 switchgrass populations based on 16 switchgrass EST-SSR markers

Source of variation	Degrees of freedom	Sum of squares	Means square	Est. var.	% variation	P value
Among populations	11	675.637	61.422	4.554	36%	0.001
Within populations	129	1022.377	7.925	7.925	64%	0.001
Total	140	1698.014		12.479	100%	0.001
$\Phi_{PT} = 0.365$						0.001
Est. var. - Estimated variation						

3.3.5. Combined Analysis

UPGMA analysis of the combined principal components revealed a cluster pattern somewhat similar to that of both the morphological and molecular cladograms. The combined analysis resulted in three distinct clades, with the lowland types (Kanlow and Timber) forming a separate clade from the upland types (Figure 3). This is similar to the results seen in the morphological (Figure 1) and the molecular (Figure 2) analyses. Within the upland groups, Carthage and High Tide formed a second clade which differs from both the morphological cluster analysis and the molecular cluster analysis (Figures 1 and 2). Carthage originated in NC while High Tide was collected in MD, the two sites being in relatively close geographic proximity to one another. The remaining upland populations, Argentina, Pathfinder, Sunburst, Turkey, Shawnee, Caddo, Brooklyn, and Shelter, comprised the third clade, similar to the morphological cluster analysis (Figure 1). The cluster analysis of the combined data set most closely resembles that of the morphological data set with one major exception. The combined data included High Tide in a clade with Carthage, whereas in the morphological analysis High Tide clustered with all the other upland populations and Carthage formed its own clade.

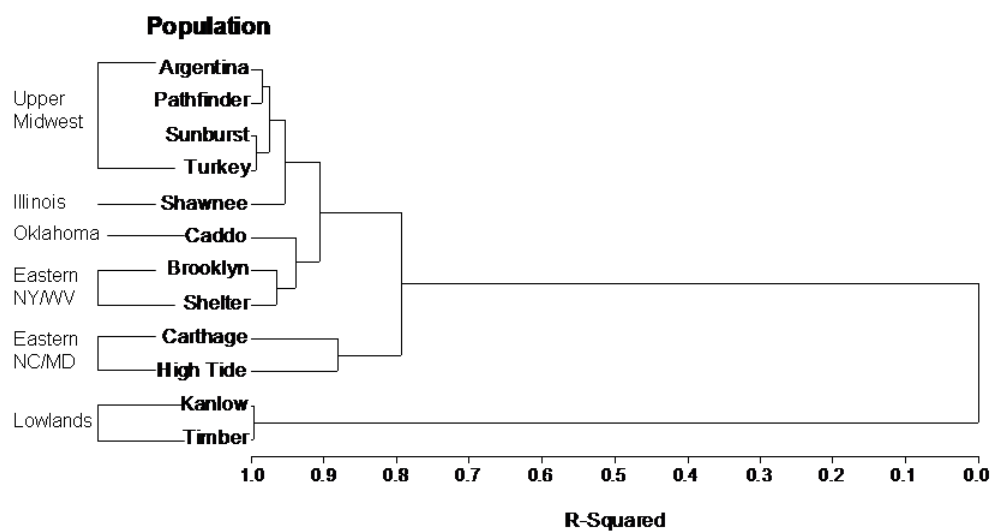
The cluster analysis of the combined data set fit most closely to geographic origin compared to all other analyses if it is assumed that the populations Argentina and Turkey are introductions of ecotypes from the upper Midwest. The upper Midwest ecotypes i.e. Sunburst, Pathfinder, and Shawnee clustered with the majority of the upland populations. Caddo, originating from OK, also grouped with the majority of the upland populations.

In a cluster analysis of SSR markers in 31 switchgrass accessions, upland ecotypes Caddo, Argentina, Turkey, Sunburst, and Shawnee were all included in the same cluster (Narasimhamoorthy et al., 2008).

The eastern upland ecotypes were split into two groups: Brooklyn and Shelter, and Carthage and High Tide. Brooklyn and Shelter are lower growing than Carthage and High Tide (personal observation) and may be the reason for the similarities observed. The lowlands formed their own clade irrespective of geographic origin, which was similar to the other analyses reported in this experiment and others (Gunter et al., 1996; Missaoui et al., 2006; Narasimhamoorthy et al., 2008). Although others have reported genetic differences among Midwest ecotypes (Casler et al., 2007a; Narasimhamoorthy et al., 2008), this is the first report of clear separation between midwestern and eastern ecotypes of switchgrass.

Although other types of analyses with different assumptions may reveal different genetic relationships, there are assumptions made with all types of genetic analyses. For example, UPGMA assumes a constant rate of nucleotide substitutions over time (Nei and Jin, 1989), while maximum-likelihood (ML) makes assumptions about the ratio of the transition rate to the transversion rate and GC content (Tateno et al., 1994). Several other genetic diversity studies have utilized both UPGMA and neighbor-joining (NJ) analyses and have found similar results from both analyses (Yokoyama et al., 2000; Zeller et al., 2000; Budak et al., 2004; Sehgal et al., 2008). Although other methods may provide insight and further discriminate genetic similarities and differences between populations, a principal component analysis supported the UPGMA results reported here (data not shown).

Figure 3. Cluster analysis using UPGMA of 12 switchgrass populations using 14 principal components, seven morphological and seven molecular components.



Distances between clusters are expressed in R^2 values, where high R^2 values indicate more similarity.

3.4. CONCLUSIONS

This is the first study to combine morphological and molecular markers to evaluate genetic diversity in switchgrass. It is also the first report to evaluate the genetic diversity of eastern switchgrass ecotypes compared to midwestern ecotypes. The combined data set corresponded best to ecotype origin and did separate upland and lowland ecotypes as well as some midwestern and eastern upland ecotypes. The distinctness between ecotypes reported here provides further evidence that regional breeding programs may be necessary to optimize genetic diversity in a given area as well as performance and biomass yield potential. The combination of morphological and EST-SSR markers were useful in differentiating between closely related germplasm sources and could have applications in identifying unknown origins of germplasm sources, plant variety protection, and/or cultivar identification.

4. BIOENERGY TRAITS OF TEN SWITCHGRASS POPULATIONS GROWN IN THE NORTHEASTERN/MID-ATLANTIC US

4.1. INTRODUCTION

Switchgrass (*Panicum virgatum* L.) is a C₄ perennial warm-season grass native to the eastern two-thirds of the United States. Switchgrass has a wide range of applications including forage, hay, habitat restoration and erosion control as well as a component in seeded native grass mixtures and buffer strips (Missaoui et al., 2005). Switchgrass has also been selected by the U.S. Department of Energy (USDOE) as a model bioenergy feedstock species due to its native status, high productivity across a wide geographic range, suitability for marginal land, low water and nutrient requirements, as well as positive environmental benefits (Sanderson et al., 1996). Switchgrass is a highly diverse species, at both the morphological and molecular levels (Casler, 2005; Casler et al., 2007; Casler et al., 2007b; Casler et al., 2004, Cortese et al., 2010; Gunter et al., 1996; Hultquist et al., 1996; Missaoui et al., 2005; Narasimhamoorthy et al., 2008). Switchgrass is an outcrossing polyploid that has been classified into two ecotypes, upland and lowland, based on habitat preference and morphology (Das et al., 2004). Upland ecotypes are commonly octaploid ($2n = 8x = 72$) and occasionally tetraploid ($2n = 4x = 36$) and are shorter, fine-stemmed, earlier maturing, and adapted to drier, more northern sites (Hultquist et al., 1996; Sanderson et al., 1996). Lowland ecotypes are tetraploid ($2n = 4x = 36$) and tend to be taller and coarse stemmed, late maturing, more resistant to rust caused by *Puccinia* spp., adapted to wetter sites, and faster growing than uplands

(Cassida et al., 2005; Hultquist et al., 1996; Lewandowski et al., 2003; Sanderson et al., 1996; Vogel, 2004).

Researchers have identified the importance of breeding for improved biomass production for specific sites (Fike et al., 2006; Fike et al., 2006b, Lewandowski et al., 2003; Vogel and Jung, 2005) and suggest that no single switchgrass cultivar is likely to have yield advantages across all locations (Casler et al., 2004; Hopkins et al., 1995). Adaptation zones of switchgrass cultivars have been established based on multi-location cultivar evaluations. These studies have shown that photoperiod, cold tolerance, heat tolerance, precipitation, and humidity are important environmental factors that limit adaptation of switchgrass populations (Casler et al., 2004; Cassida et al., 2005; Fike et al., 2006, Fike et al., 2006b, Sanderson et al., 1999). To date, much of the research on switchgrass as a bioenergy crop in the US has been conducted in the Midwest (Berdahl et al., 2005; Casler and Boe, 2003; Casler et al., 2004; Lemus et al., 2008; Vogel et al., 2002), the Southeast (Fike et al., 2006, Fike et al., 2006b, Lemus et al., 2008; Lemus et al., 2009; Muir et al., 2001, Sharma et al., 2003), and the South (Cassida et al., 2005; Cassida et al., 2005b; Fuentes and Taliaferro, 2002; Sanderson et al., 1996; Sanderson et al., 1999; Thomason et al., 2004) despite the fact that differential response of ecotypes in biomass production across latitudes have been shown. The Northeast/Mid-Atlantic region is the most populated and urbanized region of the United States and responsible for a large amount of the fossil fuels consumed in this country. It is unlikely that it will be economically feasible to transport biomass from far distances to utilize as renewable energy in the Mid-Atlantic and Northeast. Therefore it will be important to identify and breed germplasm specifically for this environment if biomass is going to be a significant

source of renewable energy for this region. New Jersey and other states throughout the region are interested in utilizing renewable energy sources (New Jersey Clean Energy Program – Board of Public Utilities, Maryland Public Interest Research Group). Although NJ is a small state, there are more than 60,700 hectares of land enrolled in the farmland preserve program that are not suitable or are not currently being used for typical agricultural crop production. New Jersey is unique in that it covers four plant hardiness zones from 7a to 5b (<http://www.usna.usda.gov/Hardzone/ushzmap.html>) and represents the northern range of the Mid-Atlantic region and the southern range of the Northeast region of the US. The objectives of this study were to evaluate the bioenergy characteristics of ten switchgrass populations grown in New Jersey and to determine which populations are best for use in biomass production in the Northeast/Mid-Atlantic region of the US.

4.2. MATERIALS AND METHODS

4.2.1. Plant Material

Switchgrass seed from 10 populations was obtained from various sources (Table 5). Seed of 9064202, Carthage, High Tide, and Timber was provided by the Natural Resources Conservation Service—United States Department of Agriculture Plant Materials Center in Cape May, NJ and represented eastern ecotypes. Seed of Cimarron, a southern ecotype, and NSU and NSL, midwestern/southern ecotypes was provided by Dr. Charles Taliaferro, Oklahoma State University. Alamo, a southern ecotype, was obtained from Turner Seed Co. (Breckenridge, TX), while Kanlow, another southern ecotype, was obtained from Ernst Conservation Seeds (Meadville, PA). Finally, Cave-in-Rock, a midwestern ecotype, was obtained from Albert Lea Seed House (Albert Lea, MN). Ecotype classifications (upland vs. lowland) are reported in Table 5. High Tide has not yet been classified as upland or lowland but has morphology consistent with the upland designation (Cortese et al., 2010).

Table 5. Ten switchgrass populations evaluated in NJ for bioenergy traits, their seed source, ecotype designation, origin, and latitude of origin

Population	Seed Source	Ecotype	Origin	Latitude ^a
Alamo	Turner Seed Co.	Lowland	Frio River, TX	28°34' N
9064202	NRCS-USDA-PMC-NJ ^b	Upland	Brooklyn, NY	40°39' N
Carthage	NRCS-USDA-PMC-NJ	Upland	Carthage, NC	35°20' N
Cave-in-Rock	Albert Lea Seed House, Inc.	Upland	Cave-in-Rock, IL	37°28' N
High Tide	NRCS-USDA-PMC-NJ	Upland ^c	Perryville, MD	39°32' N
Kanlow	Ernst Conservation Seeds	Lowland	Wetumka, OK	35°14' N
NSU	OK State University ^d	Upland	OK and southern KS	36° N
NSL	OK State University	Lowland	OK and southern KS	36° N
Cimarron	OK State University	Lowland	Central and south TX	29° N
Timber	NRCS-USDA-PMC-NJ	Lowland	NC	35° N

^a Latitudes for population origins without a city are approximate

^b Natural Resources Conservation Service-United States Department of Agriculture Plant Materials Center, Cape May, NJ

^c This population has not been characterized but exhibits characteristics very similar to the ecotype designation written

^d Samples from Oklahoma State University were obtained from Dr. Charles Taliaferro

4.2.2. Field Design and Data Collection

Seed of each population was germinated in Pro-Mix HP (K.C. Shafer, York, PA) in 30.5×38.1 cm flats. Individual plants were transplanted to 48-celled flats and maintained under greenhouse conditions for approximately 8 weeks. Temperatures ranged from 15.6°C to 26.7°C with no supplemental lighting, and plants were watered as needed. Plants were transplanted to a spaced-plant nursery in the spring of 2006 on a Holmdel sandy loam at the Rutgers University Plant Biology Research and Extension Farm at Adelphia, NJ (USDA plant hardiness zone 6b). Individual plants were spaced 0.9 m apart with 12 plants per row, two rows per replication, in a randomized complete block design with four replications for a total of 96 plants per population. The pre-emergent herbicide dithiopyr (3.5 L ha⁻¹), 48.3 kg N ha⁻¹ (as ammonium nitrate), and irrigation were applied directly after transplanting. Additional preemergent (dithiopyr) and postemergent herbicides (2,4-D, dicamba, and MCPP) were applied to control volunteer weeds in the spring of 2007 and 2008. Plants were mowed to a height of 15 cm after biomass harvest each year.

All individual plants were evaluated for winter injury, heading date, anthesis date, anthracnose disease caused by the fungus *Colletotricum navitas* (Crouch et al., 2009), lodging, tiller density, mature plant height, and biomass yield in 2007 and 2008. Winter injury, anthracnose disease, lodging, tiller density, and height were evaluated using a visual rating scale of one to 10, whereby one represented 100% winter injury, anthracnose disease, and lodging and minimum tiller density and height, and 10

represented the maximum tiller density and height and 0% winter injury, anthracnose disease, and lodging. Winter injury was evaluated in the spring of 2007 following the first growing season. No winter injury was observed in 2008. Anthracnose disease was only observed in 2008 and plants were rated on 1 August. Lodging was evaluated in late summer of both 2007 and 2008 after flowering and before harvest, while tiller density was evaluated before flowering in early June in 2007 and 2008. Plant height was rated on 18 August 2007 and 28 August 2008. Heading date was recorded as the day of year on which panicles were first visible, and anthesis date was recorded as the day of year on which 50% of florets extruded anthers. Biomass yield was determined on an individual plant basis. Twelve plants (representing subsamples) per replication from each population were harvested for a total of 48 plants per population. The same 12 plants per replication were harvested on 31 October 2007 and 2 December 2008 with a Kawasaki hedge clipper leaving a stubble height of 15 cm. The plant material was then dried at 43°C for 14 days, and weighed. The weights from each individual plant were averaged (12 per replicate), and the means of the 12 plants were reported on a per plant basis as g dried biomass plant⁻¹.

Three random samples were selected from the dried plant material of each of the first three replicates of each population in 2007 and 2008 for a total of 180 samples and sent to Dairy One (Ithaca, NY) for lignocellulosic characteristic analysis. Samples were analyzed for the following characteristics: acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin, ash, chlorine (Cl), calcium (Ca), magnesium (Mg), phosphorous (P), and potassium (K) and were presented as percentages of dry matter. Percent cellulose

was calculated as the difference between ADF and lignin, and percent hemicellulose was calculated as the difference between NDF and ADF (Hopkins et al., 1995).

4.2.3. Data Analysis

Data were analyzed using the Proc GLM procedure of the SAS statistical software program (SAS, 2004). Populations were considered to be a fixed effect, and years and reps were considered random. When year by population interactions were significant, means were compared within years (Tables 6, 7 and 8). Mean separations were based on Fisher's protected least significant difference (LSD). Differences were considered to be significant at the 5% level. Pearson's correlation coefficients and Spearman's rank correlation coefficients were determined among all data collected for both 2007 and 2008 using the CORR procedure of the SAS statistical software program.

Table 6. Mean square values for winter injury, anthracnose, lodging, tiller density, plant height, heading date, and anthesis date for ten populations of switchgrass grown in New Jersey in 2007 and 2008

		Mean Square						
Source of Variation	DF	Winter injury	Anthracnose	Lodging	Tiller density	Plant height	Heading date	Anthesis date
2007								
Rep	3	17.4339***	-	29.2481***	15.8558**	2.6930	298.0644***	32.3152
Population	9	37.7031***	-	68.2738***	53.6835***	15.0129***	12125.60***	8486.355***
2008								
Rep	3	-	26.0360***	1.0687	10.1461**	26.6373***	185.3092**	96.3708**
Population	9	-	19.9866***	30.8111***	61.4572***	71.0122***	13826.29***	5452.1655***

*Indicates significance at the 0.05 level of probability

**Indicates significance at the 0.01 level of probability

***Indicates significance at the 0.001 level of probability

Table 7. Mean square values for percent ash, chlorine, phosphorous, and potassium for ten populations of switchgrass grown in New Jersey in 2007 and 2008

Source of Variation	DF	Mean Square							
		Ash		Cl		P		K	
		2007	2008	2007	2008	2007	2008	2007	2008
Rep	3	0.000080	0.000528	0.000138*	0.0001043	0.000032	0.000103**	0.0003637	0.000290**
Population	9	0.001047	0.00177***	0.000136**	0.0000743*	0.000166***	0.000201***	0.0003268*	0.001022***

*Indicates significance at the 0.05 level of probability

**Indicates significance at the 0.01 level of probability

***Indicates significance at the 0.001 level of probability

Table 8. Mean square values for yield (kg plant⁻¹), percent cellulose, hemicellulose, lignin, calcium and magnesium for ten populations of switchgrass grown in New Jersey in 2007 and 2008

Source of Variation	DF	Mean Square					
		Yield	Cellulose	Hemicellulose	Lignin	Ca	Mg
Year	1	6331.5	0.0003157	0.035623**	0.018511**	0.000078	0.0007313**
Rep	3	317021.4**	0.0004115	0.00022949	0.0010088	0.0000021	0.00000671
Population	9	126183.3*	0.0031028*	0.00499731*	0.003048*	0.0003287**	0.0001729**
Pop x Year	9	55223.9	0.0018021	0.00319023	0.0012131	0.0000284	0.00003818

*Indicates significance at the 0.01 level of probability

**Indicates significance at the 0.0001 level of probability

4.3. RESULTS AND DISCUSSION

In this study, no significant population by year effects were observed for biomass yield, hemicellulose, cellulose, lignin, Ca, and Mg (data not shown); therefore, this data is presented as combined over years (Tables 9, 10, and 11). Population means for lodging, tiller density, mature plant height, heading date, anthesis date (Table 9), ash, chlorine, phosphorous, and potassium (Table 11) were reported separately for 2007 and 2008 due to significant population by year effects (data not shown). Several factors may explain why significant population by year effects were observed. Switchgrass plants typically reach full productive potential in the third growing season (Parrish and Fike, 2005). The data collected in 2007 corresponded to the second growing season, while the data collected in 2008 corresponded to the third. The difference in age between the plants in 2007 and 2008 may have contributed to the significant year effects. In addition, average monthly temperatures and rainfall amounts varied in 2007 and 2008 (data not shown). For example, total monthly rainfall for July and August was 18.9 cm and 7.7 cm in 2007 and 9.7 cm and 3.3 cm in 2008, while average high temperatures for June, July and August were 27.0°C, 28.2°C, and 28.6°C in 2007 and 29.7°C, 30.8°C, and 28.9°C in 2008. Therefore plants could be responding to changes in moisture and temperature between years. El-Nashaar et al. (2009) suggested that site-specific management practices as well as other environmental influences may have a large effect on mineral concentration in switchgrass populations, while environmental factors including rainfall amount were shown to have a greater impact on variation of cellulose and lignin

concentration than genotype (Cassida et al., 2005b; Lemus et al., 2002). In addition, Fike et al (2006b) observed negative correlations between biomass yield and seasonal rainfall in upland switchgrass cultivars, and Sanderson et al. (1999) stated that rainfall had large effects on switchgrass yields, where highest yields were seen in years with the greatest amounts of rainfall between April and September. Significant differences were observed between populations for all bioenergy characteristics. Results for the individual characteristics are described below.

4.3.1. Winter Injury

Winter injury was evaluated in 2007 (Table 9). No winter injury was observed in 2008. The eastern populations 9064202, High Tide, and Carthage, all upland ecotypes, showed the least amount of winter injury. The lowland populations Cimarron and Alamo showed the greatest winter injury. This is not surprising and is consistent with research that showed that moving lowland populations more than one hardiness zone north of their origin results in acute losses in survival and biomass yield (Casler et al., 2004). As seen in Table 5, Cimarron and Alamo originated at 29° and 28°34' N respectively, which falls in USDA hardiness zone 8, and the Rutgers University Plant Biology Research and Extension Farm at Adelphia, NJ is located at 40°13' N in USDA hardiness zone 6b. Winter injury was negatively correlated with both heading date ($r=-0.78$; $p\text{-value}=0.0075$) and anthesis date ($r=-0.70$; $p\text{-value}=0.024$) in 2007. As described by Vogel (2004), plants that flower late into the season remain vegetative longer into the fall and therefore are unable to reallocate resources from shoots to roots for winter survival.

Lowland populations, which are adapted to southern latitudes, flower later than upland populations and are therefore more prone to winter injury. Interestingly, all the winter injury occurred in the winter of the establishment year. Based on the observations in this study, it appears that winter injury is most likely to occur in the establishment year when plants are less mature and have smaller root systems rather than when plants are larger and more mature. However, it should be noted that winter injury can occur at any time, especially if winter stress factors are high (Berdahl et al., 2005; Casler et al., 2002).

4.3.2. Maturity

Heading date and anthesis date were highly correlated to each other (in 2007 $r=0.98$, $p\text{-value}\leq 0.0001$; in 2008 $r=0.95$, $p\text{-value}\leq 0.0001$) and reflected maturity differences between populations. Lowland populations were later maturing than upland populations, with Cimarron and NSL maturing the latest (Table 9). High Tide was the latest maturing upland population with a mean heading date of 197 and anthesis date of 229 in 2008. The earliest maturing upland population was 9064202, with a mean heading date of 182 and anthesis date of 204 in 2008 (Table 9). Populations originating from the southern US have been shown to flower later than those originating from the northern US, as photoperiod requirement is based on latitude of origin (Hopkins et al., 1995; Lemus et al., 2002; Vogel, 2004). Cultivars studied here generally followed this trend (Table 9). In addition, biomass yield was positively correlated to heading date (in 2007 $r=0.72$, $p\text{-value}=0.0186$; in 2008 $r=0.74$, $p\text{-value}=0.0150$) and anthesis date (in 2007 $r=0.74$, $p\text{-value}=0.0140$; in 2008 $r=0.83$, $p\text{-value}=0.0030$). Later maturing varieties of

switchgrass are generally more desirable for biomass production (Jakob et al., 2009) because they grow for a longer period of time before transitioning from vegetative to reproductive growth phases resulting in a longer more efficient growing season and higher biomass yields. However, widespread utilization of later maturing switchgrass varieties as a bioenergy crop is limited due to their susceptibility to winter injury (Zhang et al., 2011).

4.3.2. Anthracnose Disease

Anthracnose disease in switchgrass caused by the novel fungal species *Colletotricum navitas* (Crouch et al., 2009) was first observed at the Rutgers University Plant Biology Research and Extension Farm at Adelphia, NJ in 2006. However, the disease was not identified as *C. navitas* until 2008 and no disease incidence data was taken prior to identification. In general, the lowland populations were less susceptible to anthracnose than the upland populations (Table 9). In addition, no difference in anthracnose disease severity was observed among the five lowland populations. Among the upland varieties tested, eastern populations High Tide and 9064202 showed the least disease severity, with mean ratings of 7.1 and 6.9 respectively. NSU, an upland population that originated in the more arid southern/midwestern US, was the most susceptible population to anthracnose with a mean rating of 5.6. Interestingly, anthracnose disease was found to be negatively correlated to percent ash ($r=-0.69$, $p\text{-value}=0.025$) and positively correlated with lodging ($r=0.860$, $p\text{-value}=0.0014$) and percent hemicellulose ($r=0.73$; $p\text{-value}=0.016$) in 2008, indicating that plants more

susceptible to anthracnose disease exhibit more lodging, have less hemicellulose, and a higher ash content. The correlation between anthracnose severity and lodging is likely related to the difference between upland and lowland varieties, whereby the lowland populations evaluated in this study were less susceptible to both lodging and anthracnose disease than upland varieties; however, the correlation between anthracnose and cell wall components may be related to something different. For a disease such as anthracnose, cell wall composition can impact the ability of the pathogen to penetrate and colonize host plants (King et al., 2011). In addition, Bergstrom (2010) found that *C. navitas* and *Fusarium* spp. were superior at hydrolyzing biomass substrates in a study comparing 400 fungal strains. This may have implications as new switchgrass cultivars are developed and more is understood about the effects of fungi on cell wall components in switchgrass.

These results support the need for regionally adapted cultivars. New Jersey, located in the cool-humid Northeast region of the United States, has environmental conditions favorable to disease. Foliar pathogens have the potential to decrease biomass yields (Gravert et al., 2000; Gustafson et al., 2003; Hopkins et al., 1995; Vogel, 2004). Therefore, it will be important to determine the effects of pathogens such as anthracnose and rust on biomass yield in switchgrass, especially if it will be grown on thousands of acres as a monoculture. Future studies should be conducted on regional effects of diseases in switchgrass on biomass yield, as differential effects of disease on biomass yield of switchgrass have been demonstrated. For example, Hopkins et al. (1995) observed a significant but weak correlation between rust and biomass yield ($r=-0.12$, $p<0.05$) in the midwestern US while Cassida et al (Cassida et al., 2005) reported that rust was not correlated to yield or stand density in the South Central US.

4.3.3. Lodging

Lodging was observed in all populations in both 2007 and 2008 and differed between years (Table 9). NSL consistently had the least amount of lodging in both years. Additionally, in 2007, Carthage showed the least amount of lodging, while Cimarron showed the least amount of lodging in 2008. Upland populations Cave-in-Rock and NSU showed the most amount of lodging in both years. In general, higher lodging scores were associated with lowland populations in 2008. This was supported by a positive correlation between lodging and heading date in 2008 ($r=0.63$, $p\text{-value}=0.05$), where later flowering lowland plants showed less lodging. Lodging ratings were negatively correlated to ash content in 2007 ($r=-0.66$, $p\text{-value}=0.0390$), indicating that both low ash and less lodging can be found in populations of switchgrass. Lodging can cause yield losses by making plant biomass unavailable to harvest equipment. Various studies have reported lodging in switchgrass. Cassida et al., (2005) showed that lodging was greater in upland populations at five of six experimental locations. Upland populations were also shown to have greater lodging than lowland populations in England (Christian et al., 2002).

4.3.4. Tiller density

In 2007, upland populations Cave-in-Rock, 9064202, and High Tide had the highest mean tiller density ratings, while lowland populations Alamo and Cimarron had

the lowest mean tiller density ratings (Table 9). In 2008, Cave-in-Rock, 9064202, and Carthage had highest tiller densities while Kanlow, NSL, SL-93, and Alamo had the lowest tiller densities. In 2008, tiller densities of all upland populations were higher than all lowland populations evaluated. Within the lowland populations in both 2007 and 2008, Timber had the highest mean tiller density ratings of 6.5 and 5.8 respectively. In general, tiller density tended to decrease within each population from 2007 to 2008, which was seen in other studies, where older switchgrass plants had lower tiller densities (Cassida et al., 2005; Sharma et al., 2003). Tiller density was negatively correlated to heading date and anthesis date, respectively, in 2007 ($r=-0.79$, $p\text{-value}=0.0062$; $r=-0.71$, $p\text{-value}=0.0226$) and 2008 ($r=-0.89$, $p\text{-value}=0.0006$; $r=-0.88$, $p\text{-value}=0.0008$) as well as mature plant height in 2008 ($r=-0.64$, $p\text{-value}=0.0452$), which is likely due to the difference in plant architecture between upland and lowland cytotypes (i.e. upland ecotypes are known to have higher tiller densities than lowland ecotypes) (Vogel, 2004). Tiller density has been shown to be weakly positively correlated to biomass yield in swards of switchgrass (Boe, 2007). Therefore, selecting for increased tiller density has been recommended to increase biomass yields, particularly in a within-population selection scheme (Das et al. 2004; Mitchell et al., 2008). However, several studies that examined tiller density and biomass yield in switchgrass indicated that tiller mass is a better predictor of biomass yield than tiller density (Boe, 2007; Fike et al., 2006; Muir et al., 2001]. In this study no correlation between tiller density and biomass yield was observed (data not shown), similar to findings of Fike et al. (2006) (who determined tiller density by counting tillers 0.25m^2), and is therefore not recommended for use as an indirect selection criterion for biomass among the varieties tested.

4.3.5. Plant height

Similar to findings of Bhandari et al. (2010), differences were observed for plant height between 2007 and 2008, which may be due to the difference in plant age between the two years. Switchgrass is considered to have reached full yield potential in the third growing season (McLaughlin et al., 1999), which corresponds to data taken in 2008. Despite the difference observed between years, Timber and Kanlow were the tallest populations in both years (Table 9). The greatest differences between populations were observed in 2008, with Timber being the tallest population with a mean height rating of 8.2, while 9064202, a population selected for local habitat restoration and not biomass potential, was the shortest with a mean rating of 5.3 (Table 9). Lowland populations were taller than upland populations in 2008, consistent with described differences between the two ecotypes as well as studies showing that late-maturing lowland populations produce taller canopies (Lemus et al., 2002; Madakaze et al., 1998). Within the upland populations, eastern populations Carthage and High Tide were tallest, with 2008 mean ratings of 7.0 and 6.6 respectively. In 2008, plant height was positively correlated to heading date ($r=0.80$; $p\text{-value}=0.0060$), anthesis date ($r=0.84$; $p\text{-value}=0.0023$), and biomass yield ($r=0.75$; $p\text{-value}=0.0121$). A similar positive correlation between plant height and biomass yield ($r=0.85$, $p\text{-value}<0.0001$) was observed by Lemus et al. (2002). Therefore, our research indicates that plant height may have potential as an indirect selection criterion for improving biomass yields, more so than tiller density.

4.3.6. Biomass yield

Lowland populations Cimarron and Timber had the highest yields, with 906.9 and 803.6 g dry matter plant⁻¹, respectively (Table 9). These results differ from studies that showed Alamo as one of the best performing cultivars in the southern US (Sanderson et al., 1996; Sanderson et al., 1999). In previous studies in the southern US, lowland populations outyielded upland populations (Fike et al., 2006b; Thomason et al., 2004). In NJ, upland populations NSU and 9064202 had the lowest yields; however, the upland eastern population Carthage was high yielding with 701.5 g dry matter plant⁻¹ and not statistically different from Timber, Alamo, and Kanlow. It has been shown that early heading was associated with lower yields (Hopkins et al., 1995), and that latitude of origin has an effect on production at different latitudes (Casler et al., 2004). In this study, population 9064202 had the earliest heading date, the lowest yield (Table 9), and the northern most origin (Table 5) of the populations evaluated in this study. These results further support the idea of the need for site-specific cultivars to be utilized in bioenergy cropping systems, as it is important to utilize cultivars with photoperiod traits, morphological plasticity, and stress tolerances that correspond to the environmental characteristics of a particular region (Casler et al., 2007b). This research also indicates that both upland and northern lowland populations of switchgrass can be grown equally well with similar biomass yields in USDA plant hardiness zone 6b.

4.3.7. Lignocellulosics

Minor differences in cellulose content were observed between cultivars evaluated in this study. Lowland cultivars Timber, NSL, and Kanlow all had higher levels of cellulose than the upland populations Carthage and 9064202 (Table 10), which is similar to the findings of Cassida et al. (2005b). Hemicellulose content did not differ greatly among cultivars and ranged from 31.49% in High Tide to 26.21% in NSU (Table 10). Cellulose and hemicellulose are the polysaccharides converted to ethanol in cellulosic conversion systems; therefore high concentrations of these cell wall fractions are desirable for high ethanol yields (Girio et al., 2010; Lemus et al., 2002). Few differences were observed between cultivars for percent lignin with no observable trend between upland and lowland cultivars. As seen in Table 10, Carthage, NSU, and Alamo had higher lignin content while High Tide and Timber had the lowest lignin content. Therefore, Timber, a high yielding, high cellulose, low lignin cultivar could be promising for bioenergy production of cellulosic ethanol in NJ. Although lignin interferes with conversion of biomass to ethanol by limiting enzymatic hydrolysis of polysaccharides, it does have energy value in biomass that is combusted (Mitchell et al., 2008).

4.3.8. Ash and Minerals

A significant year by cultivar effect was observed for ash content. In 2007, mean percent ash ranged from 2.93 in NSL to 1.79 in Timber, while in 2008 it ranged from 1.85 in NSU to 0.91 in High Tide (Table 11). Higher ash contents observed in 2007 could be related to the time of sampling, where plants were harvested on 31 October in 2007

and 2 December in 2008. It has been demonstrated that switchgrass harvested later in the year has lower ash content presumably due to translocation of minerals from stems and leaves to roots (Lemus et al., 2002, McLaughlin et al., 1999). Cassida et al. (2005b) observed higher ash concentrations in upland populations, but no real pattern for ash concentration with respect to cytotype was seen in this study. Results from this study are consistent with the idea that ash concentration is primarily affected by environmental factors (Cassida et al., 2005b). In 2007, ash content was positively correlated with Ca ($r=0.64$, $p\text{-value}=0.0454$), P ($r=0.68$, $p\text{-value}=0.0310$), Mg ($r=0.65$, $p\text{-value}=0.0400$), K ($r=0.70$, $p\text{-value}=0.0257$), and Cl ($r=0.75$, $p\text{-value}=0.0126$) and with P ($r=0.63$, $p\text{-value}=0.0511$) and with Mg ($r=0.70$, $p\text{-value}=0.0237$) in 2008. Ash is the inorganic residue that remains after combustion of herbaceous biomass and is composed of alkali minerals including K, Ca, Si, Al, and Mg (Lewandowski and Kicherer, 1997; Ogden et al., 2009) so the high correlation to K and Mg observed here is not surprising. High ash content is problematic in combustion systems primarily because it causes slagging and fouling of boilers, which results in boiler damage as well as poor combustion efficiency (Lewandowski and Kicherer, 1997).

In 2007, mean chlorine content ranged from 0.34 percent in NSU to 0.22 percent in Timber and did not differ greatly between cultivars. In fact, the only difference existed between NSU and 9064202 and the remaining eight cultivars (Table 11). In 2008, mean chlorine content ranged from 0.17 percent in 9064202 to 0.12 percent in Cave-in-Rock, Alamo, and NSU. Similar to ash content, no real trend with respect to cytotype was observed for chlorine content in the populations tested in this study. Results seen here support those reported by El-Nashaar et al. (2009), who observed complex location by

cultivar interactions influencing the concentration of chlorine measured in switchgrass, where relative ranking of the populations tested was not consistent across all locations.

Calcium content was greater in upland cultivars than lowland cultivars tested in this study and ranged from 0.12 percent in NSU to 0.05 percent in Cimarron (Table 11). These results are in agreement with El-Nashaar et al. (2009), who found higher Ca concentration in upland switchgrass cultivars at three of four locations tested. Within the lowland cultivars tested in this study, no difference was observed (Table 11).

Magnesium content was highest in NSU (0.11 percent) and lowest in Cimarron, High Tide, and 9064202 (0.06, 0.05, and 0.06 respectively) as seen in Table 11. Similar to calcium, no difference in magnesium content was observed among the lowland cultivars tested in this study. As with several other minerals, no pattern in Mg content with respect to cytotype was observed in this study.

Phosphorous content in the cultivars examined in this study ranged from 0.09 percent to 0.02 percent (Table 11), similar to the findings of Christian et al. (2002). Phosphorous content was highest in NSU and Carthage in 2007 (both with means of 0.09 percent), and in Cave-in-Rock and Timber in 2008 (0.08 and 0.07 percent respectively) (Table 11). Again, as with most other minerals evaluated in this study, no real trend with respect to cytotype was observed.

Potassium content ranged from 0.82 (NSU) to 0.53 percent (High Tide) in 2007 and from 0.47 (Kanlow) to 0.10 percent (NSU) in 2008, with no trend observed with respect to cytotype (Table 11). Similarly, El-Nashaar et al. (2009) reported significant location by genotype interactions for potassium concentration with little variation

between cultivars. Christian et al. (2002) also reported few differences between cultivars for potassium concentration.

Few correlations were observed between mineral content and other traits examined in this study. Interestingly, in 2008 chlorine and potassium were positively correlated to heading date ($r=0.73$, $p\text{-value}=0.0171$; $r=0.82$, $p\text{-value}=0.0040$) and anthesis date ($r=0.68$, $p\text{-value}=0.0310$; $r=0.83$, $p\text{-value}=0.0030$) in 2008, while phosphorous was negatively correlated to heading date ($r=-0.669$, $p=0.0345$) and anthesis date ($r=-0.661$, $p=0.0374$) in 2007. These correlations are consistent with a study that showed chlorine and potassium levels in switchgrass decreased with increasing maturity, while phosphorous levels increased (Dien et al., 2006). Because all populations in the current study were harvested on the same date but varied in maturity (Table 9), upland populations were more physiologically mature at harvest than lowland populations in terms of days after anthesis. Therefore, the more physiologically mature upland populations were associated with lower levels of chlorine and potassium and higher levels of phosphorous.

In general, mineral content showed little variation among genotypes evaluated in this study with little to no observable patterns with respect to cytotype. These results are supported by findings of El-Nashaar et al. (2009), who showed a strong location effect on mineral concentration in switchgrass populations and suggested that site-specific management practices affecting soil characteristics such as chemical weed control and soil fertilization and other environmental influences may have a large effect on mineral concentrations.

Table 9. Mean winter injury, heading and anthesis dates, anthracnose disease, lodging, tiller density, plant height ratings, and biomass yield for ten populations of switchgrass grown in New Jersey in 2007 and 2008

Population ^h	Winter Injury ^a	Heading Date ^b		Anthesis Date ^c		Anthracnose Disease ^d	Lodging ^e		Tiller Density ^f		Plant Height ^g		Biomass Yield
	2007 Mean Rating	2007 Mean Day of Year	2008 Mean Day of Year	2007 Mean Day of Year	2008 Mean Day of Year	2008 Mean Rating	2007 Mean Rating	2008 Mean Rating	2007 Mean Rating	2008 Mean Rating	2007 Mean Rating	2008 Mean Rating	2007 and 2008 Mean Yield (g plant ⁻¹)
<i>Cimarron</i>	6.0 D ^j	210.5 A	216.9 A	232.5 A	244.3 A	6.8 AB	7.7 B	7.2 AB	5.6 E	5.2 D	6.0 DE	7.2 CD	906.9 A
<i>Timber</i>	6.9 BC	199.8 D	205.4 D	229.7 C	238.5 AB	6.8 AB	7.2 CD	7.0 BC	6.5 CD	5.8 C	6.9 A	8.2 A	803.6 AB
Carthage	7.1 ABC	189.9 F	196.1 E	216.8 E	229.8 BCD	6.1 CD	8.3 A	6.6 C	6.5 CD	6.9 A	6.1 DE	7.0 D	701.5 BC
<i>Alamo</i>	5.9 D	208.1 B	212.9 B	230.7 BC	242.3 A	6.7 AB	7.0 CD	6.7 C	5.0 F	4.7 E	5.8 E	7.3 C	695.1 BC
High Tide	7.2 AB	192.7 E	197.4 E	220.0 D	228.5 CDE	7.1 A	7.4 BC	6.9 BC	7.2 AB	6.0 BC	6.9 AB	6.6 E	689.2 BC
<i>NSL</i>	6.7 C	209.0 AB	216.5 A	232.7 A	243.5 A	6.7 AB	7.9 AB	7.5 A	6.2 D	5.3 D	6.5 BC	7.1 CD	676.8 BC
<i>Kanlow</i>	6.6 C	205.1 C	209.8 C	232.0 AB	237.8 ABC	6.5 BC	6.8 D	6.6 C	6.1 D	5.4 D	6.7 AB	7.8 B	668.6 BC
Cave-in-Rock	7.1 ABC	183.1 G	186.9 F	206.0 G	219.6 E	6.0 DE	5.4 E	5.7 D	6.5 CD	7.0 A	6.0 DE	6.3 F	628.5 BCD
NSU	6.6 C	183.1 G	185.4 F	209.0 F	221.8 DE	5.6 E	5.3 E	5.3 D	6.9 BC	6.2 B	6.1 DE	5.6 G	588.7 CD
9064202	7.5 A	178.9 H	181.7 G	203.0 H	203.8 F	6.9 AB	6.9 D	7.0 BC	7.4 AB	7.3 A	6.3 CD	5.3 H	430.1 D
LSD <0.05	0.5	1.6	1.7	1.8	9.5	0.4	0.5	0.4	0.5	0.4	0.4	0.3	201.1

^a Winter injury was rated on a 1-10 scale, where 1=100% injury, 10=no injury

^b Heading date was recorded as the day of year on which flower heads were first visible

^c Anthesis date was recorded as the day of year on which 50% of flowers were open

^d Anthracnose disease was rated on a 1-10 scale, where 1=100% disease, 10=no disease

^e Lodging was rated on a 1-10 scale, where 1=100% lodging, 10=no lodging

^f Tiller density was rated on a 1-10 scale, where 1=least dense, 10=most dense

^g Plant height was rated on a 1-10 scale, where 1=shortest, 10=tallest

^h Upland populations are designated by standard lettering, lowland populations by bold italic lettering

ⁱ Means with the same letter are not significantly different at $p \leq 0.05$

^j Means were rounded to the nearest tenth

Table 10. Mean percent cellulose, hemicellulose, and lignin content of ten switchgrass populations grown in New Jersey in 2007 and 2008

Cultivar ^a	Cellulose	Hemicellulose	Lignin
<i>Timber</i>	49.39 A ^b	29.09 AB	10.45 D
<i>NSL</i>	48.88 AB	28.86 AB	11.37 BCD
<i>Kanlow</i>	48.88 AB	29.91 AB	11.68 BCD
NSU	48.50 ABC	26.21 C	12.51 AB
<i>Alamo</i>	47.23 BCD	28.16 BC	12.17 ABC
<i>Cimarron</i>	47.01 BCD	30.27 AB	10.93 CD
Cave-in-Rock	46.63 CD	29.87 AB	11.71 BCD
High Tide	46.54 CD	31.49 A	10.68 D
9064202	45.84 D	29.96 AB	11.68 BCD
Carthage	46.02 D	30.21 AB	13.07 A
LSD ≤ 0.05	2.12	2.53	1.33

^aUpland populations are designated by standard lettering, lowland populations by bold italic lettering

^bMeans with the same letter are not significantly different at $P \leq 0.05$

Table 11. Mean percent ash, chlorine, calcium, magnesium, phosphorous, and potassium for ten populations of switchgrass grown in New Jersey in 2007 and 2008

Cultivar	Ash 2007 ^a	Ash 2008	Cl 2007	Cl 2008	Ca ^d	Mg	P 2007	P 2008	K 2007	K 2008
NSU ^b	2.93 A ^c	1.85 A	0.34 A	0.12 D	0.12 A	0.11 A	0.09 A	0.02 E	0.82 A	0.10 F
Cave-in-Rock	2.53 AB	1.58 AB	0.25 B	0.12 CD	0.11 AB	0.07 BC	0.07 B	0.08 A	0.68 ABCD	0.23 DE
High Tide	2.36 AB	0.91 C	0.26 B	0.13 BCD	0.11 AB	0.05 DE	0.04 D	0.03 DE	0.53 D	0.34 BC
<i>Cimarron</i>	2.28 AB	1.44 B	0.27 B	0.16 ABC	0.05 C	0.06 CDE	0.04 CD	0.03 E	0.73 AB	0.18 E
9064202	2.28 AB	1.09 C	0.33 A	0.17 A	0.10 AB	0.05 E	0.06 BC	0.04 CD	0.69 ABCD	0.32 BC
Carthage	2.25 AB	1.68 AB	0.24 B	0.15 ABCD	0.10 B	0.08 B	0.09 A	0.04 DE	0.72 ABC	0.40 AB
<i>Alamo</i>	2.20 AB	1.51 AB	0.25 B	0.12 D	0.06 C	0.07 BC	0.05 CD	0.06 BC	0.75 AB	0.22 DE
<i>Kanlow</i>	1.97 B	1.62 AB	0.23 B	0.17 AB	0.07 C	0.06 BCD	0.03 D	0.05 BCD	0.60 BCD	0.47 A
<i>NSL</i>	1.81 B	1.82 A	0.23 B	0.13 ABCD	0.06 C	0.07 BC	0.04 CD	0.03 E	0.58 BCD	0.23 DE
<i>Timber</i>	1.79 B	1.56 AB	0.22 B	0.14 ABCD	0.07 C	0.06 BCD	0.05 CD	0.07 AB	0.54 CD	0.27 CD
LSD ≤ 0.05	0.75	0.33	0.06	0.04	0.02	0.02	0.02	0.02	0.19	0.08

^a Not significant at $P \leq 0.05$

^b Upland populations are designated by standard lettering, lowland populations by bold italic lettering

^c Means with the same letter are not significantly different at $P \leq 0.05$

^d Means percent for Calcium and Magnesium are presented as averages over 2007 and 2008 since year effect was not significant

4.4. CONCLUSIONS

This is the first in depth analysis of switchgrass bioenergy traits in the Northeast/Mid-Atlantic US. Both upland and lowland populations grew well in New Jersey with adequate biomass yields [9.0 Mg ha^{-1} or more (Sanderson et al., 2012)]. Timber, an eastern lowland ecotype, was the tallest and highest yielding cultivar with minimal winter injury and anthracnose incidence, low lignin content, median tiller density, maturity, lodging, and mineral content, and high cellulose content making it a promising population for biomass production in the Northeast/Mid-Atlantic region of the US. This outcome may not apply to northern New Jersey (USDA plant hardiness zone 5b), as winter injury may be more extensive. Cimarron also had high yields and other characteristics conducive towards its use as a bioenergy crop, but showed significant winter injury in New Jersey. Carthage was the best performing upland population with minimal winter injury, high tiller density and biomass yields, and low lignin. There are currently few switchgrass cultivars that have been developed for biomass or biofuel production (Parrish and Fike, 2009). Further research including multi-location studies, sward plot studies, evaluation of new germplasm developed for bioenergy, and investigation of the effects of disease on yields of switchgrass in the Northeast/Mid-Atlantic US should be conducted if switchgrass is to be used as a bioenergy feedstock throughout the region.

5. BIOMASS ENERGY CHARACTERISTICS OF SWITCHGRASS CULTIVARS GROWN IN NEW JERSEY

5.1. INTRODUCTION

Switchgrass (*Panicum virgatum* L.), a C₄ perennial warm-season grass native to the eastern two-thirds of the United States, is emerging as a bioenergy feedstock. Switchgrass has a wide range of applications including forage, hay, habitat restoration and erosion control as well as a component in seeded native grass mixtures and buffer strips (Missaoui et al., 2005). Switchgrass has also been selected by the U.S. Department of Energy (USDOE) as a model bioenergy feedstock species for several reasons including its native status, high productivity across a wide geographic range, suitability for marginal land, low water and nutrient requirements, as well as positive environmental benefits, and is currently being grown as a bioenergy crop (Monti, 2012). Switchgrass has been shown to be a highly diverse species, both morphologically and molecularly (Casler et al. 2007a, b; Cortese et al., 2010; Zhang et al., 2011). An outcrossing polyploid, switchgrass has been classified into two ecotypes, upland and lowland, based on habitat preference and morphology (Das et al., 2004). Upland ecotypes are typically shorter, fine-stemmed, earlier maturing, and adapted to drier, more northern sites (Casler, 2012). Lowland ecotypes tend to be taller and thick stemmed, late maturing, adapted to wetter sites, more resistant to rust caused by *Puccinia* spp., and faster growing than upland ecotypes (Vogel, 2004).

Previous research has illustrated the importance of breeding improved switchgrass cultivars for specific sites (Vogel and Jung, 2001; Fike et al., 2006a) and suggests that no single switchgrass cultivar is likely to have yield advantages across multiple locations

(Casler et al., 2004; Hopkins et al., 1995). Adaptation zones of switchgrass cultivars have been established based on multi-location cultivar evaluations. These studies have shown that photoperiod, cold tolerance, heat tolerance, precipitation, and humidity are important environmental factors that limit adaptation of switchgrass populations (Casler et al., 2004; Cassida et al., 2005b; Fike et al., 2006a). To date, much of the research on switchgrass as a bioenergy crop in the US has been conducted in the Midwest, the Southeast, and the South, where differential response of ecotypes in biomass production across latitudes have been shown (Wulschleger et al., 2010). The Northeast/Mid-Atlantic region is the most populated and urbanized region of the United States and is responsible for the consumption of a large amount of fossil fuels in the USA. Because it is not economically feasible to transport biomass from far distances to utilize as a bioenergy feedstock in the Mid-Atlantic and Northeast, it will be important to identify germplasm that performs well specifically for this environment if biomass is going to be a significant source of renewable energy for the region. New Jersey and other states throughout the region are interested in utilizing renewable energy sources (New Jersey Clean Energy Program – Board of Public Utilities, Maryland Public Interest Research Group). New Jersey falls within the eastern broadleaf forest hardiness zone 6/7 gene pool for deployment of regionally adapted switchgrass germplasm, which stretches east from the 100th meridian and north from Tennessee and North Carolina to Kentucky, West Virginia, southern Pennsylvania and New York, and Massachusetts, encompassing a large portion of the eastern US (Casler, 2012). In New Jersey alone there are more than 200,000 ha of land enrolled in preservation that are not suitable or are not currently being used for commercial agricultural crop production (Hasse and Lathrop, 2010).

Biomass feedstocks like switchgrass can be converted into energy using several technologies including fermentation, direct combustion, and thermo-chemical conversion (Boateng et al., 2006). Because there is currently no existing infrastructure for a cellulosic ethanol plant or pyrolysis and/or gasification operation in the Mid-Atlantic and Northeast, direct combustion of switchgrass biomass is likely the most viable current option for conversion of this feedstock into energy. In addition to biomass yield and gross energy content, several factors can affect net energy content in biomass combustion systems. Moisture contents greater than 23% are generally considered problematic because excess moisture increases transportation costs, causes safety issues for biomass storage, and decreases combustion efficiency (Lewandowski and Kicherer, 1997). High ash content can also be problematic in combustion systems potentially causing slagging, fouling, and corrosion of boilers, again decreasing combustion efficiency (Lewandowski and Kicherer, 1997). In addition, timing of harvest has also been shown to affect biomass quality (Sanderson and Wolf, 1995; Cassida et al., 2005a). Characterization of biomass energy characteristics using wet chemistry methods is time consuming and expensive, but near-infrared reflectance spectroscopy (NIRS) has been widely utilized in quantifying the composition of agricultural products such as forages, grains, oilseeds, vegetables, sugarcane, as well as in switchgrass (Roberts et al., 2004; Vogel et al., 2011). Such characterization of biomass energy crops could be useful in speeding crop development programs and real time product analysis in the biomass conversion process. The overall objectives of this study were to evaluate biomass yield, dry matter, ash, and combustion energy content of three switchgrass cultivars over time grown in New Jersey and to

determine which cultivars and harvest times are most useful for biomass production for combustion energy systems in the Northeast/Mid-Atlantic region of the US.

5.2. MATERIALS AND METHODS

Switchgrass seed from three cultivars was obtained from different sources. Seed of Carthage (upland) and Timber (lowland) was provided by the Natural Resources Conservation Service—United States Department of Agriculture Plant Materials Center in Cape May, NJ. Seed of Alamo (lowland) was obtained from Turner Seed Company (Breckenridge, TX). Switchgrass cultivars were chosen based on overall performance and high biomass yields seen previously in New Jersey (Cortese and Bonos, 2013). Plots measuring 4.6m by 12.2m were seeded with cultivars Alamo, Carthage, and Timber, at a rate of 11.2 kg pure live seed ha⁻¹ with a drop spreader (Lesco, Cleveland, OH) at two locations in New Jersey – the Rutgers Agricultural and Research Extension Center in Upper Deerfield and the Clifford E. and Melda C. Snyder Research and Extension Farm in Pittstown. Plots were arranged in a randomized complete block design with three replicates at each location. The Upper Deerfield location was seeded on 11 June 2007 in a Chillum silt loam (Fine-silty, mixed, semiactive, mesic Typic Hapludults) and the Pittstown location was seeded on 13 June 2007 in a Quakertown silt loam (fine-loamy, mixed, active, mesic Typic Hapludults). On 15 June 2008, the Pittstown location was reseeded at the same rate used previously because of initial stand failure. Plots were irrigated for four weeks to aid establishment. Weeds were controlled with 2,4-Dichlorophenoxyacetic acid (0.7 kg ai ha⁻¹) and Dicamba (3,6-dichloro-2-methoxybenzoic acid) (0.14 kg ai ha⁻¹) applied on 16 July 2007 at Upper Deerfield and

10 July 2008 at Pittstown. All plots received 60 kg N ha⁻¹ (as ammonium nitrate) in mid-May of each post-establishment year (2008, 2009 and 2010 at Upper Deerfield, 2009 and 2010 at Pittstown), but no additional irrigation or herbicides were applied.

All plots were harvested for biomass yield with a single fall harvest. Upper Deerfield plots were harvested on 2 December 2009 and 3 December 2010, and Pittstown plots were harvested on 18 November 2009 and 21 December 2010. A single strip measuring 10.03 m² was harvested from the center of each plot with a Carter forage harvester (Brookston, IN) at a stubble height of 15 cm. Fresh weights were recorded from biomass harvested from each plot. Two 0.5 kg to 1.0 kg subsamples from each plot were dried at 43°C for 14 days and used to determine dry matter content. In addition, subsamples were taken from each plot to determine ash content and energy values. After the fall biomass yield harvest, three random subsamples consisting of 60-80 tillers were taken monthly from each plot's remaining biomass. Subsamples were hand harvested at a height of 15 cm for a total of 12 subsamples per plot of each harvest year, and were used to determine dry matter, ash, and high heating value. An effort was made to avoid harvesting shortly after a rain or snow event; however, in some cases, this was not possible and is noted in the results and discussion. Upper Deerfield subsamples were taken at the end of the third growing season on 2 December 2009, 7 January 2010, 4 February 2010, and 25 March 2010, and in the fourth season on 3 December 2010, 24 January 2011, 15 February 2011, and 24 March 2011. Pittstown subsamples were taken at the end of the second growing season on 18 November 2009, 18 December 2009, 19 January 2010, and 8 March 2010 and in the third season on 21 December 2010, 25 January 2011, 15 February 2011, and 25 March 2011. Dried subsamples were ground

with a Wiley mill to 1mm and sent to Dairy One Forage Testing Lab (Ithaca, NY) for determination of ash content (AOAC Method 942.05) and high heating value (quantified by bomb calorimetry) (<http://www.dairyone.com/Forage/Procedures/default.htm>, March 20, 2013). These same samples were also subjected to NIRS using a Unity Scientific Spectrastar TM 2400 Drawer model (Brookfield, CT). Samples were placed in a 7cm diameter holder (non-rotating) and scanned at 1 nm intervals over the wavelength range of 1200-2400 nm. Data from the Dairy One Forage Testing Lab were used as reference values to develop calibration equations and statistics using the UcalTM software package (Unity Scientific, Brookfield, CT) set at default values using the partial linear squares statistical model.

Statistical analysis was conducted using the SAS System version 9.3 (SAS Institute Inc., Cary, NC) to perform analysis of variance. Biomass yield was analyzed using the MIXED procedure of the SAS System (SAS Institute Inc., Cary, NC). Data from both years and both locations were analyzed together. Effects of year, location, their interaction, and their interactions with factors of interest were included in the model as fixed effects and removed if not significant. Dry matter content, ash content, and high heating values were analyzed using the MIXED procedure of the SAS System (SAS Institute Inc., Cary, NC) to fit a mixed effects repeated measures analysis of variance with harvest as the repeated effect. Effects of year, location, year x location, cultivar, harvest, cultivar x harvest, year x harvest, location x harvest, and year x location x harvest were included in the model as fixed effects and removed if not significant, and effects of block within year and location and year x location x block x cultivar were considered random. Data were examined for violations of assumptions by inspection of

plots of standardized residuals. Mean separations were based on Tukey's studentized range test (HSD) and were considered significant at the 0.05 level.

5.3. RESULTS AND DISCUSSION

5.3.1. Biomass Yield

Biomass yield is a complex trait influenced by many factors. Environmental effects on switchgrass yield have been shown to be great (Cassida et al., 2005a; Hopkins et al., 1995; Wulschleger et al., 2010). Regionally, factors such as rainfall, daylength, growing-degree days, and winter temperatures play a role in determining switchgrass cultivar recommendations (Wulschleger et al., 2010). In this study, a significant year by location interaction was observed for biomass yield (Table 12). In 2009, the Upper Deerfield location had a higher mean yield than Pittstown (12.9 Mg ha^{-1} vs. 9.9 Mg ha^{-1}), and in 2010 no difference between locations was observed. At Upper Deerfield, yields were greater in 2009 (12.5 Mg ha^{-1}) than in 2010 (10.0 Mg ha^{-1}), when less precipitation occurred during the growing season at this location (data not shown). At Pittstown, no difference in yield between harvest years was observed. The difference in yield between locations in 2009 may be due to difference in stand age between locations, where Upper Deerfield plots were harvested after the third growing season and Pittstown the second. Because switchgrass typically reaches full yield potential by the third growing season, yields at Pittstown were expected to increase in 2010, similar to results reported by Hoagland et al (2013). Dry conditions in 2010 may have contributed to the lack of yield increase at Pittstown; however, it is difficult to determine the degree to which stand age and environmental factors such as soil type and precipitation played a role in observed

yields. Generally, lowland cultivars are less drought tolerant, more prone to winter injury, and higher yielding than upland cultivars. However, no difference in biomass yield among cultivars or harvest years was observed, indicating that cultivars Alamo, Timber, and Carthage performed similarly across years and locations in this study. Based on these findings, both upland and lowland switchgrass cultivars can be grown successfully as bioenergy feedstocks in New Jersey. It should be noted however, that lowland cultivars have been shown to be less productive than upland cultivars near 42.2° N and north of this latitude (Casler et al., 2004; Wullschleger et al., 2010).

There is limited biomass yield data available in published literature for the cultivars tested in this study. While it is difficult to compare the yields observed here with other studies that tested the same switchgrass cultivars in other parts of the US, yields observed here fall within the range of reported yields for the same cultivars. For example, Alamo has been shown to yield 19.74 Mg ha⁻¹ in College Station, Texas, 10.88 Mg ha⁻¹ in Stephenville, Texas, 10.70 Mg ha⁻¹ in Louisiana, and 16.75 Mg ha⁻¹ in Arkansas in the southern US (Cassida et al., 2005b). In the southeast, reported yields for Alamo include 15.1 Mg ha⁻¹ in NC, 10.8-12.4 Mg ha⁻¹ in Blacksburg, VA 16.4 Mg ha⁻¹ in Orange, VA, and 17.5 Mg ha⁻¹ in WV (Fike et al., 2006b). Lemus et al. (2002) reported yields of 12.1 Mg ha⁻¹ for Alamo and 9.9 Mg ha⁻¹ for Carthage switchgrass near Chariton, Iowa. Few studies have reported switchgrass yields in the northeastern US. In Rock Springs, Pennsylvania, switchgrass yields for upland cultivar Cave-in-Rock and lowland synthetic populations ranged from 7.1 to 9.7 Mg ha⁻¹ (Sanderson, 2010). In addition, Bonos et al. (2011) reported similar yields ranging from 8.41 to 9.47 Mg ha⁻¹ for switchgrass cultivars grown in New Jersey, South Dakota, and Wisconsin.

5.3.2. Dry matter content

A significant year by location by harvest interaction was observed for dry matter content, as presented in Table 13 and Figures 4a and 4b. One of the most glaring differences between Figures 4a and 4b is the trend dry matter content follows over harvest dates. In 2009, dry matter content increased after harvest one, decreased after harvest two, and increased again after harvest three at both locations in the study. In 2010 however, dry matter content tended to increase with each consecutive harvest date. This difference between years is likely due to a rain event that preceded the third harvest in 2009 at both locations, decreasing dry matter content. Had it been dry prior to harvest three, the trend of dry matter content over harvest date in 2009 would likely have been similar to that in 2010. Overall, dry matter content tended to increase with later harvest dates in both years at both locations (Figures 4a and 4b) unless modified by weather conditions, which is consistent with previous studies (Cassida et al., 2005b; Adler et al., 2006; Hoagland et al., 2013). At both locations and in both years of the study (with the exception of harvest 3 in 2009), dry matter contents reached acceptable levels for direct harvest (less than 770 g kg⁻¹) (Lewandowski and Kicherer, 1997) by the second harvest.

In 2009, dry matter content was similar at both locations over harvest dates with the exception of harvest four, while in 2010, dry matter content differed most at harvest one. The interaction of location by harvest date for dry matter seen in Figures 4a and 4b is likely a result of differences in environmental conditions between locations at each harvest date. For example, in 2010, harvest date 1 was 3 December at Upper Deerfield and 21 December at Pittstown. Additionally, this Upper Deerfield harvest coincided with

a snow event. Therefore, it logically follows that the Upper Deerfield location had lower dry matter content than the Pittstown location at harvest one in 2010. Cassida et al. (2005b) suggested that environmental factors play the largest role in determining dry matter content. While important to note, these differences are not useful in determining optimal harvest dates at the locations in this study due to the variable nature of weather conditions.

The significant cultivar x harvest interaction observed indicates that cultivars performed differently over harvest dates (Figure 5). At harvest one, lowland cultivar Alamo, the latest maturing cultivar tested (Cortese and Bonos, 2013), had lower dry matter content than Timber and Carthage. Later maturing lowland cultivars tend to enter into dormancy more slowly than earlier maturing upland cultivars after exposure to killing frost (Mitchell and Schmer, 2012). Based on the trends observed here, later maturing cultivars, which tend to be higher yielding, have lower dry matter content at early harvest dates. This trend should be noted when harvesting biomass for combustion. In addition, this variation among cultivars decreased with later harvest, indicating that cultivar has less influence on dry matter content with later harvest dates. Despite the significant cultivar x harvest interaction, dry matter content increased in all cultivars with later harvest dates (with the exception of harvest 3 in 2009 due to the rain event). These results indicate that harvesting switchgrass in January in New Jersey allows for maximum dry matter content, and highlight the importance of not harvesting directly after a precipitation event, as this significantly affected the dry matter content of switchgrass. Dry matter contents among upland and lowland cultivars tested were comparable except

during the fall, when southern lowland cultivars like Alamo may not be fully senesced and therefore have lower dry matter content.

5.3.3. Ash content

The strong year x location effect observed (Table 14) shows that ash content averaged over harvest date was similar in 2009 and 2010 at the Upper Deerfield location but differed between years at the Pittstown location, where mean ash content was greater in 2009 than in 2010. This difference in mean ash content between years at Pittstown could be due to difference in stand age between years. At Pittstown, the data collected in 2009 corresponded to the second growing season, while the 2010 data corresponded to the third. It has been shown that ash content decreases with stand age over the first three growing seasons (Liu et al., 2013). Additionally, the difference in ash content between locations may be due to the fact that the soil at the Pittstown location has a higher clay content than the soil at the Upper Deerfield location. Ash content has been shown to be higher in bioenergy crops grown in soils with higher clay content (Burvall, 1997; Landstrom et al., 1996; Cassida et al., 2005b).

Ash content of cultivars varied across the four harvest dates in this study, indicated by a significant cultivar x harvest interaction (Figure 7). While the rank of ash content among cultivars changed across harvest dates, no difference among cultivars at each harvest date was observed; therefore, the differences in ash content between cultivars at each harvest date observed likely do not bear practical significance. Ash

content tended to decrease in all cultivars with later harvest dates, with minimum ash content (19.2 g kg^{-1}) realized by the January harvest date (harvest one, Figure 7).

The overall decrease in ash content with later harvest dates is likely due to the translocation of nutrients to the basal stem and roots, as well as leaching of minerals such as potassium from the biomass as weathering continues through the winter (Kering et al., 2013). Previous studies have reported switchgrass ash levels ranging from 3% (Adler et al., 2006) to 4.8% (Cassida et al., 2005a). Ash levels reported in this study were generally lower and therefore more preferable for use in combustion systems. It should be noted, however, that ash content is affected by environmental factors and will vary over locations and with different harvest methods.

5.3.4. Combustible Energy Content

A significant year by location interaction for combustible energy content was observed (Table 15). Combustible energy content was higher at Upper Deerfield (19.82 MJ kg^{-1}) than Pittstown (19.39 MJ kg^{-1}) in 2009, and was higher at Pittstown (20.08 MJ kg^{-1}) than Upper Deerfield (19.68 MJ kg^{-1}) in 2010. It is possible energy content increased at Pittstown from 2009 to 2010 due to the increase in stand age. Allison et al. (2012) and Liu et al. (2013) observed an increase in lignin content in switchgrass with increasing stand age. Plant tissue with increased lignin content has a higher energy content (Lewandowski and Kicherer, 1997). Based on the fact that combustible energy values were similar among all cultivars tested, across all harvest dates, and across locations in the study, the difference in combustible energy content seen in the year by

location interaction does not likely bear practical significance. Overall, energy values ranged from 19.39 to 20.08 MJ kg⁻¹ and were slightly higher than previously reported values of 17.4 MJ kg⁻¹ (McKendry, 2002), 18.3 MJ kg⁻¹ (Hoagland et al., 2013), and 18.7 MJ kg⁻¹ (Dien et al., 2006). Results observed here indicate that cultivar and harvest date do not have a significant influence on combustible energy content in switchgrass. It should be noted however, that future studies that include more cultivars, locations, and harvest dates are needed to further assess the impact of these factors on combustible energy content.

5.3.5. Near Infrared Reflectance Spectroscopy

The evaluation of biomass samples for ash and combustible energy using NIRS resulted in a calibration equation for ash with a highly significant ($P < 0.01$) R-squared value of 0.88, whereas the R-squared value for combustion energy was only 0.33. The lack of significance for the combustible energy value was likely a result of the non-significant difference among sample parameters because variation was less than 10% from minimum to maximum energy levels. With ash content, the range was more than five-fold. Overall these results suggest that for the range and types of biomass encountered in this study, NIRS could be used to rapidly analyze ash content but not combustible energy. The lack of differences among the range of genetic and environmental parameters that these samples represent would suggest that processors may need to only randomly sample a few incoming lots of biomass to ascertain the potential combustion energy of sources if 10% or less variation is acceptable.

Table 12. Analysis of variance summary for biomass yield in three switchgrass cultivars grown in Upper Deerfield and Pittstown, NJ in 2009 and 2010

	Numerator DF	Denominator DF	F Value	P Value
Year	1	8	4.26	0.0729
Location	1	8	5.38	0.0490
Year x Location	1	8	14.79	0.0049
Cultivar	2	22	2.64	0.0939

Table 13. Analysis of variance summary for dry matter content in three switchgrass cultivars grown in Upper Deerfield and Pittstown, NJ in 2009 and 2010

	Numerator DF	Denominator DF	F Value	P Value
Year	1	8	21.20	0.0017
Location	1	8	9.28	0.0159
Year x Location	1	8	1.63	0.2381
Cultivar	2	22	15.51	<0.0001
Harvest	3	90	149.81	<0.0001
Cultivar x Harvest	6	90	6.77	<0.0001
Year x Harvest	3	90	95.56	<0.0001
Location x Harvest	3	90	51.78	<0.0001
Year x Location x Harvest	3	90	4.47	0.0057

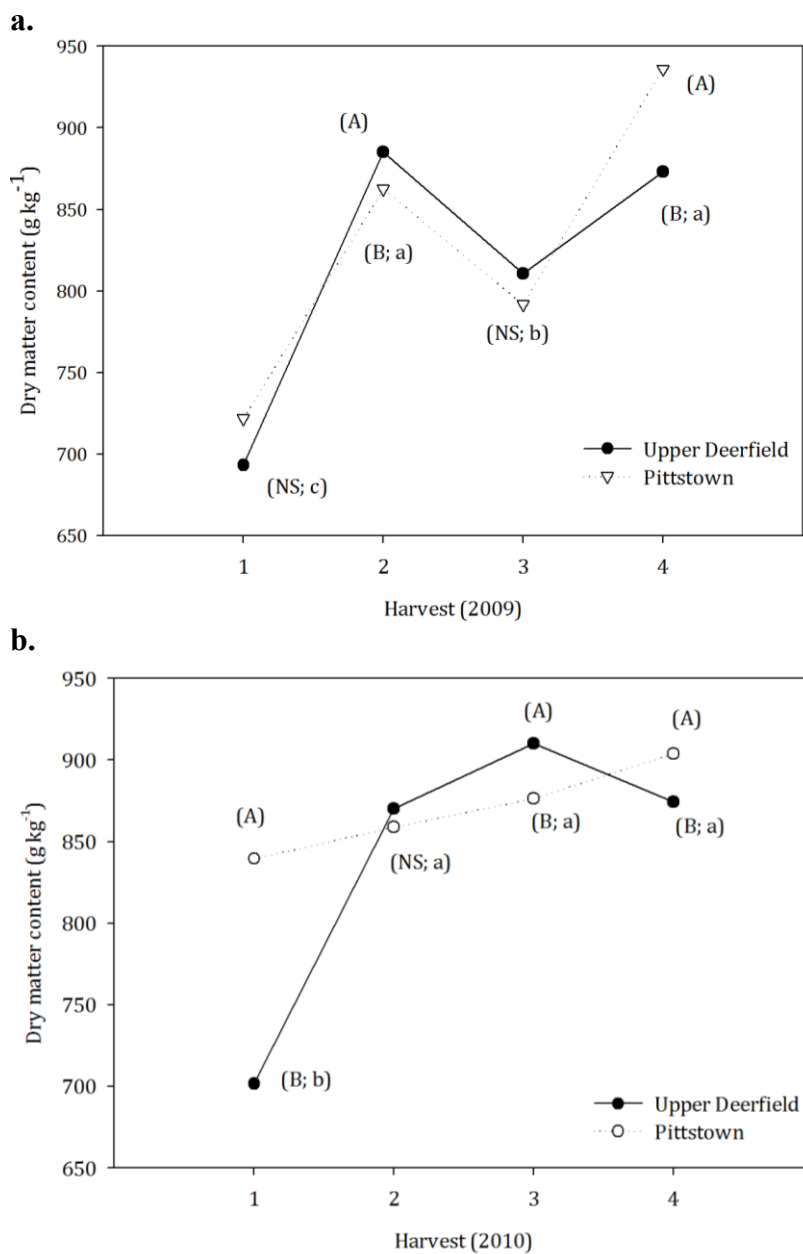
Table 14. Analysis of variance summary for ash content in three switchgrass cultivars grown in Upper Deerfield and Pittstown, NJ in 2009 and 2010

	Numerator DF	Denominator DF	F Value	P Value
Year	1	8	15.86	0.0040
Location	1	8	104.49	<0.0001
Year x Location	1	8	17.00	0.0033
Cultivar	2	22	7.19	0.0039
Harvest	2	66	3.40	0.0349
Cultivar x Harvest	4	66	2.71	0.0375

Table 15. Analysis of variance summary for combustible energy content in three switchgrass cultivars grown in Upper Deerfield and Pittstown, NJ in 2009 and 2010

	Numerator DF	Denominator DF	F Value	P Value
Year	1	8	57.42	<0.0001
Location	1	8	1.25	0.2966
Year x Location	1	8	150.29	<0.0001
Cultivar	2	22	0.69	0.5098
Harvest	3	99	1.39	0.2516
Cultivar x Harvest	6	99	0.77	0.5976

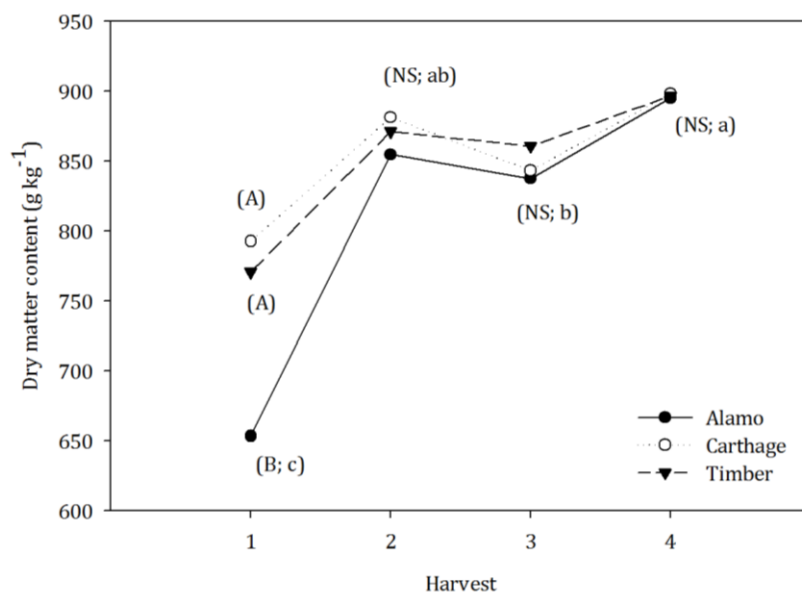
Figure 4.a. Mean dry matter content of three switchgrass varieties grown in Upper Deerfield and Pittstown, NJ in 2009 by harvest date. **b.** Mean dry matter content of three switchgrass varieties grown in Upper Deerfield and Pittstown, NJ in 2010 by harvest date.



NS - Not significant

Uppercase letters indicate difference between locations within harvest. Lowercase letters indicate difference among harvest dates ($p \leq 0.05$).

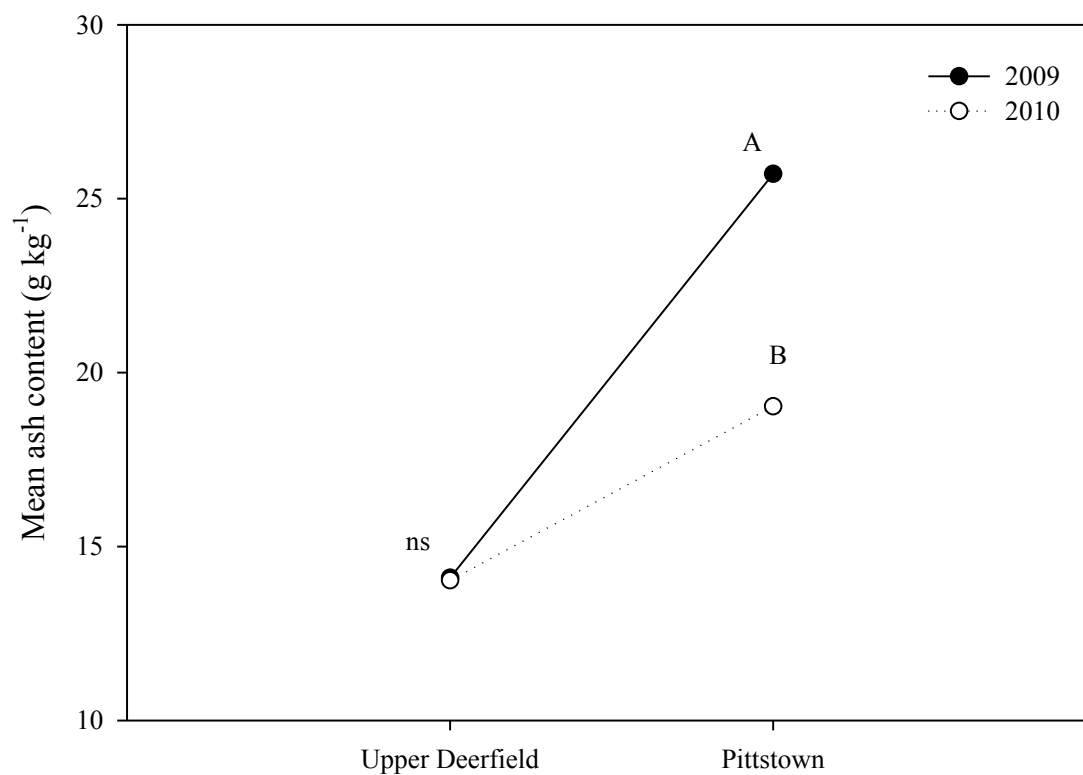
Figure 5. Mean 2009 and 2010 dry matter content of switchgrass cultivars Alamo, Carthage, and Timber grown in Upper Deerfield and Pittstown, NJ across four harvest dates.



NS - Not significant

Uppercase letters indicate differences among cultivars within each harvest. Lowercase letters indicate differences among harvest dates ($p \leq 0.05$).

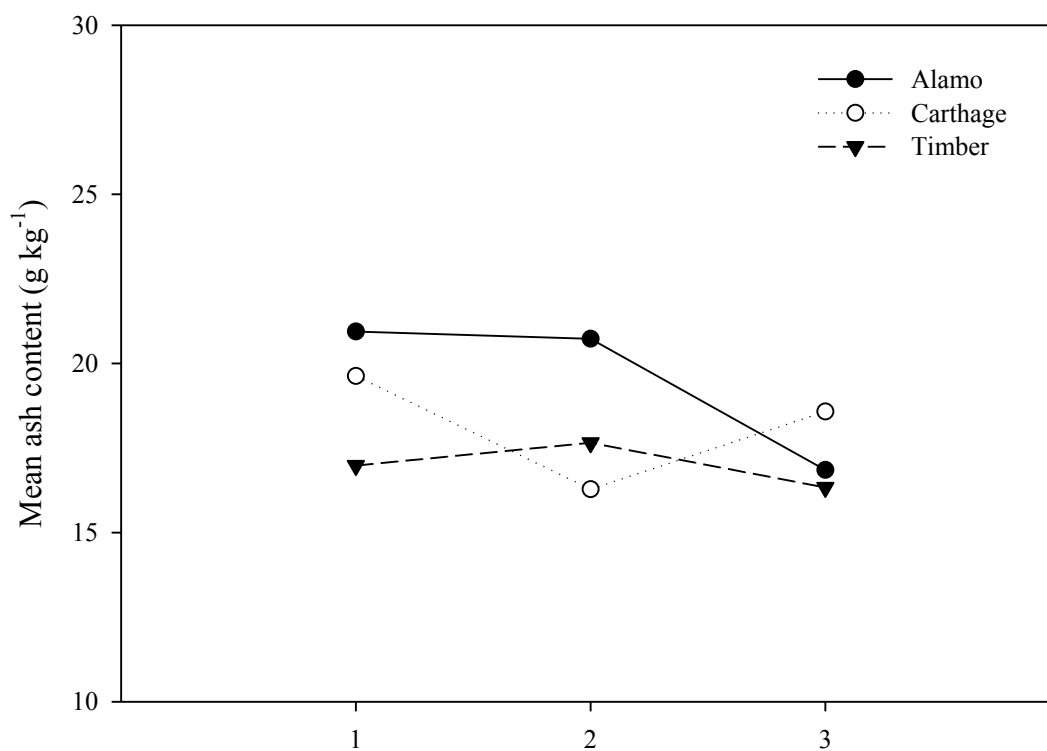
Figure 6. Mean ash content averaged across three switchgrass cultivars grown in Upper Deerfield and Pittstown, NJ in 2009 and 2010.by harvest date.



NS - Not significant

Letters indicate difference between years within each location ($p \leq 0.05$).

Figure 7. Mean 2009 and 2010 ash content of switchgrass cultivars Alamo, Carthage, and Timber grown in Upper Deerfield and Pittstown, NJ across three harvest dates.



NS - Not significant

5.4. CONCLUSIONS

This is the first study to report biomass yields, moisture content, ash content, and combustible energy content of switchgrass cultivars grown in sward plots in New Jersey and the northeastern/Mid-Atlantic US. Dry matter biomass yields differed across locations and harvest years, but were similar among cultivars tested, ranging from 9.87 Mg ha⁻¹ to 12.93 Mg ha⁻¹. Dry matter content tended to increase with later harvest dates, and reached 900 g kg⁻¹ by January at both locations with the exception of the Pittstown location in 2009. Generally, dry matter content did not differ among cultivars tested except at harvest one, where the late-maturing cultivar Alamo showed lower dry matter content. Dry matter contents observed would allow for direct or same day harvest of switchgrass biomass by January without the need for further drying, weather permitting. Ash values reached minimum levels in all cultivars tested by January at both locations. Combustible energy values did not differ among cultivars or harvest dates and showed no consistent pattern with respect to year. Therefore, harvesting switchgrass in January or later provides for minimal ash levels and higher dry matter content, which are favorable for combustion. Because yields were not taken at later harvest dates, further studies are needed to determine the optimal harvest time that would minimize dry matter loss while maintaining low moisture and ash contents. Further characterization of specific combustion characteristics would also be beneficial for making switchgrass recommendations for direct combustion. Switchgrass cultivars Alamo, Timber, and Carthage have promise as bioenergy feedstocks for use in combustion bioenergy generating systems in New Jersey and the northeastern/Mid-Atlantic US.

6. GERMINATION IN THREE POPULATIONS OF SWITCHGRASS DIVERGENTLY SELECTED FOR SEED WEIGHT

6.1. INTRODUCTION

Switchgrass (*Panicum virgatum* L.), a warm-season grass native to the US, is emerging as a popular bioenergy crop. Switchgrass contains two ecotypes, upland and lowland, which are distinguished by differences in plant phenotype. Upland ecotypes are typically adapted north of 34° N latitude and are finer stemmed, shorter, and more drought tolerant, while lowland ecotypes are adapted to warmer, wetter habitats and are taller with fewer but larger tillers, thicker stems, and larger leaves (Casler, 2012). Switchgrass is being grown as a bioenergy feedstock due to its high yield potential, perennial habit, low water and fertilizer requirements, adaptability to a wide range of soil types, ability to grow on marginal land, and ability to be propagated by seed (Parrish and Fike, 2012). However, switchgrass seed has poor germination resulting in slow and inconsistent establishment (Aiken and Springer, 1995; McLaughlin and Kszos, 2005). The inability of switchgrass to produce rapid and consistent stands is one of the major constraints to growing switchgrass as an economically viable bioenergy crop (Perrin et al., 2008). In fact, poor stand establishment is often times due to poor seed quality as well as seed dormancy (Mitchell and Vogel, 2012). As a small-seeded species that allocates most of its energy into the development of a strong root system in the establishment year, weed competition also impedes successful switchgrass establishment (McLaughlin and Kszos, 2005). Additionally, few herbicides are currently labeled for use in switchgrass grown as a bioenergy feedstock (Sanderson et al., 2006). Stand failure is not uncommon with switchgrass establishment, and is costly due to several factors: the

need to repurchase seed, and the loss of time, tillage, and land costs (Buhler et al, 1998). In order to increase the profitability and sustainability of switchgrass production, research is needed to develop switchgrass varieties with improved germination, emergence, and stand establishment. However, little research has been conducted investigating breeding efforts directed towards improving switchgrass germination and establishment (Casler, 2012). Selection for seedling shoot mass and crown node placement (Smart et al., 2003; Elbersen et al., 1999) to improve switchgrass establishment has been suggested; however, neither of these methods have been consistently effective. Selecting for reduced post-harvest seed dormancy resulted in the release of switchgrass germplasm line TEM-LoDorm, which has improved germination (Burson et al., 2009).

Many switchgrass cultivars are comprised of seed increases from remnant prairies in the central and eastern USA and have not been subjected to any directed selection efforts. In addition, switchgrass has a relatively short breeding history, with initial breeding efforts initiated in the 1950's that focused on forage quality improvement. Therefore, many wild traits such as seed dormancy persist in currently available switchgrass cultivars (Casler, 2012). Switchgrass seeds contain variable degrees of dormancy, even within the same seed lot. Dormancy mechanisms are not well understood in switchgrass, but several studies have shown breaking of dormancy and improved germination through various seed treatments (Zegada-Lizarazu *et al.*, 2012). Mechanical and chemical scarification (Jensen and Boe, 1991; Haynes *et al.*, 1997), cold stratification (Sanderson *et al.*, 1996; Shen *et al.*, 2001;), and hydrogen peroxide, sodium nitroprusside, potassium ferrocyanide (Sarath *et al.*, 2006; Sarath *et al.*, 2007), and gibberellic acid (Madakadze *et al.*, 2000) applications have been shown to increase

germination in switchgrass.

Additionally, seed size has been shown to play a role in germination and establishment in several grass species (Kneebone and Cremer, 1955; Glewen and Vogel, 1984; Aiken and Springer, 1995). In previous research, Aiken and Springer (1995) demonstrated that germination and emergence increased as seed size increased in switchgrass cultivars Alamo, Blackwell, Cave-in-Rock, Kanlow, Pathfinder, and Trailblazer. Smart and Moser (1999) found similar results, where germination was greater in heavy seed compared to light seed lots of switchgrass cultivars Blackwell and Trailblazer. Boe and Johnson (1987) suggested seed cleaning and breeding for large seeded cultivars as two possible methods for improving switchgrass establishment. They demonstrated that selection for high seed weight was effective in increasing seed weight of progeny from a bulk seed lot in an open pollinated field. However, no studies have been conducted that investigate the effects of several cycles of selection for seed weight on switchgrass germination and emergence nor have improved cultivars resulted from such selection efforts. The purpose of this study was to evaluate the effects of 1) divergent selection for seed weight on germination and emergence in three switchgrass populations over two cycles of selection and 2) cold stratification on seed germination in these divergently selected switchgrass lines.

6.2. MATERIALS AND METHODS

6.2.1. Base Populations and Selection for Seed Weight – Cycle One

Seed of switchgrass cultivars Carthage (upland) and Timber (lowland), and experimental ecotype 9064202 (Cape May Plant Materials Center accession #9064202;

upland) was provided by the Natural Resources Conservation Service—United States Department of Agriculture Plant Materials Center in Cape May, NJ. This original source of seed represented the base population from each population without selection, an unsorted check, referred to as U₁ (Unsorted [for 1st cycle]). Approximately one kg of seed from each of the three populations tested was sorted with a gravity deck (Westrup, Plano, TX) into five weight classes at NexGen Turf Research, LLC (Albany, Oregon), and only seed from the heaviest and the lightest weight classes were utilized. The act of sorting seed by weight served as the cycle of selection in this study. Seed from the heaviest weight class of each population represented cycle one heavy seed and was identified as C1H, and seed from the lightest weight classes of each population represented cycle one light seed and was identified as C1L. Three replicates of 100 seeds from all three populations of C1H, C1L, and U₁ seed with glumes removed were germinated in 7.62 x 7.62 cm petri plates on a double layer of blotter paper moistened with distilled water, with 100 seeds per petri plate, in a growth chamber in February 2008 under the following conditions: 14H light at 25°C, 10H dark at 15°C, 75% relative humidity, and light at 350 ME. Petri plates were kept evenly moist and randomly repositioned in the growth chamber daily. Germination was monitored and recorded daily for 28d. Germinated seedlings were transplanted to Pro-Mix HP (K.C. Shafer, York, PA) in 48-celled 30.5 × 38.1 cm flats when leaves reached 10 cm in length and were maintained under greenhouse conditions for approximately 8 weeks. Temperatures ranged from 15.6 to 26.7 °C with no supplemental lighting, and plants were monitored twice daily and watered as needed. Individual plants were given an identifying number, and plant heights (length from the crown to the tip of the newest leaf, with the leaf extended vertically),

leaf number, and tiller number were taken at 2, 4, and 6 weeks after transplanting on all plants throughout the spring of 2008. These plants were transplanted to the field in isolated polycross blocks according to population and weight class (C1H, C1L, and U₁) in spring of 2008 at the Rutgers University Plant Biology Research and Extension Farm at Adelphia, NJ for a total of 9 blocks. The pre-emergent herbicide dimethyl tetrachloroterephthalate was applied at a rate of 16.8 kg active ingredient ha⁻¹. Polycross blocks were spaced at least 25 meters from each other and other switchgrass plants, and were also isolated by planting field corn in all areas between blocks. The field corn grew taller than the switchgrass flowers (>60 cm), which helped to eliminate pollen spreading from one block to another and therefore cross contamination of the polycross blocks. Each isolated polycross block contained a minimum of 25 plants, and plants were spaced approximately 0.76 m apart. Plants received 56 kg N ha⁻¹ in May of 2009, and were left in the field to mature for three growing seasons. In May 2009, April 2010, May 2011, and May 2012, atrazine and pendimethilin were applied at rates of 4.48 and 1.4 kg active ingredient ha⁻¹. In the fall of 2009, an equal amount of seed from each genotype in each isolated polycross block was harvested by hand, dried at 65°C for 14 days, hand threshed on corrugated rubber, and bulked. Seed from each isolated block was then stored at 15.6°C and relative humidity levels of 40% or less for two months.

6.2.2. Selection for Seed Weight - Cycle Two

In January 2010, approximately 1 kg of seed from C1H and C1L isolated polycross blocks (harvested in 2009) of all three populations tested was sorted with the

gravity deck into heavy and light groups as previously described, which completed a second cycle of selection. Following a divergent selection scheme, heavy seed was selected from the C1H isolated polycross blocks and was referred to as C2H (cycle two heavy) seed, and light seed was selected from the C1L polycross blocks and was referred to as C2L (cycle two light) seed. Seed harvested from the U₁ polycross blocks was not sorted with the gravity deck and referred to as U₂ (Unsorted [for 2nd cycle]), and was used as an unsorted check for cycle two seed. Three replicates of 100 seeds from all three populations, two selected weight classes (C2H and C2L), and unsorted seed (U₂) with glumes removed were germinated in the growth chamber under the conditions mentioned above. Due to poor survival (specifically of the light seed classes), the growth chamber germination of all populations and weight groups was repeated in an effort to produce more seedlings. Seedlings were transplanted and maintained in the greenhouse under the conditions mentioned above until they were transplanted to the field in isolated polycross blocks according to population and weight class (C2H, C2L, and U₂) in spring of 2010 at the Rutgers University Plant Biology Research and Extension Farm at Adelphia, NJ for a total of nine blocks. The pre-emergent herbicide dimethyl tetrachloroterephthalate was applied at a rate of 16.8 kg active ingredient ha⁻¹. Polycross blocks were isolated as previously mentioned. Each isolated block contained a minimum of 9 plants (due to poor seedling survival), and plants were spaced approximately 0.76 m apart. Plants received 56 kg N ha⁻¹ in May of 2010, and in May 2011 and May 2012, atrazine and pendimethilin were applied at rates of 4.48 and 1.4 kg active ingredient ha⁻¹. Plants were left in the field to mature for two growing seasons (2010 and 2011). Cycles of selection are shown in Figure 8.

6.2.3. Germination Experiment

In the fall of 2011, an equal amount of seed from each plant in each of the 18 isolated polycross blocks (C1H, C1L, U₁, C2H, C2L, and U₂) was harvested (when seed from the top of panicles started to shatter), threshed, dried, bulked, and stored as above so that all seed was produced and harvested in the same year. Five replicates of 100 randomly selected seeds (with glumes removed) from all three populations and six weight classes were weighed to determine effects of selection on seed weight. Three replicates of 100 seeds (with glumes removed) from all three populations and six weight classes were germinated in the growth chamber under the conditions mentioned previously with the addition of a surface sterilization step. Seed was rinsed in a 10% bleach solution for 10 min and then triple rinsed with sterilized distilled water prior to placement in petri plates. In addition, three replicates of 100 seeds (with glumes removed) from all three populations and all six weight classes were exposed to a cold stratification treatment prior to germination in the growth chamber with non-stratified seed. Seed was surface sterilized as above, kept moist with sterile distilled water, and maintained at 5°C for 14 days prior to placement in petri plates. Germination was monitored in the growth chamber as above, and germination percentage and germination rate index (GRI) and corrected germination rate index (CGRI), similar to that described by Evetts and Burnside (1972), were calculated for all populations and weight classes. The germination experiment was repeated as above with all seed lots in a second run 30d after the start of run 1.

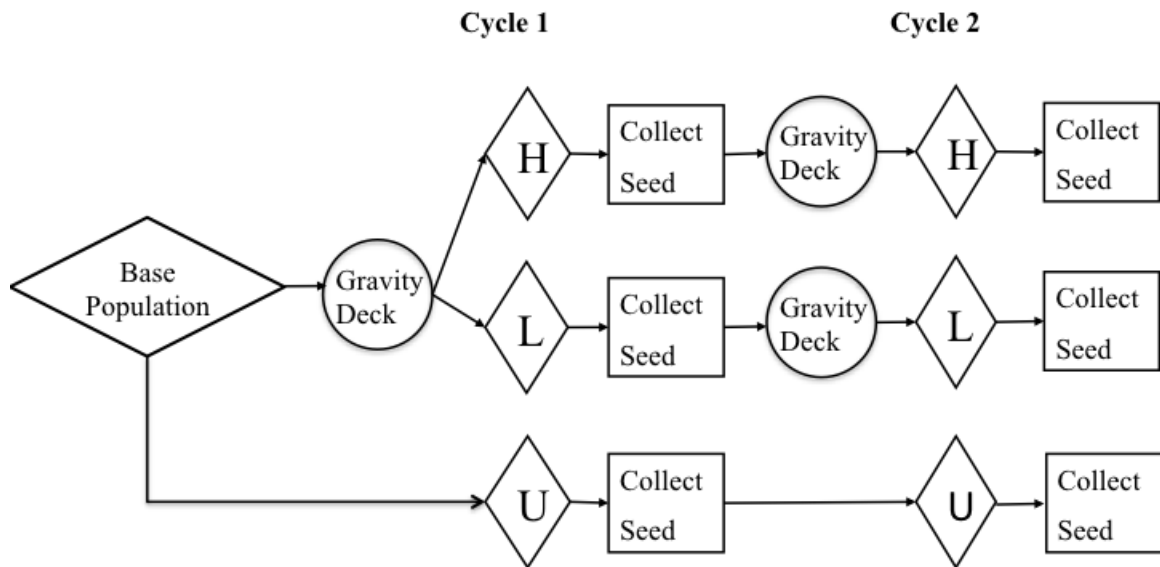
Statistical analysis was conducted using the SAS System version 9.3 (SAS Institute Inc., Cary, NC) to perform analysis of variance. A completely randomized 3 x 3 x 2 x 2 factorial design with three replicates was used. Plots consisted of population (9064202, Carthage, and Timber), seed weight (heavy, light, and unsorted) and cycle (one and two), and treatment (cold stratification and non-stratification). 100 Seed weights of each population were analyzed using the MIXED procedure of the SAS System (SAS Institute Inc., Cary, NC). Effects of cycle, weight class, and cycle x weight class were included in the model as fixed effects, while effect of cycle x weight x rep was considered random. Data were examined for violations of assumptions by inspection of plots of standardized residuals. The DIFF function of the LSMEANS procedure was used to compare means, and significance was established at $P \leq 0.05$. Percent germination, germination rate index, and CGRI were analyzed using the MIXED procedure of the SAS System (SAS Institute Inc., Cary, NC). Effects of cycle, population, cycle x population, weight, weight x population, treatment, treatment x cycle, treatment x population, treatment x weight, treatment x cycle x population, treatment x weight x cycle, and treatment x weight x population were included in the model as fixed effects, and effects of cycle x population x weight, cycle x population x weight x treatment, and cycle x population x weight x stratification x run were considered random. Data were examined for violations of assumptions by inspection of plots of standardized residuals. The DIFF function of the LSMEANS procedure was used to compare means, and significance was established at $P \leq 0.05$.

6.2.4. Emergence and Mature Plant Data

Plant height, leaf number, and tiller number were recorded for all germinated seedlings from all lots of Cycle one seed at 2, 4, and 6 weeks after transplanting to Pro-Mix in 2008 as mentioned above. This data was used to calculate a plant height rate index, leaf number rate index, and tiller number rate index. Indices were calculated using a formula similar to the germination rate index described by Maguire (1962), where the sum of plant height, leaf number, and tiller number for each measurement was divided by the number of days after transplant for each measurement. Rate indices for plant height, leaf number, and tiller number were analyzed using the Proc GLM procedure of the SAS statistical software program.

In the fall of 2012, individual plant height and total tiller number were recorded for all plants in all nine cycle one polycross blocks. These plants were grown in the field for three years. This data along with plant height, leaf number, and tiller number from each corresponding seedling taken in spring of 2008 were used to determine phenotypic correlation coefficients. Data were analyzed using the Proc CORR procedure of the SAS statistical software program.

Figure 8. Selection scheme used in divergent selection divergent selection for seed weight in three ecotypes of switchgrass over two cycles with an unsorted check.



Diamonds represent seed lots, rectangles represent isolated polycross blocks, and circles represent sorting with the gravity deck. H=heavy, L=light, U=unsorted.

6.3. RESULTS

6.3.1. Seed Weight

Mean 100 seed weights and standard deviations of seed used to generate plants for isolated polycross blocks of all cultivars (sorted with the gravity deck as well as the unsorted checks), cycles, and weight groups tested are presented in Table 16. In all populations tested, selection intensity for seed weight increased with selection, as the difference in mean 100 seed weight between the heavy, light, and unsorted weight groups was greater in the cycle 2 seed than the cycle 1 seed. However, mean 100 seed weight did not always change as expected after selection. In population 9064202, mean 100 seed weight of U₂ was heavier than U₁ (Table 16). In cultivar Carthage, the C2H seed was actually lighter than C1H seed, and U₂ seed was lighter than U₁ seed. Finally, in cultivar Timber, C2H seed was slightly lighter than C1H, and U₂ seed was lighter than U₁ seed.

Despite the difference in seed weight seen in the seed lots used to generate plants for the isolated polycross blocks in some populations tested, seed weight response to two cycles of divergent selection varied among cultivars tested. In experimental selection 9064202, a significant cycle x weight class interaction was observed (Table 17). The degree to which mean 100 seed weight changed from cycle one to cycle two differed among weight groups (Figure 9). Mean 100 seed weight increased in the light and unsorted weight classes, with the greatest increase seen in the light weight class. Meanwhile, C2H mean 100 seed weight did not differ from C1H. Among weight classes in cycle one, C1H seed had the highest mean 100 seed weight. Among weight classes in cycle two however, C2L seed had the highest mean 100 seed weight.

Cultivar Carthage showed a significant cycle x weight class interaction as well (Table 17). Similar to selection 9064202, mean 100 seed weight increased from cycle one to cycle two in the light and unsorted weight groups with the greatest increase seen in the light weight class (Figure 10). However, no difference in mean 100 seed weight was observed among weight classes in cycle two. Among weight classes in cycle one, C1H seed had the highest mean 100 seed weight. Among weight classes in cycle two however, no difference in mean 100 seed weight among weight classes was seen.

No significant cycle x weight class interaction was observed in cultivar Timber, nor were the main effects of cycle or weight class significant.

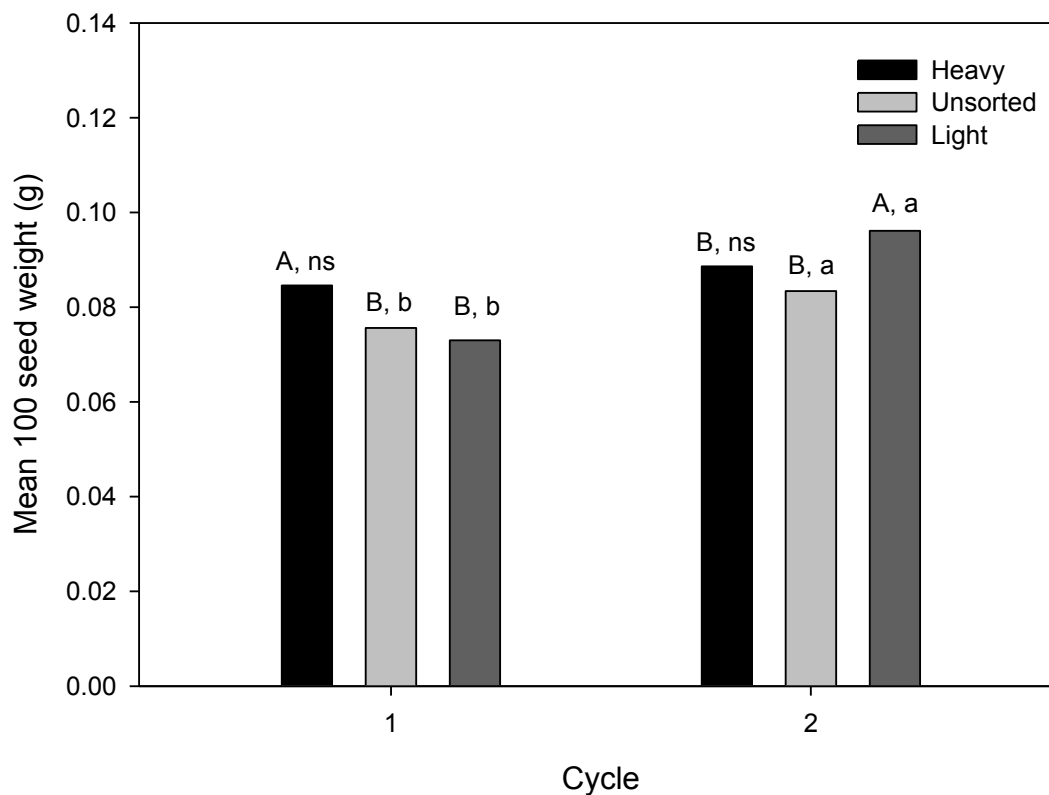
Table 16. Mean 100 seed weights for switchgrass seed from three parent populations separated into light and heavy weight classes on a gravity deck and an unsorted check for two cycles used to generate plants for isolated polycross blocks

Population	Weight class	Cycle	Mean 100 seed weight	Standard deviation
9064202	Heavy	1	0.0769	0.0042
9064202	Light	1	0.0509	0.0043
9064202	Unsorted	1	0.0739	0.0006
9064202	Heavy	2	0.1029	0.0026
9064202	Light	2	0.0332	0.0019
9064202	Unsorted	2	0.0870	0.0036
Carthage	Heavy	1	0.1746	0.0021
Carthage	Light	1	0.1373	0.0013
Carthage	Unsorted	1	0.1603	0.0056
Carthage	Heavy	2	0.1577	0.0039
Carthage	Light	2	0.0398	0.0022
Carthage	Unsorted	2	0.1142	0.0036
Timber	Heavy	1	0.1163	0.0096
Timber	Light	1	0.1218	0.0041
Timber	Unsorted	1	0.1129	0.0041
Timber	Heavy	2	0.0809	0.0046
Timber	Light	2	0.0425	0.0066
Timber	Unsorted	2	0.0661	0.0035

Table 17. Analysis of variance for mean 100 seed weights (g) in progeny of three switchgrass populations divergently selected for seed weight over two cycles

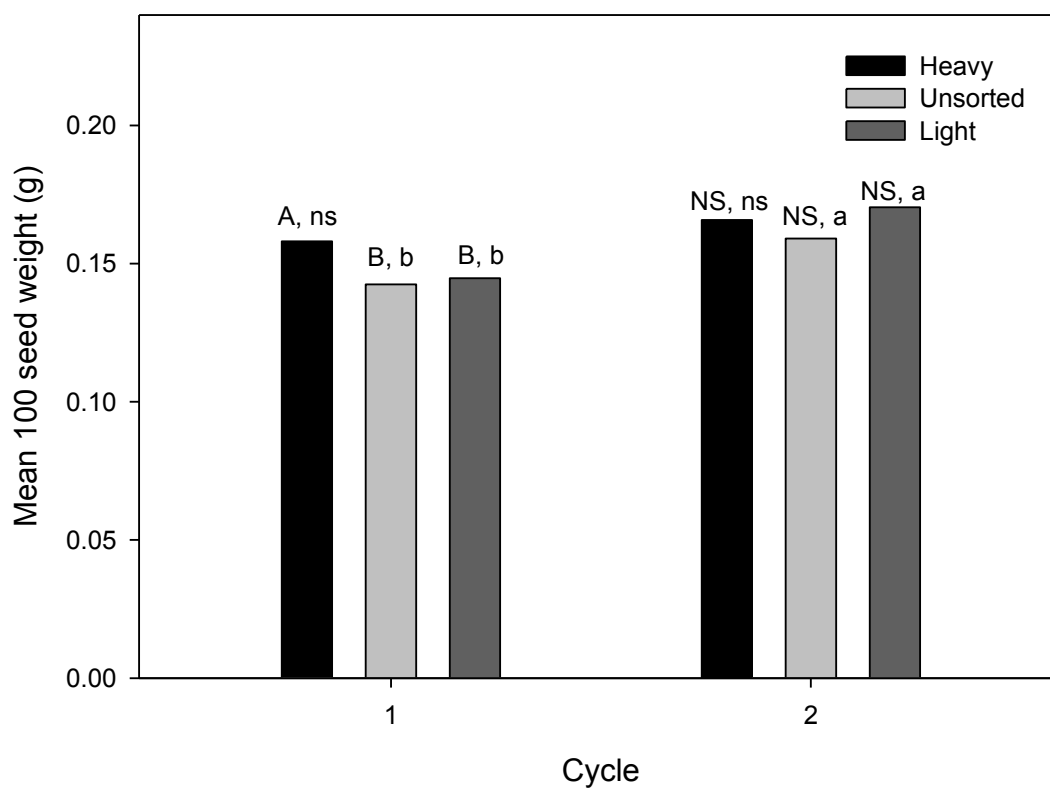
Population	Effect	Numerator	Denominator	F value	P value
		DF	DF		
9064202	Cycle	1	24	35.95	<0.0001
	Weight class	2	24	4.68	0.0193
	Cycle x Weight class	2	24	9.05	0.0012
Carthage	Cycle	1	24	44.16	<0.0001
	Weight class	2	24	6.65	0.0050
	Cycle x Weight class	2	24	4.31	0.0252
Timber	Cycle	1	24	2.93	0.0997
	Weight class	2	24	2.71	0.0871
	Cycle x Weight class	2	24	2.42	0.1100

Figure 9. Mean 100 seed weight in progeny of experimental population 9064202 divergently selected for seed weight over two cycles of selection.



Upper case letters indicate significant differences among weight classes within each cycle. Lower case letters indicate significant difference between cycle one and cycle two within each weight class.

Figure 10. Mean 100 seed weight in progeny of cultivar Carthage divergently selected for seed weight over two cycles of selection.



Upper case letters indicate significant differences among weight classes within each cycle. Lower case letters indicate significant difference between cycle one and cycle two for each weight class

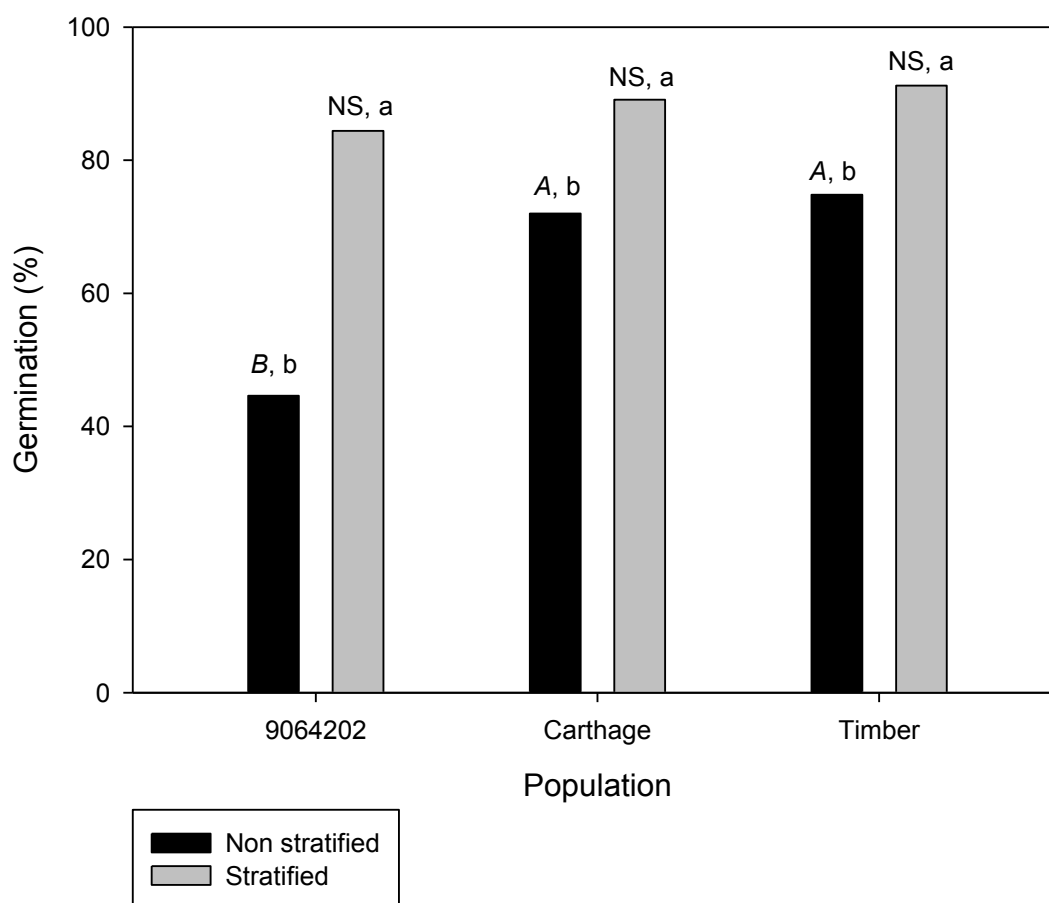
6.3.2. Percent Germination

A significant population x treatment interaction was observed for percent germination (Table 18), indicating that the three populations tested in this study responded differently to the stratification treatment. While percent germination increased in all populations tested with the cold stratification treatment, population 9064202 showed the greatest increase in percent germination, from 44.6% to 84.4% (Figure 11). Among non-stratified populations, Timber and Carthage showed higher percent germination than population 9064202 (Figure 11). No difference was observed for percent germination among populations that received a cold stratification treatment (Figure 11). Additionally, a significant cycle x treatment interaction was observed (Table 18), where cycles responded differently to stratification treatment. Percent germination increased with the stratification treatment in both cycles of selection. In cycle one, mean percent germination in non-stratified seed was 57.4%, which increased significantly to 70.1% in cycle 2. Mean percent germination in stratified seed was 87.5% for cycle 1 and 89% for cycle 2; however the difference was not significant (Figure 12).

Table 18. Analysis of variance summary for percent germination in three switchgrass populations divergently selected for seed weight over two cycles and subjected to a cold stratification treatment

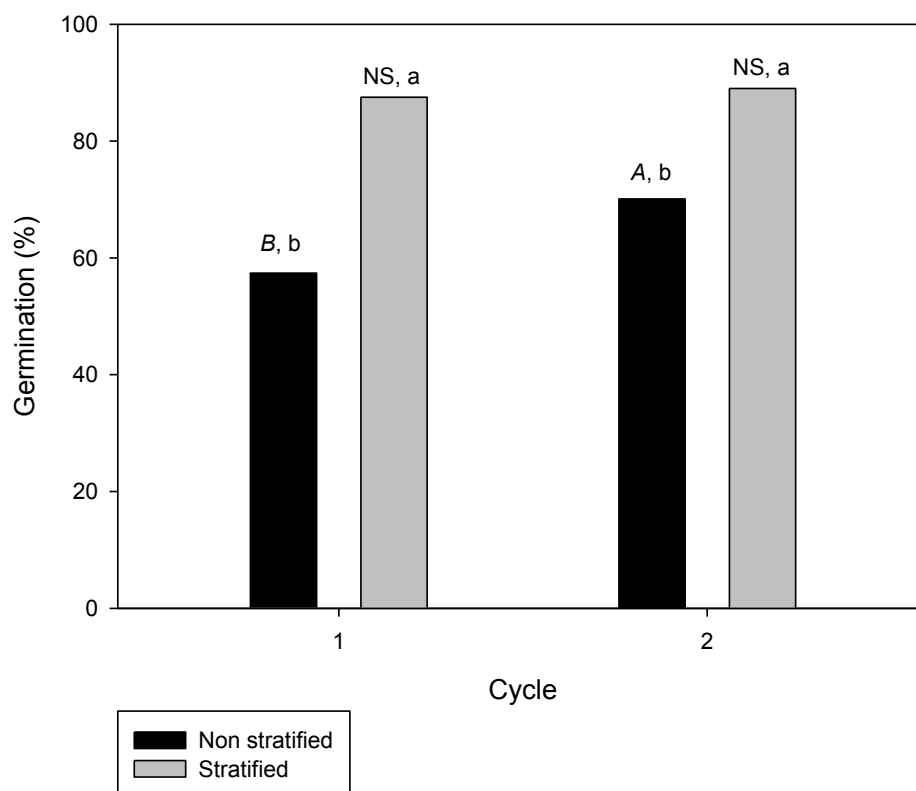
Effect	Numerator DF	Denominator DF	F Value	P Value
Cycle	1	4	13.41	0.0216
Cultivar	2	4	36.12	0.0028
Cycle x Cultivar	2	4	2.55	0.1933
Weight	2	4	1.09	0.4201
Cycle x Weight	2	4	0.51	0.6326
Cultivar x Weight	4	4	1.91	0.2728
Stratification	1	4	161.30	0.0002
Cycle x Stratification	1	4	8.57	0.0429
Cultivar x Stratification	2	4	15.97	0.0124
Weight x Stratification	2	4	1.01	0.4430
Cycle x Cultivar x Stratification	2	4	4.47	0.0955
Cycle x Weight x Stratification	2	4	0.60	0.5921
Cultivar x Weight x Stratification x Cycle	4	4	0.51	0.7344

Figure 11. Mean percent germination in three cultivars of switchgrass divergently selected for seed weight over two cycles of selection given no stratification and a cold stratification treatment.



Upper case letters indicate significant differences among cultivars for non-stratified (*italics*) and stratified seed. Lower case letters indicate significant difference between non-stratified and stratified seed within each population.

Figure 12. Mean percent germination averaged across three cultivars of switchgrass divergently selected for seed weight over two cycles of selection given no stratification and a cold stratification treatment.



Upper case letters indicate significant differences between cycle for non-stratified (*italics*) and stratified seed. Lower case letters indicate significant difference between non-stratified and stratified seed within each cycle.

6.3.3. Germination Rate

Results for GRI were similar to those for CGRI. No significant interactions were observed for GRI or CGRI. However, the main effects of cycle, treatment, and cultivar were significant for both GRI and CGRI (Tables 19 and 20). Weight class did not have effect on GRI or CGRI. Cycle one showed a lower mean GRI and CGRI than cycle two (Table 21). Cold stratified seed had a greater mean GRI and CGRI than non-stratified seed (Table 21). Mean GRIs and CGRIs for cultivars Carthage and Timber were greater than the mean GRI and CGRI for population 9064202 (Figures 13 and 14).

Table 19. Analysis of variance summary for germination rate index (GRI) in three switchgrass populations divergently selected for seed weight over two cycles and subjected to a cold stratification treatment

Effect	Numerator DF	Denominator DF	F Value	P Value
Cycle	1	4	14.76	0.0184
Cultivar	2	4	35.58	0.0028
Cycle x Cultivar	2	4	1.61	0.3076
Weight	2	4	0.28	0.7674
Cycle x Weight	2	4	0.35	0.7248
Cultivar x Weight	4	4	0.82	0.5720
Stratification	1	4	211.21	0.0001
Cycle x Stratification	1	4	1.04	0.3657
Cultivar x Stratification	2	4	0.43	0.6760
Weight x Stratification	2	4	0.27	0.7783
Cycle x Cultivar x Stratification	2	4	0.25	0.7931
Cycle x Weight x Stratification	2	4	0.40	0.6968
Cultivar x Weight x Stratification x Cycle	4	4	0.03	0.9979

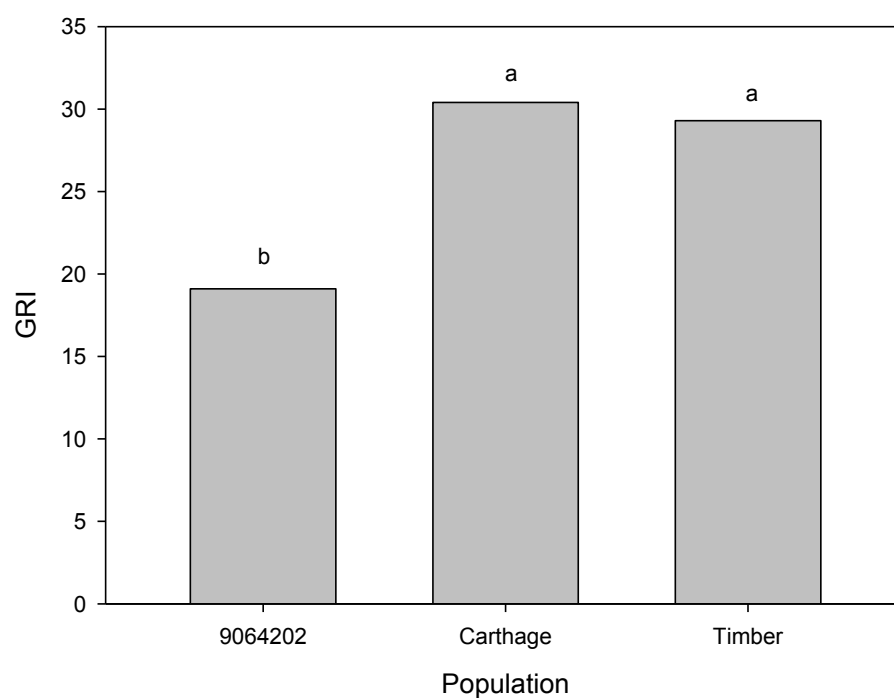
Table 20. Analysis of variance summary for corrected germination rate index (CGRI) in three switchgrass populations divergently selected for seed weight over two cycles and subjected to a cold stratification treatment

Effect	Numerator DF	Denominator DF	F Value	P Value
Cycle	1	4	14.44	0.0191
Cultivar	2	4	26.87	0.0048
Cycle x Cultivar	2	4	3.12	0.1528
Weight	2	4	1.15	0.4039
Cycle x Weight	2	4	0.58	0.5990
Cultivar x Weight	4	4	1.16	0.4457
Stratification	1	4	164.57	0.0002
Cycle x Stratification	1	4	0.81	0.4201
Cultivar x Stratification	2	4	2.02	0.2479
Weight x Stratification	2	4	0.41	0.6878
Cycle x Cultivar x Stratification	2	4	0.01	0.9924
Cycle x Weight x Stratification	2	4	0.23	0.8061
Cultivar x Weight x Stratification x Cycle	4	4	0.19	0.9318

Table 21. Mean GRI and CGRI of three switchgrass populations divergently selected for seed weight over two cycles and subjected to a cold stratification treatment

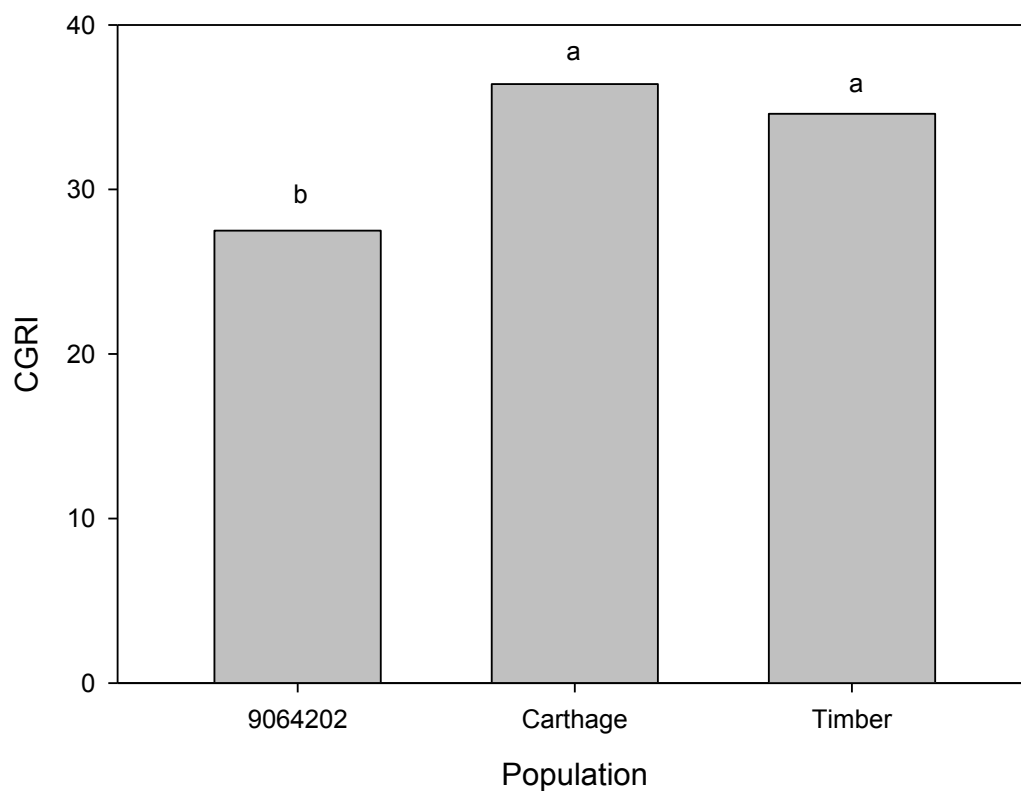
Effect		Mean GRI	Mean CGRI
Cycle	Cycle1	23.9	30.8
	Cycle 2	28.6	34.8
P value		0.0184	0.0002
Stratification	Non-Stratified	17.5	26.1
	Stratified	35.1	39.6
P value		0.0001	0.019

Figure 13. Mean germination rate index (GRI) in three cultivars of switchgrass divergently selected for seed weight over two cycles of selection given no stratification and a cold stratification treatment.



Means with the same letter are not significantly different at $p \leq 0.05$

Figure 14. Mean corrected germination rate index (CGRI) in three cultivars of switchgrass divergently selected for seed weight over two cycles of selection given no stratification and a cold stratification treatment.



Means with the same letter are not significantly different at $p \leq 0.05$

6.3.4. Emergence and Mature Plant Data

Table 22 includes the total plant number for each population and weight class. The total number of seeds germinated to produce the plants in cycle one polycross blocks was lower than the number needed to produce plants in cycle two polycross blocks. Additionally, no correlations ($p \leq 0.05$) were observed between mature plant height or mature plant tiller number and seedling height, seedling leaf number, or seedling tiller number for each cultivar and weight class tested, nor was any correlation observed between mature plant height or tiller number and seedling growth rate index, seedling leaf rate index, or seedling tiller rate index (data not shown).

No differences ($p \leq 0.05$) were observed among weight classes for seedling tiller rate index in all cultivars tested (data not shown). Among selection 9064202 weight classes, light seedlings had higher height rate indices than heavy seedlings. Among Carthage weight classes, both unsorted and heavy seedlings had higher seedling height rate indices and leaf rate indices than light seedlings. Among Timber weight classes, heavy seedlings had higher seedling height rate indices than light and unsorted seedlings. Timber heavy seedlings had greater leaf rate indices than unsorted seedlings.

Table 22. Total number of plants per isolated polycross block and number of seeds germinated to derive each polycross block

Isolated polycross block	Total plant number	Number of seeds germinated	Ratio of plants to seeds
90964202 C1L	22	300	0.073
90964202 U ₁	51	300	0.170
90964202 C1H	46	300	0.153
90964202 C2L	9	900	0.010
90964202 U ₂	18	900	0.020
90964202 C2H	21	900	0.023
Carthage C1L	29	300	0.097
Carthage U ₁	58	300	0.193
Carthage C1H	73	300	0.243
Carthage C2L	20	900	0.022
Carthage U ₂	37	900	0.041
Carthage C2H	93	600	0.155
Timber C1L	112	300	0.373
Timber U ₁	150	300	0.500
Timber C1H	182	300	0.607
Timber C2L	30	900	0.033
Timber U ₂	22	900	0.024
Timber C2H	29	900	0.032

6.4. DISCUSSION

Two cycles of divergent selection for seed weight in switchgrass cultivars Carthage and Timber and experimental population 9064202 did not alter seed weight in progeny as predicted. After one cycle of selection, mean 100 seed weight was highest in the heavy seed weight classes in populations 9064202 and Carthage. However, mean 100 seed weight increased in all weight groups in these populations after a second cycle of selection. Additionally, lowland cultivar Timber showed no difference in mean 100 seed weight, suggesting that factors other than genetics play a role in determining seed weight in these populations. The lack of expected weight change in these seed lots may be due to drift, as the mean 100 seed weights in unsorted check parental populations (which did not undergo selection for weight) were altered in all populations tested, and seed weights in progeny populations were altered in 9064202 and Carthage. Given these results, it logically follows that seed weight class did not have a significant effect on percent germination, GRI, or CGRI in these populations. Boe and Johnson (1987) stated that selection for heavy seed in switchgrass resulted in a genetic gain for seed weight. While data presented by Boe and Johnson (1987) revealed highly significant positive linear relationship between mean 100-seed weights of four weight categories and 100-seed weights from progeny of these weight categories, no comparison was made between the parental seed weight and that of the progeny, nor among the progeny 100-seed weights. Therefore, it is difficult to determine the importance of genetic or environmental effects on seed weight or whether one cycle of selection for seed weight increased seed weight in progeny. The Boe and Johnson (1987) study did, however, provide evidence supporting

the highly variable nature of seed weight in switchgrass, as individual plants differed in their ability to produce heavy seed. Several studies have demonstrated that seed weight is a complex trait often highly influenced by the environment (Silvertown, 1989; Greipsson and Davy, 1995; Boe, 2003; Fang et al., 2004; Casler 2006). Few studies have investigated heritability of seed weight in switchgrass. Boe (2003) reported significant environmental effects of both location and year on seed weight in upland switchgrass cultivars Sunburst and Summer. However, based on narrow-sense heritability estimates, Boe (2003) suggested that selection for seed weight would be successful in both cultivars tested. Based on the results seen here, one cycle of selection for heavy seed successfully increased mean 100 seed weight in populations 9064202 and Carthage, but did not alter 100 seed weight in cultivar Timber. A second cycle of divergent selection for seed weight resulted in an increase in the light and unsorted weight classes and no change in the heavy weight class. The lack of gain in heavy seed from cycle one to cycle two in these populations indicates that maximum seed weight may have been realized after one cycle of selection; however further cycles of selection would be needed to confirm this. Similarly, the lack of decrease in mean 100 seed weights in the light weight classes suggests minimum seed weight may have been realized after one cycle of selection. However, the increase in mean 100 seed weight in the generation 2 (U_2) unsorted weight class suggests a general trend towards increase in mean seed weight with advancement of cycles and indicates that other factors such as the environment may influence seed weight. Kassel et al. (1985) found that 100-seed weights differed between harvest year, where mean 100 seed weight was greater in the warmer, drier year in switchgrass cultivars Blackwell and Pathfinder. Additionally, Boe (2003) reported a significant difference in mean 100 seed weight between harvest years, location, as well as

environment (competitive vs. non-competitive) in switchgrass cultivars Sunburst and Summer in South Dakota, and suggested that mean 100 seed weight likely changes in response to moisture conditions during seed development.

The significant population x stratification interaction observed for percent germination is not surprising given the variation among populations tested, particularly the genetic backgrounds of these populations/cultivars. Consistent with these findings, percent germination has been shown to vary among switchgrass ecotypes (Seepaul et al, 2011). Population 9064202 is a seed increase of a collection made in Brooklyn, NY, and has not been subjected to cycles of selection for agronomic traits. Given that cold stratification has been shown to break primary dormancy in switchgrass, (Moser and Vogel, 2004) population 9064202 likely has retained more dormancy than cultivars that have undergone selection for characteristics of interest, such as Timber and Carthage. Logically, as cultivars are subjected to cycles of selection, dormancy is reduced because seeds that do not germinate (that have a high degree of dormancy) are not selected (Casler, 2012). The significant cycle x treatment effect observed for percent germination provides stronger evidence that dormancy decreases with cycles of selection, as cold stratification increased percent germination over non-stratified seed by 52.4% with one cycle of selection and 27.0% with two cycles of selection. No difference in percent germination between cycle one and cycle two was observed for cold stratified seed. As cold stratification essentially eliminates dormancy, it is likely that dormancy is a major factor involved in poor seed germination, and therefore what is selected against in the switchgrass breeding process. The lack of significance of the effect of seed weight class on percent germination suggests that while seed weight was altered, selecting for seed

weight alone is not sufficient to bring about the desired improvement in overall switchgrass germination.

The significant cycle effect seen for germination rate (represented by GRI and CGRI) regardless of seed weight supports the increase in germination rate with two cycles of selection in switchgrass, as was seen for percent germination. Completing one cycle of selection for seed weight in switchgrass cultivars Carthage and Timber and experimental population 9064202 was sufficient to bring about an increase in GRI and CGRI across all weight groups. Because germination rate increased across all switchgrass cultivars and weight groups tested, the simple act of elimination of non-germinated seeds (inherent in any breeding program using seed as a source of breeding material) is likely sufficient to bring about improvements in germination rates in switchgrass. Similarly, the cold stratification treatment improved GRI and CGRI, indicating that seed with less dormancy germinates more quickly than seed with higher degrees of dormancy. Further testing is needed to determine whether these increases would continue with further cycles of selection. Among populations tested in this study, the two cultivars (Carthage and Timber) showed higher mean GRIs and CGRIs than the experimental selection 9064202, similar to results seen for percent germination in non-stratified seed. As with percent germination, the variation in germination rate among the populations is likely due to the fact that the two cultivars have undergone more cycles of selection than the experimental variety, and therefore have retained less dormancy than the experimental variety. As with percent germination, selection for heavy seed as demonstrated in this study did not sufficiently increase germination rate in the three switchgrass populations tested.

The lack of correlation between mature plant characteristics and seedling traits indicates that seedling height, leaf number, and tiller number are not helpful for predicting mature plant size. Additionally, no clear pattern between seed weight and measures of seedling development were observed, similar to results seen by Smart and Moser (1999) and Zhang and Maun (1991), where only slight differences in morphological development of switchgrass seedlings were observed among seeds selected for size difference.

6.5. CONCLUSIONS

Based on previous research demonstrating both improved germination and emergence in larger, heavier switchgrass seed (Aikin and Springer, 1995; Smart and Moser, 1999), and high narrow-sense heritability for seed weight in two switchgrass cultivars (Boe, 2003), we hypothesized that selecting for heavier seed would result in an increase in percent germination and CGRI and selecting for light seed would show the opposite trend. However, selection for seed weight alone using the selection methods described above was not sufficient to improve overall germination and germination rate in the populations tested in this study, as no significant effect of seed weight was observed for any factors tested. Cycles of selection, regardless of seed weight, improved percent germination and germination rate in non-stratified seed of all switchgrass cultivars tested.

Dormancy is another factor that has been shown to affect germination in

switchgrass, and is a major hurdle that must be overcome if switchgrass is to be cultivated on a large scale. As previously demonstrated, cold stratification improved percent germination and germination rate in the three switchgrass populations tested. While the efficacy of cold stratification of switchgrass seed in reducing dormancy is recognized, this may not be a practical solution for improving germination. Shen et al. (2001) showed that the process of drying switchgrass seed for mechanical planting greatly reduced the benefits of the cold stratification treatment. Additionally, gains from cold stratification were smaller after a second cycle of selection for seed weight, demonstrating that dormancy reduction is inherent in the switchgrass breeding process.

It is clear that there is a need for better understanding of factors affecting switchgrass germination and establishment so that switchgrass cultivars with improved establishment capacity can be developed. Based on the results of this study, breeding efforts directed at reducing dormancy, rather than increasing seed weight, should be developed and implemented in order to improve switchgrass germination and establishment.

7. GENOTYPE X ENVIRONMENT AND BROAD-SENSE HERITABILITY ESTIMATES OF LIGNOCELLULOSIC CHARACTERISTICS OF SWITCHGRASS CLONES GROWN ON MARGINAL AND PRIME SOILS IN NEW JERSEY

7.1. INTRODUCTION

Switchgrass (*Panicum virgatum* L.) is a perennial a warm-season grass native to the US emerging as a bioenergy crop. Switchgrass has become a popular bioenergy feedstock due to its native status, broad adaptation to a wide range of environmental conditions (Parrish and Fike, 2005), environmental benefits, amenability to multiple conversion methods, and potential to produce high biomass yields on marginal cropland with few inputs (Bouton, 2008). Several studies have demonstrated that switchgrass cultivars vary widely in performance across different adaptation zones (Casler, 2010). Therefore, it is likely that the performance and biomass yields of switchgrass cultivars will differ on marginal land versus prime land. Switchgrass is expected to be produced on marginal land; however, few studies have investigated its performance on soils not suitable for agronomic crop production (Tulbure et al. 2012). Mulkey et al (2006) evaluated the effects of N rate and harvest date on switchgrass grown on land enrolled in the conservation reserve program in South Dakota, and determined that N rates of 56 kg ha⁻¹ with one post killing frost harvest annually was effective for biomass production. Schmer et al (2008) reported switchgrass biomass yields of 5.2 to 11.1 Mg ha⁻¹ on land with marginal characteristics in the Midwestern US. Varvel (2008) conducted a study comparing switchgrass and corn biomass yields and estimated ethanol yields on a low fertility field representative of marginal soils in the conservation Reserve Program. They

reported estimated ethanol yield in switchgrass to be the same as or greater than that of corn (calculated from both grain and stover) given the same fertilizer rate. However, no switchgrass yield in kg ha^{-1} was reported.

Currently, few switchgrass cultivars with improved biomass feedstock traits are commercially available despite evidence of gains in biomass yield (Casler, 2012). Outside of biomass yield, lignocellulosic characteristics including cellulose, hemicellulose, and lignin content are major determinants of feedstock quality. Feedstocks high in cellulose and hemicellulose and low in lignin are desirable in fermentation systems, while those high in lignin are valuable in direct combustion systems. As the concept of cellulosic ethanol is relatively recent, little information is available about the nature of genetic variation for these traits in switchgrass germplasm, and progress in improvement of lignocellulosic characteristics has been slow (Murray et al., 2008). If switchgrass is to be adapted as a bioenergy feedstock, breeders will need to develop new cultivars capable of producing high quality yields on marginal land. Switchgrass breeding is typically performed on prime farmland, yet little is known about the performance of improved cultivars on marginal soils. Breeders must know if selection in non-target environments (prime farmland) will effectively improve performance in target environments (Rose et al, 2007).

Quantitative traits such as biomass yield are often more strongly influenced by environmental factors than qualitative traits (Sleper and Poehlman, 2006). Plant breeders utilize various methods to estimate the extent to which the environment influences traits of interest. Broad-sense heritability refers to the amount of genetic variance expressed as a proportion of the total phenotypic variance, and estimates the relative importance of

genetic vs. non-genetic effects on a trait of interest (Bernardo, 2002). Broad-sense heritability estimates can assist plant breeders in determining the best selection methods to utilize. Traits with higher heritability estimates can usually be improved more rapidly than those with low estimates (Nyquist, 1991). Additionally, stability analysis allows for the identification of genotypes that are stable or unstable over different environments (Bernardo, 2002). Identification of switchgrass genotypes that exhibit stability in both prime and marginal locations, or genotypes with improved performance in marginal soils will aid switchgrass breeders in developing high-yielding, high quality cultivars for use as bioenergy feedstocks on marginal land. The objectives of this study were to 1) examine genotype by environment effects and 2) determine broad-sense heritability estimates on cellulose, hemicellulose, and lignin content in switchgrass clones grown on marginal and prime soils.

7.2. MATERIALS AND METHODS

7.2.1. Plant Material

In summer of 2007, five switchgrass genotypes from 10 different switchgrass cultivars and germplasm populations (Table 23) were randomly chosen from a spaced-plant breeding nursery that was established at the Rutgers University Plant Biology Research and Extension Farm at Adelphia, NJ. Each genotype was evenly divided into six ramets and planted to a Freehold sandy loam in summer 2008. Plants received 56.1 kg N ha⁻¹ after transplanting. In spring 2009, each ramet was further divided evenly into three ramets for a total of 18 ramets per genotype and 900 individual ramets. Plants were

maintained in 10 cm pots in Pro-Mix HP (K.C. Shafer, York, PA) for 4 weeks before planting to three locations in New Jersey, one on prime farmland and two on marginal land sites (Tables 24 and 25). The prime site was located at the Rutgers University Plant Biology Research and Extension Farm at Adelphia, NJ. The Adelphia site has a Class II prime land designation (USDA) with a Freehold sandy loam soil, and was planted on 29 June, 2009. One of the marginal sites was located in Somerset, NJ. The Somerset location is designated as Class IV marginal land (USDA) with a Klinesville channery loam soil, and was planted on 7 July 2009. The second marginal site was located in Jackson, NJ at the Pine Barrens Golf Club. The Jackson location has a Class V marginal land designation (USDA) with an Evesboro sand soil and was planted 30 June, 2009. At each location, individual plants were spaced 0.9 m apart with 10 plants per row in a randomized complete block design with six replications for a total of 300 plants per location. All clones received 56.1 Kg N ha⁻¹ upon transplant and in June 2010.

Table 23. Ecotype and origin of ten switchgrass populations and cultivars grown at three locations in New Jersey in 2009 and 2010

Population	Ecotype	Origin
9064202	Upland	Brooklyn, NY
Alamo	Lowland	Frio River, TX
Carthage	Upland	Carthage, NC
Cave-in-Rock	Upland	Cave-in-Rock, IL
High Tide	Upland	Perryville, MD
Kanlow	Lowland	Wetumka, OK
NSU	Upland	OK and southern KS
NSL	Lowland	OK and southern KS
Cimarron	Lowland	Central and south TX
Timber	Lowland	GA, FL, and TX

Table 24. Location, soil type, and land classification of three New Jersey sites where thirty switchgrass clones were evaluated for lignocellulosic traits in 2009 and 2010

Site	Soil Type	Land Classification
Rutgers Plant Biology Research Farm, Adelphia, NJ	Freehold sandy loam	Class II-Prime
Farmer's Field, Somerset, NJ	Kleinsville shale	Class IV-Marginal
Pine Barrens Golf Course, Jackson, NJ	Evesboro sand	Class V-Marginal

Table 25. Soil characteristics of three New Jersey sites where thirty switchgrass clones were evaluated for lignocellulosic traits in 2009 and 2010

Site	pH	P ^a	K ^a	Mg ^a	Ca ^a	EC ^b	Sand ^c	Silt ^c	Clay ^c	Gravel content ^c	SOM ^{cd}
Adelphia	6.31	495	253	363	1234	0.08	76	17	7	0.40	1.10
Somerset	5.35	40	297	878	2064	0.09	36	43	21	14.04	1.82
Jackson	5.50	504	89	46	788	-	-	-	-	-	-

^a Kg ha⁻¹

^b Electrical conductivity (mmho cm⁻¹)

^c (%)

^d Soil organic matter

7.2.2. Data Collection

Dried plant material from a subset of clones consisting of three randomly chosen ramets from all 10 switchgrass cultivars and five replicates at all locations (450 total plants) harvested in 2009 and 2010 was used to determine cellulose, hemicellulose, and lignin content. Each individual plant was harvested by hand at a height of 10 cm, dried at 43 °C for 14 days and ground through a 1mm screen on a Wiley mill (Thomas-Wiley Mill Co., Philadelphia, PA). Harvest dates for the 2009 growing season were 11 March 2010 at Somerset, 18 March 2010 at Adelphia, and 19 March 2010 at Jackson. Harvest dates for the 2010 growing season were 4 January 2011 at Somerset, 17 February 2011 at Jackson, and 4 March 2011 at Adelphia. All 2009 samples (450) and 100 randomly chosen samples from 2010 (550 total) were analyzed for neutral detergent fiber (NDF) and acid detergent fiber (ADF) utilizing the filter bag system method described for use with an Ankom 2000 Fiber Analyzer (ANKOM Technology Corp., Fairport, NY) (Anonymous, 2011a; Anonymous 2011b) with the following modifications: samples were processed in a flask for 75 min under constant agitation at 97-100°C for both NDF and ADF procedures. Acid detergent lignin (ADL) was determined using the procedure described by the ANKOM ADL procedure (Anonymous, 2013). Samples were run in duplicate and mean values were used to calculate cellulose and hemicellulose contents as follows: cellulose was calculated as the difference between ADF and ADL, and hemicellulose was calculated as the difference between NDF and ADF (Hopkins et al., 1995). Lignin was reported as ADL. Cellulose, hemicellulose, and lignin content for the

remaining 350 samples from 2010 were based on near infrared reflectance spectroscopy calibrated with wet chemistry from the 550 samples described above. Samples were scanned using a Unity Scientific Spectrastar™ 2400 Drawer model (Brookfield, CT) to collect reflectance measurements between 1200 and 2400 nm, recorded at 1 nm intervals. Calibration equations were calculated using the partial linear squares statistical model in the Ucal™ software package (Unity Scientific, Brookfield, CT). For the 350 2010 samples, coefficients of determination (R^2) and standard errors of the calibration and cross validation were 0.92, 1.13, and 1.23 for NDF; 0.97, 0.67, and 1.37 for ADF; and 0.98, 0.19, and 0.94 for ADL.

7.2.3. Data Analysis

Statistical analysis was conducted using the SAS System version 9.3 (SAS Institute Inc., Cary, NC) to perform analysis of variance. Genotype x environment interactions for cellulose, hemicellulose, and lignin content data were analyzed using the MIXED procedure of the SAS System (SAS Institute Inc., Cary, NC). Effects of clone, year, location, clone x year, clone x location, year x location, and clone x year x location were included in the model as fixed effects and removed if not significant, and effects of block within year and location and year x location x block x clone were considered random. Data were examined for violations of assumptions by inspection of plots of standardized residuals. When analysis detected significant interaction effects, differences were assessed using the SLICE option in the LSMEANS statement and were considered significant at the 0.05 level. When the analysis indicated a significant clone x year x

location interaction, data from each location were analyzed separately using the MIXED procedure of the SAS System (SAS Institute Inc., Cary, NC). Effects of clone, year, and clone x year were included in the model as fixed effects, and effect of block within year was considered random. Differences were assessed using the DIFF option in the LSMEANS statement and were considered significant at the 0.05 level. Kendall's tau and Spearman's rank correlation coefficients were determined using the PROC CORR procedure of the SAS System (SAS Institute Inc., Cary, NC).

Broad sense heritability estimates for cellulose, hemicellulose, and lignin content were determined from restricted maximum likelihood variance and covariance components using the MIXED procedure of the SAS System (SAS Institute Inc., Cary, NC). All effects were considered random because years were not chosen with respect to expected climatic conditions (Gordon et al., 1972), locations were not chosen with respect to specific ecological properties, and no information on the characteristics evaluated in the switchgrass clones was known before the initiation of the study. Heritability was calculated on a clonal mean (Hc) basis as well as on a single-plant basis (Hsp) using the following formulas:

$$Hc = \sigma^2_c / (\sigma^2_c + \sigma^2_{cy}/y + \sigma^2_{cl}/l + \sigma^2_{cr(l)}/rl + \sigma^2_{cly}/ly + \sigma^2_{e}/rly)$$

$$Hsp = \sigma^2_c / (\sigma^2_c + \sigma^2_{cy} + \sigma^2_{cl} + \sigma^2_{cr(l)} + \sigma^2_{cly} + \sigma^2_e)$$

where σ^2_c = the total genetic variance of clones, σ^2_{cy} = clone x year variance, σ^2_{cl} = clone x location variance, $\sigma^2_{cr(l)}$ = clone x replication within location variance, σ^2_{cly} = clone x year x location variance, and σ^2_e = experimental error (clone x year x replication within location). Letters in the denominator refer to the number of replications (five),

replications (locations) (15), locations (three), and years (two) (Poehlman and Sleper, 2006).

7.3. RESULTS AND DISCUSSION

7.3.1. Cellulose

7.3.1.1. Genotype x Environment Interactions

A significant clone x location effect was observed for cellulose content (Table 26). The interaction was likely due to changes in rank among locations. Kendall's tau correlation coefficients were highest for 2009 Adelphia and 2009 Somerset and 2009 Somerset and 2009 Jackson environments, indicating fewer inversions of clone rankings for cellulose content when comparing these environments. However, Kendall's tau correlation coefficients were lowest for the 2010 Somerset and 2010 Jackson environments (Table 27). In spite of the clone x location effect, clone Timber 3 ranked among the top five for cellulose content at all three locations (Figure 15). Clone Timber 2 ranked among the top five clones at both Adelphia and Jackson, and despite ranking 14th at Somerset with a mean of 49.85, was not statistically different from the fifth ranked clone. Clone Carthage 1 ranked among the top five clones at Adelphia and Somerset, and despite ranking 17th at Jackson with a mean of 45.29 was not statistically different than the clone with the fifth highest cellulose content. Clone NSL 4 was among the top five clones at the two marginal locations, and ranked eight highest at Adelphia with a mean of 48.96 that did not differ statistically from the fifth highest clone. Clone 9064202 3 ranked among the lowest five clones for cellulose content at all three locations, while

clones Alamo 1 (ranked 13th highest at Jackson with a mean of 45.66) and 90642023 2 (ranked 24th at Jackson with a mean 44.04 and did not differ statistically from the 26th ranked clone) were among the lowest five clones at Adelphia and Somerset and clone NSU 1 (ranked 14th at Adelphia with a mean of 47.82, but not statistically different than the 26th clone) ranked among the bottom five clones at the marginal sites. A significant clone x year effect was also observed (Table 26), similar to the findings of Cassida et al. (2005b), who observed a significant genotype x year effect for cellulose in nine switchgrass genotypes grown at five locations for three years. Despite the clone x year effect, clones Timber 3 and NSL 4 ranked among the top five clones for cellulose content across years, while clone 9064202 3 was among the bottom five (Figure 15). Lowland clones tended to have higher cellulose content than uplands, consistent with findings of Cassida et al. (2005b), and Cortese and Bonos (2013). Additionally, a significant year x location effect was seen, where mean percent cellulose content did not differ among locations in 2009, but differed in 2010. Mean percent cellulose was highest at the prime location (Adelphia), followed by the Jackson and Somerset locations (43.0 and 40.3%, respectively) (Figure 16). The 2009 growing season was wetter than average at all locations, while the 2010 growing season was drier than usual (data not shown). It is possible that the dry conditions in 2010 contributed to the difference in cellulose content observed among locations, as the combination of both marginal soils and water stress may have resulted in lower cellulose contents. Drought has been shown to reduce grass cell wall concentrations through delayed stem development (Wilson, 1983), and Cassida et al. (2005) reported that the two driest locations of a five location study had lower mean cellulose contents in a study investigating nine switchgrass populations. Additionally, cell

wall component concentration tends to increase with later harvest dates (Lemus et al., 2002), so it is likely the difference in harvest dates among locations in 2010 contributed to the interaction (Somerset – January; Jackson - February; Adelphia – March). The fact that some clones consistently ranked among the top five or bottom five clones for cellulose content across locations and years as mentioned previously, highlights the potential for identification of clones with high cellulose content that could be used in a switchgrass breeding program. Further testing of the effectiveness of selection for cellulose content in switchgrass is needed. While no reports of stability across multiple environments of cellulose content in switchgrass clones currently exist in the literature to our knowledge, Claessens et al. (2004) reported that two clones chosen for either high or low cellulose content in timothy (*Phleum pratense* L.) consistently had high or low cellulose content when planted in a randomized complete block arrangement and evaluated for two years. Additionally, in a study that evaluated nine switchgrass populations in sward plots, Cassida et al. (2005b) found that switchgrass cultivar Alamo and population NL942 were stable for cellulose content across five locations and two years, while populations SL931 and NL931 were not stable across environments.

Table 26. Analysis of variance summary for percent cellulose in thirty switchgrass clones grown at three locations in New Jersey in 2009 and 2010

Source	Numerator DF	Denominator DF	F Value	P Value
Year	1	24	133.22	<0.0001
Location	2	24	43.56	<0.0001
Year x Location	2	24	30.68	<0.0001
Clone	29	716	10.73	<0.0001
Clone x Year	29	716	2.71	<0.0001
Clone x Location	57	716	1.67	0.0021

Table 27. Kendall's tau and Spearman's rank (in parentheses) correlation coefficients for cellulose content of fifty switchgrass clones grown at three locations in New Jersey in 2009 and 2010

	2009 Adelphia	2009 Somerset	2009 Jackson	2010 Adelphia	2010 Somerset	2010 Jackson
2009 Adelphia	-	0.57*** (0.74***)	0.41** (0.59**)	0.42** (0.57**)	0.25* (0.35)	0.30* (0.41*)
2009 Somerset		-	0.56*** (0.75***)	0.40** (0.54**)	0.35** (0.52**)	0.43** (0.56**)
2009 Jackson			-	0.32* (0.42*)	0.23 (0.39*)	0.23 (0.37*)
2010 Adelphia				-	0.23 (0.34)	0.30* (0.41*)
2010 Somerset					-	0.22 (0.29)

***Indicates significance at $p < 0.0001$

**Indicates significance at $p < 0.01$

*Indicates significance at $p < 0.05$

Figure 15. Ranking of thirty switchgrass clones grown at three locations in NJ in 2009 and 2010 based on mean percent cellulose content.

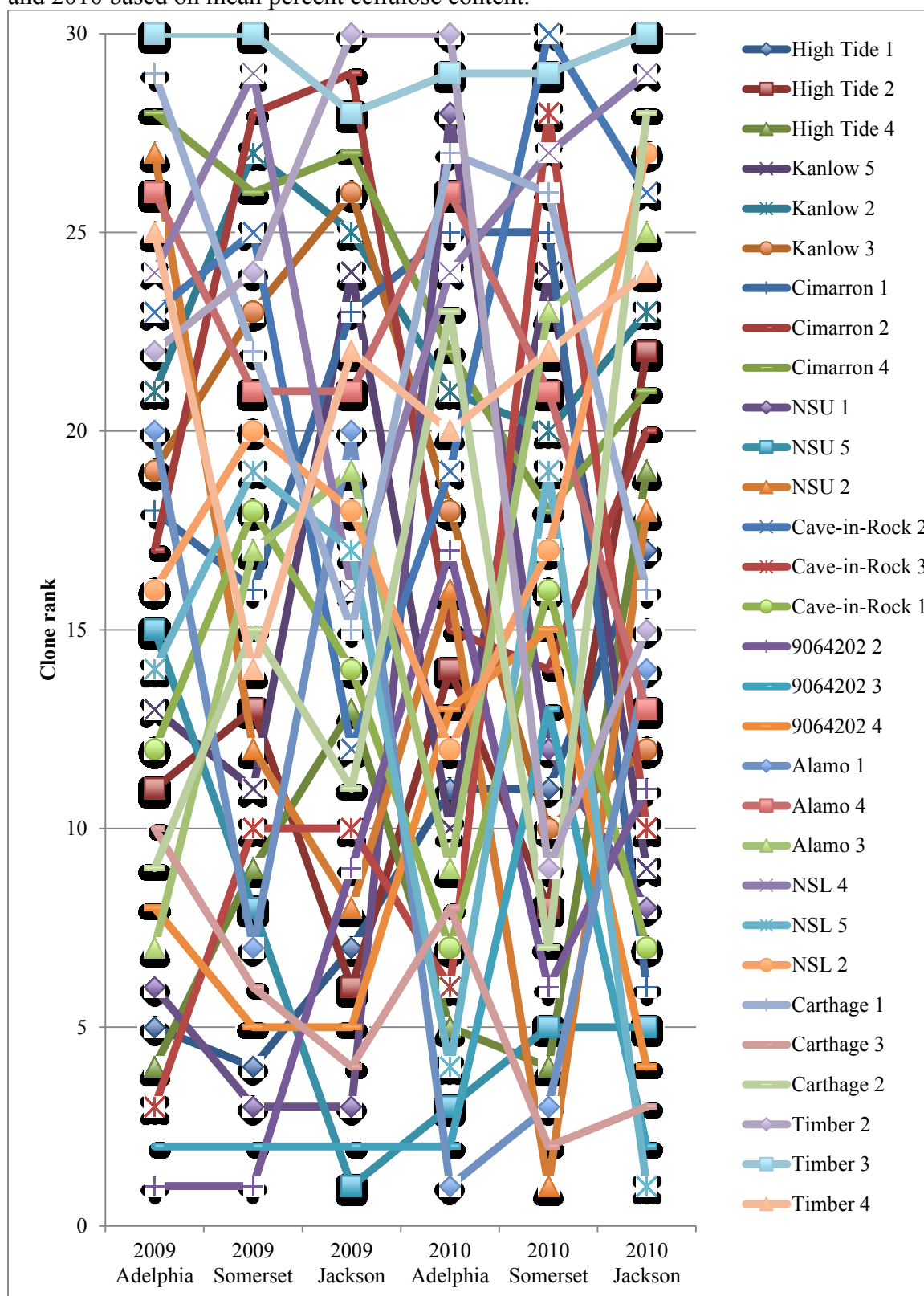
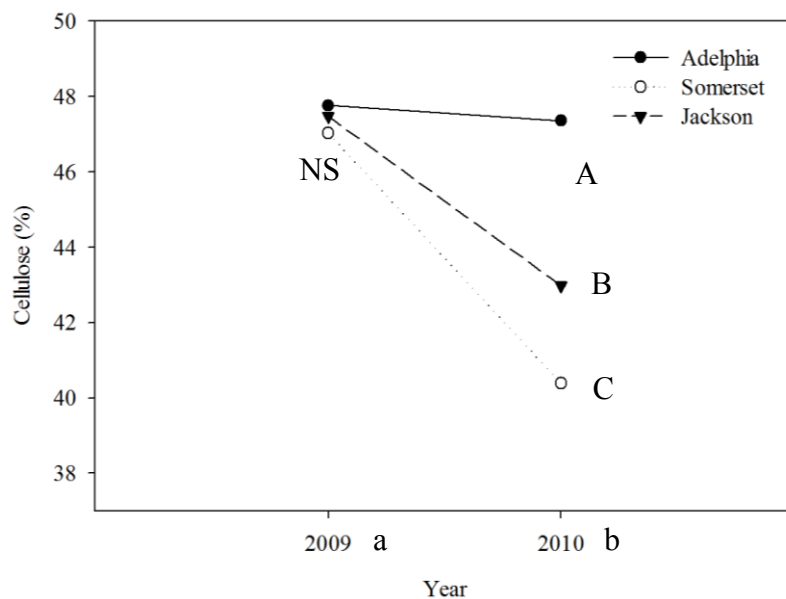


Figure 16. Mean percent cellulose content of thirty switchgrass clones grown in three locations in NJ in 2009 and 2010.



NS - Not significant

Upper case letters indicate differences among locations within each year. Lower case letters indicate differences between years.

7.3.1.2. Broad-sense Heritability Estimates

Broad-sense heritability estimate for cellulose content in switchgrass clones evaluated at three locations over two years on a clonal basis was 0.70, while on a single plant basis was only 0.18 (Table 28). The low single plant heritability estimate indicates that selection for cellulose content in this population of switchgrass would be ineffective if based on non-replicated single plants in one environment, as clonal variation for cellulose content within environment was observed. Broad-sense heritability estimates for cellulose content at each location were slightly lower than the overall estimate (Adelphia- $H_c = 0.68$, $H_{sp} = 0.21$; Somerset $H_c = 0.68$, $H_{sp} = 0.24$; Jackson $H_c = 0.58$, $H_{sp} = 0.21$) and did not differ greatly (Table 29), indicating similar amounts of genetic contribution to phenotypic expression of cellulose content in these three environments. These heritability estimates (on a clonal basis) indicate that selection should improve cellulose content in switchgrass, however, the genotype x environment interaction should be accounted for. While no reports of heritability estimates or effectiveness of selection for cellulose content in switchgrass were found in the literature, Carpenter and Casler (1990) investigated the effects of one cycle of divergent selection for several lignocellulosic traits in smooth brome grass (*Bromus inermis* Leyss.) and found no significant difference in cellulose content between the high and low cellulose progeny populations, nor a difference between either the high or low populations and an unselected check population.

Table 28. Analysis of variance and broad-sense heritability estimates (H) for cellulose content (percent) of thirty switchgrass clones grown at three locations in New Jersey in 2009 and 2010

Source of Variation	DF	SS	MS	F value	P value	Variance Component ^a
Year	1	2935.68	2935.68	444.56	<0.0001	
Location	2	1925.52	962.76	145.79	<0.0001	
Rep(Location)	12	252.14	21.01	3.18	0.0003	
Year x Location	2	1370.35	685.17	103.76	<0.0001	
Year x Rep(Location)	12	267.61	22.3	3.38	0.0001	
Clone	29	1836.03	63.31	9.59	<0.0001	1.5484
Clone x Year	29	468.74	16.16	2.45	<0.0001	0.6420
Clone x Location	57	537.05	9.42	1.43	0.0313	0.3096
Clone x Rep(Location)	344	1816.28	5.28	0.8	0.9788	0.0000
Clone x Year x Location	57	389.62	6.84	1.04	0.4141	0.2147
Error=Clone x Year x Rep(Loc)	315	2080.12	6.60			5.9205
$H_c^b = 0.70^c$						
$H_{sp}^d = 0.18$						

^aVariance components were determined using Restricted Maximum Likelihood Estimation using the PROC MIXED procedure in SAS (Cary, NC)

^b H_c = heritability estimate on a clonal basis; 95% confidence interval 0.83-0.58 calculated

^c95% confidence interval for $H_c = 0.77$ -0.64; computed according to Knapp and Bridges (1987)

^d H_{sp} = heritability estimate on a single-plant basis

Table 29. Broad-sense heritability estimates of percent cellulose, hemicellulose, and lignin of thirty clones at three locations in New Jersey in 2009 and 2010

		Adelphia	Somerset	Jackson
Cellulose	<i>Hc</i>	0.68 (0.82-0.55) ^a	0.68 (0.79-0.57)	0.58 (0.68-0.49)
	<i>Hsp</i>	0.21	0.24	0.21
Hemicellulose	<i>Hc</i>	0.55 (0.80-0.30)	0.01 (0.23-0.23)	0.00 (0.20-0.20)
	<i>Hsp</i>	0.11	0.00	0.00
Lignin	<i>Hc</i>	0.32 (0.50-0.14)	0.16 (0.24-0.09)	0.32 (0.42-0.22)
	<i>Hsp</i>	0.08	0.03	0.10

^a 95% confidence interval for *Hc* computed according to Knapp and Bridges (1987)

7.3.2. Hemicellulose

7.3.2.1. Genotype x Environment Interactions

A significant clone x location x year effect was observed for hemicellulose content (Table 30). Kendall's tau and Spearman's rank correlation coefficients were relatively low for all combinations of environments with the highest values of 0.44 (2009 Adelphia and 2009 Somerset) and 0.43 (2009 Somerset and 2009 Jackson) (Table 31). In keeping with these low correlation coefficients, very few clones were consistently ranked over all environments for hemicellulose content. Claessens et al. (2004) found similar results in a study that evaluated hemicellulose content over two years in timothy clones identified for either high or low hemicellulose content. While hemicellulose content differed among the high and low clones in the first year of evaluation, the low hemicellulose clones showed higher but not statistically different cellulose content than the high hemicellulose clones. Similarly, one cycle of divergent selection for hemicellulose content was shown to be ineffective in smooth bromegrass, as no difference in hemicellulose content was seen between high hemicellulose and low hemicellulose progeny populations (Carpenter and Casler, 1990).

Within years, only clone Cave-in-Rock 2 ranked among the bottom five clones for hemicellulose content at all three locations in 2009, and clone 9064202 3 ranked among the top five clones at all three locations in 2010 (Figure 17). Interestingly, clone Timber 4 ranked among the top five clones at Somerset and Jackson in 2009 but among the bottom five clones at all three locations in 2010, demonstrating a large crossover in ranking. Averaged across locations, clone Kanlow 5 ranked among the top five clones

for hemicellulose content in both 2009 and 2010, while no clones consistently ranked among the bottom five clones in both years. As was seen among locations, a substantial change in ranking was observed for clones Cave-in-Rock 1 and NSU 5 across years. Cave-in-Rock 1 and NSU 5 were among the bottom five clones for hemicellulose content in 2009 but ranked in the top five in 2010. These crossovers suggest that hemicellulose content is strongly influenced by environmental factors, similar to findings of Lemus et al. (2002). Additionally, a significant year x location effect was observed for hemicellulose content (Table 30). In 2009, mean hemicellulose content was higher at the Adelphia and Jackson sites than the Somerset location, while in 2010, mean hemicellulose content at Jackson was greater than at Adelphia and Somerset (Figure 18). Differences in hemicellulose content among locations and years were not consistent with differences in harvest date. Reports of hemicellulose content in switchgrass are limited, especially with regards to genotype x environment effects. Fritz et al. (1991) reported no difference in hemicellulose content between two switchgrass strains divergently bred for IVDMD (in-vitro dry matter digestibility), and stated that the relationship between IVDMD and cell wall components is poorly understood. Schmer et al. (2012) evaluated four upland switchgrass cultivars in ten locations over five years and saw a significant cultivar by year interaction and cultivar effect for pentose sugars, which comprise hemicellulose, at three of the ten locations evaluated, as well as a significant cultivar by location interaction at two locations, a significant cultivar effect at three locations, and a significant year effect at all locations in the study for xylose content, another component of hemicellulose. The authors concluded that due to the year to year and field to field variability seen in switchgrass biomass composition, cellulosic biorefineries should test

plant material prior to use.

Table 30. Analysis of variance summary for percent hemicellulose content in thirty switchgrass clones grown at three locations in New Jersey in 2009 and 2010

Source	Numerator DF	Denominator DF	F Value	P Value
Year	1	24	86.34	<0.0001
Location	2	24	22.72	<0.0001
Year x Location	2	24	16.05	<0.0001
Clone	29	660	4.58	<0.0001
Clone x Year	29	660	3.59	<0.0001
Clone x Location	57	660	1.36	0.0451
Clone x Year x Location	57	660	1.44	0.0216

Table 31. Kendall's tau and Spearman's rank (in parentheses) correlation coefficients for hemicellulose content of fifty switchgrass clones grown at three locations in New Jersey in 2009 and 2010

	2009 Adelphia	2009 Somerset	2009 Jackson	2010 Adelphia	2010 Somerset	2010 Jackson
2009 Adelphia	-	0.44** (0.61**)	0.20 (0.32)	0.28* (0.38*)	0.09 (0.13)	0.04 (0.04)
2009 Somerset		-	0.43** (0.58**)	0.23 (0.33)	0.03 (0.02)	-0.07 (-0.11)
2009 Jackson			-	0.12 (0.18)	-0.05 (-0.08)	-0.15 (-0.24)
2010 Adelphia				-	0.23 (0.35)	0.25 (0.33)
2010 Somerset					-	0.28* (0.39*)

***Indicates significance at $p < 0.0001$

**Indicates significance at $p < 0.01$

*Indicates significance at $p < 0.05$

Figure 17. Ranking of thirty switchgrass clones grown at three locations in NJ in 2009 and 2010 based on mean percent hemicellulose content.

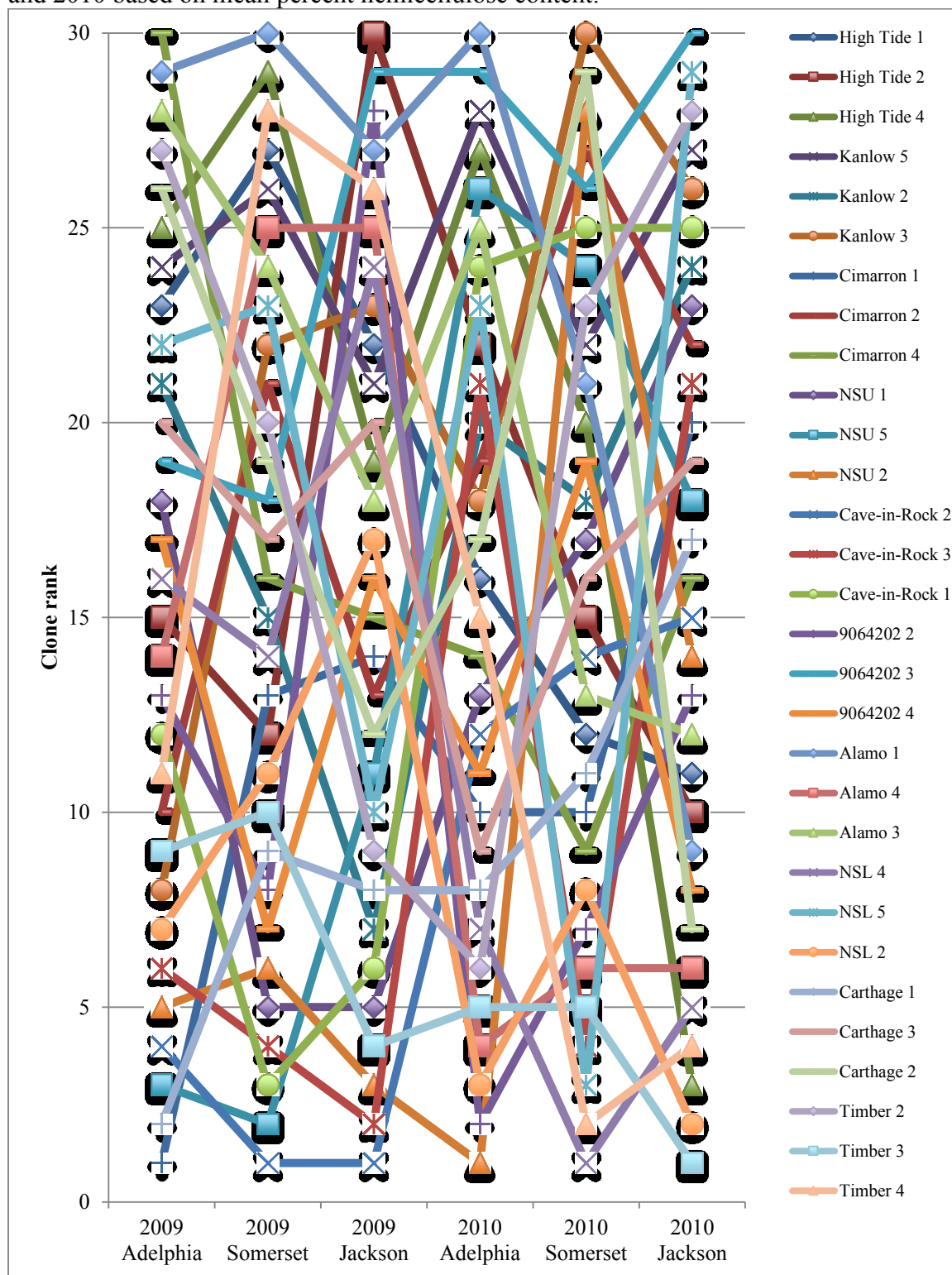
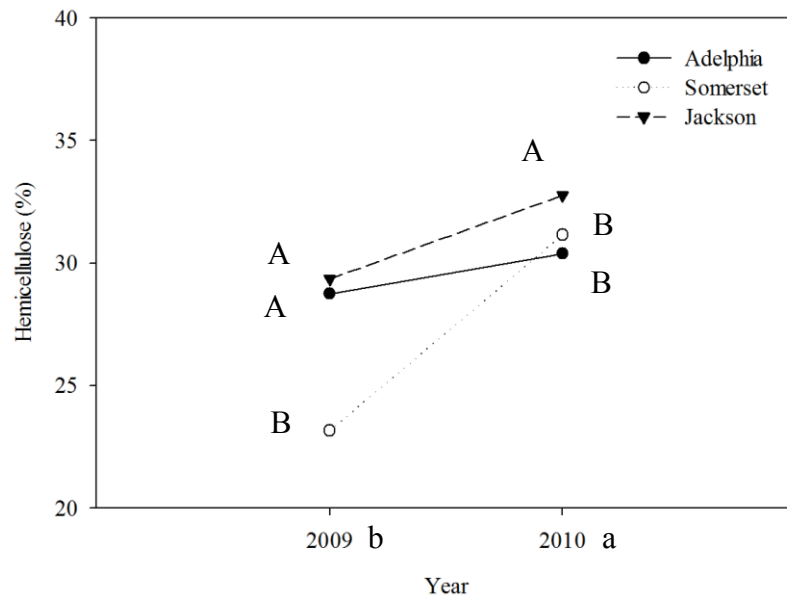


Figure 18. Mean percent hemicellulose content of thirty switchgrass clones grown in three locations in NJ in 2009 and 2010.



Upper case letters indicate differences among locations within each year. Lower case letters indicate differences between years.

7.3.2.2. Broad-sense Heritability Estimates

The broad-sense heritability estimate for hemicellulose content in switchgrass clones evaluated at three locations over two years on a clonal basis of 0.22 was considerably lower than that observed for cellulose content. On a single plant basis, broad-sense heritability for hemicellulose content in this population was only 0.03 (Table 32). As with cellulose, the low single plant heritability estimate indicates a strong influence of microenvironment on hemicellulose content. The low overall heritability estimates seen for hemicellulose indicate that selection for hemicellulose content in this switchgrass population would be slow, as the genetic contribution to phenotype is small. Broad-sense heritability estimates for hemicellulose content at each location were varied. At the prime location, H_c was estimated to be 0.55, while at the marginal locations of Somerset and Jackson, H_c estimates were 0.01 and 0.00, respectively (Table 29). These differences in heritability estimates between prime and marginal locations suggest that selection for hemicellulose content should be carried out in multiple environments and should include both prime and marginal sites. Land can be classified as marginal by the United States Department of Agriculture for various reasons, including low fertility, susceptibility to flooding or erosion, and slope (Natural Resources Conservancy Service, U.S. Department of Agriculture). It is possible the differences in soil characteristics between the prime and marginal locations contributed to the different heritability estimates. Determining the cause of these differences is beyond the scope of this study. To our knowledge, no reports of heritability of hemicellulose content in switchgrass exist in the literature. Murray et al. (2008) investigated lignocellulosic traits in a sorghum

population derived from a cross between a sweet sorghum cultivar and a grain sorghum inbred line and reported heritability estimates of 0.50 and 0.45 in sorghum stem and leaf tissue, respectively. Despite the relatively high heritability estimates, the authors found that selection for biomass yield should be more effective than selection for cell wall traits due to the fact that variation in biomass yield had a greater effect on theoretical ethanol yield than cell wall traits.

Table 32. Analysis of variance and broad-sense heritability estimates (H) for hemicellulose content (percent) of thirty switchgrass clones grown at three locations in New Jersey in 2009 and 2010

Source of Variation	DF	SS	MS	F value	P value	Variance Component ^a
Year	1	3993.00	3993.00	481.03	<0.0001	
Location	2	1931.64	965.82	116.35	<0.0001	
Rep(Location)	12	616.97	51.41	6.19	<0.0001	
Year x Location	2	1474.75	737.37	88.83	<0.0001	
Year x Rep(Location)	12	547.45	45.62	5.50	<0.0001	
Clone	29	993.70	34.27	4.13	<0.0001	0.2608
Clone x Year	29	815.61	28.12	3.39	<0.0001	1.1817
Clone x Location	57	589.99	10.35	1.25	0.1236	0.0000
Clone x Rep(Location)	344	2449.77	7.12	0.86	0.9181	0.0000
Clone x Year x Location	57	644.82	11.31	1.36	0.0526	0.5702
Error=Clone x Year x Rep(Loc)	315	2623.10	8.30			0.2608
$H_c^b = 0.22^c$						
$H_{sp}^d = 0.03$						

^aVariance components were determined using Restricted Maximum Likelihood Estimation using the PROC MIXED procedure in SAS (Cary, NC)

^b H_c = heritability estimate on a clonal basis

^c95% confidence interval for $H_c = 0.36$ -0.07; computed according to Knapp and Bridges (1987)

^d H_{sp} = heritability estimate on a single-plant basis

7.3.3. Lignin

7.3.3.1. Genotype x Environment Interactions

As was observed for cellulose content, a significant clone x location effect was observed for lignin content (Table 33), similar to findings of Cassida et al. (2005b) who reported a significant genotype x location interaction for lignin content in nine switchgrass genotypes evaluated at five sites for three years. Similar to results seen for hemicellulose content, Kendall's tau correlations coefficients were generally low indicating a large number of rank inversions, with the highest coefficients observed between the 2009 Somerset and 2009 Jackson environments, the 2009 Adelphia and 2009 Jackson environments, and the 2010 Adelphia and 2010 Somerset environments (Table 34). These low correlation coefficients further support the strong environmental effects mentioned previously. Clone Timber 2 consistently ranked among the bottom five clones over locations and years (Figure 19). Little information is available on stability of lignin content in switchgrass clones across environments. Sarath et al. (2008) investigated lignin content in six clones from each of two switchgrass populations divergently selected for IVDMD planted at one location harvested once. While lignin content of the population means was similar to previous reports of means from the same populations evaluated in different environments, differences among genotypes within each population were seen. In an evaluation of three forage grasses divergently selected for lignin and etherified ferulates, Casler et al. (2008) highlighted some of the difficulties inherent in evaluating clones for characteristics with large amounts of environmental and laboratory variation. "While clonal repeatability is high on samples collected within an experimental nursery,

repeatability is reduced when the clones are transplanted to a new experiment, likely reducing both broad- and narrow-sense heritability in selection for lignin or etherified ferulates.” Therefore, results seen here are not surprising given the amount of variation observed.

While no clone consistently ranked among the top five for lignin content across all locations, clones Cave-in-Rock 3 and 9064202 2 were among the top five at Adelphia and Somerset, while clone NSU 2 was among the top five at Adelphia and Jackson. Clones NSU 5 and 9064202 4 ranked among the top five clones at both marginal locations. Clones Timber 2 and Cimarron 4 were among the bottom five clones for lignin content at all locations, while clone Cimarron 2 ranked among the bottom five clones at both marginal sites (Figure 19). A significant clone x year effect was also observed (Table 33). Only clone NSU 5 consistently ranked among the top five clones for lignin content across years. In contrast, clones Cimarron 2 , Kanlow 5, Timber 2, and Cimarron 4 were all ranked in the bottom five clones for lignin content across years (Figure 19). Interestingly, Cimarron, Kanlow and Timber are all lowland ecotypes. Lemus et al. (2002) reported a similar trend in a study that investigated 20 switchgrass cultivars grown in Iowa over four years, where lowland switchgrass cultivars had lower lignin content than upland cultivars. Hopkins (1995a) evaluated lignin content at heading (plant material was harvested in June-August) in 20 switchgrass populations grown in three locations for two years and similarly found no population that ranked consistently among the top five across all years and locations. As was seen with cellulose and hemicellulose, a significant year x location effect was observed for lignin content (Table 33), also reported by Cassida et al. (2005b). Mean lignin content did not differ among locations in 2009, but

in 2010 was greater at Adelphia and Jackson than at Somerset (Figure 20), consistent with the later harvest dates at Adelphia and Jackson. Cassida et al. (2005b) stated that environmental effects (location and year) were more influential on variation in switchgrass lignocellulosic composition than cultivar, which is supported by results seen here.

While the factors affecting ethanol yield of cellulosic feedstocks are not well understood, feedstocks high in cellulose and hemicellulose and low in lignin are desirable (Vogel et al., 2011). Among the 30 clones evaluated, clone Timber 2 consistently ranked among the highest clones for cellulose content and lowest for lignin content, making it an ideal clone for use in a cellulosic feedstock breeding program. While this clone shows optimal lignocellulosic characteristics, other traits, especially biomass yield, disease resistance, and lodging resistance, must be considered. Several studies that have investigated cell wall characteristics in switchgrass and other feedstocks have demonstrated that while considerable variation for these traits exists in current germplasm, it remains unclear whether the benefits of selection for high cellulose/hemicellulose and low lignin outweigh the benefits of selecting for high biomass yield in terms of ethanol yield (Vogel et al., 2013; Sarath et al., 2011; Murray et al., 2008).

Table 33. Analysis of variance summary for percent lignin in thirty switchgrass clones grown at three locations in New Jersey in 2009 and 2010

Source	Numerator DF	Denominator DF	F Value	P Value
Year	1	24	55.66	<0.0001
Year x Location	2	24	5.14	0.0139
Clone	29	714	6.40	<0.0001
Clone x Year	29	714	4.31	<0.0001
Clone x Location	57	714	1.57	0.0060

Table 34. Kendall's tau and Spearman's rank (in parentheses) correlation coefficients for lignin content of fifty switchgrass clones grown at three locations in New Jersey in 2009 and 2010

	2009 Adelphia	2009 Somerset	2009 Jackson	2010 Adelphia	2010 Somerset	2010 Jackson
2009 Adelphia	-	0.37** (0.51**)	0.42** (0.61**)	0.10 (0.12)	0.17 (0.25)	0.01 (0.00)
2009 Somerset		-	0.48** (0.67***)	-0.12 (-0.20)	0.13 (0.23)	0.16 (0.21)
2009 Jackson			-	0.10 (0.11)	0.34** (0.48**)	0.12 (0.16)
2010 Adelphia				-	0.41** (0.57**)	0.13 (0.21)
2010 Somerset					-	0.20 (0.29)

***Indicates significance at $p < 0.0001$

**Indicates significance at $p < 0.01$

*Indicates significance at $p < 0.05$

Figure 19. Ranking of thirty switchgrass clones grown in three locations in NJ in 2009 and 2010 based on mean lignin content.

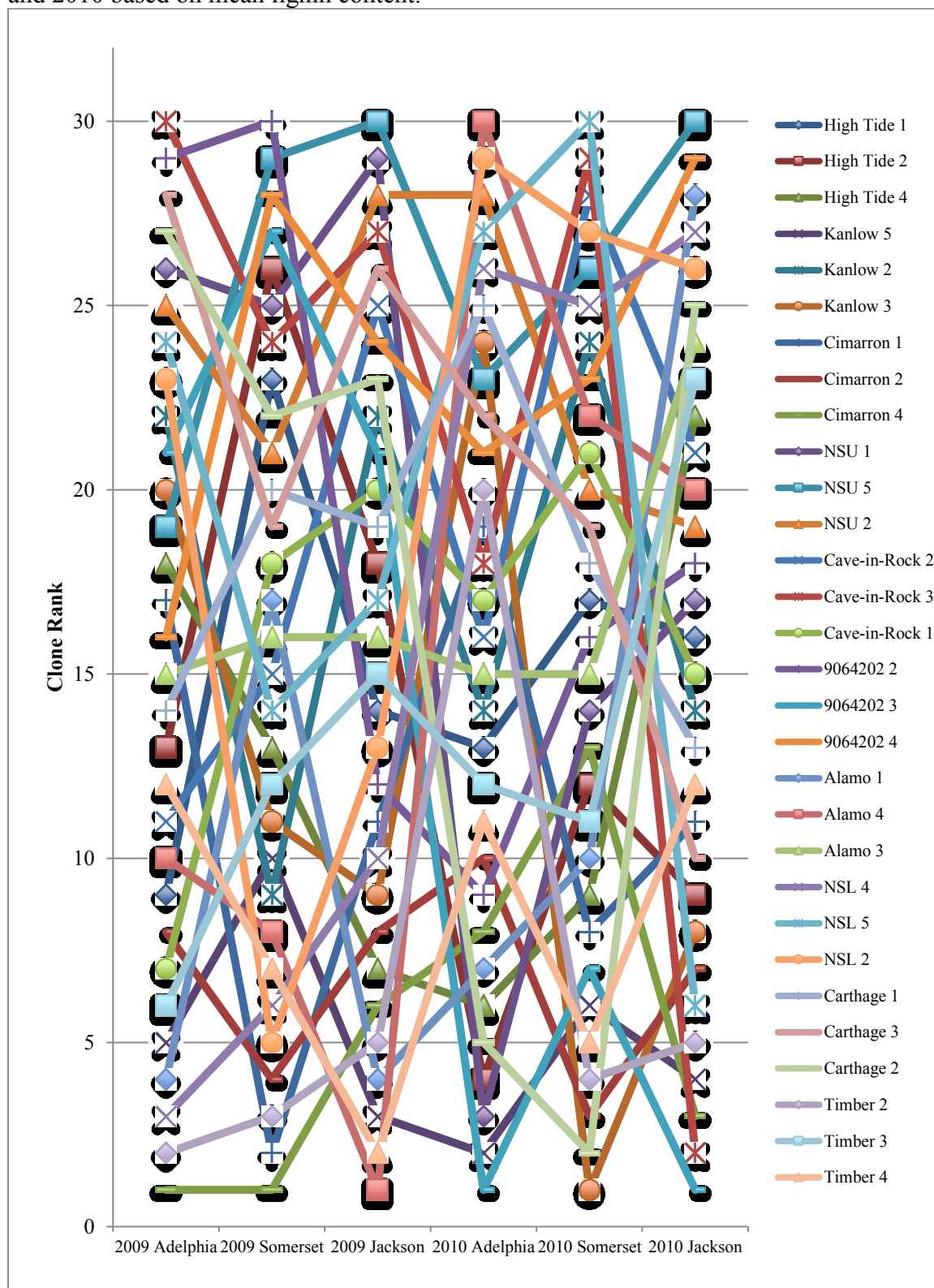
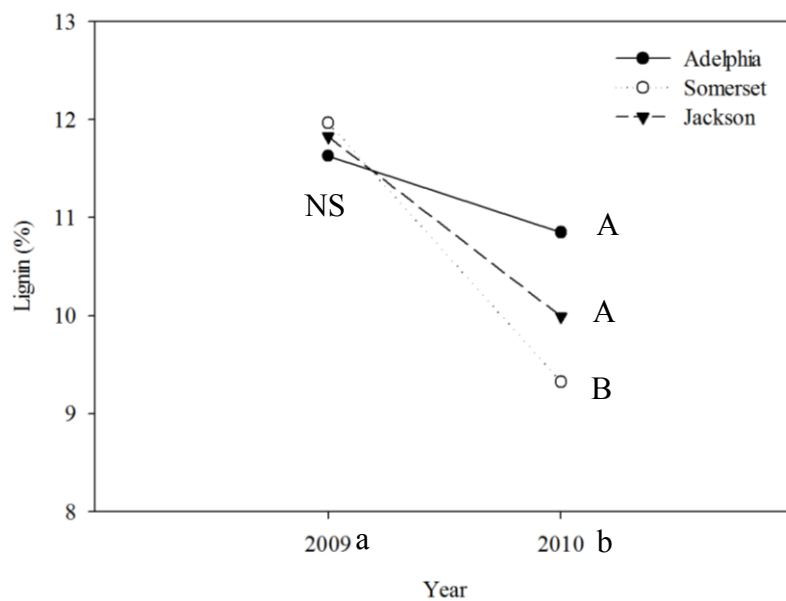


Figure 20. Mean percent lignin content of thirty switchgrass clones grown in three locations in NJ in 2009 and 2010.



NS - Not significant

Upper case letters indicate differences among locations within each year. Lower case letters indicate differences between years.

7.3.3.2. Broad-sense Heritability Estimates

Broad-sense heritability estimate for lignin content in switchgrass clones evaluated at three locations over two years on a clonal basis was 0.32, while on a single plant basis was only 0.05 (Table 35). Similar to results for cellulose and hemicellulose, the low single plant heritability estimate indicates a strong influence of microenvironment on lignin content in the switchgrass population evaluated. As was seen with hemicellulose, the low overall heritability estimates seen for lignin indicate that selection for lignin content in this switchgrass population would be slow, contrary to findings of Casler and Boe (2003), who reported no significant cultivar x environment interaction for lignin in six switchgrass populations grown at two locations over three years. Broad-sense heritability estimates for lignin content at each location varied. At Adelphia and Jackson, heritability estimates on a clonal basis were the same as the overall estimate of 0.32, while at Somerset, a lower estimate of 0.16 was observed (Table 29). The differences observed between the two marginal locations indicate that various marginal locations should be included when selecting for lignin content. It is possible the differences in soil characteristics between the two marginal locations contributed to the different heritability estimates; however one would have expected to see differential heritability estimates among all locations if this were the case. Determining the cause of these differences is beyond the scope of this study. While no studies investigating the effects of selection for lignin content in switchgrass were found in the literature, Carpenter and Casler (1990) reported that one cycle of divergent selection for lignin content in smooth brome grass was successful, whereby high lignin populations had

greater lignin than low lignin populations. However, no difference between either the high or low lignin populations and an unselected check were observed. Additionally, Casler et al. (2008) reported broad-sense heritability estimates for Klaser lignin content in clonal populations of smooth brome grass ($H = 0.47$), reed canarygrass ($H = 0.72$), and orchardgrass ($H = 0.82$); however Klaser lignin content has been shown to differ from lignin content determined from ADL (Dien et al., 2006; Vogel et al., 2013). Vogel et al. (2013) investigated the effects of six cycles of divergent selection for IVDMD in nine half-sib switchgrass families and found that lignin decreased with selection for increased IVDMD and lignin increased with selection for low IVDMD. However, the relationship between lignin content and ethanol yield is not straightforward, as Sarath et al. (2011) demonstrated that less than half of the variation observed among switchgrass genotypes for ethanol yield was due to differences in lignin content.

Table 35. Analysis of variance and broad-sense heritability estimates (H) for lignin content (percent) of thirty switchgrass clones grown at three locations in New Jersey in 2009 and 2010

Source of Variation	DF	SS	MS	F value	P value	Variance Component ^a
Year	1	610.44	610.44	185.52	<0.0001	
Location	2	27.02	13.51	4.11	0.0174	
Rep(Location)	12	199.52	16.63	5.05	<0.0001	
Year x Location	2	98.75	49.37	15.01	<0.0001	
Year x Rep(Location)	12	130.35	10.86	3.30	0.0002	
Clone	29	585.76	20.20	6.14	<0.0001	0.2469
Clone x Year	29	406.60	14.02	4.26	<0.0001	0.6917
Clone x Location	57	290.09	5.09	1.55	0.0109	0.0665
Clone x Rep(Location)	344	1133.15	3.29	1.00	0.4966	0.0000
Clone x Year x Location	57	262.98	4.61	1.40	0.0385	0.2722
Error=Clone x Year x Rep(Loc)	315	1029.87	3.29			3.3007
$H_c^b = 0.32^c$						
$H_{sp}^d = 0.05$						

^aVariance components were determined using Restricted Maximum Likelihood Estimation using the PROC MIXED procedure in SAS (Cary, NC)

^b H_c = heritability estimate on a clonal basis

^c95% confidence interval for $H_c = 0.42$ - 0.22 ; computed according to Knapp and Bridges (1987)

^d H_{sp} = heritability estimate on a single-plant basis

7.4. CONCLUSIONS

This is the first study investigating lignocellulosic characteristics of switchgrass clones grown at both prime and marginal sites. Significant clone x location, clone x year, and year x locations interactions observed for cellulose and lignin contents, as well as significant clone x location x year effects seen for hemicellulose content indicate environment influences expression of these lignocellulosic traits in the switchgrass population tested here. Unlike cellulose, low broad-sense heritability estimates seen for hemicellulose and lignin content and the crossover in ranking observed among some clones for hemicellulose content support the importance of genotype x environment interactions on these characteristics. However, the relative ranking of switchgrass clones across locations and years revealed clones that were consistently high or low in cellulose and hemicellulose content at all three locations as well as clones that were consistently low in lignin content at all locations. Some clones were identified that were consistently ranked among the top or bottom five clones at both marginal locations for cellulose and lignin content, which supports the idea of breeding for site-specifically adapted cultivars. Given the high degree of genetic diversity found within currently available switchgrass cultivars and the fact that these cultivars have not undergone extensive cycles of selection for improvement (Casler, 2012), the variable nature of the results in this study are not surprising and support studies demonstrating the existence of both specifically and broadly adapted switchgrass germplasm (Casler and Boe, 2003). While further studies investigating effect of selection environment (especially soil characteristics affecting land classification), narrow-sense heritability, and realized gains from selection in switchgrass

populations grown on both prime and marginal sites are needed, results from this study support the need for evaluation of switchgrass germplasm across multiple years and environments that include both prime and marginal sites in order to develop cultivars with optimal lignocellulosic characteristics. Additionally, despite previous reports that demonstrated selection effectively altered cell wall components in switchgrass, it is evident that a better understanding of what selection criteria and testing methods are most effective in improving lignocellulosic traits and ethanol yields of bioenergy feedstocks is needed.

8. GENOTYPE X ENVIRONMENT AND BROAD-SENSE HERITABILITY ESTIMATES OF AGRONOMIC CHARACTERISTICS OF SWITCHGRASS CLONES GROWN ON MARGINAL AND PRIME SOILS IN NEW JERSEY

8.1. INTRODUCTION

Switchgrass (*Panicum virgatum* L.) is a perennial C₄ grass native to the eastern two thirds of the US, identified as a model bioenergy crop by the US Department of Energy in 1991 based on its native status, soil conservation and environmental benefits, broad adaptability to a wide range of environments, and potential for high yields on marginal soils with few inputs (Parrish and Fike, 2005; Bouton, 2009). Switchgrass as a species contains two ecotypes, upland and lowland, which are distinguished by differences in plant phenotype. Upland ecotypes are typically adapted north of 34° N latitude and are finer stemmed, shorter, and more drought tolerant, while lowland ecotypes are adapted to warmer, wetter habitats and are taller with fewer but larger tillers, thicker stems, and larger leaves (Casler, 2012). While switchgrass is currently utilized as a bioenergy feedstock, there are few improved cultivars available that have been developed as feedstocks, and those that do exist have undergone few cycles (five to six) of selection from wild germplasm (Casler, 2012). Ideally, switchgrass will be grown on marginal soils not suited for traditional agricultural crops, but little research has investigated its performance on marginal soils (Tulbure et al., 2012). Mulkey et al. (2006) investigated effects of harvest date and N rate on switchgrass grown on land enrolled in the Conservation Reserve Program in South Dakota, and determined that one post killing frost harvest annually with N rates of 56 kg ha⁻¹ was effective for biomass

production. In the Midwest US, Schmer et al. (2008) reported switchgrass biomass yields of 5.2 to 11.1 Mg ha⁻¹ on land with marginal characteristics. Varvel (2008) conducted a study comparing switchgrass and corn biomass yields and estimated ethanol yields on a low fertility field representative of marginal soils in the Conservation Reserve Program. He reported estimated ethanol yield in switchgrass to be the same as or greater than that of corn (calculated from both grain and stover) given the same fertilizer rate. However, no switchgrass yield in kg ha⁻¹ was reported. Land can be classified as marginal (category IV, V, VI, or VII) based on several characteristics including soil fertility, erosion, steep slope, poor drainage, or severe salinity (Klingebeil and Montgomery, 1961). Furthermore, several studies have demonstrated that switchgrass cultivars vary widely in performance across different adaptation zones (Casler, 2010), with significant cultivar x environment interactions reported for biomass yield (Casler and Boe, 2003). Therefore, it is likely that the performance and biomass yields of switchgrass cultivars will differ on marginal land versus prime land. While it has already been demonstrated that regional switchgrass cultivars should be developed, breeders need a better understanding of the effects of marginal soil on switchgrass feedstock characteristics in order to effectively develop improved cultivars. Most switchgrass breeding is carried out on research stations with prime soils. Breeders must know if selection in non-target environments (prime farmland) will effectively improve performance in target environments (Rose et al., 2007). Therefore, the following study was conducted in order to 1) examine genotype by environment effects and 2) determine broad-sense heritability estimates on resistance to anthracnose disease caused by *Colletotrichum navitas* and rust disease caused by *Puccinia* sp., plant height, tiller number, lodging, and biomass yield in switchgrass clones

grown on marginal and prime soils in New Jersey.

8.2. MATERIALS AND METHODS

8.2.1. Plant Material

In summer of 2007, five switchgrass genotypes from 10 different switchgrass cultivars and germplasm populations (Table 23) were randomly chosen from a spaced-plant screening nursery that was established at the Rutgers University Plant Biology Research and Extension Farm at Adelphia, NJ. Each genotype was evenly divided into six ramets and planted to a Freehold sandy loam in summer 2008. Plants received 56.1 kg N ha⁻¹ after transplanting. In spring 2009, each ramet was further divided evenly into three ramets for a total of 18 ramets per genotype and 900 individual ramets. Plants were maintained in 10 cm pots in Pro-Mix HP (K.C. Shafer, York, PA) for 4 weeks before planting to three locations in New Jersey, one on prime farmland and two on marginal land sites (Tables 24 and 25). The prime site was located at the Rutgers University Plant Biology Research and Extension Farm at Adelphia, NJ. The Adelphia site has a Class II prime land designation (USDA) with a Freehold Sandy Loam soil, and was planted on 29 June, 2009. One of the marginal sites was located in Somerset, NJ. The Somerset location is designated as Class IV marginal land (USDA) with a Klinesville channery loam soil, and was planted on 7 July 2009. The second marginal site was located in Jackson, NJ at the Pine Barrens Golf Club. The Jackson location has a Class V marginal land designation (USDA) with an Evesboro sand soil and was planted 30 June, 2009. At each location, individual plants were spaced 0.9 m apart with 10 plants per row in a

randomized complete block design with six replications for a total of 300 plants per location. All clones received 56.1 Kg N ha⁻¹ upon transplant and in June 2010 and 2011.

8.2.2. Data Collection

All individual clones at all locations were evaluated for anthracnose disease caused by *Colletotrichum navitas*, rust disease caused by *Puccinia* sp., plant height, tiller number, lodging, and biomass yield in 2010 and 2011. Anthracnose disease and rust disease were evaluated using a visual rating scale of 1 to 10, whereby 1 represented 100 % anthracnose and rust disease, and 10 represented 0 % anthracnose and rust disease. Anthracnose disease was determined on 26 July 2010 and 28 July 2011 at all three locations, and rust disease on 2 October 2010 and 5 October 2011 at all three locations. Plant height was determined by measuring individual plants from the plant base to the top of an average panicle. Plant heights were recorded when plants were no longer actively growing on 11 November 2010 and 13 September 2011 at all locations. Tiller number per plant was determined by manually counting all vegetative and reproductive tillers on all individual plants, and was recorded after plants stopped producing new tillers on 18 November 2010 at Somerset and 19 November 2010 at Adelpia and Jackson, and 10 September 2011 at Jackson and Somerset and 14 September 2011 at Adelpia. Lodging was evaluated using a visual rating scale of 1 to 10, whereby 1 represented 100 % lodging, and 10 represented 0 % lodging. Lodging was determined on 9 September 2010 and 5 October 2011 at all locations. Biomass yield was determined on an individual plant basis. Each individual plant was harvested by hand at a height of 10 cm. The plant material was then dried at 43 °C for 14 days and weighed. The 2010 biomass yields were

determined from harvests made on the following dates: 4 January 2011 at Somerset, 17 February 2011 at Jackson, and 4 March 2011 at Adelphia, while 2011 yields were determined from harvests on 20 December 2011 at Jackson, 9 January 2012 at Somerset, and 31 January 2012 at Adelphia.

8.2.3. Data Analysis

Statistical analysis was conducted using the SAS System version 9.3 (SAS Institute Inc., Cary, NC) to perform analysis of variance. Genotype x environment interactions for anthracnose disease, rust disease, plant height, tiller number, lodging, and biomass yield data were analyzed using the MIXED procedure of the SAS System (SAS Institute Inc., Cary, NC). Effects of clone, year, location, clone x year, clone x location, year x location, and clone x year x location were included in the model as fixed effects and removed if not significant, and effects of block within year and location and year x location x block x clone were considered random. Data were examined for violations of assumptions by inspection of plots of standardized residuals. When analysis detected significant interaction effects, differences were assessed using the SLICE option in the LSMEANS statement and were considered significant at the 0.05 level. When the analysis indicated a significant clone x year x location interaction, data from each location were analyzed separately using the MIXED procedure of the SAS System (SAS Institute Inc., Cary, NC). Effects of clone, year, and clone x year were included in the model as fixed effects, and effect of block within year was considered random. Differences were assessed using the DIFF option in the LSMEANS statement and were

considered significant at the 0.05 level. Kendall's tau and Spearman's rank correlation coefficients were determined using the PROC CORR procedure of the SAS System (SAS Institute Inc., Cary, NC).

Broad sense heritability estimates for anthracnose disease, rust disease, plant height, tiller number, lodging, and biomass yield were determined from restricted maximum likelihood variance and covariance components using the MIXED procedure of the SAS System (SAS Institute Inc., Cary, NC). All effects were considered random because years were not chosen with respect to expected climatic conditions (Gordon et al., 1972), locations were not chosen with respect to specific ecological properties, and no information on the characteristics evaluated in the switchgrass clones was known before the initiation of the study. Heritability was calculated on a clonal mean (Hc) basis as well as on a single-plant basis (Hsp) using the following formulas:

$$Hc = \sigma^2_c / (\sigma^2_c + \sigma^2_{cy}/y + \sigma^2_{cl}/l + \sigma^2_{cr(l)}/rl + \sigma^2_{cly}/ly + \sigma^2_e/rly)$$

$$Hsp = \sigma^2_c / (\sigma^2_c + \sigma^2_{cy} + \sigma^2_{cl} + \sigma^2_{cr(l)} + \sigma^2_{cly} + \sigma^2_e)$$

where σ^2_c = the total genetic variance of clones, σ^2_{cy} = clone x year variance, σ^2_{cl} = clone x location variance, $\sigma^2_{cr(l)}$ = clone x replication within location variance, σ^2_{cly} = clone x year x location variance, and σ^2_e = experimental error (clone x year x replication within location). Letters in the denominator refer to the number of replications (six), replications (locations) (18), locations (three), and years (two) (Poehlman and Sleper, 2006).

8.3. RESULTS AND DISCUSSION

8.3.1. Anthracnose

8.3.1.1. Genotype x Environment Interactions

A significant clone x year x location effect was observed for anthracnose disease; therefore, each location was analyzed separately (Table 36). Additionally, a significant year x location effect was observed, where the Adelphia location had the highest mean anthracnose rating in 2010 but the lowest in 2011, while Jackson had the lowest mean anthracnose rating in 2010 but the highest in 2011. These differences indicate differential anthracnose disease pressure across the year-location combinations in this study. At Adelphia, significant clone x year effect was observed, which was due to a change in rank among clones between 2010 and 2011. In fact, none of the same clones were ranked among the top five clones at the prime location in 2010 and 2011 (high ratings indicate disease resistance). However, clones NSU 5, NSU 1, and NSU 2 ranked in the bottom five clones for anthracnose, with consistently high susceptibility to anthracnose across years in this study (Table 37). At the Somerset location, a similar clone x year effect was observed. Clone Timber 4 ranked among the top five clones for anthracnose ratings in both 2010 and 2011, while clones NSU 5, NSU 1, NSU 2, and 9064202 5 consistently ranked among the bottom five clones (Table 37). The Jackson location demonstrated the largest change in mean anthracnose rating between years (6.1 in 2010 and 8.1 in 2011). In fact, the highest mean rating in 2010 was only 7.5 (seen in clones Carthage 5 and

Timber 4). Similar to other locations, a change in clone rank for anthracnose disease between evaluation years was seen at Jackson. Clones Timber 4 and Cimarron 3 ranked among the top five clones for anthracnose disease, while clone NSU 2 ranked among the bottom 5 in both 2010 and 2011. No clone consistently ranked among the top five across all years and locations, suggesting environmental influence on anthracnose disease resistance in the switchgrass population used in this study. However, clone Timber 4 did rank among the top five clones for all years and locations except at Adelphia in 2011, where it ranked eighth (Table 37). Clone NSU 2 ranked among the bottom five clones for anthracnose disease at all locations in 2010 and 2011, and clones NSU 1 and NSU 5 ranked among the bottom five clones at all locations and years except for Jackson in 2010. Clone Cimarron 5 exhibited a substantial crossover in ranking between Somerset in 2010 where it was among the top five clones and Adelphia in 2011 where it was among the bottom five clones. At other locations and years, Cimarron 5 ranked 30th at Adelphia in 2010, 30th at Somerset in 2011, 39th at Jackson in 2010, and 12th at Jackson in 2011. Kendall's tau and Spearman's rank correlation coefficients were high for 2010 Adelphia and 2010 Jackson and all combinations of 2011 locations (Table 38), indicating few inversions in clone rank when comparing these environments. However, correlation coefficients were low for all other comparisons. This variation across years and locations suggests that anthracnose disease resistance is affected by environmental factors..

Table 36. Analysis of variance summary for anthracnose disease in fifty switchgrass clones grown at three locations in New Jersey in 2010 and 2011

Source	Numerator DF	Denominator DF	F Value	P Value
Year	1	30	241.98	<0.0001
Location	2	30	0.77	0.4715
Year x Location	2	30	25.39	<0.0001
Clone	49	1423	33.51	<0.0001
Clone x Year	49	1423	4.58	<0.0001
Clone x Location	98	1423	4.84	<0.0001
Clone x Year x Location	98	1423	1.89	<0.0001

Table 37. Clones with the highest and lowest mean anthracnose rating (1=100% disease, 10=no disease) of fifty switchgrass clones grown at three locations in New Jersey in 2010 and 2011

Location Year	Adelphia				Somerset				Jackson			
	2010		2011		2010		2011		2010		2011	
	Clone	Mean anthrac- nose	Clone	Mean anthrac- nose	Clone	Mean anthrac- nose	Clone	Mean anthrac- nose	Clone	Mean anthrac- nose	Clone	Mean anthrac- nose
Highest 5	High Tide 4	8.8	Timber 5	8.8	High Tide 4	8.2	Cimarron 2	8.8	Carthage 5	7.5	Cimarron 1	9.0
	High Tide 5	8.8	Cimarron 2	8.7	Timber 4	7.8	Timber 3	8.8	Timber 4	7.5	Kanlow 5	9.0
	High Tide 3	8.5	Cimarron 5	8.7	Carthage 3	7.7	Timber 4	8.8	Timber 1	7.3	NSL 3	9.0
	Timber 4	8.5	NSL 3	8.7	C-i-R 4	7.5	Cimarron 3	8.7	Alamo 4	7.0	Timber 4	9.0
	Carthage 5	8.0	Timber 2	8.6	High Tide 1	7.5	Alamo 5	8.6	Cimarron 3	7.0	Cimarron 3	8.8
Lowest 5	C-i-R ^a 5	4.7	Carthage 4	5.0	NSU 1	4.8	NSU 1	6.0	9064202 4	5.0	NSU 1	6.7
	NSU 5	4.2	C-i-R 1	4.3	NSU 2	4.7	9064202 5	5.7	NSU 2	5.0	9064202 5	6.6
	NSU 1	3.3	NSU 5	4.0	Cimarron 5	4.5	NSU 2	5.7	High Tide 2	4.8	NSU 5	6.2
	NSU 2	3.2	NSU 1	3.3	NSU 5	4.5	C-i-R 1	5.2	NSU 4	4.5	C-i-R 1	5.8
	9064202 5	3.0	NSU 2	3.3	9064202 5	4.3	NSU 5	5.0	9064202 1	3.8	NSU 2	5.3
LSD		1.0		1.0		1.1		0.7		1.3		0.8

^a C-i-R – Cave-in-Rock

Table 38. Kendall's tau and Spearman's rank (in parentheses) correlation coefficients for anthracnose disease of fifty switchgrass clones grown at three locations in New Jersey in 2010 and 2011

	2010 Adelphia	2010 Somerset	2010 Jackson	2011 Adelphia	2011 Somerset	2011 Jackson
2010 Adelphia	-	0.17 (0.20)	1.00*** (1.00***)	0.19 (0.27)	0.39** (0.55***)	0.41 *** (0.54***)
2010 Somerset		-	0.17 (0.20)	0.23* (0.30*)	0.26** (0.36**)	0.18 (0.26)
2010 Jackson			-	0.19 (0.27)	0.39** (0.55***)	0.41 *** (0.54***)
2011 Adelphia				-	0.59*** (0.74***)	0.55*** (0.70***)
2011 Somerset					-	0.65*** (0.79***)

***Indicates significance at $p < 0.0001$

**Indicates significance at $p < 0.01$

*Indicates significance at $p < 0.05$

8.3.1.2. Broad-sense Heritability Estimates

The broad-sense heritability estimate for anthracnose in switchgrass clones evaluated at three locations over two years was 0.78 on a clonal basis, and 0.32 on a single plant basis (Table 39). While a significant portion of the variance was due to the effect of clone, the lower single plant heritability estimate demonstrates the large amount of clonal variation observed within environment for anthracnose disease and suggests that evaluation of germplasm for anthracnose resistance should include clonal replicates. Broad-sense heritability estimates for anthracnose disease at each location were varied. At the prime location, H_c was estimated to be 0.91, while at the marginal locations of Somerset and Jackson, H_c estimates were 0.70 and 0.59, respectively (Table 40). Single plant heritability estimates were higher at the prime location than the marginal locations as well (Adelphia 0.62, Somerset 0.33, Jackson 0.20). These differences in heritability estimates between locations suggest that selection for anthracnose resistance should be carried out in multiple environments and should include both prime and marginal sites. Additionally, it is suggested that germplasm should be clonally replicated and evaluated in multiple environments in order to identify clones with superior anthracnose resistance.

Table 39. Analysis of variance and broad-sense heritability estimates (H) for anthracnose disease of fifty switchgrass clones grown at three locations in New Jersey in 2010 and 2011

Source of Variation	DF	SS	MS	F value	P value	Variance Component ^a
Year	1	712.79133	712.79133	968.42	<.0001	
Location	2	5.055619	2.52781	3.43	0.0328	
Rep(Location)	15	38.864127	2.590942	3.52	<.0001	
Year x Location	2	152.514269	76.257134	103.61	<.0001	
Year x Rep(Location)	15	51.066852	3.404457	4.63	<.0001	
Clone	49	1277.625685	26.073994	35.43	<.0001	0.5806
Clone x Year	49	175.133488	3.574153	4.86	<.0001	0.1196
Clone x Location	98	366.834142	3.743206	5.09	<.0001	0.1838
Clone x Rep(Location)	727	609.530075	0.838418	1.14	0.0407	0.0662
Clone x Year x Location	98	143.640952	1.465724	1.99	<.0001	0.1307
Error=Clone x Year x Rep(Loc)	709	521.8477	0.74			0.7213
$H_c^b = 0.78^c$						
$H_{sp}^d = 0.32$						

^aVariance components were determined using Restricted Maximum Likelihood Estimation using the PROC MIXED procedure in SAS (Cary, NC)

^b H_c = heritability estimate on a clonal basis

^c95% confidence interval for $H_c = 0.79$ -0.76; computed according to Knapp and Bridges (1987)

^d H_{sp} = heritability estimate on a single-plant basis

Table 40. Broad-sense heritabilities of biomass field characteristics of fifty switchgrass clones grown at Adelphia, Somerset, and Jackson, NJ in 2010 and 2011

		Adelphia	Somerset	Jackson
Anthracnose	<i>Hc</i> ^a	0.91 (0.93-0.89) ^b	0.70 (0.73-0.66)	0.59 (0.66-0.52)
	<i>Hsp</i> ^c	0.62	0.33	0.20
Rust	<i>Hc</i>	0.55 (0.63-0.46)	0.42 (0.46-0.37)	0.67 (0.73-0.61)
	<i>Hsp</i>	0.16	0.16	0.25
Plant Height	<i>Hc</i>	0.97 (0.98-0.96)	0.92 (0.93-0.90)	0.96 (0.96-0.95)
	<i>Hsp</i>	0.77	0.65	0.79
Lodging	<i>Hc</i>	0.85 (0.88-0.81)	0.80 (0.83-0.77)	0.86 (0.90-0.82)
	<i>Hsp</i>	0.43	0.43	0.44
Tiller Number	<i>Hc</i>	0.86 (0.92-0.81)	0.89 (0.90-0.87)	0.88 (0.91-0.84)
	<i>Hsp</i>	0.37	0.61	0.46
Biomass Yield	<i>Hc</i>	0.94 (0.95-0.92)	0.85 (0.86-0.84)	0.84 (0.90-0.79)
	<i>Hsp</i>	0.64	0.61	0.37

^a*Hc* = heritability estimate on a clonal basis

^b95% confidence interval for *Hc* computed according to Knapp and Bridges (1987)

^c*Hsp* = heritability estimate on a single-plant basis

8.3.2. Rust Disease

8.3.2.1. Genotype x Environment Interactions

As was observed for anthracnose disease, a significant clone x year x location effect was observed for rust disease (Table 41), similar to the findings of Hopkins et al. (1995a), who observed a significant effect of population x year x location for disease (which included rust and anthracnose) in 20 switchgrass populations evaluated at three locations for two years. Disease pressure was greater in 2011 than in 2010 as indicated by the lower overall mean ratings, and among locations was greater at Adelphia and Jackson than at Somerset, similar to the findings of Uppalapati et al (2013), who reported differential rust ratings between evaluation years in a study that investigated rust incidence on clones from switchgrass cultivars Alamo, Kanlow, Summer, and Cave-in-Rock in OK. A significant clone x year effect was observed for rust ratings at all locations, similar to anthracnose ratings. However, each location showed at least one clone that ranked in the top 5 or bottom five in both years of the study. At Adelphia, clone NSU 5 ranked in the top five clones for rust rating in both 2010 and 2011, while clone NSL 2 ranked in the bottom five clones in both years. At Somerset, clone Timber 4 consistently ranked among the top ten percent of clones for rust resistance, and clone 9064202 2 consistently ranked in the bottom ten percent for rust. Finally, at Jackson, clone Kanlow 3 was among the best five clones for rust resistance, while clones 9064202 2 and High Tide 4 were among the worst five clones (Table 42). No clones consistently ranked in the top or bottom ten percent for rust resistance across all years and locations in

this study, nor were any substantial crossovers in rank observed (Table 42). Similarly, Uppalapati et al. (2013) failed to identify rust resistant clones from a two year field evaluation of ten genotypes each from cultivars Alamo, Kanlow, Summer, and Cave-in-Rock. The low Kendall's tau and Spearman's rank correlation coefficients for rust (Table 43) support the importance of environmental effects on rust resistance in this population of switchgrass evaluated in the six environments of this study.

Table 41. Analysis of variance summary for rust disease in fifty switchgrass clones grown at three locations in New Jersey in 2010 and 2011

Source	Numerator DF	Denominator DF	F Value	P Value
Year	1	30	12.13	0.0015
Location	2	30	149.91	<0.0001
Year x Location	2	30	5.00	0.0133
Clone	49	1423	29.40	<0.0001
Clone x Year	49	1423	2.59	<0.0001
Clone x Location	98	1423	4.03	<0.0001
Clone x Year x Location	98	1423	1.70	<0.0001

Table 42. Clones with the highest and lowest mean rust rating (1=100% disease, 10=no disease) of fifty switchgrass clones grown at three locations in New Jersey in 2010 and 2011

Location	Adelphia				Somerset				Jackson			
Year	2010		2011		2010		2011		2010		2011	
	Clone	Mean rust	Clone	Mean rust	Clone	Mean rust	Clone	Mean rust	Clone	Mean rust	Clone	Mean rust
Highest 5	Kanlow 2	8.0	C-i-R 1	6.7	Kanlow 3	9.3	Carthage 4	7.8	NSL 1	9.2	Timber 2	6.8
	NSL 4	8.0	NSU 2	6.5	NSL 4	9.3	NSU 2	7.8	Alamo 4	9.0	Cimarron 1	6.7
	NSU 5	8.0	NSU 3	6.5	Timber 3	9.3	NSU 5	7.8	Kanlow 3	8.8	NSL 4	6.7
	Timber 2	8.0	NSU 5	6.5	Timber 4	9.3	Timber 4	7.8	Timber 4	8.7	NSL 3	6.5
	C-i-R ^a 3	7.7	Carthage 3	6.3	NSL 3	9.2	C-i-R 1	7.7	Cimarron 3	8.2	Kanlow 3	6.4
Lowest 5	NSL 2	6.0	Kanlow 1	3.8	C-i-R 2	7.7	High Tide 4	5.2	9064202 3	5.5	High Tide 3	4.5
	Carthage 1	5.8	Kanlow 3	3.8	NSU 4	7.7	9064202 3	4.8	High Tide 4	5.3	9064202 1	4.3
	Cimarron 5	5.8	NSL 2	3.8	9064202 2	7.5	9064202 2	4.7	9064202 4	5.0	High Tide 5	4.3
	9064202 4	5.3	Cimarron 4	3.5	C-i-R 3	7.3	9064202 5	4.2	9064202 5	4.7	9064202 2	4.2
	9064202 2	4.5	Timber 5	3.4	Cimarron 5	6.8	9064202 4	3.8	9064202 2	3.3	High Tide 4	4.0
LSD		1.0		1.3		0.7		0.9		1.3		0.9

^a C-i-R – Cave-in-Rock

Table 43. Kendall's tau and Spearman's rank (in parentheses) correlation coefficients for rust disease of fifty switchgrass clones grown at three locations in New Jersey in 2010 and 2011

	2010 Adelphia	2010 Somerset	2010 Jackson	2011 Adelphia	2011 Somerset	2011 Jackson
2010 Adelphia	-	0.21* (0.27*)	0.17 (0.23)	0.28** (0.42**)	0.37** (0.50**)	0.25* (0.31*)
2010 Somerset		-	0.40*** (0.52***)	-0.13 (-0.17)	0.17 (0.24)	0.16 (0.20)
2010 Jackson			-	-0.10 (-0.16)	0.25* (0.33*)	0.32** (0.44**)
2011 Adelphia				-	0.28** (0.40**)	0.10 (0.14)
2011 Somerset					-	0.35** (0.49**)

***Indicates significance at $p < 0.0001$

**Indicates significance at $p < 0.01$

*Indicates significance at $p < 0.05$

8.3.2.2. Broad-sense Heritability Estimates

The broad-sense heritability estimate for rust disease in switchgrass clones evaluated at three locations over two years on a clonal basis of 0.61 was lower than that observed for anthracnose disease. On a single plant basis, broad-sense heritability for rust in this population was only 0.14 (Table 44), indicating a strong influence of microenvironment on rust resistance. The low single plant heritability estimate indicates that selection for rust in this population of switchgrass would be ineffective if based on non-replicated single plants in one environment, as clonal variation for rust resistance within environment was observed, similar to findings of Uppalati et al. (2013). As with anthracnose, broad-sense heritability estimates for rust at each location were varied (Table 40). In this case, the Jackson location demonstrated the highest heritability estimate ($H_c = 0.67$, $H_{sp} = 0.25$), followed by the prime location ($H_c = 0.55$, $H_{sp} = 0.16$) and the Somerset location ($H_c = 0.42$, $H_{sp} = 0.16$) which had the least disease pressure, indicating differential amounts of genetic contribution to phenotypic expression of rust resistance in these three environments.

The low heritability estimates combined with the lack of consistent ranking of any clone across all locations and environments in this study suggests a strong environmental influence on rust resistance in the population of switchgrass evaluated, similar to findings of Gustafson et al. (2003), who reported the presence of non-additive genetic variation for rust disease based on significant variation observed among individuals within each of four switchgrass populations that were evaluated at two locations in South Dakota over two years. As was suggested with anthracnose, evaluation of switchgrass germplasm for

rust resistance should be carried out on replicated clones in multiple environments in order to identify clones to be used in breeding programs. It is possible that the sample size of five genotypes per population or cultivar did not allow for the identification of stable genotypes in this study, as Gustafson et al. (2003) also reported significant among family variation that suggests the presence of additive genetic variation for rust.

Table 44. Analysis of variance and broad-sense heritability estimates (H) for rust disease of fifty switchgrass clones grown at three locations in New Jersey in 2010 and 2011

Source of Variation	DF	SS	MS	F value	P value	Variance Component ^a
Year	1	1121.7989	1121.7989	1530.46	<.0001	
Location	2	839.4001	419.7001	572.59	<.0001	
Rep(Location)	15	40.5906	2.7060	3.69	<.0001	
Year x Location	2	13.6362	6.8181	9.3	0.0001	
Year x Rep(Location)	15	59.5049	3.9670	5.41	<.0001	
Clone	49	540.7346	11.0354	15.06	<.0001	0.1901
Clone x Year	49	156.4220	3.1923	4.36	<.0001	0.0655
Clone x Location	98	298.2453	3.0433	4.15	<.0001	0.0663
Clone x Rep(Location)	727	655.8096	0.9021	1.23	0.0028	0.0909
Clone x Year x Location	98	215.1569	2.1955	3	<.0001	0.2397
Error=Clone x Year x Rep(Loc)	709	519.6823	0.7330			0.7272
$H_c^b = 0.61^c$						
$H_{sp}^d = 0.14$						

^aVariance components were determined using Restricted Maximum Likelihood Estimation using the PROC MIXED procedure in SAS (Cary, NC)

^b H_c = heritability estimate on a clonal basis

^c95% confidence interval for $H_c = 0.64$ - 0.58 ; computed according to Knapp and Bridges (1987)

^d H_{sp} = heritability estimate on a single-plant basis

8.3.3. Plant Height

8.3.3.1. Genotype x Environment Interactions

Similar to the disease data, a significant clone x year x location effect was observed for plant height (Table 45). As expected, clones were shorter at the two marginal locations than the prime location. Mean plant height was 121.1 cm in 2010 and 188.5 cm in 2011 at Somerset, 169.0 cm in 2010 and 180.3 cm in 2011 at Jackson, and 223.0 cm in 2010 and 220.2 cm in 2011 at Adelphia. Additionally, at each location, a significant clone x year effect was observed. Much of the rank changes between years occurred among clones with median height at each location, while the tallest and shortest five clones were similar (Table 46). Despite the significant clone x year x location effect, some clones consistently ranked among the tallest or shortest ten percent. Clone NSL 5 was among the top five clones for plant height at all years and locations, while clones 9064202 2 and 9064202 3 were among the bottom five for plant height. The consistent ranking of clones is further supported by the high Kendall's tau and Spearman's rank correlation coefficients seen in Table 47.

Table 45. Analysis of variance summary for plant height in fifty switchgrass clones grown at three locations in New Jersey in 2010 and 2011

Source	Numerator DF	Denominator DF	F Value	P Value
Year	1	30	165.94	<0.0001
Location	2	30	412.93	<0.0001
Year x Location	2	30	121.10	<0.0001
Clone	49	1423	121.30	<0.0001
Clone x Year	49	1423	1.83	0.0005
Clone x Location	98	1423	3.55	<0.0001
Clone x Year x Location	98	1423	1.91	<0.0001

Table 46. Clones with the highest and lowest mean plant height of fifty switchgrass clones grown at three locations in New Jersey in 2010 and 2011

Location	Adelphia				Somerset				Jackson			
Year	2010		2011		2010		2011		2010		2011	
	Clone	Mean height	Clone	Mean height	Clone	Mean height	Clone	Mean height	Clone	Mean height	Clone	Mean height
Highest 5	NSL 5	280.2	NSL 5	278.2	NSL 5	174.2	NSL 5	232.2	Kanlow 3	225.2	Kanlow 3	232.6
	Cimarron 4	272.0	Cimarron 4	266.2	Cimarron 2	159.8	NSL 3	225.2	Cimarron 2	219.8	NSL 5	224.0
	Cimarron 2	270.8	Kanlow 3	263.3	Cimarron 3	159.0	Alamo 4	225.0	Cimarron 3	216.7	Cimarron 3	221.2
	NSL 1	268.7	Alamo 4	260.2	NSL 4	156.2	Alamo 3	224.8	NSL 5	215.3	Kanlow 1	220.2
	Kanlow 3	266.3	Alamo 2	259.3	Alamo 3	151.0	Timber 2	223.4	NSL 4	212.3	Alamo 5	218.0
Lowest 5	C-i-R ^a 5	174.7	Carthage 3	177.8	NSU 5	97.2	9064202 5	145.5	9064202 4	112.7	NSU 5	143.8
	9064202 1	170.2	9064202 3	164.3	9064202 4	91.3	9064202 1	145.0	9064202 1	110.2	NSU 2	133.2
	9064202 5	159.5	9064202 5	162.3	9064202 3	91.2	9064202 4	144.7	9064202 5	106.5	NSU 1	130.3
	9064202 3	142.5	NSU 1	143.2	Carthage 5	88.3	9064202 3	136.5	9064202 3	95.5	9064202 3	123.5
	9064202 2	125.7	9064202 2	122.5	9064202 2	72.5	9064202 2	120.8	9064202 2	88.7	9064202 2	105.2
LSD		24.2		18.6		18.8		16.0		15.7		16.0

^a C-i-R – Cave-in-Rock

Table 47. Kendall's tau and Spearman's rank (in parentheses) correlation coefficients for plant height of fifty switchgrass clones grown at three locations in New Jersey in 2010 and 2011

	2010 Adelphia	2010 Somerset	2010 Jackson	2011 Adelphia	2011 Somerset	2011 Jackson
2010 Adelphia	-	0.63*** (0.83***)	0.74*** (0.91***)	0.76*** (0.92***)	0.73*** (0.91***)	0.72*** (0.90***)
2010 Somerset		-	0.70*** (0.88***)	0.68*** (0.87***)	0.68*** (0.87***)	0.62*** (0.82***)
2010 Jackson			-	0.77*** (0.93***)	0.76*** (0.92***)	0.76*** (0.92***)
2011 Adelphia				-	0.75*** (0.92***)	0.79*** (0.93***)
2011 Somerset					-	0.74*** (0.91***)

***Indicates significance at $p < 0.0001$

**Indicates significance at $p < 0.01$

*Indicates significance at $p < 0.05$

8.3.3.2. Broad-sense Heritability Estimates

The broad-sense heritability estimate for plant height in switchgrass clones evaluated at three locations over two years was 0.97 on a clonal basis and 0.72 on a single plant basis (Table 48), which is similar to findings of Casler (2005), who reported a broad-sense heritability estimate for plant height of 0.90 in 49 switchgrass populations collected from prairie remnants in the northern US evaluated at two locations for two years, and Eberhart and Newell (1959) who reported an estimate of 0.90 based on the 30 plant mean of 48 populations collected primarily from Nebraska. Broad-sense heritability estimates for plant height at each location were similar to the overall estimate (Adelphia-Hc = 0.97, Hsp = 0.77; Somerset Hc = 0.92, Hsp = 0.65; Jackson Hc = 0.96, Hsp = 0.79) and did not differ greatly (Table 40), indicating similar amounts of genetic contribution to phenotypic expression of plant height in these three environments. Casler et al. (2004) demonstrated a large population x latitude effect in 20 switchgrass populations evaluated at five locations over two years, so the similar heritability estimates seen here are not surprising given the three locations in this study all have similar latitudes. The high heritability estimates indicate that selection for plant height in this switchgrass population would be effective in either the prime or marginal locations used in this study.

While variation within cultivar/family for plant height in switchgrass has been demonstrated, plant height is largely a factor of cytotype and latitude of origin (Casler et al., 2004). Given the high heritability estimate and high rank correlation coefficients, it is

likely that progress can be made in breeding switchgrass cultivars for plant height using single plants in either the prime or marginal locations used in this study. However, the lack of consistent ranking of tall clones among locations may warrant the use of multiple locations, as plant height has been correlated with biomass yield (Price and Casler, 2014) and may be a useful indirect selection criteria for improving biomass yield in switchgrass.

Table 48. Analysis of variance and broad-sense heritability estimates (H) for plant height of fifty switchgrass clones grown at three locations in New Jersey in 2010 and 2011

Source of Variation	DF	SS	MS	F value	P value	Variance Component ^a
Year	1	265293.46	265293.46	1766.87	<.0001	
Location	2	1341198.69	670599.34	4466.22	<.0001	
Rep(Location)	15	38220.46	2548.03	16.97	<.0001	
Year x Location	2	390997.89	195498.94	1302.03	<.0001	
Year x Rep(Location)	15	10717.57	714.51	4.76	<.0001	
Clone	49	1509303.80	30802.12	205.14	<.0001	860.73
Clone x Year	49	22753.77	464.36	3.09	<.0001	0.00
Clone x Location	98	88133.13	899.32	5.99	<.0001	17.81
Clone x Rep(Location)	727	265988.88	365.87	2.44	<.0001	109.58
Clone x Year x Location	98	49269.34	502.75	3.35	<.0001	58.35
Error=Clone x Year x Rep(Loc)	709	106455.80	150.15			149.66
$H_c^b = 0.97^c$						
$H_{sp}^d = 0.72$						
^a Variance components were determined using Restricted Maximum Likelihood Estimation using the PROC MIXED procedure in SAS (Cary, NC)						
^b H_c = heritability estimate on a clonal basis						
^c 95% confidence interval for $H_c = 0.97$ -0.97; computed according to Knapp and Bridges (1987)						
^d H_{sp} = heritability estimate on a single-plant basis						

8.3.4. Tiller Number

8.3.4.1. Genotype x Environment Interactions

Significant year x location, clone x location, and clone x year effects were observed for tiller number (Table 49). At all locations, mean tiller number was higher in 2011 than 2010, which corresponded to the second and third growing seasons, respectively. As expected, the prime location had the highest mean tiller number in both 2010 and 2011, followed by the Somerset location and Jackson location, respectively (Figure 21). Across all locations, clone NSU 4 consistently ranked among the top five clones for tiller number, while clone NSU 1 was among the bottom five clones (Table 50). In addition to these two clones, the two marginal locations (Somerset and Jackson) shared additional rankings. Clones Carthage 1, Carthage 3, and Timber 1 were among the top five clones for tiller number at these locations, while clones Kanlow 3 and 9064202 3 were among the bottom five (Table 50). Despite the clone x year effect, clones NSU 4, NSU 3, and NSL 4 ranked among the top five clones for tiller number across years, while clones 9064202 5, 9064202 2, and NSU 1 were among the bottom five (Table 51). Kendall's tau and Spearman's rank correlation coefficients across all environments were relatively high for tiller number, further supporting similar clone performance across all environments tested in the study (Table 52).

Table 49. Analysis of variance summary for tiller number in fifty switchgrass clones grown at three locations in New Jersey in 2010 and 2011

Source	Numerator DF	Denominator DF	F Value	P Value
Year	1	30	327.21	<0.0001
Location	2	30	305.70	<0.0001
Year x Location	2	30	15.00	<0.0001
Clone	49	1424	28.74	<0.0001
Clone x Year	49	1424	2.35	<0.0001
Clone x Location	98	1424	2.96	<0.0001
Clone x Year x Location	98	1424	1.01	0.4571

Table 50. Clones with the highest and lowest mean tiller number of fifty switchgrass clones grown at three locations in New Jersey averaged over 2010 and 2011

Location	Adelphia		Somerset		Jackson	
	Clone	Mean tiller number	Clone	Mean tiller number	Clone	Mean tiller number
Highest 5	NSL 4	300.4	NSU 3	251.8	NSU 4	206.6
	Timber 3	243.1	Carthage 1	214.3	Carthage 1	172.1
	NSU 3	238.0	NSU 4	188.4	Alamo 1	166.1
	NSU 4	233.7	Timber 1	180.7	Timber 1	166.0
	Alamo 2	227.1	Carthage 3	178.3	Carthage 3	164.8
Lowest 5	NSU 5	103.8	Cave-in-Rock 1	77.5	9064202 5	53.5
	9064202 5	95.1	Cave-in-Rock 5	77.0	9064202 3	53.2
	9064202 2	87.5	Kanlow 3	74.4	Kanlow 3	49.0
	Cave-in-Rock 5	87.5	9064202 3	68.6	9064202 1	48.8
	NSU 1	64.1	NSU 1	60.9	NSU 1	37.8
LSD		45.9		22.3		30.5

Table 51. Clones with the highest and lowest mean tiller number of fifty switchgrass clones grown in New Jersey in 2010 and 2011 averaged over three locations

2010			2011	
	Clone	Mean tiller number	Clone	Mean tiller number
Highest 5	NSU 4	184.9	NSU 3	268.7
	Alamo 1	174.0	Carthage 1	255.7
	Timber 1	168.4	NSL 4	234.4
	NSU 3	162.4	NSU 4	234.2
	NSL 4	161.7	Carthage 3	231.4
Lowest 5	9064202 5	64.4	Kanlow 3	96.0
	NSU 5	63.3	9064202 2	94.4
	9064202 3	60.6	Cave-in-Rock 5	93.1
	9064202 2	60.2	9064202 5	90.8
	NSU 1	41.6	NSU 1	66.9
LSD		23.7		34.0

Table 52. Kendall's tau and Spearman's rank (in parentheses) correlation coefficients for tiller number of fifty switchgrass clones grown at three locations in New Jersey in 2010 and 2011

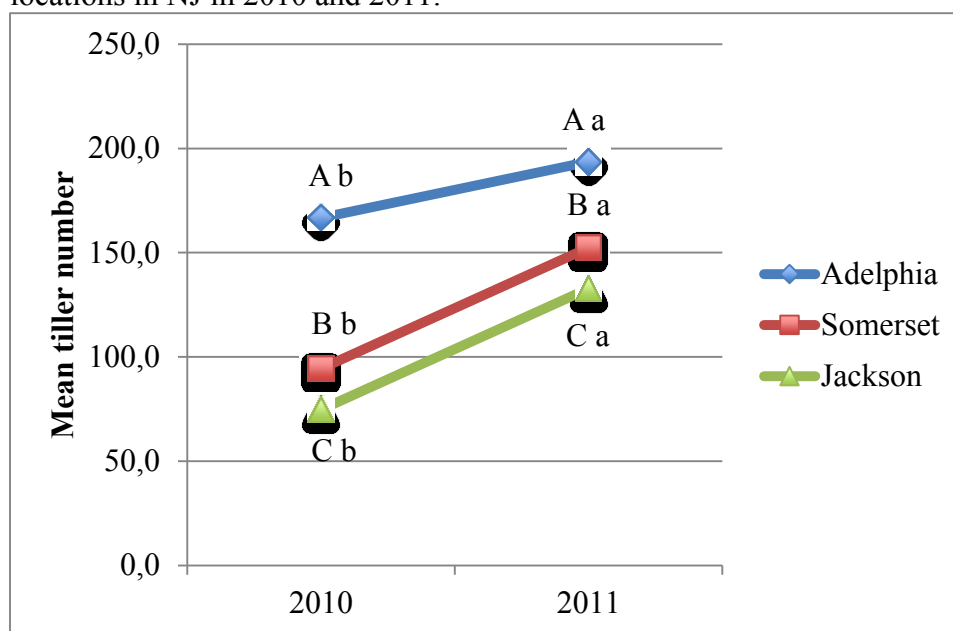
	2010 Adelphia	2010 Somerset	2010 Jackson	2011 Adelphia	2011 Somerset	2011 Jackson
2010 Adelphia	-	0.58*** (0.75***)	0.54*** (0.72***)	0.54*** (0.74***)	0.49*** (0.65***)	0.46*** (0.63***)
2010 Somerset		-	0.69*** (0.87***)	0.56*** (0.74***)	0.69*** (0.87***)	0.62*** (0.81***)
2010 Jackson			-	0.44*** (0.61***)	0.69*** (0.86***)	0.70*** (0.88***)
2011 Adelphia				-	0.52*** (0.71***)	0.45*** (0.63***)
2011 Somerset					-	0.68*** (0.86***)

***Indicates significance at $p < 0.0001$

**Indicates significance at $p < 0.01$

*Indicates significance at $p < 0.05$

Figure 21. Mean tiller number averaged across fifty switchgrass clones grown at three locations in NJ in 2010 and 2011.



Upper case letters indicate significant ($p \leq 0.05$) differences among locations within each year. Lower case letters indicate differences between years within each location.

8.3.4.2. Broad-sense Heritability Estimates

The clonal based broad-sense heritability estimate for tiller number was high ($H_c = 0.86$), while the estimate on a single plant basis ($H_{sp} = 0.36$) was low, indicating an influence of microenvironment on tiller number in the switchgrass population evaluated (Table 53). The low single plant heritability estimate indicates that selection for tiller number in this population of switchgrass would be more effective if replicated clones were utilized. As with plant height, heritability estimates of tiller number at each location were similar to the overall estimate (Table 40), indicating similar levels of genetic contribution to phenotypic expression of plant height in these three environments.

Despite the high heritability estimate seen for tiller number, significant clone \times location and clone \times year effects, identification of only one clone consistently ranked in the top five for tiller number suggests that selection for high tiller number should be carried out in multiple environments in order to be effective. It is important to note that broad-sense heritability estimates total genetic variation, which includes additive, dominance, and epistatic effects. Narrow sense heritability estimates are more useful for plant breeders because they estimate the ratio of additive genetic variance to the phenotypic variance of individuals in a population, which determines the response to selection (Nyquist, 1991; Poehlman and Sleper, 2006). Price and Casler (2014) reported an overall narrow-sense heritability estimate of 0.06 for tiller number in a tetraploid upland switchgrass population. As switchgrass is a cross-pollinated polyploid species, it is likely that dominance and epistatic variance exist and result in an upward bias of broad-sense heritability estimated from variance components (Newell and Eberhart,

1961). Therefore, it is possible that the high heritability estimate observed for tiller number was elevated due to large amounts of dominance and epistatic variance as opposed to additive variance.

Table 53. Analysis of variance and broad-sense heritability estimates (H) for tiller number (total tillers plant⁻¹) of fifty switchgrass clones grown at three locations in New Jersey in 2010 and 2011

Source of Variation	DF	SS	MS	F value	P value	Variance Component ^a
Year	1	1012140.50	1012140.50	1679.79	<.0001	
Location	2	1849349.53	924674.77	1534.63	<.0001	
Rep(Location)	15	70083.58	4672.24	7.75	<.0001	
Year x Location	2	83572.25	41786.13	69.35	<.0001	
Year x Rep(Location)	15	36234.24	2415.62	4.01	<.0001	
Clone	49	2438105.77	49757.261	82.58	<.0001	1211.71
Clone x Year	49	189356.10	3864.41	6.41	<.0001	125.64
Clone x Location	98	493650.84	5037.25	8.36	<.0001	95.03
Clone x Rep(Location)	727	2044989.57	2812.92	4.67	<.0001	1123.13
Clone x Year x Location	98	167893.42	1713.20	2.84	<.0001	189.64
Error=Clone x Year x Rep(Loc)	710	427802.59	602.54			603.96
$H_c^b = 0.86^c$						
$H_{sp}^d = 0.36$						
^a Variance components were determined using Restricted Maximum Likelihood Estimation using the PROC MIXED procedure in SAS (Cary, NC)						
^b H_c = heritability estimate on a clonal basis						
^c 95% confidence interval for $H_c = 0.86$ -0.85; computed according to Knapp and Bridges (1987)						
^d H_{sp} = heritability estimate on a single-plant basis						

8.3.5. Lodging

8.3.5.1. Genotype x Environment Interactions

A significant clone x year x location effect was observed for lodging (Table 54), similar to most other traits evaluated in this study. While all locations had clones that showed little to no lodging (ratings closest to 10), the prime location showed the most lodging (ratings closest to 1), with mean lodging ratings of 5.5 in 2010 and 5.3 in 2011. Lodging has been shown to be correlated to plant height (Casler et al., 2004), so it is not surprising that the prime location, where plants were tallest, had the most lodging. Despite the clone x year x location effect, some clones consistently ranked in the top five or bottom five for lodging at all years and locations in the study. Clone 9064202 3 had little lodging across years and locations (Table 55); however, this clone would not likely be utilized due to its poor yield and disease performance. Clones High Tide 2 and NSL 3 ranked among the top five clones at the prime location, but not at the marginal locations. However, mean lodging ratings for these clones were still relatively high at the marginal sites (above and 8.6 and 8.4, respectively). Clones Kanlow 4 and Carthage 3 ranked among the bottom five clones for lodging at five of the six year location environments in this study (Table 55). Clone Cave-in-Rock 5 demonstrated a substantial change in rank for lodging across environments in this study, whereby it ranked among the bottom five clones at Adelphia in both years, but ranked among the top five clones at the Somerset location in 2010. Kendall's tau and Spearman's rank correlation coefficients for lodging were high for the 2010 Jackson and 2011 Somerset environments, the 2011 Adelphia and

Jackson environments, the 2010 and 2011 Adelphia environments, and the 2010 and 2011 Jackson environments, but moderately low for other environments (Table 56).

Table 54. Analysis of variance summary for lodging in fifty switchgrass clones grown at three locations in New Jersey in 2010 and 2011

Source	Numerator DF	Denominator DF	F Value	P Value
Year	1	30	12.13	0.0015
Location	2	30	149.91	<0.0001
Year x Location	2	30	5.00	0.0133
Clone	49	1423	29.40	<0.0001
Clone x Year	49	1423	2.59	<0.0001
Clone x Location	98	1423	4.03	<0.0001
Clone x Year x Location	98	1423	1.70	<0.0001

Table 55. Clones with the highest and lowest mean lodging rating of fifty switchgrass clones grown at three locations in New Jersey in 2010 and 2011

Location Year	Adelphia				Somerset				Jackson			
	2010		2011		2010		2011		2010		2011	
	Clone	Mean lodging	Clone	Mean lodging	Clone	Mean lodging	Clone	Mean lodging	Clone	Mean lodging	Clone	Mean lodging
Highest 5	High Tide 2	8.5	9064202 3	9.2	9064202 5	9.7	9064202 3	9.3	High Tide 4	9.3	9064202 3	9.7
	NSL 2	8.5	9064202 1	7.7	NSU 3	9.3	9064202 5	8.7	9064202 3	9.2	9064202 1	9.0
	NSL 3	8.5	9064202 4	7.3	Timber 5	9.3	High Tide 4	8.7	Timber 5	9.2	C-i-R 1	9.0
	Carthage 2	8.2	NSL 3	7.0	C-i-R 5	9.2	High Tide 2	8.4	C-i-R 1	8.8	High Tide 1	9.0
	Timber 5	8.2	High Tide 2	6.8	NSU 2	9.2	Timber 5	8.4	C-i-R 3	8.8	High Tide 4	9.0
Lowest 5	9064202 2	3.2	Carthage 4	3.7	Kanlow 1	6.2	Kanlow 5	5.7	Kanlow 1	5.3	Alamo 1	5.3
	Carthage 4	3.2	Alamo 1	3.3	NSL 5	6.0	Kanlow 4	5.5	NSL 5	5.0	Carthage 3	5.3
	C-i-R ^a 5	3.2	C-i-R 4	3.3	Kanlow 3	5.8	Carthage 3	5.3	Kanlow 4	4.7	C-i-R 4	5.2
	Kanlow 4	3.2	C-i-R 5	3.3	Kanlow 4	5.5	NSL 5	5.3	Carthage 3	4.5	Kanlow 4	5.2
	Carthage 3	1.7	Carthage 3	1.8	Alamo 1	5.2	Cimarron 5	5.2	Kanlow 5	3.8	Carthage 4	4.4
LSD		1.6		1.7		0.8		1.1		1.4		1.4

^a C-i-R – Cave-in-Rock

Table 56. Kendall's tau and Spearman's rank (in parentheses) correlation coefficients for lodging of fifty switchgrass clones grown at three locations in New Jersey in 2010 and 2011

	2010 Adelphia	2010 Somerset	2010 Jackson	2011 Adelphia	2011 Somerset	2011 Jackson
2010 Adelphia	-	0.26** (0.34*)	0.40*** (0.52***)	0.63*** (0.78***)	0.42*** (0.54***)	0.54*** (0.67***)
2010 Somerset		-	0.56*** (0.75***)	0.28** (0.38**)	0.47*** (0.68***)	0.35** (0.49**)
2010 Jackson			-	0.47** (0.63***)	0.65*** (0.82***)	0.60*** (0.76***)
2011 Adelphia				-	0.52*** (0.70***)	0.65*** (0.80***)
2011 Somerset					-	0.56*** (0.72***)

***Indicates significance at $p < 0.0001$

**Indicates significance at $p < 0.01$

*Indicates significance at $p < 0.05$

8.3.5.2. Broad-sense Heritability Estimates

The broad sense heritability estimate for lodging in the fifty switchgrass clones evaluated in this study on a clonal basis was 0.83, while on a single plant basis was 0.34 (Table 57), and was higher than the broad-sense heritability estimate of 0.51 reported by Casler (2005). As was seen with anthracnose, rust, and tiller number, the low single plant heritability estimate demonstrates a high degree of clonal variation for lodging within environment. Therefore, evaluation trials for low lodging should include replicated clones in order for selection to be most effective. Broad-sense heritability estimates for lodging at each location were high and similar to the overall estimate (Table 40), indicating that genetics is a large determinant of phenotypic value for lodging of an individual in this population and that selection for low lodging should be successful. The high rank correlation coefficients along with high heritability estimates and consistent ranking of three clones suggests a genetic component involved in lodging resistance in this switchgrass population, and that selection for low lodging should be most successful if performed on replicated plants in any of the environments in this study.

Table 57. Analysis of variance and broad-sense heritability estimates (H) for lodging of fifty switchgrass clones grown at three locations in New Jersey in 2010 and 2011

Source of Variation	DF	SS	MS	F value	P value	Variance Component ^a
Year	1	61.59	61.59	47.33	<.0001	
Location	2	1425.94	712.97	547.85	<.0001	
Rep(Location)	15	40.98	2.73	2.1	0.0085	
Year x Location	2	41.76	20.88	16.04	<.0001	
Year x Rep(Location)	15	101.85	6.79	5.22	<.0001	
Clone	49	2011.90	41.06	31.55	<.0001	0.9791
Clone x Year	49	175.43	3.58	2.75	<.0001	0.0703
Clone x Location	98	556.47	5.68	4.36	<.0001	0.2596
Clone x Rep(Location)	727	1108.34	1.53	1.17	0.0171	0.1174
Clone x Year Location	98	240.62	2.46	1.89	<.0001	0.1917
Error=Clone x Year x Rep(Loc)	709	922.69	1.30			1.2946
$H_c^b = 0.83^c$						
$H_{sp}^d = 0.34$						
^a Variance components were determined using Restricted Maximum Likelihood Estimation using the PROC MIXED procedure in SAS (Cary, NC)						
^b H_c = heritability estimate on a clonal basis						
^c 95% confidence interval for $H_c = 0.85$ - 0.82 ; computed according to Knapp and Bridges (1987)						
^d H_{sp} = heritability estimate on a single-plant basis						

8.3.6. Biomass Yield

8.3.6.1. Genotype x Environment Interactions

As expected, biomass yields were highest at the prime location (845.0 g in 2010, 1071.9 in 2011) and lowest at the Jackson location (228.9 g in 2010, 446.6 g in 2011), which has the lowest land classification (Class V) of all sites in this study. Additionally, biomass yields increased from 2010 to 2011 (which correspond to the second and third growing seasons, respectively) at all locations in this study, demonstrating that switchgrass does not reach full yield potential after two growing seasons in New Jersey. A significant clone x year x location effect was observed for biomass yield (Table 58), similar to findings of Casler and Boe (2003). Additionally, Rose et al. (2007) demonstrated a large genotype x environment effect of yield environment in lowland half-sib switchgrass families selected for biomass yield in high yield and low yield environments. Despite the interaction seen here, some of the highest yielding clones and lowest yielding clones showed little change in rank across all years and locations in this study. Clones NSL 4 and NSL 2 ranked among the top five clones for five of the six environments evaluated, while clones NSU 1, 9064202 5, Cave-in-Rock 5, and 9064202 2 ranked in the bottom five clones for biomass yield at the majority of environments in the study (Table 59). Kendall's tau and Spearman's rank correlation coefficients for biomass yield ranged from moderately high to high (Table 60). Rank correlation coefficients were highest when comparing years within each location and lowest for the 2011 Adelphia and 2011 Jackson comparison, suggesting that switchgrass breeding for

for biomass yield would be improved by testing clones at both prime and marginal locations.

Table 58. Analysis of variance summary for biomass yield in fifty switchgrass clones grown at three locations in New Jersey in 2010 and 2011

Source	Numerator DF	Denominator DF	F Value	P Value
Year	1	30	138.84	<0.0001
Location	2	30	360.06	<0.0001
Year x Location	2	30	1.25	0.3012
Clone	49	1410	44.25	<0.0001
Clone x Year	49	1410	2.86	<0.0001
Clone x Location	98	1410	11.26	<0.0001
Clone x Year x Location	98	1410	1.29	0.0355

Table 59. Clones with the highest and lowest mean biomass yield of fifty switchgrass clones grown at three locations in New Jersey in 2010 and 2011

Location	Adelphia				Somerset				Jackson			
Year	2010		2011		2010		2011		2010		2011	
	Clone	Mean yield ^a	Clone	Mean yield	Clone	Mean yield	Clone	Mean yield	Clone	Mean yield	Clone	Mean yield
Highest 5	NSL 4	2439.4	NSL 4	3890.0	NSL 5	634.5	NSL 4	1268.3	Alamo 2	479.0	NSL 2	867.5
	NSL 2	1605.4	Timber 2	2186.0	NSL 4	627.0	NSL 2	1171.7	Alamo 1	455.6	Alamo 2	811.5
	NSL 1	1541.2	NSL 1	2076.7	NSL 2	556.8	NSL 1	957.5	NSL 4	419.4	Alamo 1	790.3
	Timber 3	1524.0	Cimarron 4	2061.7	Alamo 4	510.5	NSU 3	956.7	Alamo 4	413.9	Cimarron 3	777.8
	Cimarron 4	1433.4	Timber 3	2017.0	Alamo 1	471.7	Cimarron 3	953.3	NSL 2	398.8	NSL 5	736.3
Lowest 5	9064202 3	145.8	NSU 5	204.7	9064202 3	82.3	C-i-R 5	135.7	C-i-R 1	55.3	C-i-R 1	110.2
	NSU 1	117.3	NSU 1	116.8	9064202 2	78.0	9064202 5	135.2	9064202 3	42.8	9064202 5	105.2
	9064202 5	116.3	9064202 5	103.0	NSU 1	77.7	NSU 5	123.7	9064202 5	33.0	NSU 2	105.2
	C-i-R ^b 5	114.5	9064202 2	95.7	9064202 1	74.0	9064202 1	116.2	9064202 1	29.5	9064202 1	99.7
	9064202 2	65.5	C-i-R 5	90.6	C-i-R 5	72.3	9064202 2	91.3	NSU 1	24.5	NSU 1	71.5
LSD		359.0		623.2		103.3		173.7		113.9		202.2

^a g plant⁻¹

^b C-i-R – Cave-in-Rock

Table 60. Kendall's tau and Spearman's rank (in parentheses) correlation coefficients for biomass yield of fifty switchgrass clones grown at three locations in New Jersey in 2010 and 2011

	2010 Adelphia	2010 Somerset	2010 Jackson	2011 Adelphia	2011 Somerset	2011 Jackson
2010 Adelphia	-	0.68*** (0.86***)	0.63*** (0.81***)	0.80*** (0.94***)	0.68*** (0.86***)	0.54*** (0.73***)
2010 Somerset		-	0.73*** (0.89***)	0.64*** (0.84***)	0.79*** (0.94***)	0.65*** (0.82***)
2010 Jackson			-	0.55*** (0.75***)	0.68*** (0.86***)	0.81*** (0.94***)
2011 Adelphia				-	0.63*** (0.84***)	0.49*** (0.68***)
2011 Somerset					-	0.63*** (0.80***)

***Indicates significance at $p < 0.0001$

**Indicates significance at $p < 0.01$

*Indicates significance at $p < 0.05$

8.3.6.2. Broad-sense Heritability Estimates

The broad-sense heritability estimate for biomass yield in switchgrass clones evaluated at three locations over two years on a clonal basis was 0.71, while on a single plant basis was 0.31 (Table 61). While heritability estimates are a property of the population and locations used to determine them, they tend to be similar in different populations (Falconer and Mackay, 1996). The heritability estimate for yield in this study is similar to those previously reported. Eberhart and Newell (1959) reported broad-sense heritability estimates of 0.78 for 36 switchgrass populations grown at one location for two years. In a study involving 49 prairie remnant populations collected from the northern US, Casler (2005) reported a broad-sense heritability of biomass yield of 0.63. Broad-sense heritability estimates for biomass yield at each location varied slightly. The prime location had the highest clonal based and single plant heritability estimates for biomass yield of 0.94 and 0.64, while estimates at the marginal locations of Somerset and Jackson were somewhat lower. At Somerset, $H_c = 0.85$ and $H_{sp} = 0.61$, while at Jackson, $H_c = 0.84$ and $H_{sp} = 0.37$ (Table 40). The high value of the clone x location variance component seen in Table 55 suggests that breeding cultivars specifically adapted to the locations tested in this study may be successful. The low overall single plant heritability estimate indicates that evaluation trials for switchgrass biomass would be more effective in replicated clones were included.

Despite the high heritability estimate and similar ranking of some clones for high yield, the significant genotype x environment effects and lower rank correlation coefficients for the prime vs. marginal environments suggests that a clonally replicated

multi-location evaluation of germplasm would be most efficient in selecting for high yield in this population of switchgrass.

Table 61. Analysis of variance and broad-sense heritability estimates (H) for biomass yield (g plant⁻¹) of fifty switchgrass clones grown at three locations in New Jersey in 2010 and 2011

Source of Variation	DF	SS	MS	F value	P value	Variance Component ^a
Year	1	24709096.4	24709096.4	642.6	<.0001	
Location	2	127717005.7	63858502.9	1660.8	<.0001	
Rep(Location)	15	3217781.2	214518.7	5.6	<.0001	
Year x Location	2	438698.9	219349.5	5.7	0.0035	
Year x Rep(Location)	15	2519381.8	167958.8	4.4	<.0001	
Clone	49	183363834.9	3742119.1	97.3	<.0001	76560.0
Clone x Year	49	11891733.2	242688.4	6.3	<.0001	7859.2
Clone x Location	98	92897132.9	947929.9	24.7	<.0001	64838.0
Clone x Rep(Location)	727	92664980	127462.1	3.3	<.0001	45609.0
Clone x Year x Location	98	10113660.4	103200.6	2.7	<.0001	11483.0
Error=Clone x Year x Rep(Loc)	696	26762313.3	38451.6			38890.0
$H_c^b = 0.71^c$						
$H_{sp}^d = 0.31$						

^aVariance components were determined using Restricted Maximum Likelihood Estimation using the PROC MIXED procedure in SAS (Cary, NC)

^b H_c = heritability estimate on a clonal basis

^c95% confidence interval for $H_c = 0.72$ - 0.71 ; computed according to Knapp and Bridges (1987)

^d H_{sp} = heritability estimate on a single-plant basis

8.4. CONCLUSIONS

This is the first study investigating disease, plant height, tiller number, lodging, and biomass yield characteristics of upland and lowland switchgrass clones grown at both prime and marginal sites. Significant clone x location x year interactions observed for all characteristics evaluated except tiller number, as well as significant clone x location, clone x year, and year x location effects seen for tiller number indicate environment influences expression of these traits in the switchgrass population tested here. Broad-sense heritability estimates for all traits were high on a clonal basis, but estimates on a single plant basis were low for all but plant height, indicating that adding clonal replicates to evaluation of germplasm in this population of switchgrass would improve selection efficiency. As clonal replication is costly in terms of time, space, and expense, a breeder must decide whether the benefits outweigh the cost. Few clones consistently ranked among the top or bottom 10%, further supporting the importance of genotype x environment interactions on these characteristics. Therefore, the use of multiple locations including both prime and marginal sites is recommended for evaluation of each trait in this study, with the exception of plant height. As with clonal replication, the use of multiple locations in breeding is costly, and breeders must determine the best way to reach breeding objectives given the resources available. Additionally, breeders typically select for an ideotype, which is a plant with an agglomeration of desirable traits, as opposed to selecting for several individual plants each expressing one desirable trait. In this study, no one clone possessed stable expression of all desired traits evaluated, and selection of clones with ideal expression of one trait may result in the inclusion of non-

desired alleles for another when choosing breeding material. Further studies investigating effect of selection environment, narrow-sense heritability, and realized gains from selection in switchgrass populations grown on both prime and marginal sites are needed, as switchgrass is relatively young from a breeding perspective. Results from this study support the need for evaluation of switchgrass germplasm across multiple years and environments that include both prime and marginal sites in order to develop cultivars with optimal agronomic characteristics.

9. CONCLUDING REMARKS

This dissertation investigated several aspects concerning the use of switchgrass as a bioenergy feedstock in the northeastern/Mid-Atlantic US. When the work was initiated, information on the performance of switchgrass in the northeastern and Mid-Atlantic US was limited. The first study aimed to assess genetic diversity and determine differences among and between 12 switchgrass populations grown in New Jersey by examining both morphological and molecular characteristics, and to determine whether morphological, molecular, and/or combined data sets could detect ecotype and/or geographical differences at the population level, as basic knowledge of germplasm diversity is important when initiating a breeding program. It was found that most (64%) of the molecular variation in switchgrass populations studied exists among individuals within populations, with lesser amounts between populations (36%), which is common for heterogeneous outcrossing species and not surprising given that most switchgrass populations have been subjected to little to no cycles of selection. This diversity within populations provides great opportunity for the development of improved cultivars, and the combination of morphological and molecular markers used may be useful in future applications such as genetic diversity studies, plant variety protection, cultivar identification, and/or identifying geographic origin of germplasm.

A second study evaluated biomass characteristics of ten switchgrass populations grown in New Jersey to determine which populations are best for use in biomass production, as little information on performance of switchgrass was available. Ten populations of switchgrass were evaluated for: winter injury, anthracnose disease caused

by *Colletotrichum navitas*, lodging, tiller density, height, heading and anthesis date, and biomass yield as well as cellulose, hemicellulose, lignin, ash, chlorine, calcium, magnesium, phosphorous, and potassium. This study helped determine that both upland and lowland ecotypes perform well in New Jersey, and population Timber was identified as having the best combination of characteristics evaluated. Results showed that lignocellulosic and mineral contents did not differ greatly among populations, suggesting that breeding for these characteristics would not likely be successful. Additionally, a new disease of switchgrass, *Colletotrichum navitas*, was discovered during this study (Crouch et al., 2009). However, the degree to which this and other diseases such as rust impact biomass quality remains to be determined. Future studies that assess the impacts of disease on both biomass yield and quality are needed.

The third investigation in this dissertation evaluated the performance of three switchgrass cultivars (Alamo, Carthage, and Timber) grown for bioenergy at two locations in New Jersey. Switchgrass biomass yields ranged from 8.94 to 13.21 Mg ha⁻¹ and were similar among cultivars and locations tested. Results indicated that Alamo, Carthage, and Timber are high yielding, low ash switchgrass cultivars that could be promising choices for biomass production in the northeastern and Mid-Atlantic US, and that dry, low-ash biomass can be harvested throughout the winter if weather conditions permit. Additional work is needed to determine the optimal harvest time for switchgrass in this region of the US. While mineral and ash content of switchgrass declines over time, so does biomass yield. A study investigating both biomass yield and quality over time would help optimize harvest practices for the northeastern/Mid-Atlantic US.

The fourth study in this work was implemented in an effort to improve the

establishment capacity of switchgrass, an area where little research has been done and is persistently problematic. Two cycles of divergent selection for seed weight in switchgrass cultivars Carthage and Timber and experimental population 9064202 resulted in an increase in 100 seed weight in all weight classes in populations Carthage and 9064202 and no change in Timber. Selection for seed weight alone was not sufficient to improve overall germination and germination rate in the populations tested in this study. Cycles of selection improved percent germination and germination rate (regardless of seed weight) in non-stratified seed of all switchgrass populations tested. Cold stratification increased percent germination and germination rate in all populations tested. Results indicate that breeding efforts directed at reducing dormancy, rather than increasing seed weight, should be developed and implemented in order to improve switchgrass germination and establishment. Switchgrass, due to its relatively unimproved status, has retained characteristics like seed dormancy and shattering. While breeders aim to speed the improvement process through selection, switchgrass breeders may have to be content with allowing the process of domestication to reduce dormancy and improve germination and establishment capacity, as selection for or against any particular trait has been successful in accomplishing this goal thus far.

In a fifth study, thirty switchgrass clones were evaluated for cellulose, hemicellulose, and lignin content at one prime and two marginal soil locations in New Jersey in 2009 and 2010 in order to 1) examine genotype by environment effects and 2) determine broad-sense heritability estimates on lignocellulosic traits. Significant genotype x environment interactions were observed for all traits evaluated, and were particularly substantial for hemicellulose content. Broad-sense heritability estimates

ranged from 0.70 for cellulose content to 0.22 for hemicellulose content. While no clones were identified as stable across all environments for any trait evaluated, some clones consistently ranked in the top or bottom five for each trait across locations and years. Results support the existence of both specifically and broadly adapted switchgrass germplasm, and demonstrate the need for evaluation of switchgrass germplasm across multiple years and environments (including both prime and marginal sites) in order to develop cultivars with optimal lignocellulosic characteristics. Previous research has suggested that while selection for cell wall traits in switchgrass have been successful, the effects of the differential cell wall components on ethanol yield vary. Therefore, switchgrass breeders are faced with some difficulties. First, we must be able to accurately quantify traits of interest. Several studies have pointed out inconsistencies and inadequacies of the various laboratory methods for determining cell wall components. There is a need for the development of a universally accepted protocol for determining cell wall components. Second, we must be able to identify the optimal bioenergy feedstock ideotype, especially in terms of cell wall components. Third, we need to identify whether these traits are heritable, and whether they are linked to other traits such as biomass yield. Based on what is known currently, switchgrass breeders should focus their efforts on improving biomass yield, as yield is often the limiting factor in terms of economic success. Perhaps with a better understanding of cell wall components, breeding objectives in switchgrass may change.

The final study in this dissertation examined genotype by environment effects and determined broad-sense heritability estimates on six agronomic traits in fifty switchgrass clones grown on marginal and prime soils in New Jersey. Significant genotype x

environment interactions were observed for all traits evaluated. Broad-sense heritability estimates ranged from 0.97 for plant height to 0.61 for rust. Stability analysis revealed few stable clones for each of the traits evaluated, and few clones consistently ranked among the top or bottom 10%. Therefore, the use of multiple locations including both prime and marginal sites is recommended for evaluation of traits in this study, with the exception of plant height, in order to develop cultivars with improved agronomic characteristics. While studies here indicated differential effects of environment on lignocellulosic and agronomic traits in switchgrass, future studies investigating the effects of selection in target (marginal soil) vs. non-target (prime soil) environments in switchgrass are needed. If switchgrass is to be produced on marginal land, breeders must determine the most effective ways to develop cultivars that perform well on these types of soils.

If switchgrass is to become a successful bioenergy feedstock crop in the US, gains in knowledge, particularly information that will affect breeding methods, will have to be made. Most likely, switchgrass cultivars will be developed for use in targeted regions of the US. While this body of work provides a foundation that has allowed for the development of cultivar recommendations, management practices, breeding strategies, and improved germplasm for the utilization of switchgrass as a bioenergy feedstock in the northeastern and Mid-Atlantic US, work in these areas should be continued to allow for the development and release of improved switchgrass cultivars.

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