INVESTIGATING HYBRIDIZATION POTENTIAL, COMPONENTS OF FITNESS, AND VOLUNTEERISM IN WILD AND CULTIVATED *Panicum virgatum* L. (SWITCHGRASS)

DISSERTATION

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ABSTRACT

Extensive gene flow between cultivated plants and wild relatives can be of concern because crop alleles may persist in wild populations and dilute the native gene pool or confer traits that enhance lifetime fitness, perhaps increasing the wild populations' tendency toward weediness. Switchgrass (*Panicum virgatum* L.) is a warm-season North American perennial that is widely planted for forage and soil conservation. Certain switchgrass cultivars have undergone a limited degree of breeding for use as a biofuel crop and could be planted on a large scale in the near future. However, very little research has examined the potential impact that mass plantings will have on wild populations. The goals of my research were to examine the potential for crop-to-wild gene flow and associated fitness effects by studying ploidy levels, flowering phenology, fitness components, and volunteer establishment. First, I determined ploidy levels of eight wild populations in Ohio and three in Illinois. Eight populations were tetraploid (4x), one was octaploid (8x), and two had mixed ploidy. In 2008 and 2009, I planted two common garden experiments at Ohio State University in Columbus, Ohio, with plants from three wild Ohio populations and seven cultivars: Kanlow (4x), Advanced Kanlow (4x), Summer (4x), Shawnee (8x), Trailblazer (8x), and two Advanced Octaploid strains (8x). I then compared the height and numbers of florets, filled seeds, and shoots of two- and three-year-old plants. Ohio native biotypes were similar to each other in all measured characteristics. Flowering times of native biotypes and the cultivars overlapped, but the degree of overlap varied, with Kanlow-type plants flowering much later than the rest. Kanlow-type plants were taller and produced four times as many florets as native biotypes, while Kanlow and Summer produced twice the number of filled seeds as the native biotypes. All other cultivars were similar to the native plants, except one Advanced Octaploid strain, which produced more shoots. I also examined the frequency, morphology, and flowering phenology of volunteers growing in the common gardens in 2011. Ploidy and ecotype frequencies were similar between common garden plants and volunteers, which were common. At least 20 of the 36 volunteers examined in detail were derived from cultivars rather than native plants. Hybridization was also evident, as 48% of volunteers had mixed features between Kanlow and other biotypes and two were "lowland ecotype" octaploids, a combination not present in the parents (only Kanlow types were "lowland"). Volunteer flowering overlapped with all biotypes and was bimodal. Taken together, my results show that gene flow between cultivars and wild relatives is possible due to shared ploidy and overlapping flowering times. Certain cultivars with much higher fecundity, like Kanlow, might eventually become common in Ohio if they escape widely and become feral or hybridize with wild plants. Volunteer switchgrass plants also could facilitate gene flow between cultivated and wild populations. Future research should focus on whether mass plantings of certain switchgrass cultivars could result in the displacement or dilution of local switchgrass genetic diversity or possible weed problems resulting from feral and crop-wild hybrid populations.

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DEDICATION

I dedicate this to my wonderful family, whose love
and encouragement means the world to me.

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In Illinois, Kelly Neal, Stewardship Project Manager with the Illinois Nature Preserve Commission, gave me permission to sample switchgrass populations that were part of protected natural areas. Bill Glass, biologist at Midewin Federal Preserve in Illinois, directed me to several remnant populations in Grundy and Will Counties, and Art Rohr, volunteer at Goose Lake State Natural Area, acted as my guide through many of them. Joe Giacone, Site Superintendent at Goose Lake Prairie, Jeff Wepprecht, Site Superintendent at Des Plaines State Fish & Wildlife Area, and Char McDade, receptionist at Goose Lake Prairie, also provided valuable assistance and support.

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CHAPTER 1

THE POTENTIAL FOR GENE FLOW BETWEEN CULTIVATED AND WILD SWITCHGRASS (*PANICUM VIRGATUM* L.): PLOIDY LEVELS AND FLOWERING PHENOLOGY.

ABSTRACT

Extensive pollen-mediated gene flow between cultivated plant species and wild relatives can be of concern because crop genes may persist in wild populations and dilute the native gene pool, which could reduce genetic diversity or even displace the native type. Switchgrass (*Panicum virgatum* L.), a warm-season perennial that is native to much of North America, is a forage grass that is undergoing selection and breeding for use as a biofuel crop. It is an outcrossing species that occurs mainly as either a tetraploid (4x) or octaploid (8x). Past research has shown that individuals with different ploidy levels do not cross, although plants with the same ploidy and different geographic origins have been successfully hybridized for commercial purposes. With the recent surge of interest in biofuel technology, a possible future scenario with switchgrass is very widespread cultivation and the insertion of transgenes into the genome to improve yield or provide more efficient conversion to ethanol. To date, reports of the potential for gene flow and hybridization between common cultivars and wild populations are scarce. I investigated the flowering phenology of three Ohio native biotypes and seven cultivar biotypes – Kanlow (4x), an advanced Kanlow strain (4x), Summer (4x), Shawnee (8x), Trailblazer (8x), and two experimental advanced octaploid strains (8x) – in a common garden experiment in Columbus, Ohio, in 2010 and 2011. Ohio native biotypes flowered earlier, on average, than the cultivars, but there was a considerable overlap in flowering times. Both years, the closely related tetraploids, Kanlow and Advanced Kanlow, flowered later than the other biotypes and barely overlapped with the natives. Flowering dates varied across years, resulting in greater overlap in 2010 than in 2011 between the native and cultivated biotypes, including the late-flowering Kanlows. This suggests that flowering phenology depends, in part, on environmental cues. I also assessed the ploidy levels of 10-21 plants from each of eight Ohio and three Illinois native remnant switchgrass populations using flow cytometry. Eight native populations were 4x, one was mostly 4x, and two were either 8x or mostly 8x. Mixed ploidy also has been reported in accessions from remnant populations in five other Midwestern states. Thus, gene flow between cultivated switchgrass and native remnant populations is possible, provided ploidy levels match. In summary, my findings suggest that growers may not be able to use ploidy level or delayed flowering of current cultivars as methods to reduce potential gene flow. Instead, breeding for inducible flowering or sterility would be a better way to avoid gene flow from novel cultivars to wild populations. Several research groups are pursuing this strategy, given that seed set is not needed for high biomass yields.

INTRODUCTION

Gene flow between closely related plant species is a common event in natural settings, and hybrid zones between close relatives with sympatric ranges have frequently been documented. Cultivated plants can also hybridize with related species. For example, major crops such as wheat, maize, rice, sorghum, and sunflower all have wild relatives with which they can cross (Ellstrand et al. 1999). Evidence suggests the introduction and persistence of crop alleles in wild populations is quite common (Ellstrand et al. 1999). However, concerns may arise if gene flow occurs between two populations when one is small, fragmented, or isolated, because a decline in genetic diversity due to gene swamping and hybridization may cause local extinctions (Levin et al., 1996). For example, Beatty et al. (2010) used single nucleotide polymorphism genotyping to examine the extent of hybridization between two species of *Pyrola* in areas where *P. grandiflora* populations were common and *P. minor* populations were sparse or had a patchy distribution. Beatty et al. sampled 5 populations of *P. minor,* 3 populations of *P. grandiflora*, and 2 populations of putative hybrids that were intermediate in phenotype. The authors discovered extensive unidirectional gene flow and introgression from the common *P. grandiflora* populations to the less common *P. minor* populations, probably due to pollen swamping. DeWoody et al. (2008) used isozymes to demonstrate that hybridization between the rare, threatened *Oenothera wolfii* (evening primrose) and the horticultural species *O. glazioviana* is more prevalent than previously thought. The authors concluded that genetic swamping by the garden escape is as much of a risk to the threatened primrose's continuance as anthropogenic habitat destruction.

Successful gene flow between populations depends in part on the absence of mating barriers. Plants that flower at different times of the year, for instance, will not have an opportunity to share pollen. Likewise, differing chromosome numbers between closely related species, or even within the same species, may inhibit reproduction. The main reason for failed inter- and intraspecific crosses, including those between different ploidy levels, seems to be endosperm breakdown (Brink and Cooper 1947). The endosperm of a seed requires a 2:1 maternal-to-paternal dosage in order to develop normally (Johnston et al. 1980). Past research has shown that when the maternal parent is the higher ploidy plant, the endosperm develops and seeds are smaller but can still be viable. Conversely, when the maternal plant is the lower ploidy plant, endosperm formation is abnormal and usually results in shriveled or aborted seeds (*in* Martinez-Reyna and Vogel, 2002).

To investigate the potential for gene flow between a cultivated species and its wild relative, I studied *Panicum virgatum* L., or switchgrass, an outcrossing species that is native to much of North America and is broadly adapted across a wide range of growing conditions, perhaps owing to genetic variability (Parrish and Fike, 2005). Switchgrass has been cultivated for use as a forage grass and is used in the USDA's Conservation Reserve Program (CRP), a land retirement program established by the Food Security Act of 1985 (Mulkey et al. 2006). CRP offers farmers the option of receiving payments and cost-share assistance for taking eligible agricultural land out of production and dedicating it to long-term resource conservation. Almost half of the 12,500,000 hectares currently enrolled in the CRP in the U.S. occur in the Midwest (www.fsa.usda.gov) (Table 1.1). Now, with the recent surge of interest in biofuel technology, switchgrass has been selected as a model biofuel feedstock (Wright 2007). It is particularly attractive as a biofuel crop for several reasons: it requires fewer nutritive inputs, it tolerates drought well, it establishes from seed, it is perennial, and it is native to much of North America (McLaughlin and Kszos, 2005). Because switchgrass can establish well on poor soil (Moser and Vogel, 1995), there is less pressure on farmers to plant it in agricultural fields that are dedicated to food production. Indeed, although several plant species have been considered as potential biofuel feedstocks (e.g., maize, *Zea mays*; wheat, *Triticum aestivum*; and sorghum, *Sorghum bicolor*), switchgrass is becoming known as the main herbaceous biofuel feedstock (Bouton, 2007). Consequently, switchgrass may be on the verge of being planted much more widely (Figure 1.1). In addition, transgenic cultivars with improved yield, increased drought tolerance, and more efficient conversion to ethanol are under development (e.g., Tobias et al. 2008, Wang et al. 2010, www.isb.vt.edu).

Switchgrass has a basal chromosome number of n = 9, and an early report by Nielson (1944) documented a range of ploidy levels, from 2n = 2x = 18 to 2n = 12x = 108. More recent reports have documented mainly 4x, 6x, and 8x individuals in wild populations and germplasm accessions (Hopkins et al. 1996, Hultquist et al. 1997, Zhang et al. 2011a), and switchgrass cultivars are well documented as being either tetraploids or octaploids (Hultquist et al. 1996, Zalapa et al. 2011). Martinez-Reyna and Vogel (2002) investigated whether switchgrass plants with different ploidy levels could hybridize by conducting a series of controlled interploidy crosses. In 4x (maternal) by 8x crosses, which resulted in a 2:2 maternal:paternal endosperm ratio, seeds were small and shriveled. In 8x (maternal) by 4x crosses, which had a 4:1 maternal:paternal endosperm ratio, seeds were smaller and the endosperm had a floury appearance. Martinez-Reyna and Vogel (2002) concluded that postfertilization incompatibility exists between different switchgrass ploidies, preventing successful crosses, although seed viability tests were not included in the study.

Along with differences in ploidy, switchgrass occurs as either an upland or lowland ecotype. Lowland ecotypes typically occur in more southern latitudes and are adapted to more mesic soils, while upland ecotypes are generally found in more northern latitudes and tend to inhabit drier environments (Parrish & Fike 2005). Hultquist et al. (1996) discovered a genetic difference in the chloroplast DNA between ecotypes: lowland types have a restriction site that is missing in upland cpDNA. Several morphological characteristics seem to be associated with ecotype, such as relative plant height, width of shoots, leaf color, and rhizome form (Porter 1966, Beaty et al. 1978, Zhang et al. 2011a). Zhang et al. (2011a) analyzed nuclear DNA, cpDNA, ploidy levels and phenotypes of 480 plants from 67 accessions and discovered that ~10% of plants were ecotype hybrids. Many of the hybrid plants had a chloroplast genome typical of one ecotype and a phenotype typical of the other ecotype, but, usually, switchgrass ecotypes can be distinguished based on morphology (Parrish and Fike 2005).

A major difference between ecotypes seems to be flowering phenology. As with many other grasses, switchgrass is strongly influenced by photoperiod. Response to photoperiod is highly heritable in grasses (Watson and McLean 1991, McLean and Watson 1992, Talbert et al. 1983). Photoperiod response in grasses can also vary a great deal within species that are distributed across a wide geographical range, and ecotypes at more northern latitudes tend to show more sensitivity to changes in day-length than their southern counterparts (Olmsted, 1944). For example, Van Esbroeck et al. (2003) grew switchgrass clones from Illinois and Texas in a greenhouse experiment under ambient light and extended light conditions. The authors discovered that the northern-latitude cultivars had an extended vegetative phase and delayed flowering in the extended light environment. However, even in the higher light environment, the Illinois cultivars flowered sooner than the Texas cultivars. The Texas cultivars did not respond differently to changes in photoperiod.

Switchgrass vegetative development responds to number of growing degree days (GDD), and there is variation across latitudes. For instance, Sanderson et al. (1999) grew several varieties from northern and southern latitudes in field plots in Texas and discovered that northern-latitude cultivars matured earlier compared to those originating from more southern latitudes. Generally speaking, northern-latitude varieties mature rapidly when moved south, and southern-latitude cultivars delay reproductive maturity and increase biomass when moved northward (Sanderson & Wolf 1995; Vogel et al. 1985). In fact, past research suggests agriculturalists should not grow switchgrass cultivars more than one hardiness zone away from their zone of origin, to avoid large fluctuations in biomass production (Casler et al. 2004).

Although there have been many studies about the agronomy of switchgrass cultivars, there are relatively few reports about the ecology of wild populations, and investigations into the potential for gene flow and hybridization between cultivars and wild biotypes are scarce. There is little information about the frequencies of different ploidy levels in wild populations. Hultquist et al. (1997) sampled seed accessions from 28 Midwestern populations and tested 5 individuals per accession. They found a mixture of tetraploids and octaploids in 17 populations, and might have found more mixed populations if their sample sizes were larger. My research adds to existing knowledge about mixed-ploidy switchgrass populations in the Midwest. There are no reports of the flowering phenology of cultivated *vs.* wild biotypes. This much-needed information would help determine if it is possible for biofuel crops to share genes with wild switchgrass populations.

There are conflicting opinions and reports about the potential for lignocellulosic biofuel crops to negatively impact wild populations and the environment. Some researchers have expressed concern that biofuel plants used in monoculture could escape cultivation and become a weedy invader, because of life history traits that are similar to invasive plants and because of the potential scale of cultivation that is being proposed (Raghu et al., 2006; DiTomaso et al., 2007). Once an escaped cultivar becomes a feral invader, it could potentially reduce species diversity in local communities, like some other invasive plants (e.g., Hovick et al. 2011, Greene and Blossey 2012). Others believe that since forage cultivars were recently derived from native wild strains, and because breeding cycles take years to complete, it is likely switchgrass cultivars are still genetically similar to their wild counterparts (Casler et al. 2007).

Researchers have used molecular markers to investigate the level of genetic diversity in switchgrass cultivars with an aim toward improving breeding or conserving germplasm (Gunter et al. 1996), determining whether mass cultivar plantings could swamp local gene pools (Casler et al. 2007), and detecting ecotype or geographical differences between populations or genetic pools (Cortese et al. 2010, Zalapa et al. 2011). For example, Casler et al. (2007) analyzed 46 Midwestern prairie remnant populations (including two on the eastern edge of Ohio) and 11 forage cultivar populations using random amplified polymorphic DNA markers. Results showed higher genetic variation within both prairie and cultivated populations than between populations, but low marker variability between cultivars and remnant prairie populations. The low genetic differentiation between the groups led the authors to conclude that prairie remnants in the Midwest would not be "contaminated" by the introduction of non-local switchgrass cultivars into the area (Casler et al., 2007). Hopkins et al. (1995) investigated genetic variability for "forage quality" traits among 23 Midwestern native populations and 5 cultivars. They found significant variability in flowering date, forage yield, and vegetative *in vitro* digestibility (lignin/cellulose/hemicellulose content) among wild switchgrass populations, in contrast with cultivar populations, which varied significantly only in flowering date. Zalapa et al. (2011) used microsatellite markers to see whether genetic differentiation exists between cultivars of different ecotypes. The authors tested individuals from 18 cultivars and found distinct differentiation between upland and lowland ecotypes. Zhang et al. (2011b) expanded on the work of Zalapa et al. (2011), using microsatellite markers to investigate genetic differentiation in terms of ploidy and ecotype using 34 cultivars and seed accessions representing 33 wild populations across the eastern and central U.S. Zhang et al. (2011b) found clear differentiation between switchgrass ecotypes and ploidy levels. Although wild switchgrass populations have already been exposed to cultivars *via* forage cropping systems and CRP lands, these reports suggest switchgrass populations, including those from which cultivars originated, have maintained distinct gene pools to some degree. Current investigations into genetic differentiation among wild populations in Ohio and Illinois and between wild populations and several cultivars are underway at Ohio State University, and they include the populations used in my study. Early results show distinct population differentiation between cultivars and wild populations, and between Ohio and Illinois populations (E. Mutegi, pers. comm.).

My goal was to investigate ploidy levels in wild populations and examine the flowering phenology of several wild and cultivated biotypes to (1) characterize within-population ploidy structure, and (2) test for overlapping flowering times between cultivars and wild plants. I sampled individuals from 11 native switchgrass populations in remnant prairies in Ohio and Illinois, and I documented the flowering phenology of cultivated and non-cultivated Ohio switchgrass growing in a common garden in Columbus, Ohio. My study provides important information about the potential for switchgrass cultivars to hybridize with wild biotypes, potentially leading to genetic swamping or the introduction of fitness-related traits that are new to local populations. My results also contribute to the literature on mixed-ploidy populations of native prairie species (e.g., Keeler 1992, Brown & Young 2000).

METHODS

Seed Sources

Native Populations

Native remnant prairie populations were identified in Ohio and Illinois. I selected one switchgrass population in each of 11 remnant prairies – 8 in Ohio and 3 in Illinois. I chose populations that contained a minimum of 50 switchgrass plants. Although most of the original prairie in both states has been converted to other uses, some areas remain (Jack McDowell, Franklin County Metro Parks, Ohio, pers. comm.; Bill Glass, Midewin Federal Preserve, Illinois, pers. comm.). A "remnant" prairie is defined here as one whose individuals are presumed to be descendents of those that were present at the site prior to European settlement. Railroad rights-of-way and federally- or state-owned land are examples of areas within which remnant prairies may be found. All sites included in this research are considered remnant prairies by local historians and environmental specialists.

Ohio sites were chosen to represent three major historical prairie regions in the state: the Erie Plains, or Firelands (northern Ohio), the Sandusky Plains (north-central Ohio), and the Darby Plains (south-central Ohio) (Figure 1.2, Table 1.2). In Erie County, the National Aeronautics and Space Administration (NASA) owns ten square miles of prairie and wooded areas that surround its facilities. The natural areas there are managed by environmental specialist John Blakeman, who assisted me in locating two widely separated switchgrass populations. In Marion County, Claridon Railroad Prairie is owned by CSX Corporation and occurs as a narrow strip of remnant prairie adjacent to an active rail line. Green Camp Railroad Prairie is also a narrow strip of land that was once owned by CSX Corporation until it was donated to the Marion County Historical Society. In Crawford County, Daughmer Bur Oak Savannah, once a privately owned 15 hectare savannah, was recently purchased by the Ohio Department of Natural Resources Division of Natural Areas and Preserves. About 4 of the hectares are remnant prairie and were traditionally used for sheep grazing. The populations in Madison and Clark Counties occur alongside a newly constructed bicycle/walking trail that has replaced a rail line. These prairies are not actively managed, but are overseen by Jack McDowell, Madison County Parks & Recreation Director and Franklin County Metro Parks prairie specialist (Table 1.2).

Illinois sites were chosen for their role in conservation efforts. In Will County, Des Plaines Conservation Area and Hitt's Siding Prairie are state-owned and managed by the Illinois Nature Preserve Commission. Midewin National Tallgrass Prairie is federally owned land that formerly was used as an army depot and now is managed by the United States Forest Service. Bill Glass, biologist at Midewin Federal Preserve, directed me to pockets of remnant prairie located within the larger sites.

The 11 populations are geographically distinct; the populations nearest each other – Plum Brook Site A & B, and Botkin Road & Neil East – are 3 km and 4 km apart, respectively. The sites are surrounded by a mix of agricultural fields and wooded areas (A. Stottlemyer, pers. obs.). With 4,000 agricultural hectares converted to CRP grasslands in Marion County alone (J. Leslie, NRCS conservationist, pers. comm.), it is likely native switchgrass populations have been exposed at some time to pollen from cultivated switchgrass swards.

Cultivars

I obtained seeds of four traditional cultivars and three experimental "advanced" strains from Dr. Kenneth Vogel, plant breeder and geneticist with the USDA-ARS at the University of Nebraska:

- Kanlow germplasm originates from a 1957 collection in Oklahoma lowlands (USDA-NRCS, 2011).
- Shawnee was developed by a single selection cycle for increased yield and decreased disease, using Cave-in-Rock as the base population (Vogel et al. 1996). Cave-in-Rock originated from southern Illinois.
- Summer is the result of selections made from a wild collection near Nebraska City, NE, in the early 1960's. Its primary use is as a forage grass. It was developed at the South Dakota Agricultural Experiment Station (USDA-NRCS, 2007).
- Trailblazer is a "synthetic" variety created by crossing two experimental strains, 'EY' and 'FF', that were themselves developed from collections made in 1953 in Nebraska and Kansas. Its main purpose was to obtain a forage variety with increased digestibility (Vogel et al. 1991).
- Kanlow-winter survival (aka 'Advanced Kanlow') was developed from a single selection of Kanlow for increased winter survival (K. Vogel, pers. comm.).
- NE Late Syn HYLD-HDMD #2590 (aka 'Advanced Octaploid 1') is a synthetic strain originating from a composite of two Nebraska populations. It has gone through 4 selection cycles for increased yield and digestibility, and its intended use is mainly in pasture systems (K. Vogel, pers. comm.).

- EY x FF HDMD C3 Syn 2 (aka ‘Advanced Octaploid 2’) is a synthetic strain developed through three cycles of selection. It is a composite of two experimental strains that were developed in the 1950’s. It has been shown experimentally to have decreased lignin content, but also decreased winter survival. There are no plans to release it commercially in the future (K. Vogel, pers. comm.).

Ploidy Assessment

I used flow cytometry to investigate the ploidy levels of wild switchgrass populations in Ohio and Illinois, as an alternative to using light microscopy to count chromosomes. Flow cytometry is most often used to examine animal cells, but it has proven to be a useful tool in determining the DNA content and ploidy level of plants (e.g., Dolezel et al. 2007, Zalapa et al. 2011, Bennert et al. 2011). For example, Hultquist et al. (1997) used flow cytometry to show that switchgrass tetraploids contained ~3 pg of nuclear DNA, while octaploids had ~6 pg of DNA. With flow cytometry, nuclei suspended in solution pass into the instrument where they are pressurized, pushed through a nozzle, and focused into a stream small enough to allow only one nucleus through at a time. The nuclei, treated with a staining solution, encounter a laser, fluoresce and scatter light. The pattern of light scatter is used to quantify the size of the nucleus (Chalmers 2011). Data are displayed in a histogram, and the mean peak fluorescence is compared to either an internal or an external standard (external standards are used to determine ploidy level, while internal standards are used to more precisely determine DNA content in picograms; Dolozel et al. 2007).

I collected ~500 seeds from each of 20 widely spaced switchgrass individuals at each of the 11 remnant populations in 2009 and 2010. In 2011, seeds were germinated in a greenhouse and young leaf tissue was excised for ploidy tests, with the goal of sampling 15-20 plants per population. To prepare the samples for flow cytometry, I adapted protocols described in the literature (see Zalapa et al. 2011, Hopkins et al. 1996, and Michaelson et al. 1991). I prepared a buffer solution containing 45 mM magnesium chloride, 30 mM sodium citrate, 20 mM 4-morpholinepropane sulfonate (MOPS), and 1 mg/mL Triton X-100 (Galbraith et al. 1983). For each individual, I placed ~50 mg of leaf tissue in a glass petri dish on ice. I added 500 μL of the buffer solution and gently chopped the tissue for 2 minutes. After allowing the slurry to sit for 5 minutes, I passed the solution through a 30-micron mesh filter into a 2-mL centrifuge tube. I added 500 μL of 50 μg/mL propidium iodide staining solution (PI) to the sample, and let it sit on ice for 20 minutes. I then centrifuged each sample at 1500 rpm for 5 minutes and pipetted 500 μL of solution off the top of the sample, to increase the speed with which the sample is read by the flow cytometer. Finally, I added 3 μL RNase (diluted 1:9 RNase:buffer) and heated the sample to 37 ºC for 15 minutes to remove any PI-stained RNA from the samples. Samples were refrigerated and analyzed within 24 hours of initial tissue harvest.

Samples were examined using a BD FACS LSR-II flow cytometer with a 488 nm laser (DHLRI Core Labs, OSU Biological Sciences Research Tower, Columbus, Ohio) and analyzed using FACSDiva v6.1.2 (BD Biosciences). Fluorescence was measured with a 610/20 nm band pass filter, and single nuclei were isolated using a PI fluorescence area and width plot. I used either Kanlow (4x) or Cave-in-Rock (8x) as an external standard, both of which have previously been examined using flow cytometry (Zalapa et al. 2011). Mean fluorescence peaks were based on a per-sample minimum of 1,000 nuclei (as in Hopkins et al. 1996) and a coefficient of variation (CV) of ≤ 5% (as in Dolezel et al. 2007). CV is a measure of peak width and is equal to the standard deviation of the peak/mean channel position of the peak, x 100 (Figure 1.3). Wider peaks result from greater variation in the amount of fluorescence among nuclei per sample. To determine the ploidy level of each sample, I used the equation (from Dolezel et al. 2007):

$$\text{Sample ploidy (integer)} = \text{Reference ploidy} \times \frac{\text{mean position of the sample peak}}{\text{mean position of the reference peak}}$$

Costich et al. (2010) discovered a high incidence of aneuploidy in switchgrass, particularly in octaploids, whose chromosome counts ranged from 7x=63 to 9x=81. Therefore, I included all individuals whose calculated ploidy was within ± 10% of the expected ploidy (e.g., 4x = 4.0). Calculated ploidy levels of 3.6-4.4 were classified as tetraploids, those with levels of 5.4-6.6 were marked as hexaploids, and those with levels of 7.2-8.8 were classified as octaploids. As a quality control measure, I re-screened 20 individuals selected at random (~10% of the total) and compared both sets of results, which were consistent. I also re-tested any individuals that produced ambiguous or unexpected ploidy results.

Flowering Phenology

I established two adjacent common gardens at Waterman Agricultural Research Station on the Ohio State University campus in 2008 and 2009 (Table 1.3). Prior to planting, the field was tilled. In 2008, I planted individuals that originated from seeds collected from one Ohio native switchgrass population (OSU Marion Restored Prairie) and four cultivars. I used cultivars that originated from areas that are, at most, one hardiness zone away from Ohio. I used a randomized complete block design with one plant of each biotype in each of 16 blocks and 2 m between rows and columns in the grid. I planted young seedlings (3 weeks after emergence) directly into the soil at the plot in late June (hereafter "cohort 1"). In 2009, I established a larger common garden experiment adjacent to cohort 1 with three different wild populations from north central Ohio and seven cultivars, including three advanced strains (hereafter "cohort 2") (Table 1.3). The randomized complete block design included 10 individuals in each of 20 blocks (one individual per biotype), for N = 20. Three-week-old seedlings were planted in early June, with 2 m between rows and columns in the grid, as in cohort 1.

In both years, newly planted seedlings that died were replaced within 5 days of the initial planting. Newly planted seedlings were watered twice a week for eight weeks, to aid in establishment. In 2008 and 2009, plots were hand-weeded around the base of each plant, and aisles were sprayed with glyphosate as needed to eliminate weeds. In 2010, cohort 2 was hand-weeded, but cohort 1 was not, as those plants had become large enough that small ground weeds around the base of each plant were deemed to have no effect on growth. Also, in 2010, a few switchgrass volunteers were found growing in the aisles in both gardens and were retained for future observation (Chapter 3). For this reason, glyphosate, a broad-spectrum herbicide, was only used for spot-treatment of monocot weeds (e.g., other grasses), and 2,4-D herbicide, which specifically targets dicot plants, was sprayed in the aisles of both sites to control broad-leaf weeds. Early in the season, aisles were mowed periodically as an additional weed control, but this was stopped when volunteers were identified. In 2011, the volunteer population increased substantially, so that only 2,4-D was used for weed control.

I recorded whether the plants in each cohort flowered during their first year (Table 1.4), and recorded flowering times beginning in their second year. Because results from the two common gardens were very consistent, I only surveyed cohort 2 in 2011. Only 9 of the 19 Shawnee plants in cohort 2 flowered in 2011, possibly due to problems with shoot lodging and poor panicle growth. Similarly, all other biotypes except Kanlow and Advanced Kanlow had 1-2 fewer plants flower in 2011. I surveyed flowering phenology on a weekly basis beginning with the first floral initiation in July. To record flowering, I visually inspected each plant, categorizing it as follows:

Early flowering	onset to 50% of shoots are flowering
Full flowering	50-100% of shoots are flowering
Late flowering	less than 50% of shoots are flowering
Done flowering	No shoots are flowering

The survey continued until the last plant had finished flowering (mid-late October for the Kanlow biotypes). When calculating the proportion of flowering plants for each biotype, I weighted the weekly values by multiplying the mean number of flowering plants per biotype in each category thus:

Early flowering:	Proportion of plants x 0.25
Full flowering:	Proportion of plants x 1
Late flowering	Proportion of plants x 0.25

Weighted flowering was used to construct the flowering phenology graphs in Figure 1.4, in order to describe peak flowering times more accurately for each biotype.

Data Analysis

To compare flowering phenology, means with standard errors were calculated for the week of initial and peak flowering, and for total number of flowering weeks for each biotype. The two common gardens were analyzed separately. Analysis of Variance was used to test for significance of biotype, block and year on initial and peak flowering dates, and total flowering weeks. Tukey's HSD was used to separate means within each year (SAS v 9.2, SAS Institute, Cary, North Carolina).

For each cohort, the ANOVA used in SAS to test the significance of year (2009 and 2010 for cohort 1, 2010 and 2011 for cohort 2) and interactions on each parameter was:

Source	df	Error terms used in SAS
Biotype	9	Biotype*Year; Biotype*Block; Biotype*Year*Block
Year	1	Biotype*Year; Year*Block; Biotype*Year*Block
Block	19	Biotype*Block; Year*Block; Biotype*Year*Block
Biotype*Block	168	Biotype*Year*Block
Biotype*Year	9	Biotype*Year*Block
Year*Block	19	Biotype*Year*Block
Biotype*Year*Block	139	

Tests for both cohorts showed there were significant differences for biotypes within years, so a second ANOVA was used to separate means for each cohort's data set using Tukey's HSD. The second ANOVA did not take year into account and was used to test parameters with each year's data separately:

Source	df	Error terms used in SAS
Biotype	9	Biotype*Block
Block	19	Biotype*Block
Biotype*Block	168, 142[1]	

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[1] In 2011, several plants lodged or didn't produce mature inflorescences. Numbers of plants in 2010 = 197; numbers in 2011 = 171. Thus, degrees of freedom is different in 2010 and 2011 for the separate tests.

RESULTS

Ploidy Assessment

Nine of the 11 populations were uniformly either tetraploid or octaploid, while two populations were mixed-ploidy (Table 1.5). The majority of tested individuals (79%) were tetraploids. The site located at Midewin National Tallgrass Prairie in Illinois had mostly tetraploids with two octaploids, and the site at Plum Brook Research Station Site B in Ohio had mostly octaploids with one tetraploid and two hexaploids.

First-Year Flowering

Almost all plants in cohort 1 and cohort 2 flowered in the establishment year. All native Ohio plants and most cultivar plants flowered in cohort 1 in 2008. In cohort 2, all but one Ohio native and one Advanced Ocatploid plant flowered in 2009 (Table 1.4).

Flowering Phenology

Flowering patterns were very similar between cohorts: flowering began each year in July, with Ohio native biotypes initiating flowering earliest, and ended in October with Kanlow and Advanced Kanlow being the last to finish flowering. Plants initiated flowering later in the season in 2009 (Figure 1.4a) and 2011 (Figure 1.4d) than they did in 2010 (Figure 1.4b, c). Because of the similarity in flowering phenology within biotypes in the two cohorts, results for cohort 2 only are reported and discussed below.

Flowering of all biotypes overlapped in both years, but the degree of overlap varied across years (Figure 1.4c, d). Initial and peak flowering times also varied, as did the total number of flowering weeks among biotypes and across years (Table 1.6). Native biotypes were very similar to each other in flowering phenology. As a group, the native biotypes overlapped the most with non-Kanlow cultivars Shawnee, Summer, Trailblazer, and the two Advanced Octaploid strains, although this was much more pronounced in 2010 than 2011 (Figure 1.5). In both years, Summer, an upland tetraploid from Nebraska, was the most similar to the Ohio native biotypes in flowering phenology (Figure 1.4c, d).

Interactions between biotypes, years, and blocks were significant for the measured parameters. Biotype x year, biotype x block, and year x block interactions were present for initial flowering date. Biotype x year and biotype x block interactions were present for peak flowering date, and a biotype x year interaction was present for total number of flowering weeks (Table 1.7).

When data for 2010 and 2011 were analyzed separately, ANOVA showed significant differences among biotypes for initial and peak flowering, as well as total number of weeks spent flowering. There were no significant block effects (Table 1.8). These results were used to separate means using Tukey’s HSD (Table 1.6).

DISCUSSION

All switchgrass cultivars overlapped in flowering phenology to some degree with the native Ohio populations in the common garden, which shows the potential exists for gene flow between cultivars and wild populations. Variation in flowering across years highlights the need to use caution when deciding which cultivars to plant based on flowering phenology. Additionally, the discovery of a mixed-ploidy population each in Illinois and Ohio emphasizes the fact that multiple ploidy levels can be found in natural areas, even in places where the majority of individuals are of a single ploidy.

Ploidy Assessment

All but two of the sampled populations were homogeneous in terms of ploidy level, and most were tetraploids. The two heterogeneous populations were dominated by either tetraploids (Midewin National Tallgrass Prairie) or octaploids (NASA Plum Brook Research Station Site B). Both Hultquist et al. (1997) and Zhang et al. (2011b) found evidence of many mixed ploidy populations in the Midwest (Figure 1.6). Their studies used seed accessions, which are not as reliable as visiting the site and making individual plant collections personally. For example, Zhang et al. (2011b) found that one of the wild accessions in their study was actually a composite of 92 accessions collected across 3 states. Nevertheless, they provide evidence, at least at the state level, that many switchgrass populations contain individuals of varying ploidy levels.

It has been reported that a post-zygotic mating barrier keeps switchgrass plants of different ploidy levels from inter-mating (Martinez-Reyna and Vogel 2002). It might be tempting, therefore, to use ploidy level as a means of reducing the potential for gene flow by planting cultivars that have a different ploidy than the nearby wild populations. However, the presence of a mixed ploidy population in Ohio and Illinois shows that tetraploids, hexaploids and octaploids do co-occur. Although I sampled a narrower geographic range and fewer populations in Illinois, additional sampling in Illinois and Ohio could reveal more cases of mixed-ploidy. This would be consistent with what has previously been found in the Midwest (Figure 1.6). Thus, even when the majority of native plants within a population are of a single ploidy level, as with those in this study, the presence of even a few individuals of different ploidy levels means there will always be the potential for pollen transfer and gene flow with nearby cultivated plants. Any plans to plant switchgrass cultivars based on their proximity to opposite ploidy wild populations should be approached with caution.

Recent DNA analyses suggest that long-term switchgrass evolution was probably linked to ecotype (Zhang et al. 2011b). Zhang et al. (2011b) determined that switchgrass in the U.S. likely originated in the southeast. As populations spread north and northwest, they adapted to differences in soil moisture, growing degree days, and photoperiod. All the northern populations represented in Figure 1.6 are upland ecotypes, while the southern populations are lowland types, supporting the hypothesis that ecotype is linked to the geographic spread and the evolution of switchgrass.

Flowering Phenology

Flowering times overlapped among all biotypes to some degree. Native Ohio plants overlapped most with non-Kanlow type cultivars. The lowland Kanlow types from

Oklahoma consistently initiated flowering much later in the growing season than the other biotypes, which supports earlier reports of delayed flowering and increased biomass in southern ecotypes when they are grown at more northern latitudes (Vogel et al. 1985, Newell 1968). Early and late flowering plants will experience less pollen flow, and therefore, less potential gene flow, than plants whose flowering overlaps more heavily. Nevertheless, gene flow could still occur between these biotypes.

Variation in flowering across years emphasizes the fact that flowering phenology must be viewed in the context of the environment in which the plants are growing. For instance, in 2009, some Kanlow plants were still flowering in mid-October when a hard frost froze their flowers and anthers. In 2011, there were unusually high levels of rainfall in central Ohio in March, April and May, which may have contributed to the delay in floral initiation and peak flowering of most biotypes (http://www.erh.noaa.gov/iln/climo/summaries/wetspring2011.php). Such variation in flowering phenology means wild and cultivated populations might experience more overlap in flowering some years than others, indicating that single-year data for flowering phenology is not sufficient if decisions are being made about what cultivars to plant to avoid flowering overlap with wild populations. Selbo and Snow (2005) surveyed flowering times of three native and three non-native *Andropogon gerardii* populations in Ohio and found that three populations that had been burned early in the season also flowered earliest. Selbo and Snow hypothesized that increased soil surface temperatures in cleared areas may have caused earlier shoot development and earlier floral initiation than populations in non-burned areas. Sherry et al. (2007) tested the effect of increased precipitation and air temperature on several prairie species, including switchgrass. They discovered that early-flowering species initiated floral production in advance of control plots, while later-flowering species delayed flowering compared to the control. In that study, switchgrass plants flowered an average of 17 days earlier in the warming treatment and 29 days earlier in the warming/increased precipitation treatment than the control (Sherry et al. 2007).

There is also genetic variation within biotypes, as not all individuals within a biotype flowered simultaneously. Variation in flowering time in many grasses seems to be under genetic control (Talbert et al. 1983, Watson & McLean 1991, McLean & Watson, 1992). Hussey et al. (1998) tested the heritability of switchgrass maturity by selecting 12 very early and 12 very late flowering Alamo plants, and 42 randomly selected Alamo plants, from a nursery in Texas. The groups were kept separate from each other, and individuals within each group were allowed to inter-mate. Parents and progeny were planted in a field trial and flowering times were recorded. The authors found that panicle emergence for parent plants differed by 22 days compared to the control group (10 days earlier or 12 days later), and the F_1 generation differed by 21.5 days. There is a high degree of genetic variability both within and between switchgrass strains for many agronomic traits (Vogel et al. 1985), although recent research has shown that there is generally greater within-population genetic variation, at least in cultivars (Zalapa et al. 2011). The biotypes used in my research showed less variation within a type than among different types, which suggests the different biotypes, including cultivars, may be locally adapted, although a larger sample size might reveal greater within-population variation. Even minor variation in flowering times between individuals in a single population increases the total overlap between populations. Thus, large swards of switchgrass cultivar plants are likely to have a greater amount of overall flowering with nearby wild populations than smaller swards.

Like the Ohio biotypes, the wild Illinois switchgrass populations that were sampled for ploidy tests typically initiate flowering in July (Bill Glass, Biologist, Midewin Federal Preserve, pers. comm.). Therefore, it seems likely there will be at least some overlap in flowering of cultivars and wild populations. Some cultivars will probably have a greater degree of overlap with local populations than others. Given that ploidy levels of most of the Ohio and Illinois biotypes match those of several of the cultivars, this increases the likelihood that gene flow could occur between them if they grow near each other.

Potential for Gene Flow

Early results from molecular markers show definite genetic differentiation between cultivars and wild populations in Ohio (E. Mutegi, pers. comm.). This supports the idea that forage crops and CRP plantings have not impacted native Ohio populations in terms of gene flow and hybridization, at least not to the degree that the genetic structure of wild populations has been compromised by those groups. In contrast, early data analyses suggest gene flow has occurred in Illinois between cultivars and the wild populations used in this study (E. Mutegi, pers. comm.). Illinois has many more hectares of CRP land, compared to Ohio (Table 1.1), which could help explain why Illinois wild populations appear to have already experienced hybridization with cultivars. In addition, controlled greenhouse experiments have shown that switchgrass cultivars can cross with other species that are near relations (C. Auer, pers. comm.). Thus, cultivars have the potential to hybridize with other grasses, as well.

It is important to remember that biofuel crops will be "improved" varieties of traditional cultivars, in agricultural terms, and some future cultivars will likely contain transgenes that confer traits that alter fitness, including survival. Biofuel crops may also be planted on a much larger scale than forage crops or CRP lands, which could lead to pollen swamping, gene swamping, and increased hybridization. Hybrid offspring with increased or decreased fitness compared to parent wild genotypes could cause localized extinctions of "pure" wild populations. Switchgrass populations would see a decline in the overall genetic variation that exists in the species, making them more vulnerable to stochastic environmental effects and reducing the amount of variability with which plant breeders have to work.

Transgenes can be used to reduce the risk of gene flow, by conferring sterility on plants. For example, Chuck et al. (2011) transformed Alamo switchgrass plants using *Corngrass1* (*Cg1*), a maize gene that promotes the production of juvenile cell walls and juvenile morphology, in an attempt to increase the starch content in switchgrass for energy production. Transformed plants had juvenile cell walls and morphology and produced up to 250% more starch. In addition, plants never flowered in the two years of the study. Other transgene biocontainment strategies include male sterility, maternal inheritance, and transgene excision. Male sterility, in particular, has been used successfully in agriculture; pollen development is disrupted through genetic manipulation (Moon et al. 2010). Using transgenes to inhibit flowering or induce sterility could greatly reduce the probability of gene flow between cultivars and wild plants, although more research is needed to ensure these containment strategies persist over the long-term, since switchgrass is a long-lived perennial plant.

While biofuel switchgrass is expected to be widely cultivated, the biotype that is used will depend on its economic value and its success in the local environment. For example, Eastern Kentucky State University's Center for Renewable and Alternative Fuel Technologies (CRAFT) is conducting research to determine which switchgrass varieties yield the most glucose, fructose and xylose, as well as what strains will grow best in the different topographic regions of Kentucky (http://www.craft.eku.edu/cellulosic-derived-biofuels-initiative). Since there is no single cultivar currently being bred for exclusive use across the U.S., it will be necessary to approach risk assessment for potential gene flow between switchgrass cultivars and wild relatives on a case-by-case basis.

These results, combined with previous research described in Table 1.9, show that switchgrass populations in the Midwest consist of either 4x, 6x, or 8x plants, either mixed or in separate populations. This extensive examination of ploidy levels across two states and multiple Midwestern regions provides important information about the existing native switchgrass populations. If biofuel switchgrass of the same ploidy level and with similar flowering phenology is sympatric to wild populations, gene flow could occur between the two groups, allowing enhanced cultivar genes to escape cultivation and enter wild populations.

TABLES AND FIGURES

State	**CRP land, in hectares**
Illinois	419,692
Indiana	115,730
Iowa	674,237
Kansas	1,107,590
Michigan	92,714
Minnesota	662,095
Missouri	551,503
Nebraska	437,054
North Dakota	1,071,682
Ohio	139,308
South Dakota	469,959
Wisconsin	161,436
Subtotal	**5,903,000**
U.S. Total	**12,613,345**

Table 1.1. Number of hectares of farmland in the Conservation Reserve Program in the Midwestern United States in November, 2011. (www.fsa.usda.gov/Internet/FSA_File/apportstate091311.pdf)

Map Code	County	Population	Location	Pop. Size, ha
OH1	Erie	NASA Plum Brook Prairie Site A	N41º20.736' W82º39.776'	0.1
OH2	Erie	NASA Plum Brook Prairie Site B	N41°22.156' W82°38.872'	0.4
OH3	Marion	Claridon Railroad Prairie	N40°37.218' W83°01.287'	1.1
OH4	Crawford	Daughmer Bur Oak Savannah	N40°43.913' W83°05.557'	0.5
OH5	Marion	Green Camp Railroad Prairie	N40°32.905' W83°11.486'	0.1
OH6	Madison	Neil East Railroad Prairie	N39°50.347' W83°34.386'	0.2
OH7	Clark	Botkin Road Railroad Prairie	N39°51.257' W83°31.848'	0.3
OH8	Clark	South Charleston Railroad Prairie	N39°49.108' W83°38.572'	0.5
IL1	Will	Midewin Nat'l Tallgrass Prairie	N41°20.837' W88°08.316'	2.8
IL2	Will	Des Plaines Conservation Area	N41°23.351' W88°11.677'	1.3
IL3	Will	Hitt's Siding Nature Preserve	N41°18.151' W88°10.488'	0.1

Table 1.2. Location and approximate size of native switchgrass populations sampled for ploidy assessment. Seeds were collected from individuals in each population in 2009 and 2010. “Map code” corresponds to Figure 1.1.

	Number of Plants			
Biotype	2008	2009	Origin	H.Z.
Shawnee	11	19	IL	6
Summer	14	20	NE	5
Trailblazer	12	20	Syn., NE & KS	5,6
Kanlow	13	20	OK & KS	6,7
Advanced Kanlow[1]	--	20	OK & KS	6,7
Advanced Octaploid 1[1]	--	19	Synthetic	5
Advanced Octaploid 2[1]	--	20	Syn., NE & KS	5,6
Local Native Restored[2]	13	--	OSU Marion	6
Ohio Native 1[2]	--	20	Green Camp RR	6
Ohio Native 2[2]	--	20	Daughmer BOS	6
Ohio Native 3[2]	--	19	Claridon RR	6

Biotype	Ploidy	Ecotype[3]	Use
Shawnee	8x	U	forage
Summer	4x	U	forage
Trailblazer	8x	U	forage
Kanlow	4x	L	forage
Advanced Kanlow	4x	L	yield/ winter survival
Advanced Octaploid 1	8x	U	yield/ ↓ lignin
Advanced Octaploid 2	8x	U	↓ lignin
Local Native Restored	4x	U	restoration
Ohio Native 1	4x	U	n/a
Ohio Native 2	4x	U	n/a
Ohio Native 3	4x	U	n/a

[1] Seeds of experimental strains were obtained from Dr. Kenneth Vogel, USDA-ARS, University of Nebraska.

[2] I determined the ploidy level (N = 10-20) for Ohio native biotypes using flow cytometry and the ecotype (N = 5) by examining rhizome morphology (see Chapter 3 for methods).

[3] L = lowland ecotype; U = upland ecotype

Table 1.3. Cultivars and wild populations used in two common garden experiments (2008 = cohort 1, 2009 = cohort 2) at Ohio State University's Waterman Farm. 'H.Z.' refers to the USDA hardiness zone in which the biotype originated (Synthetic ["Syn."] biotypes are the result of multiple crosses with several parent types).

Cohort 1 **Biotype**	**Sample Size**	**Prop. Of Plants that Flowered**	**Mean Prop. of Flowering Shoots**	**Mean No. of Shoots**	**s.e.**	**Mean Height, cm**	**s.e.**
Ohio Native	13	1	0.61	25	2.73	88.2	4.05
Summer	16	1	0.88	20	2.48	97.2	3.29
Shawnee	16	1	0.65	40	4.86	99.4	5.83
Trailblazer	15	0.85	0.62	33	3.93	87.7	7.97
Kanlow	16	1	0.55	20	1.36	119.4	6.64

Cohort 2 **Biotype**	**Sample Size**	**Prop. Of Plants that Flowered**	**Mean Prop. of Shoots that Flowered**	**Mean No. of Shoots**	**s.e.**	**Mean Height, cm**	**s.e.**
Ohio Native 1	19	0.95	0.65	24	2.97	95.1	3.69
Ohio Native 2	20	1	0.61	31	2.44	95.7	3.85
Ohio Native 3	20	1	0.59	24	2.61	100.7	2.58
Summer	20	1	0.67	35	2.08	101.2	3.83
Shawnee	20	1	0.53	26	2.98	107.3	4.02
Trailblazer	20	1	0.77	43	3.97	110.7	2.58
Kanlow	20	1	0.80	21	2.03	135.9	4.52
Advanced Kanlow	20	1	0.66	33	2.26	123.4	3.39
Adv Octaploid 1	20	0.95	0.63	24	3.24	97.8	4.39
Adv Octaploid 2	20	1	0.70	45	4.65	106.1	4.70

Table 1.4. Establishment-year data for cohort 1 and cohort 2, collected in autumn of 2008 and 2009, respectively.

State	Population	n	DNA Ploidy (proportion)		
			4x	**6x**	**8x**
Ohio	NASA Plum Brook Prairie Site A	20			1.00
	NASA Plum Brook Prairie Site B	18	0.06	0.11	0.83
	Claridon Railroad Prairie	20	1.00		
	Daughmer Bur Oak Savannah	18	1.00		
	Green Camp Railroad Prairie	10	1.00		
	Neil East Railroad Prairie	15	1.00		
	Botkin Road Railroad Prairie	18	1.00		
	South Charleston Railroad Prairie	18	1.00		
Subtotal		**137**	**0.73**	**0.01**	**0.26**
Illinois	Midewin National Tallgrass Prairie	21	0.90		0.10
	Des Plaines Conservation Area	15	1.00		
	Hitt's Siding Prairie Nature Preserve	15	1.00		
Subtotal		**51**	**0.96**	**0.00**	**0.04**
Total		**188**	**0.79**	**0.01**	**0.20**

Table 1.5. Results of ploidy assessment of individuals from 8 remnant prairies in Ohio and 3 remnant prairies in Illinois. “n” is the number of individuals included in the final analysis.

2010	**Week of Initial Flowering**		**Week of Peak Flowering**		**Total Number of Flwrng Weeks**	
Biotype	**Week:**	**Tukey**	**Week:**	**Tukey**	**mean**	**Tukey**
Ohio Native 1 (Green Camp)	28 (Jul 11)	a	29 (Jul 18)	a	3.4	abc
Ohio Native 2 (Daughmer)	28 (Jul 11)	a	29 (Jul 18)	ab	3.7	bcd
Ohio Native 3 (Claridon)	28 (Jul 11)	ab	29 (Jul 18)	abc	3.7	bcd
Summer	29 (Jul 18)	bc	30 (Jul 25)	bcd	2.8	ab
Shawnee	29 (Jul 18)	c	30 (Jul 25)	cde	2.7	ab
Trailblazer	30 (Jul 25)	c	30 (Jul 25)	de	2.5	a
Advanced Octaploid 1	30 (Jul 25)	c	30 (Aug 1)	e	2.9	abc
Advanced Octaploid 2	30 (Jul 25)	c	30 (Jul 25)	de	3.8	cd
Advanced Kanlow	32 (Aug 8)	d	34 (Aug 22)	f	5.2	e
Kanlow	34 (Aug 22)	e	35 (Aug 29)	f	4.5	de

2011	**Week of Initial Flowering**		**Week of Peak Flowering**		**Total Number of Flowering Weeks**	
Biotype	**Week:**	**Tukey**	**Week:**	**Tukey**	**mean**	**Tukey**
Ohio Native 1 (Green Camp)	29 (Jul 17)	a	30 (Jul 24)	a	1.9	a
Ohio Native 2 (Daughmer)	29 (Jul 17)	a	30 (Jul 24)	a	2.5	abc
Ohio Native 3 (Claridon)	30 (Jul 24)	a	30 (Jul 24)	a	2.6	abcd
Summer	30 (Jul 24)	a	31 (Jul 31)	a	2.4	ab
Shawnee	31 (Jul 31)	b	33 (Aug 14)	b	3.3	bcd
Trailblazer	31 (Jul 31)	b	33 (Aug 14)	b	3.5	d
Advanced Octaploid 1	32 (Aug 7)	b	33 (Aug 14)	b	2.8	abcd
Advanced Octaploid 2	32 (Aug 7)	b	33 (Aug 14)	b	3.1	bcd
Advanced Kanlow	36 (Sep 4)	c	37 (Sep 11)	c	3.1	bcd
Kanlow	36 (Sep 4)	c	37 (Sep 11)	c	3.4	cd

Table 1.6 Average week of initial and peak flowering, and total flowering weeks for each biotype in cohort 2, measured in 2010 and 2011 (± 1 SE). Tukey's HSD was used for separation of means; letters indicate significant differences at $P < 0.05$.

Initial Flowering

Source	d.f.	SS	MS	*F* Value	*P* value
Biotype	9	1355.45	150.61	276.93	< 0.0001
Year	1	225.44	225.44	414.54	< 0.0001
Block	19	11.77	0.62	1.14	0.3197
Biotype*Year	9	31.32	3.48	6.4	< 0.0001
Year*Block	19	18.98	0.99	1.84	0.0239
Biotype*Block	168	132.033	0.79	1.45	0.0120

Peak Flowering

Source	d.f.	SS	MS	*F* Value	*P* value
Biotype	9	1582.25	175.81	349.32	< 0.0001
Year	1	189.79	189.79	377.11	< 0.0001
Block	19	8.85	0.47	0.93	0.5522
Biotype*Year	9	42.45	4.72	9.37	< 0.0001
Year*Block	19	7.59	0.40	0.79	0.711
Biotype*Block	168	161.96	0.96	1.92	< 0.0001

Total Flowering

Source	d.f.	SS	MS	*F* Value	*P* value
Biotype	9	89.54	9.95	9.93	< 0.0001
Year	1	35.03	35.03	34.98	< 0.0001
Block	19	14.00	0.74	0.74	0.7769
Biotype*Year	9	67.74	7.53	7.52	< 0.0001
Year*Block	19	11.82	0.62	0.62	0.8855
Biotype*Block	168	137.28	0.82	0.82	0.8962

Table 1.7. ANOVA table showing the effect of biotype, year, and block on initial and peak flowering dates, as well as the number of weeks spent flowering for 10 biotypes in a common garden (cohort 2) in Columbus, Ohio. N = 29-40.

2010

Initial Flowering

Source	d.f.	SS	MS	*F* Value	*P* Value
Biotype	9	614.2	68.24	104.58	< 0.0001
Block	19	18.33	0.96	1.48	0.0988

Peak Flowering

Source	d.f.	SS	MS	*F* Value	*P* Value
Biotype	9	774.37	86.04	111.61	< 0.0001
Block	19	8.15	0.43	0.56	0.9314

Total Flowering

Source	d.f.	SS	MS	*F* Value	*P* Value
Biotype	9	128.83	14.31	13.76	< 0.0001
Block	19	21.56	1.13	1.09	0.3645

2011

Initial Flowering

Source	d.f.	SS	MS	*F* Value	*P* Value
Biotype	9	777.35	86.37	123.1	< 0.0001
Block	19	15.8	0.83	1.18	0.2784

Peak Flowering

Source	d.f.	SS	MS	*F* Value	*P* Value
Biotype	9	863.27	95.92	131.07	< 0.0001
Block	19	7.85	0.41	0.56	0.9255

Total Flowering

Source	d.f.	SS	MS	*F* Value	*P* Value
Biotype	9	40.71	4.52	6.18	< 0.0001
Block	19	6.11	0.32	0.44	0.9796

Table 1.8. ANOVA table showing the effect of biotype and block on initial and peak flowering dates, as well as number of weeks spent flowering for 10 biotypes in a common garden (cohort 2) in Columbus, Ohio. Data for 2010 and 2011 were analyzed separately. N = 29-40.

Ploidy:	4x only	8x only	4x and 8x	4x, 6x and 8x
Hultquist et al. (1997)				
Iowa	2	--	6	--
Illinois	3	--	--	--
Minnesota	1	--	2	--
Missouri	4	1	4	--
Nebraska	--	--	3	--
South Dakota	--	--	2	--
Current Study				
Illinois	2	--	1	--
Ohio	6	1	--	1
Total Populations	**18**	**2**	**18**	**1**

Table 1.9. Numbers of switchgrass populations with tetraploid and/or octaploid plants or tetraploid/hexaploid/octaploid mixtures, based on 5 individuals sampled from seed accessions collected from 28 Midwestern prairies (Hultquist et al. 1997) and 10-21 individuals sampled from each of 11 prairies in Ohio and Illinois (current study).

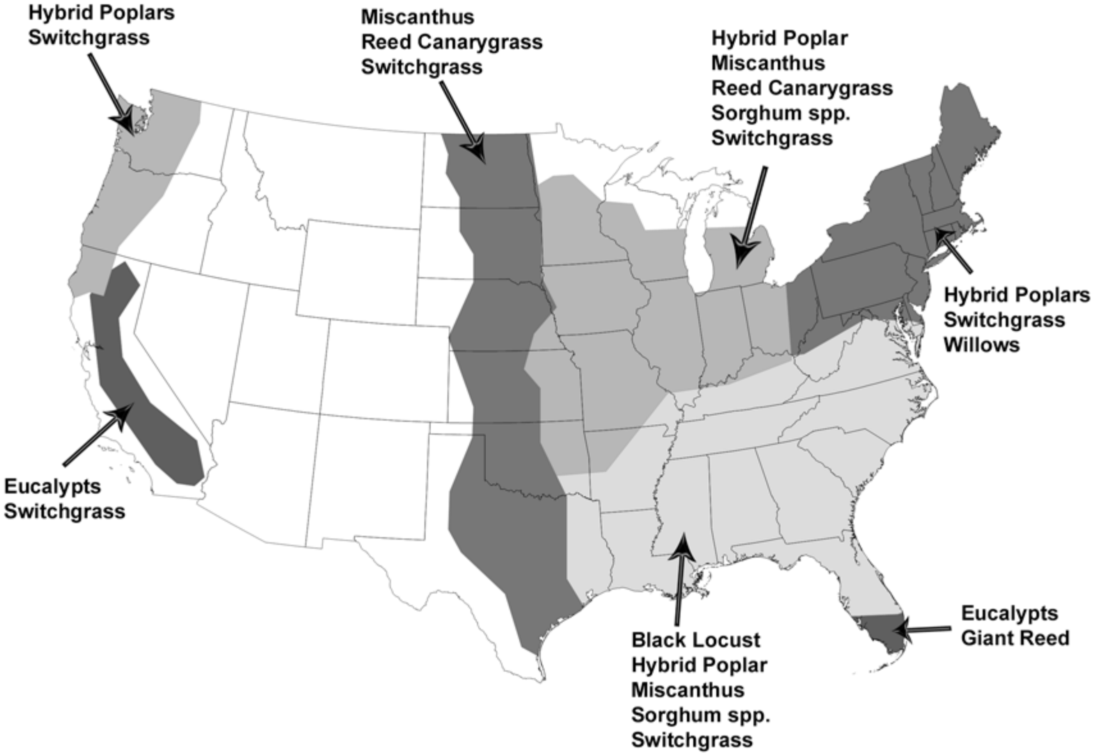


Figure 1.1. Lignocellulose biofuel crops and their potential regions of cultivation (*in* DiTomaso et al. 2007, adapted from the U.S. Department of Energy 2007).

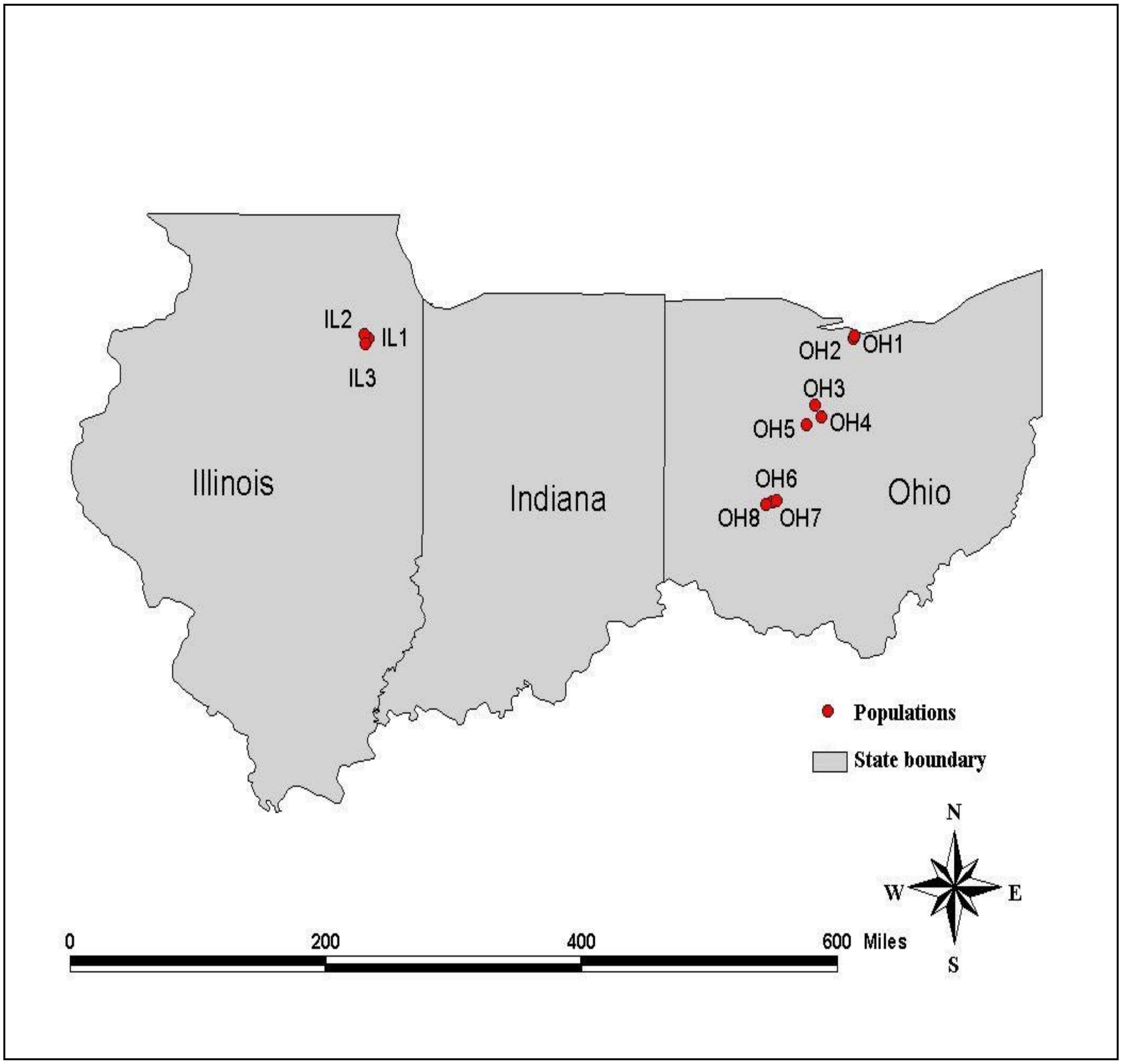
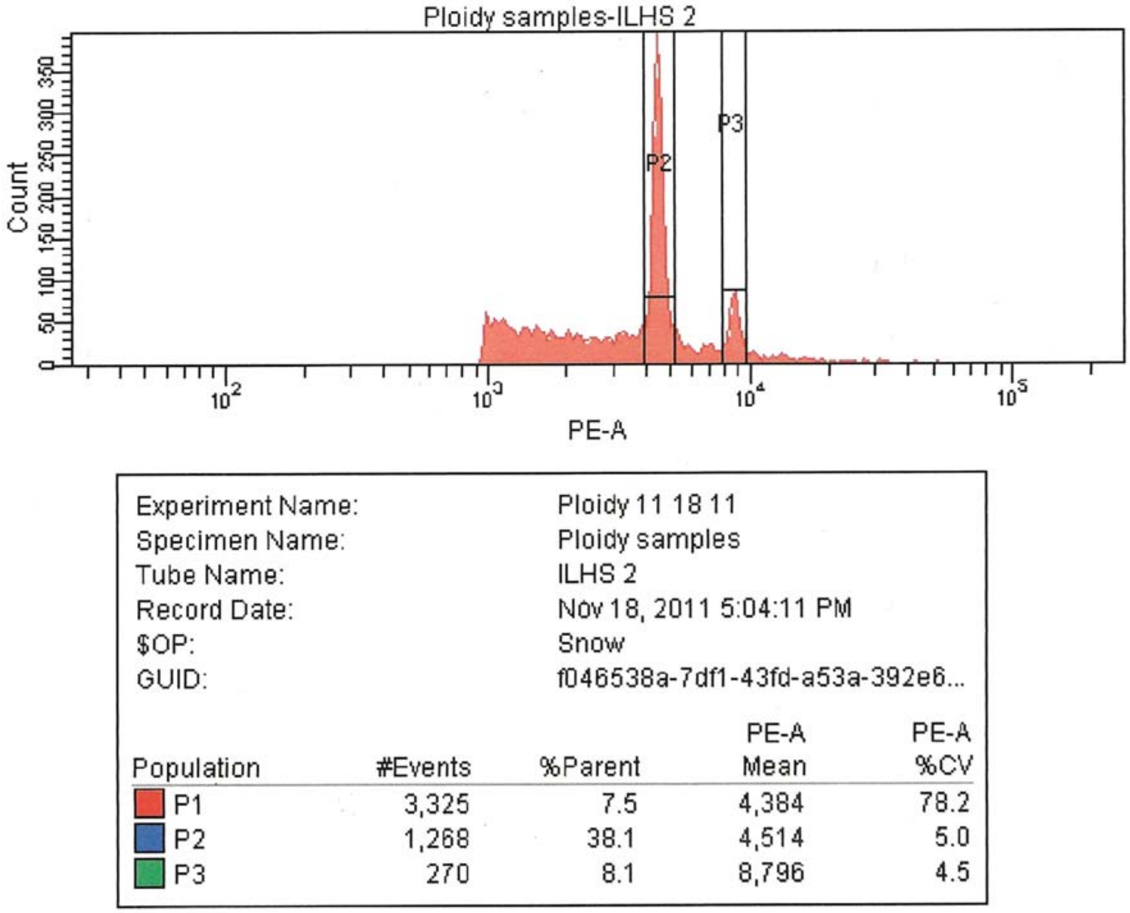


Figure 1.2 Map showing the locations of the remnant prairies that were sampled for ploidy assessment. Eight switchgrass populations were sampled in Ohio, and three populations were sampled in Illinois. See Table 1.2 for site descriptions.

(a)



Experiment Name: Ploidy 11 18 11
Specimen Name: Ploidy samples
Tube Name: ILHS 2
Record Date: Nov 18, 2011 5:04:11 PM
$OP: Snow
GUID: f046538a-7df1-43fd-a53a-392e6...

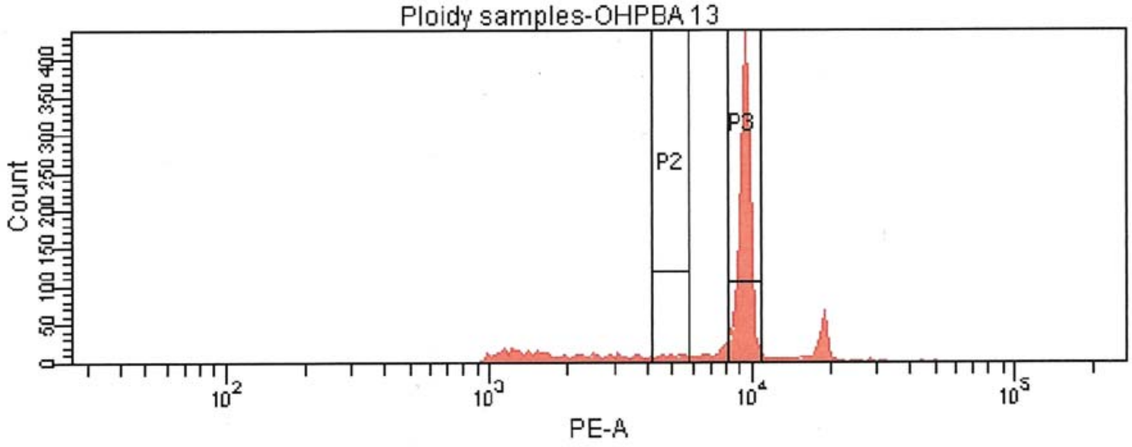
Population	#Events	%Parent	PE-A Mean	PE-A %CV
🟥 P1	3,325	7.5	4,384	78.2
🟦 P2	1,268	38.1	4,514	5.0
🟩 P3	270	8.1	8,796	4.5

Figure 1.3. Output from BD FACS LSR II, showing the mean florescence of nuclei stained with propidium iodide (PI) solution. “PE-A” refers to the mean florescence value on a logarithmic scale, and “Count” refers to the mean number of nuclei detected by the instrument. The smaller peaks are a result of nuclei that were stained while in the process of mitosis and have double the number of chromosomes of a somatic cell in diplophase. (a) A tetraploid individual from Hitt’s Siding Nature Reserve in Illinois has a mean PE-A of 4514 and a CV of 5%. (b) An octaploid individual from Plum Brook Research Station Site A in Ohio has a mean PE-A of 9520 and a CV of 4.6%.

continued

Figure 1.3 continued

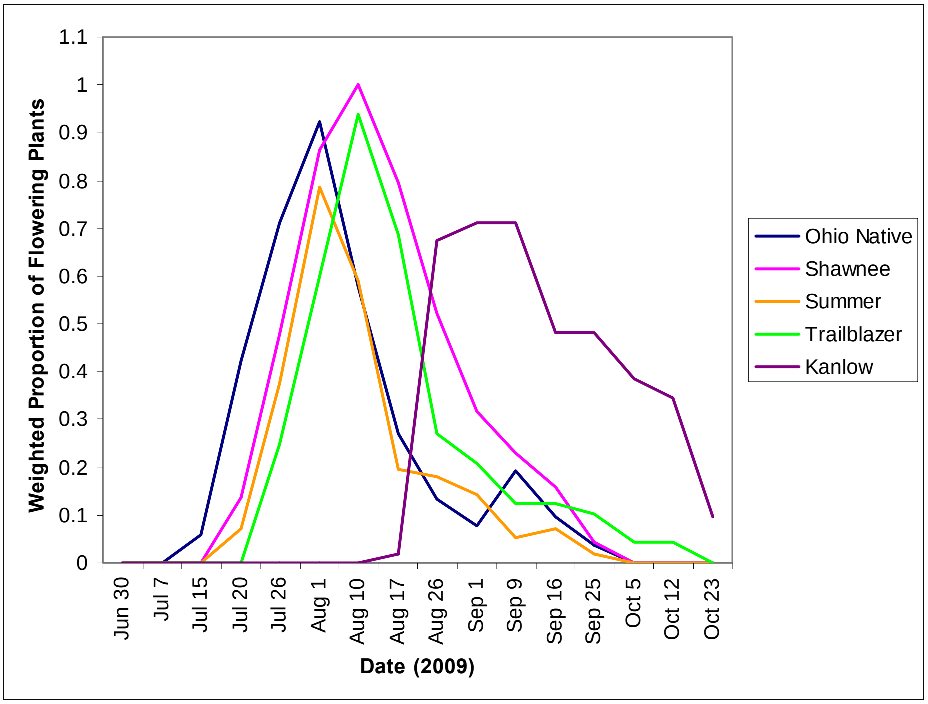
(b)



Experiment Name: Ploidy 11 18 11
Specimen Name: Ploidy samples
Tube Name: OHPBA 13
Record Date: Nov 18, 2011 4:33:14 PM
$OP: Snow
GUID: 15fe3eef-bf56-411b-a890-ed1fc...

Population	#Events	%Parent	PE-A Mean	PE-A %CV
🟥 P1	2,009	9.7	8,665	55.3
🟦 P2	70	3.5	4,933	9.2
🟩 P3	1,206	60.0	9,520	4.6

(a)



(b)

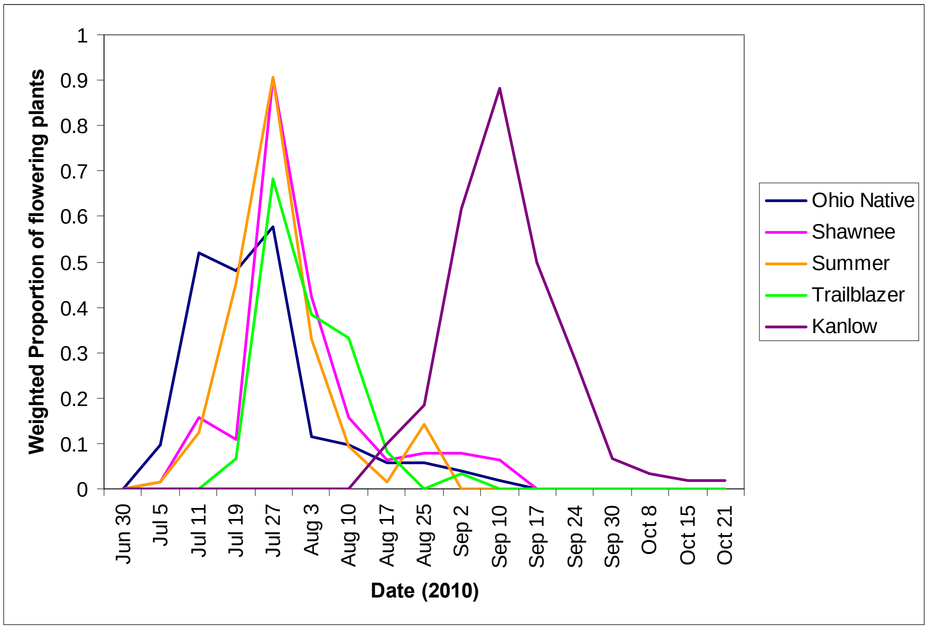
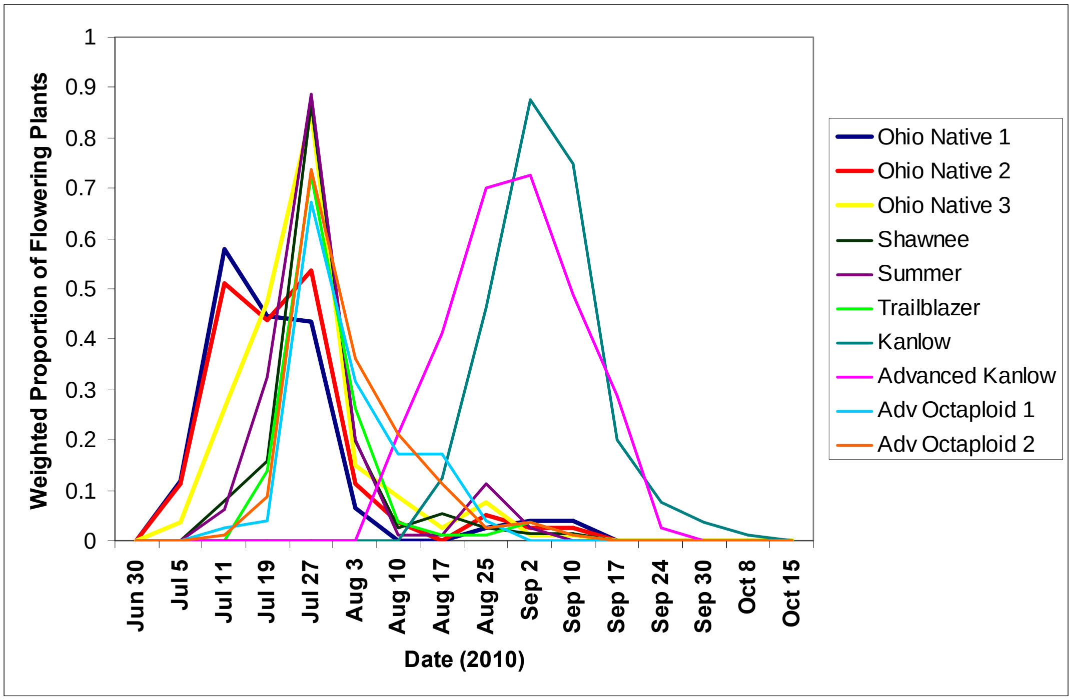


Figure 1.4. Flowering phenology of switchgrass biotypes planted in two common gardens in 2008 and 2009. Flowering was recorded weekly from July to October each year for 3 years. (a) 1st year data of cohort 1, collected in 2009. (b) 2nd year data of cohort 1, collected in 2010. (c) 1st year data of cohort 2, collected in 2010. (d) 2nd year data of cohort 2, collected in 2011. N = 13-16 for 2008, and N = 19-20 for 2009.

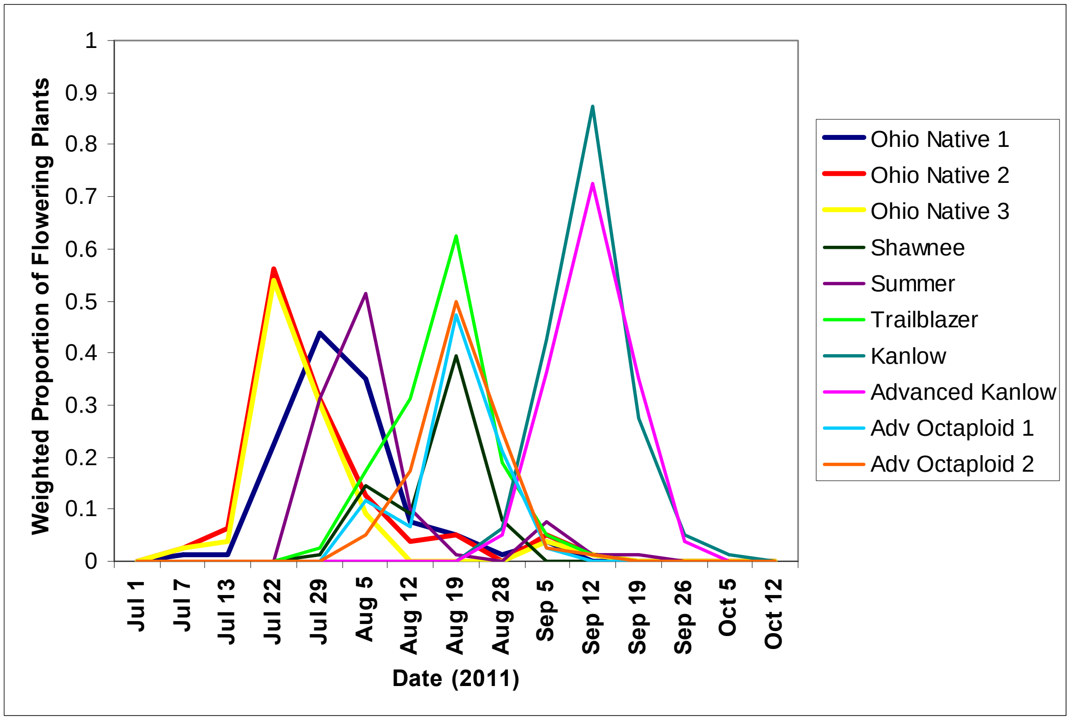
continued

Figure 1.4 continued

(c)



(d)



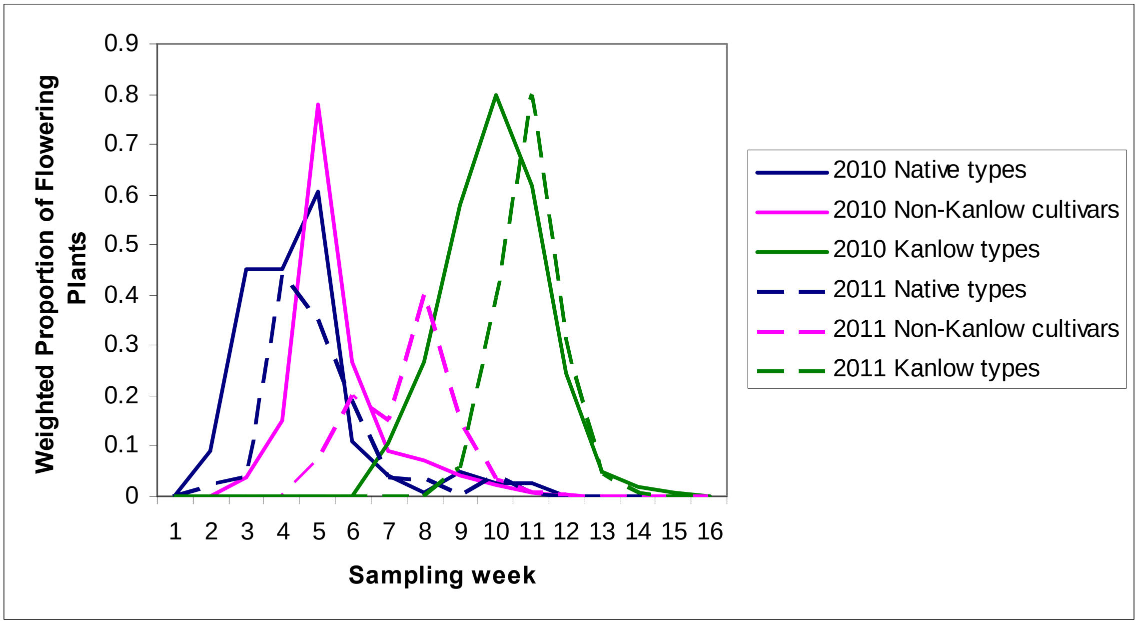


Figure 1.5. Comparison of the flowering phenology of switchgrass biotypes grown in a common garden established in 2009 in Columbus, Ohio. Figure shows the 10 biotypes grouped into 3 categories: Ohio Native types (3), Non-Kanlow type cultivars (5), and Kanlow types (2). N=59 Native plants, 98 non-Kanlow plants, and 40 Kanlow plants.

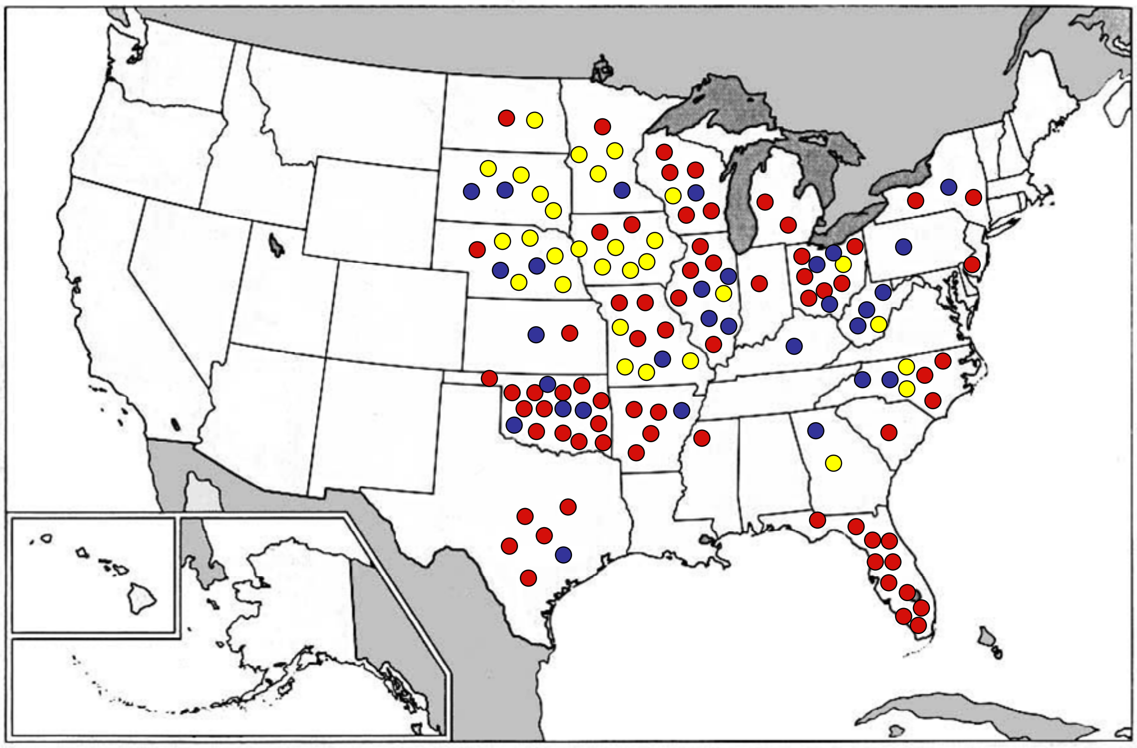


Figure 1.6. U.S. map showing the states in which switchgrass populations with different ploidy levels have been determined. Red circles = tetraploid populations, blue circles = octaploid populations, and yellow circles = mixed-ploidy populations. The level of "state" is the highest resolution; circles are arranged randomly within each state. Ploidy level data were obtained from Hopkins et al. (1996), Hultquist et al. (1997), Zhang et al. (2011b), Zalapa et al. (2011), and this study.

CHAPTER 2

FITNESS-RELATED TRAITS OF CULTIVATED *VS.* WILD SWITCHGRASS (*PANICUM VIRGATUM* L.): IMPLICATIONS FOR WIDESPREAD PLANTING OF BIOFUEL CULTIVARS.

ABSTRACT

There has been much interest in developing alternative energy sources in recent decades. Switchgrass (*Panicum virgatum* L.) has received particular notice. Its ability to tolerate drought and low nutrient levels, as well as its status as a native North American prairie species, makes it an attractive candidate for biofuels. Initially bred for forage, switchgrass is now being bred for use either in direct combustion or for conversion to ethanol, through selective enhancement of certain desirable “biofuel” traits, such as increased ethanol yields and increased biomass. In addition, it is becoming increasingly likely that transgenes will be used to improve traits like drought tolerance. In the near future, cultivated switchgrass will likely be planted on a massive scale sympatric to wild populations, but few studies have addressed the impact such plantings might have on native populations or as newly introduced populations, and there are no published reports that compare fitness-related traits of cultivars to wild biotypes. My goals were to compare certain fitness-related traits among several cultivated and wild switchgrass biotypes. In

2009, I planted 20 individuals from each of 7 cultivars: Summer (tetraploid, 4x), Shawnee (octaploid, 8x), Trailblazer (8x), Kanlow (4x), an advanced Kanlow strain (4x), and advanced octaploid strains 1 and 2 (both 8x), along with 3 Ohio native populations (biotypes, all 4x) in a common garden experiment at Ohio State University in Columbus, Ohio. All but two individuals survived over the winter. I compared seed and floret production, plant height, and numbers of reproductive and vegetative shoots in 2010. My results showed that Ohio native plants were significantly shorter than all cultivars except Summer. Shoot production was similar for all biotypes, except for Advanced octaploid 2, which produced 67% more reproductive shoots per plant, on average, than all biotypes except Trailblazer. Vegetative shoot production was negligible, making up < 5% of the total number of shoots for all biotypes. Ohio native types produced similar numbers of florets – an average of 88,000 per plant – as those of all biotypes except Kanlow and the advanced Kanlow strain. These two cultivars produced more than three times the number of florets as all other biotypes (381,000 per plant), which was highly significant. Kanlow and Summer produced twice the number of seeds per plant, on average, as the Ohio native plants. My results show that, while there is less concern for the octaploid cultivars to displace native plants or become established, the tetraploids Kanlow, Advanced Kanlow, and Summer could eventually displace native plants and establish feral populations if they escape cultivation, given suitable environmental conditions. They could also hybridize with wild plants of matching ploidy, and persistent crop genes could dilute the native gene pool and increase the potential for invasiveness in wild populations.

INTRODUCTION

Renewable energy sources have become increasingly important as an alternative energy source to diminishing fossil fuel reserves and as a way to reduce dependence on foreign oil sources. Ethanol production through conversion of sugar, grain (starch), or straw (lignocellulose) has been studied extensively in both the public and private sectors in recent decades. Sugar cane and maize are considered first-generation biofuels. Brazil, one of the world's biggest ethanol producers, converts sugar cane to ethanol through fermentation. The U.S., also at the forefront of biofuel production, harvested more maize for ethanol in 2011 than for livestock feed (USDA, 2012). However, recent research has revealed that the sugar cane process releases large amounts of air pollutants, more than gasoline or diesel production (Tsao et al. 2011), and interest in grain ethanol has waned, due in part to perceived negative consequences of producing maize for biofuels. As more agricultural land is devoted to maize for ethanol production, fewer hectares will be earmarked for maize for food. Also, there is concern that more land will be cleared and nutrient use will increase, which could lead to increased runoff to water supplies and increased soil erosion (Robertson et al. 2008). More recently, second-generation lignocellulosic biofuels have been intensively studied in order to make them an attractive, economical alternative to fossil fuels. Switchgrass (*Panicum virgatum* L.), in particular, has emerged as a viable lignocellulosic biofuel crop for several reasons: (1) it requires fewer nutritive inputs, (2) it tolerates drought well, (3) it establishes from seed, (4) it is perennial, and (5) it is native to much of North America (McLaughlin & Kszos, 2005). Because switchgrass can establish well on poorer soil, it is supposed that the biofuel crop will be less likely to encompass agricultural hectares that are currently dedicated to food production. It is becoming known as the main herbaceous biofuel feedstock (Bouton, 2007).

Switchgrass is a warm-season perennial grass native to most of the contiguous United States and parts of Canada. In the first half of the 20th century, switchgrass began to be selectively bred for use as a forage crop, and several commercial cultivars are available. Several decades ago, switchgrass cultivars began to be used in the Conservation Reserve Program (CRP). Farmers enter into contract and are paid a subsidy to remove qualified land from cultivation and plant approved species; environmental benefits include soil conservation and decreased nutrient runoff (Mapemba et al. 2007). Recently, switchgrass was identified as a potential cellulosic biofuel feedstock candidate, for use either in direct combustion (i.e., as a supplement to coal-burning) or for conversion to ethanol (i.e., as an alternative to traditional fuels) (Vogel, 1996; Tillman, 2000). In 1978, the DOE Office of Biomass initiated the Bioenergy Feedstock Development Program (BFPD), managed by Oak Ridge National Laboratory in Tennessee (Lewandowski et al., 2003; Parrish and Fike, 2005). By 1992, BFPD began to focus exclusively on switchgrass as their biofuel of choice. The BFPD stopped receiving funding in 2002, but the groups involved in that project continue to research ways to bring switchgrass into production as a biofuel feedstock.

Past research has shown that, while some traits are highly heritable in switchgrass and many cultivars are locally adapted to certain environments (Sanderson et al. 1999), other traits, such as those that promote biomass production, show genotype x environment interactions (Hopkins et al. 1995a and 1995b, Sanderson et al. 1999). Casler and Boe (2003) investigated genotype x environment interactions for several cultivar traits in switchgrass. They planted 6 cultivars at each of two locations and harvested plants for 4 years. Casler and Boe (2003) discovered that switchgrass biomass varied by year, harvest date, site, and cultivar. They also concluded that switchgrass cultivars vary in their suitability for biofuels because of differences in fiber and lignin content, but certain genotypes could be selectively bred for biofuel use. Casler et al. (2004) examined genotype x environment interactions with an emphasis on latitudinal differences by using individuals from 20 cultivar populations that originated from latitudes ranging from Texas to South Dakota. They planted the switchgrass plants at 5 locations and measured 10 agronomic traits. Casler et al. (2004) found heritable variation among cultivars related to adaptations at their latitude of origin, but they also discovered a strong genotype x environment interaction for biomass yield and survival, indicating that it will be difficult to find a single cultivar that is optimal for biofuel use across a broad latitudinal range.

To date, the focus of research into lignocellulosic biofuel production has primarily been from an agro-economic perspective. For example, recent research efforts have focused on increasing switchgrass harvest yield and altering lignin, cellulose and hemicellulose content in cell walls, to make the after-harvest fuel conversion process more efficient and economical (Vogel and Jung, 2001; Gressel, 2008). A decrease in lignin, in particular, would decrease the amount of byproduct, or slag, during fuel conversion and increase the ethanol yield. Federal legislation in the U.S. and the European Union (EU) has propelled agronomic-based research and development even further. A 2007 U.S. legislative mandate calls for 16 billion gallons of cellulosic ethanol to be produced per year by 2022, and the EU has mandated that 10% of all transport fuel be from renewable sources by 2020 (Robertson et al. 2008). To that end, research scientists with the USDA have focused on improving a select group of existing cultivars, such as the upland octaploid Cave-in-Rock, and the lowland tetraploids Kanlow and Alamo (Cave-in-Rock is already being co-fired with coal to produce electricity in Chillicothe, Iowa: http://www.iowaswitchgrass.com/about~accomplishments.html). Groups like the Samuel Roberts Noble Foundation, Ceres Inc., and a few academic research laboratories in the U.S. are actively studying ways to make transgenic switchgrass economically feasible. Ceres, Inc., under the name Blade Energy Crops, is selling what it calls "the world's first switchgrass varieties developed specifically for bioenergy production." While the varieties being sold are not transgenic, the company promises "large increases over traditional cultivars and improved vigor and establishment" (http://www.bladeenergy.com/switchgrass2.aspx).

In contrast, very little research has been carried out that investigates the potential negative impacts to natural and restored areas or to wild switchgrass populations if biofuel switchgrass cultivars are introduced on a very large scale across much of the U.S. There is a high potential for pollen-mediated gene flow, particularly if ploidy levels of cultivars and native biotypes match (Chapter 1), which could introduce novel traits into wild populations, diluting the native gene pool and possibly increasing the tendency towards invasiveness. There is also the potential for seed-mediated gene flow in places where biofuel crops are planted near natural areas, which could cause biofuel plants to escape cultivation and possibly become weedy or invasive themselves. In addition, switchgrass cultivars can cross with related grass species, at least in a controlled greenhouse setting (C. Auer, pers. comm.). Interspecific gene flow could introduce crop alleles, including genetically modified genes (transgenes), to other grass species.

Whether crop traits persist in wild populations depends on the survival and fecundity of hybrids and the fitness effects of the particular genes in question (Shivrain et al., 2009). For example, Pellegrino et al. (2009) determined that hybrids between two rare orchid species have such low fitness they pose no threat to gene swamping or introgression. Conversely, Johnston et al. (2003) investigated fitness characteristics of two iris species and their hybrid offspring using different light and water scenarios, and concluded that the hybrid plant had high relative fitness compared to the parent types. Concern arises in cases where non-neutral crop traits could alter the fitness of the wild relative, perhaps leading to increased weediness or local extinction of the original wild type. Snow et al. (2010) found that crop alleles persisted in wild radish populations after ten years, a concern if cultivated radish were genetically modified to enhance its fitness (i.e., insertion of a transgene for insect resistance). Hegde et al. (2006) determined that introgression and increased fitness in hybrid California radish has led to local extinctions of the original progenitors.

Added to these concerns is the fact that transgenes can enter the wild gene pool and persist over generations, possibly conferring a fitness benefit to hybrid offspring. For instance, Yang et al. (2011) compared fitness-related traits of crop-wild hybrid rice plants over multiple generations that were either positive or negative for two (*CpT1* and *Bt*) transgenes. They crossed transgenic rice plants with weedy relatives, producing second- and third-generation progeny. Yang et al. discovered that, under heavy insect pressure, F_3 hybrid transgenic plants had 79% less insect damage and 47% more seeds than F_3 non-transgenic individuals. Snow et al. (2003) used male-sterile wild sunflower genotypes to create BC_1 transgenic and non-transgenic progeny, in order to determine whether the *Bt* transgene would reduce herbivory or increase fecundity in wild plants. Snow et al. found that transgenic plants had a fitness advantage in a field experiment, producing more seeds per plant than non-transgenic control plants. Laughlin et al. (2009) compared the fecundity of wild squash (*Cucurbita pepo*) with virus-resistance transgenic BC_2 and BC_3 hybrids in a field experiment. Under virus pressure, second- and third-generation transgenic hybrids had higher fecundity than non-transgenic plants. Laughlin et al. (2009) also discovered there was no apparent fitness cost of the transgene when virus pressure was absent. Godfree et al. (2004) used field data and a community-based model to estimate the likelihood that transgenic clover, *Trifolium subterraneum*, would become established in a pasture community if it escaped cultivation. The authors compared growth characteristics of transgenic clover and a non-transgenic cultivar of the same species and concluded that the transgenic plant would likely decline over time in a perennial pasture community, but could persist in a grazed, annual pasture, given optimal climatic conditions.

Measuring individual fitness allows us to estimate the potential for successful establishment of a particular genotype. The more viable offspring an individual can produce, the greater the chance of parental alleles remaining in the population gene pool. Fitness estimates can therefore be used to determine the likelihood that hybrid plants will keep pace with, or even outperform, their progenitors in the long run. For instance, Mercer et al. (2007) compared the fitness of wild *Helianthus annuus* with three crop/wild hybrid genotypes, two of which carried an herbicide-resistant transgene. They discovered that the fitness of hybrid genotypes increased relative to the wild type when exposed separately to herbicide treatments and competition with wheat. Campbell et al. (2008) compared reproductive and lifetime fitness of *Ipomopsis aggregata* and *I. tenuituba* with F_1 hybrids, F_2 hybrids, and backcrossed genotypes at different field sites. The authors were expecting lower fitness in hybrids due to genetic incompatibilities. They found that both maternal effects and environment influenced fitness, but second-generation hybrid plants had higher than expected fitness, with a mean number of seeds produced that was similar to the number produced by parents and F_1 plants.

Given the potential for hybridization (Chapter 1), gene flow between wild switchgrass populations and cultivated plants may provide an avenue for crop traits to enter the local gene pool. This is particularly troublesome because wild switchgrass populations tend to be fragmented or isolated due to the historical land-use changes of natural prairies. Severe declines in genetic diversity due to gene swamping and hybridization may cause local extinctions (Levin et al., 1996). Added to this is the possibility that switchgrass cultivars that express competitively superior traits may escape cultivation and become established in natural areas, which could also cause localized extinctions of wild relatives. To date, there are no published reports that compare fitness-related traits among cultivated and wild switchgrass biotypes. Measuring reproductive traits that could potentially enhance fitness (e.g., number of inflorescences, seed/floret production) of native and cultivated switchgrass plants will provide insight into whether introgressed genes should be of concern. In addition, measuring certain vegetative traits, such as plant height and shoot number, can shed light on the relative competitive ability of the different switchgrass types. Here, I compared fitness-related traits of individuals from three wild Ohio switchgrass populations and seven cultivars (including three advanced strains) to gain insights about the possible spread and persistence of the cultivars. I hypothesized that there would be significant differences in measured traits between some or all of the switchgrass cultivars and wild populations. Wild populations are expected to be similar to one another.

METHODS

Cultivars bred for forage and biomass production may have been selected for traits that enhance vegetative yield and competitive ability, such as greater height and faster shoot production. Some cultivars may also produce more seeds than wild plants, although greater seed yield has not been a goal of breeders. To compare the growth and fecundity of wild and cultivated types in the absence of competition, I established two adjacent, common gardens at Waterman Farm in 2008 and 2009 (Table 2.1). Prior to planting, the field was tilled. In 2008, I planted individuals from one Ohio native switchgrass population (OSU Marion Restored Prairie) and four cultivars (seeds were obtained from Dr. Kenneth Vogel, switchgrass breeder and geneticist, USDA, Nebraska). I used a randomized complete block design with one plant of each biotype in each of 16 blocks and 2 m between rows and columns in the grid. I planted young seedlings (3 weeks after emergence) directly into the soil at the plot in late June. In 2009, I established a larger common garden experiment adjacent to the 2008 cohort with three different wild populations from north central Ohio and seven cultivars, including three advanced strains (Table 2.1). The randomized complete block design included 10 individuals in each of 20 blocks (one individual per biotype), for N = 20. The 3-week-old seedlings were planted in early June, again with 2 m between rows and columns in the grid. Ten days after planting, I measured seedlings' height for potential covariate analysis, should there be a correlation between initial and final plant height.

In both years, newly planted seedlings that died were replaced with cohorts within 5 days of the initial planting. After that time, any seedlings that died were not replaced.

Newly planted seedlings were watered twice a week for eight weeks, to aid in establishment. In 2008 and 2009, plants were hand-weeded around the base, and aisles were sprayed with glyphosate as needed to eliminate all weeds. In 2010, the 2009 cohort was hand-weeded around each base, but the 2008 cohort was not hand-weeded, as those plants had become large enough that small ground weeds around the base of each was deemed to have no effect on growth. Also, in 2010, a few switchgrass volunteers were found growing in the aisles, so 2,4-D herbicide was used in both sites to control dicot weeds. For monocot weed control, a combined treatment of spot-spraying with glyphosate and mowing between rows and columns was used, in order not to kill the volunteers. In 2011, the volunteer population grew substantially, so that only 2,4-D was used in aisles (no glyphosate was used).

Plant survival from establishment year to the second year was high in both cohorts (95% for cohort 1; 99% for cohort 2). In both cohorts, a few plants appeared stunted at the end of their first year. Also, one individual in each cohort had morphological characteristics of a biotype that was different than what was expected, which indicates the wrong type was planted. Finally, when inflorescences were collected in autumn of 2010, a few individuals had already begun to shed seeds and they had no inflorescences with all florets intact. These anomalies were eliminated from analysis. Overwintering survival was close to 100% for both cohorts. The final sample size for each biotype is listed in Table 2.1.

I measured the following characteristics for both cohorts:

- **Plant height:** In 2009, I measured the height of cohort 1, and in 2010, I measured the height of both cohorts. Height was determined by measuring the tallest reproductive shoot from its base to the top of the inflorescence.
- **Number of reproductive and vegetative shoots:** In 2009, all shoots were counted for cohort 1; in 2010, these plants were too large to make complete counts feasible, so plants of both cohorts were quartered and ¼ of reproductive and vegetative shoots were counted, and the total shoot numbers were estimated per plant.
- **Number of florets:** Three representative mature inflorescences were collected from each individual at the end of the growing season. In 2009, I collected panicles from cohort 1, and in 2010, I collected panicles from both cohorts. Florets were initially used as a proxy for seed number because of concerns that plants might be pollen-limited. In 2009, florets for cohort 1 were counted and total number of florets per plant was extrapolated. In 2010, because of the increased sample size, floret weight was used to estimate floret number. Florets were stripped from the three inflorescences of each individual, combined into one group and weighed. Three plants from each biotype were randomly selected, and 50 florets were counted and weighed. This was repeated five times to get an average weight for 50 florets per plant. The 15 weights (3 plants x 5 repetitions) were averaged to obtain a mean weight for 50 florets per biotype. A correlation test confirmed that floret weight and number were highly correlated ($P < 0.0001$; $r^2 = 0.975$ for 4x seeds and $P < 0.0001$; $r^2 = 0.980$ for 8x seeds.

Numbers of florets per plant were estimated based on the weight per floret.

- **Number of Normal Seeds:** Fifty florets from each of 3 plants per biotype were also dissected to determine the proportion of florets that had normal seeds, based on color and hardness. Germinable switchgrass seeds have light tan colored seed coats, are rounded in shape, and resist being flattened when pressed against a hard surface. Similar criteria have been used in other studies (e.g., Hesse et al. 2007).

Statistical Analyses

The two common gardens were analyzed separately. A general linear model analysis of variance (SAS v9.2, SAS Institute) was used to test the significance of biotype and block on the following parameters:

Vegetative Traits:
Height
Vegetative shoot number
Total shoot number

Reproductive Traits:
Flowering shoot number
Floret number
Seed number

For each parameter, the ANOVA used in SAS was:

Source	df	Error MS
Biotype	9	Biotype*Block
Block	19	Biotype*Block
Biotype*Block	167	--

Since only one individual was represented in each block, there was no error mean square term for the biotype or the block. Therefore, I used the Biotype*Block interaction term as the error mean square to calculate *F* values for biotype and block (I didn't test for a biotype x block interaction because I did not have multiple entries). Tukey's HSD test was used to separate means, based on the ANOVAs (SAS v9.2).

RESULTS

The two common gardens produced very similar results. Therefore, only the results for cohort 2 will be reported in this section. Results for cohort 1 are reported in the Appendix (Figures A.1-A.4; Table A.1). Regression analysis showed that the effect of initial plant height on final height was not significant ($P = 0.9505$). Biotype and block effects were significant for all parameters (Table 2.2). For all traits, differences among the three native populations were not statistically significant, based on Tukey (Figures 2.1-2.5).

Reproductive Traits

Kanlow and Advanced Kanlow produced more than three times the numbers of florets per plant, on average, as all other biotypes, and four times as many florets as the native biotypes (Table 2.3, Figure 2.1). Kanlow, along with Summer, also produced twice the number of seeds per plant as native biotypes, even though seed set was generally low in the Kanlow types (Table 2.4). The rest of the cultivars, including Advanced Kanlow, produced a similar number of seeds, compared to the natives (Table 2.3, Figure 2.2).

Advanced Octaploid 2 had significantly more reproductive shoots than native biotypes (Figure 2.3). Because all the biotypes produced so few vegetative shoots, differences in total shoot production were very similar to differences in reproductive shoot number. Tukey's HSD test showed that significant differences in total shoot number among biotypes were the same as those of flowering shoots (data not shown).

Vegetative Traits

Kanlow and Advanced Kanlow (Kanlow types) were significantly taller than all other biotypes, and the three Ohio native types were significantly shorter than all others, except Summer (Figure 2.4). Kanlow and advanced Kanlow were 77% taller and 59% taller, respectively, than the combined average of the three native populations (Table 2.3).

Very few vegetative shoots were produced across all biotypes, compared to flowering shoots. Also, several individual plants produced no vegetative shoots, which caused the variation within biotypes to be relatively high. However, there were a few biotypes for which vegetative shoot production was significant: Advanced Octaploid 2 produced more shoots, on average, than three of the other cultivars (Figure 2.5).

DISCUSSION

Some cultivars, like Kanlow, Advanced Kanlow, and Summer, have the potential to displace native switchgrass populations if they escape cultivation, due to higher fitness, while others, like the upland octaploids, are of less concern. Given the right conditions, cultivars with higher fitness could also displace other grassland species, or they could establish populations in disturbed areas such as roadsides, which would facilitate future hybridization events. Although this study was limited to a single site with two years of data in a non-competitive environment, the results highlight the need for more research into fitness-related traits of cultivars *vs.* wild populations. Research is currently underway at Ohio State University that compares fitness-related traits of certain cultivated and wild switchgrass biotypes that are in competition with other grass species (D. Palik, pers. comm.).

Reproductive Traits

Kanlow, Advanced Kanlow, and Summer all had relatively higher fecundity than the native biotypes. Cultivars with higher fitness could out-compete wild plants, eventually displacing native genotypes and causing declines in local population size, either through hybridization with wild relatives or through escape and establishment as feral populations.

Kanlow and Advanced Kanlow plants could have been pollen-limited due to later flowering times than the other biotypes (Chapter 1), but Kanlow still produced more than twice the number of seeds per plant, on average, as native plants. Given the variation in the timing of flowering within a biotype (Chapter 1), large swards of Kanlow plants are likely to overlap in flowering with wild relatives to a greater extent than what was seen in the common garden experiment, increasing the opportunity for hybridization. Summer, which was similar to wild plants in height, numbers of shoots, and numbers of florets, produced many more seeds than the wild plants. It should be noted that the sample size used to estimate seed numbers was small, and an increased sample size could make the results more meaningful, but Summer produces very full, compact seed heads (A. Stottlemyer, pers. obs.), so these results are not surprising. Thus, several cultivars have the potential to negatively impact wild populations through pollen swamping and gene flow because introgressed genes could alter the fitness of wild plants. Novel genes, like transgenes, could introduce traits that promote greater biomass production or improved drought tolerance, giving hybrid offspring an advantage over truly wild plants. Additionally, a cultivar like Kanlow could rapidly become feral and outcompete native plants, given a similar competitive ability.

Vegetative Traits

Kanlow and Advanced Kanlow cultivars were much taller than all other plants in the common garden. The other cultivars, except for Summer, were also somewhat taller than native plants. Height could potentially increase the distance with which seeds are dispersed, thus increasing the possibility of escape and the establishment of feral populations. Taller plants could also limit native seed dispersal. Davies and Sheley (2007) tested the effect of neighboring plant height on the ability of two other species to disperse seeds, and discovered that, as neighboring plant height increased, seed dispersal of both species decreased. Increased height could also give a competitive advantage, as taller plants could outcompete shorter individuals for light. This could provide a selective advantage to taller plants, depending on the impact of shading on reproductive output in switchgrass.

Vegetative shoot production was negligible for all biotypes. The lack of vegetative shoots could have been a result of the lack of competition in the common garden. The environment was very suitable for individual plants to achieve maximum growth and reproductive potential. Low competition for resources might have caused plants to expend more energy on sexual reproduction. One Illinois switchgrass population I observed varied across two years in the number of reproductive and vegetative shoots produced; in the second year, the population produced mostly vegetative shoots and very few inflorescences (A. Stottlemyer, pers. obs.), although the reason for this is not known.

Fitness-Related Traits: Implications

Switchgrass cultivars with high fitness may pose a risk to natural areas and recently disturbed sites. Pollen-mediated gene flow could confer unwanted traits like increased yield or drought tolerance on native switchgrass genotypes or related species, which may increase the population’s tendency toward invasiveness. Seed-mediated gene flow could introduce novel genotypes into wild populations or into new areas, establishing feral populations that could displace native plants. In this study, Kanlow, Advanced Kanlow, and Summer all had increased fecundity relative to native populations. These results emphasize the importance of switchgrass fitness studies, in order to better understand and predict possible future scenarios if switchgrass is planted on a large scale.

Floret production is an indirect measure of male fitness. Kanlow and Advanced Kanlow plants produced about 4 times the numbers of florets as native genotypes, and about 3 times as many as the other cultivars. Although flowering times did not overlap as strongly between these groups, the amount of pollen transfer from the Kanlow types could effectively swamp wild populations, more so if Kanlow is in wide-spread use as a cultivar. Large amounts of pollen could negatively impact related species, too. In a review paper about interspecific pollen transfer, Morales and Traveset (2008) explain that the effects of 'heterospecific pollen deposition" (HPD) vary, but there are documented cases of conspecific pollen loss as a direct result of HPD. Although the paper reviews cases involving animal pollinators, wind pollination could potentially produce a similar effect.

The large numbers of switchgrass seeds produced per plant increases the chance of gene flow *via* seed dispersal, particularly if biofuel switchgrass is planted across a wide swath of the Midwest and central U.S. Natural areas such as recently restored prairies and native remnant sites, as well as disturbed sites like roadside ditches, will be susceptible to the establishment and persistence of feral switchgrass populations, although areas with established plant communities may be more resistant to invasion. Since switchgrass seeds are not important for ethanol conversion, a possible scenario is seed spillage during biomass transfer. Switchgrass plants were found growing alongside highway ramps in Grundy County, Illinois (A. Stottlemyer, pers. obs.). Once plants are established from seed, further gene flow can occur if feral populations have overlapping flowering with indigenous plants (Chapter 1).

Although no hybrid switchgrass offspring were used here, this study can provide a baseline from which to compare results of future hybrid studies. Research that includes hybrids is especially important because hybrid performance is not easily predicted (e.g., Hegde et al. 2006 *vs.* Pellegrino et al. 2009). Added to the uncertainty surrounding hybrids is the fact that switchgrass is a candidate for genetic modification. Researchers want to insert certain desired traits such as increased yield and drought tolerance into the switchgrass genome. Greater biomass and better establishment could increase the likelihood that cultivars will become feral and persist in natural areas or become invasive. Controlled hybridization experiments between switchgrass and several closely related species are currently underway (C. Auer, pers. comm.), and this will provide insight into the likelihood that transgenes used in switchgrass will introgress into other grass species.

The plants used in this study were grown in a controlled, non-competitive environment as a means for obtaining much-needed information that compares some of the reproductive and growth strategies of cultivated and wild switchgrass biotypes. Future research on the ecology of switchgrass that includes responses to competition or other environmental stresses will be very useful. It would also be informative to measure fitness-related traits in switchgrass from native populations that occur across a wide geographic range, since biofuels are expected to be grown across several U.S. regions.

TABLES AND FIGURES

	Number of Plants			
Biotype	**2008**	**2009**	**Origin**	**H.Z.**
Shawnee	11	19	IL	6
Summer	14	20	NE	5
Trailblazer	12	20	Syn. EY & FF	5,6
Kanlow	13	20	OK & KS	6,7
Advanced Kanlow[1]	--	20	OK & KS	6,7
Advanced Octaploid 1[1]	--	19	Synthetic	5
Advanced Octaploid 2[1]	--	20	Syn. EY & FF	5,6
Local Native Restored[2]	13	--	OSU Marion	6
Ohio Native 1[2]	--	20	Green Camp RR	6
Ohio Native 2[2]	--	20	Daughmer BOS	6
Ohio Native 3[2]	--	19	Claridon RR	6

Biotype	Ploidy	Ecotype[3]	Use
Shawnee	8x	U	forage
Summer	4x	U	forage
Trailblazer	8x	U	forage
Kanlow	4x	L	forage
Advanced Kanlow	4x	L	yield/ winter survival
Advanced Octaploid 1	8x	U	yield/ ↓ lignin
Advanced Octaploid 2	8x	U	↓ lignin
Local Native Restored	4x	U	restoration
Ohio Native 1	4x	U	n/a
Ohio Native 2	4x	U	n/a
Ohio Native 3	4x	U	n/a

[1] Seeds of experimental strains were obtained from Dr. Kenneth Vogel, USDA-ARS, University of Nebraska.

[2] I determined the ploidy level (N = 10-20) for Ohio native biotypes using flow cytometry and the ecotype (N = 5) by examining rhizome morphology. See Chapters 1 & 3 for methods.

[3] L = lowland ecotype; U = upland ecotype

Table 2.1. Characteristics of cultivars and wild populations planted in a common garden in Columbus, Ohio.

ANOVA Results **Source:**	**df**	**Type III SS**	**Mean Square**	**F value**	**P value**
Plant Height					
Biotype	9	225975.35	25108.37	89.80	<0.0001
Block	19	9003.92	473.89	1.69	0.0413
Total Shoots					
Biotype	9	374218.74	41579.86	9.87	<0.0001
Block	19	280247.50	14749.87	3.50	<0.0001
Flowering Shoots					
Biotype	9	323596.87	35955.21	9.61	<0.0001
Block	19	245381.43	12914.81	3.45	<0.0001
Vegetative Shoots					
Biotype	9	2047.54	227.50	2.88	0.0034
Block	19	3260.73	171.62	2.18	0.0048
Floret Number					
Biotype	9	2.43×10^{12}	2.71×10^{11}	17.20	<0.0001
Block	19	6.54×10^{11}	3.44×10^{10}	2.19	0.0047
Normal Seed Number					
Biotype	9	1.39×10^{11}	1.55×10^{10}	6.02	<0.0001
Block	19	1.29×10^{11}	6.82×10^{9}	2.64	0.0005

Table 2.2. ANOVA table showing the effect of biotype and block on several fitness-related traits of cohort 2 measured in 2010 after two seasons of growth. N = 17-20.

Trait	General Results	Magnitude (relative to natives)
Height		
	Kanlow was much taller	**by 77%**
	Adv. Kanlow was much taller	**by 59%**
	4 octaploids were somewhat taller	**by 18-25%**
	Summer not different	---
Number of Flowering Shoots		
	Both Kanlow types not different	---
	4 octaploids similar in general	---
	Except Adv Octaploid 2 had more shoots	**by 62%**
Number of Florets (affects male fitness)	**Kanlow had more florets**	**by 338%**
	Adv. Kanlow had more florets	**by 327%**
	All others similar	---
Number of Normal Seeds (might be pollen-limited)	**Kanlow had more seeds**	**by 136%**
	Summer had more seeds	**by 134%**
	Others had few significant differences	---
	Adv. Kanlow not different	---

Table 2.3. Summary of cultivar performance in the common garden experiment relative to native populations. Based on significant differences ($P < 0.05$) shown in Figures 2.1-2.5. Note: The three native populations were not significantly different from each other in any of these traits. See Table 2.1 for cultivar and native seed sources.

Biotype	**Proportion of Normal Seeds (of 50)**	**s.e.**
4x		
Ohio Native 1	0.94	2.52
Ohio Native 2	0.93	1.76
Ohio Native 3	0.25	6.74
Summer	0.90	1.53
Kanlow	0.32	5.03
Advanced Kanlow	0.22	4.93
8x		
Trailblazer	0.72	4.58
Shawnee	0.75	2.67
Adv Octaploid 1	0.66	5.69
Adv Octaploid 2	0.75	3.18

Table 2.4. Proportion of 50 florets that contained normal seeds for 10 biotypes grown in a common garden in Columbus, Ohio in 2010 after two seasons of growth. N = 3 plants per biotype.

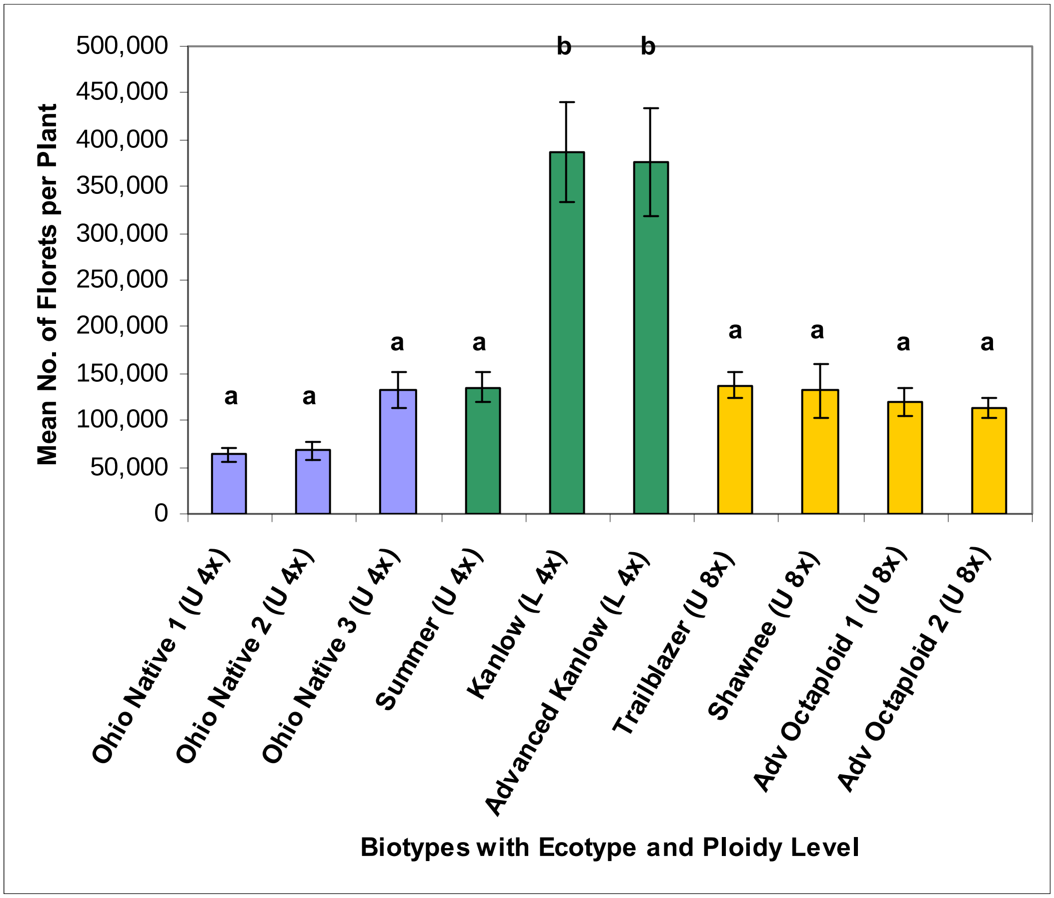


Figure 2.1. Estimated number of florets produced per plant for 3 Ohio native biotypes and 7 cultivars, including 3 advanced strains. Means ± 1 SE. Data were collected in 2010 after two seasons of growth (N = 17-20). Letters indicate significant differences in floret production ($P < 0.05$).

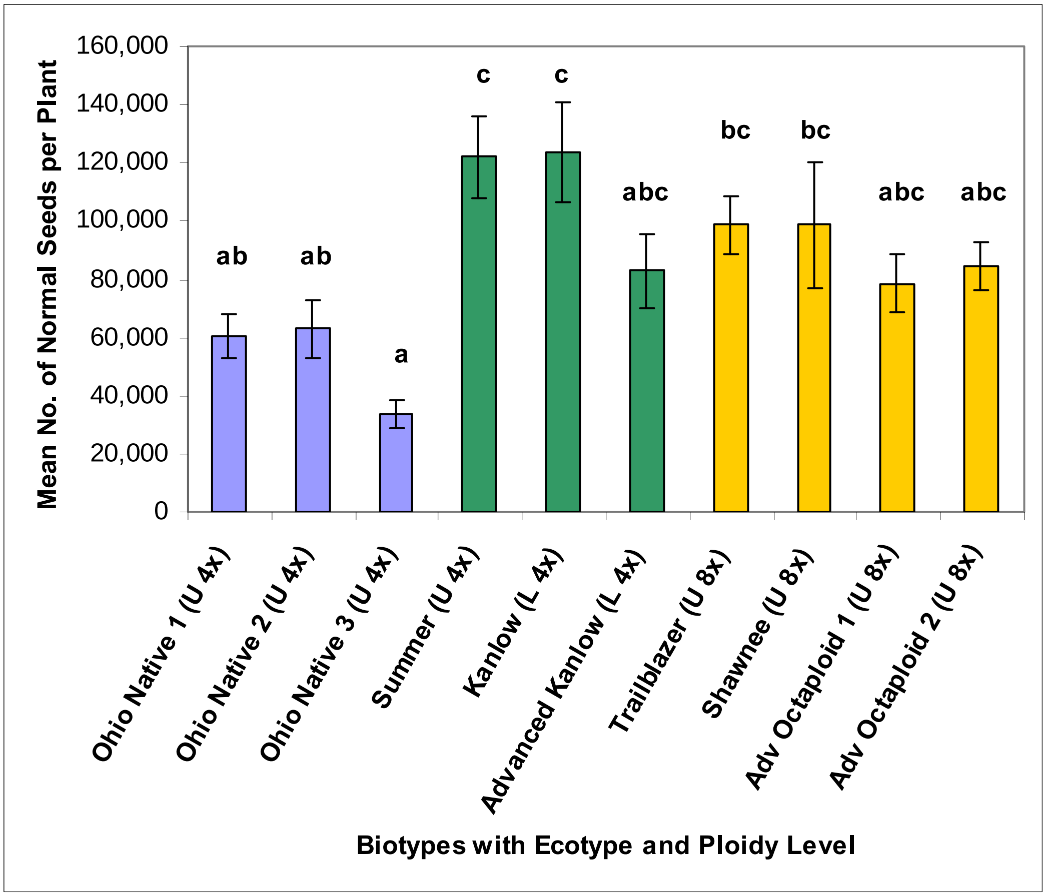


Figure 2.2. Estimated number of normal seeds produced per plant for 3 Ohio native biotypes and 7 cultivars, including 3 advanced strains. Means ± 1 SE. Data were collected in 2010 after two seasons of growth (N = 17-20). Letters indicate significant differences in floret production (*P* < 0.05).

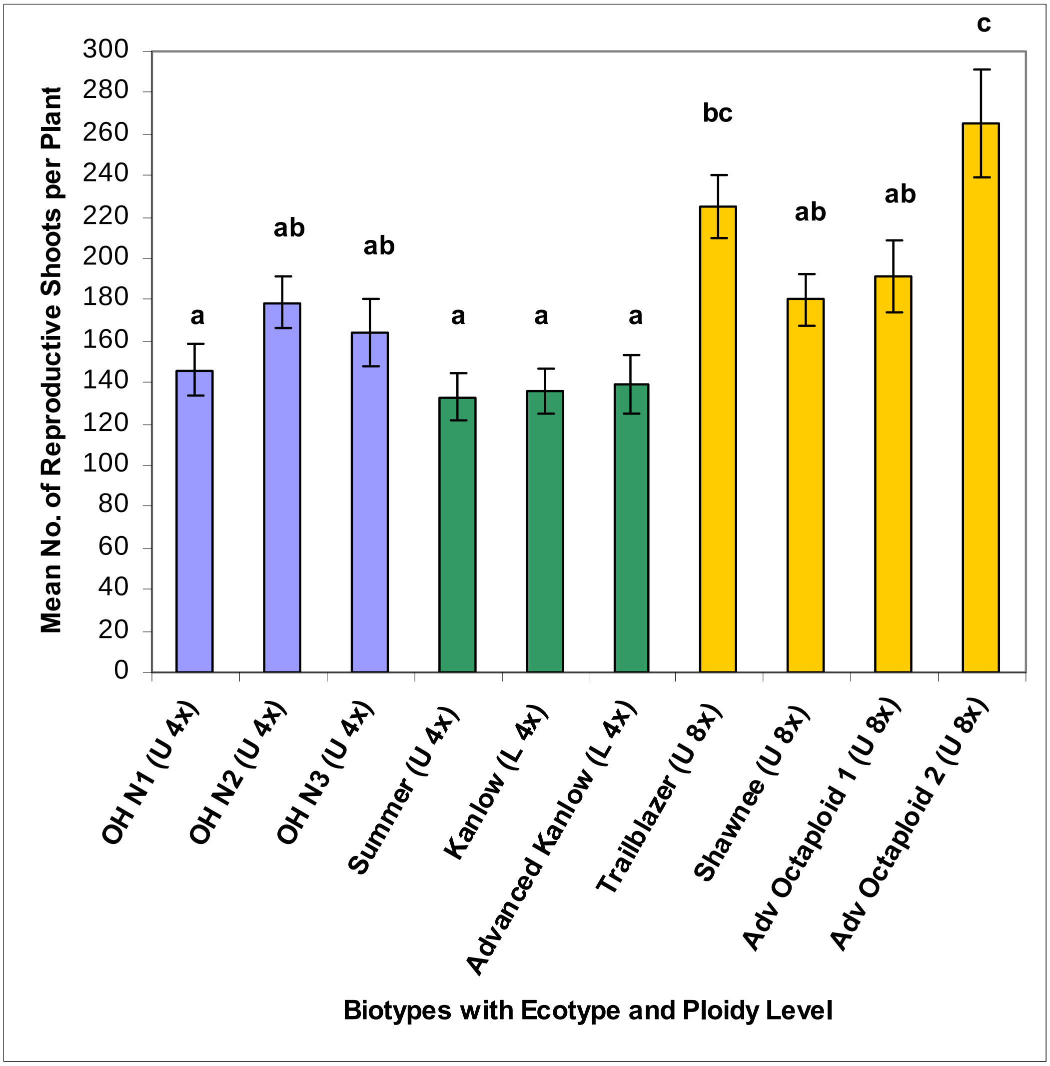


Figure 2.3. Mean number of reproductive shoots for three Ohio native switchgrass populations and seven cultivars, including three advanced strains. Plants were measured in 2010 after two seasons of growth (N = 19-20). U = Upland ecotype, L = Lowland ecotype, 4x = tetraploid, 8x = octaploid. Means ± 1 SE. Letters indicate significant differences among biotypes (*P* < 0.05).

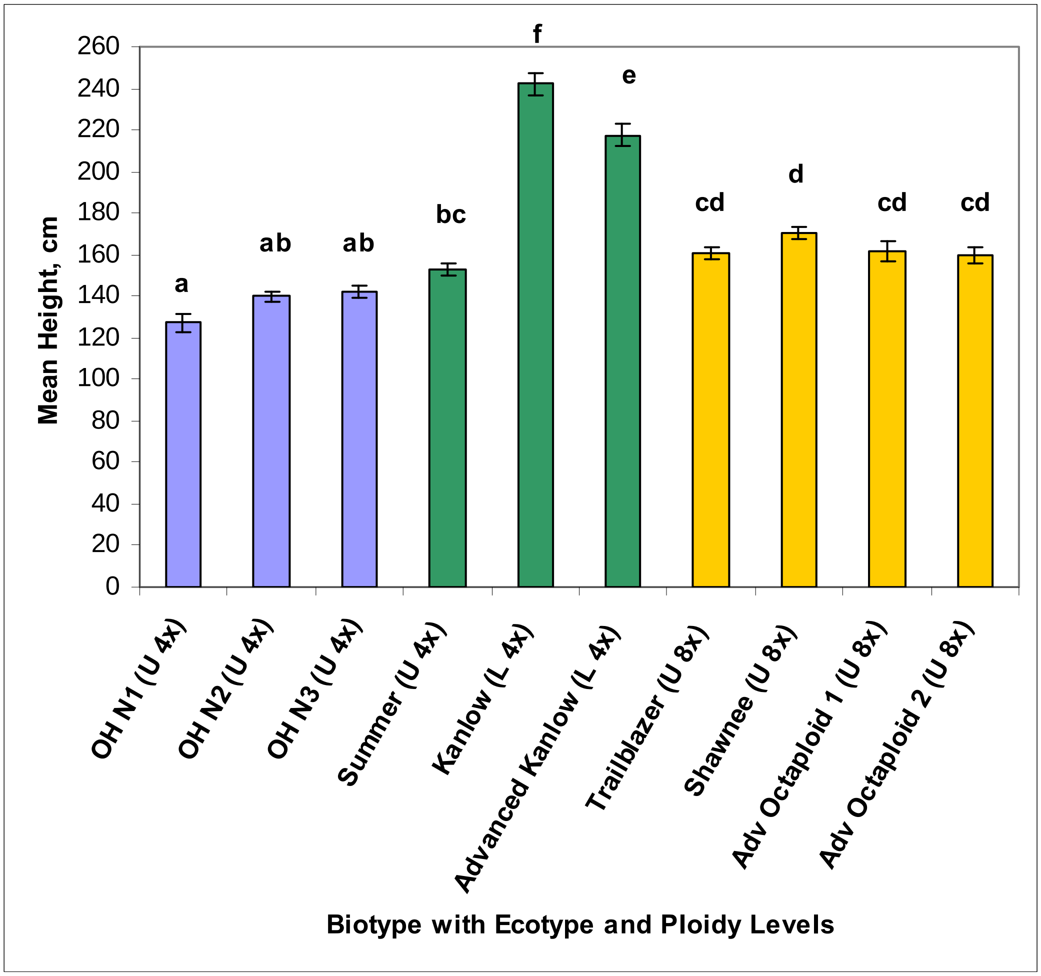


Figure 2.4. Height of three Ohio native switchgrass populations and seven cultivars, including three advanced strains, measured in 2010 after two seasons of growth in a common garden (N = 19-20). U = Upland ecotype, L = Lowland ecotype, 4x = tetraploid, 8x = octaploid. Means ± 1 SE. Letters indicate significant differences among biotypes ($P < 0.05$).

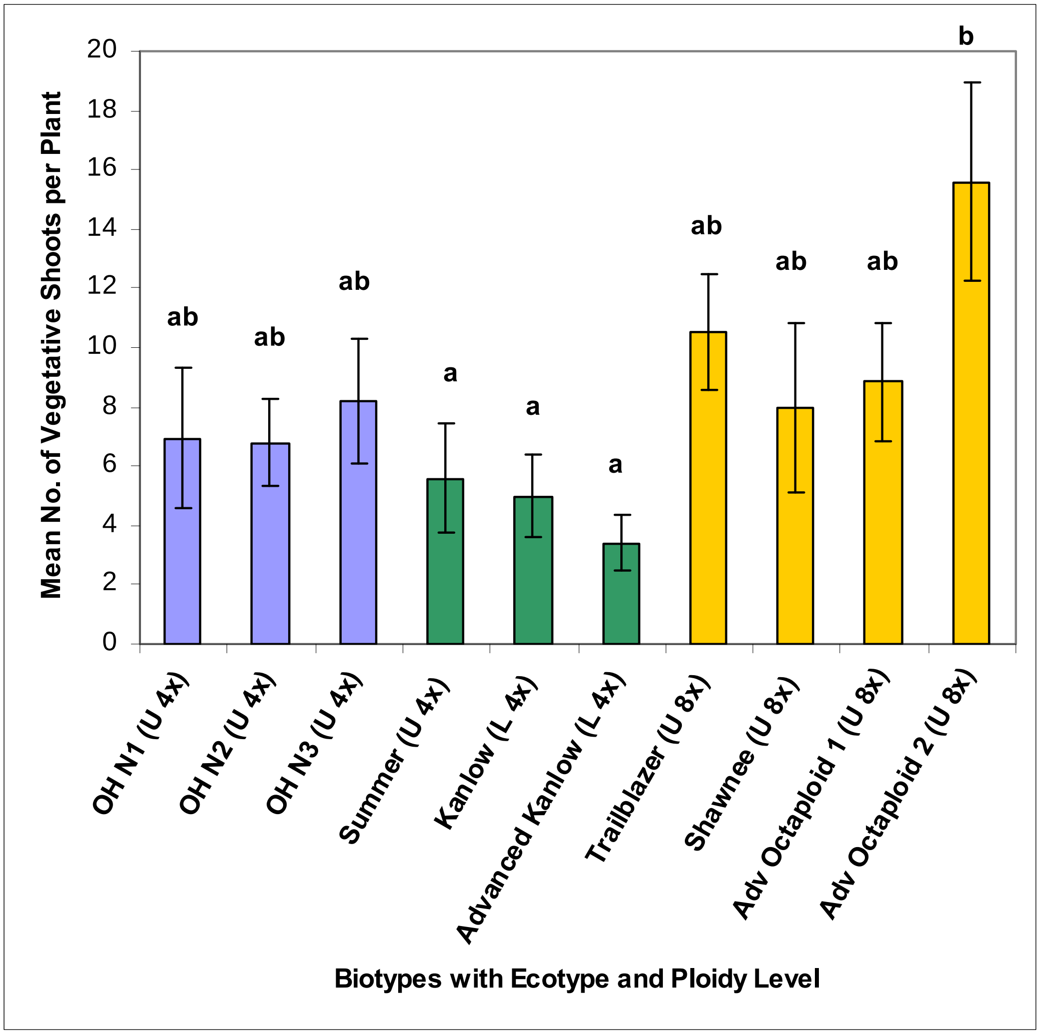


Figure 2.5. Mean number of vegetative shoots for three Ohio native switchgrass populations and seven cultivars, including three advanced strains. Plants were measured in 2010 after two seasons of growth (N = 19-20). U = Upland ecotype, L = Lowland ecotype, 4x = tetraploid, 8x = octaploid. Means ± 1 SE. Letters indicate significant differences among biotypes ($P < 0.05$).

CHAPTER 3

SWITCHGRASS VOLUNTEERS (*PANICUM VIRGATUM* L.) IN A COMMON GARDEN OF MIXED SWITCHGRASS VARIETIES: ASSESSING MORPHOLOGY, PLOIDY, FLOWERING PHENOLOGY, AND RELATIVE ABUNDANCE.

ABSTRACT

When crops and wild relatives co-occur, they frequently interact, either through pollen-mediated gene flow, which can lead to hybridization, or seed-mediated gene flow, which can lead to cultivar escape and ferality. Volunteer plants facilitate both hybridization and crop escape, which can be of concern if unwanted traits are conferred on native individuals or if feral populations become weedy. Switchgrass (*Panicum virgatum* L.) is an outcrossing perennial species that is native to much of North America. It is also bred for forage and, more recently, as a biofuel crop. Biofuel cultivars could be planted on a very wide scale in the near future, yet very little research has investigated the potential for volunteers from massive swards of switchgrass cultivars to establish feral populations. In 2011, I examined a volunteer switchgrass population growing in two adjacent common garden experiments that were planted in 2008 and 2009 in Columbus, Ohio, as part of a larger project comparing switchgrass cultivars with wild biotypes. These experiments included a total of 272 3- or 4-year-old plants, representing upland 4x

Ohio native biotypes, an upland 4x cultivar (Summer), four upland 8x cultivars (Shawnee, Trailblazer, Advanced octaploids 1 and 2), and two lowland 4x cultivars (Kanlow and Advanced Kanlow). I sampled 50 ¼-m^2 plots along 10 transects to document volunteer frequency and sub-sampled the plots to test ploidy level ($n = 37$) and rhizome morphology ($n = 36$), which can be used to distinguish lowland *vs*. upland ecotypes. I also selected 100 volunteers throughout the common gardens that were approaching maturity to survey flowering phenology and examine leaf color, both of which vary among cultivars. I compared these traits to those of plants in the extant groups in the common gardens. Volunteer plants were common and were more frequent in the 2008 common garden, where sample plots averaged 16 volunteers per square-meter. Ploidy level and ecotype proportions of volunteers were very similar to those of the extant population, and at least 20 of 36 volunteers examined in detail were derived from cultivars rather than native biotypes. I also found evidence for hybridization among the parent generation: 48% of the volunteers with blue-green leaves did not have other Kanlow features and two volunteers were lowland octaploids, a combination not present in the extant population. Volunteer flowering overlapped with the biotypes and was bimodal, with a first-wave of flowering that overlapped mainly with native biotypes and non-Kanlow type cultivars, and a second wave that overlapped with Kanlow and Advanced Kanlow. Taken together, these results show that many volunteers came from the cultivars rather than the native parents, as evidenced by the presence of octaploid and/or lowland ecotype individuals, those with Kanlow-type leaf color, and the late flowering of some volunteers. Evidence of pollen- and seed-mediated gene flow from cultivars to volunteer populations suggests cultivars are capable of producing hybrid offspring and are also likely to produce feral populations in suitable environmental conditions.

INTRODUCTION

Seed- and pollen-mediated gene flow frequently happen in areas where crop plants and their wild relatives co-occur. Pollen-mediated gene flow occurs naturally under the right conditions, although the evolutionary impact associated with such hybridization varies considerably across taxa (Ellstrand 1999). Seed-mediated gene flow with highly domesticated crops should not be a concern because crop plants are heavily dependent on humans for survival. Problems may arise, however, if hybridization leads to the introgression of crop alleles or if crop genotypes with increased fitness form feral populations or out-compete native plants. Volunteer plants (crop seeds that germinate in subsequent years after the initial crop is harvested) facilitate gene flow because they form a “genetic bridge” from cultivated plants to wild populations. They often facilitate a cultivar’s becoming feral, because they can remain continuously in the same fields for long periods, providing many opportunities for hybridization (Gressel, 2005). They are important to the crop-wild-hybrid complex because they increase the amount of space or time in which crops and wild relatives interact (Reagon & Snow 2006).

Ferality is defined as a domesticated species’ return to the wild by de-domestication (Gressel, 2005). This can occur fairly rapidly if escaped domesticated plants back-cross with wild relatives, because dominant, wild-type traits have a higher mutation rate than the typical recessive traits selected for agriculture (Gressel, 2005). Switchgrass cultivars are not far removed from wild relatives, and thus are not very domesticated. Nevertheless, non-local varieties with enhanced traits or transgenes could behave like a dedomesticated species if they escape cultivation. Snow et al. (2010)

carried out a multi-year study to investigate the long-term persistence of crop alleles in hybrid radish (*Raphanus raphanistrum* x *Raphanus sativus*) populations and discovered that the initial lower fitness of hybrids was overcome quickly with the evolution of wild-type pollen fertility. After 10 years, crop alleles persisted in the hybrids, and hybrid fitness was similar to that of wild plants. This has implications for transgenic cultivated plants, because they introduce novel genes that have never before been present in wild counterparts. Transgenes, which confer dominant traits, may persist in wild populations and provide a selective advantage to hybrid offspring, increasing the possibility that feral populations will become invasive.

Once cultivar plants become established outside cultivation, they can evolve to become weedy or invasive (Ellstrand et al. 2010). Here, weedy plants are those that cause direct environmental or economic harm, or plants that occupy disturbed or ruderal habitats, while invasive plants displace native vegetation (Snow and Campbell, 2005). For example, a variety of barnyard grass (*Echinochloa crus-galli* var. *oryzicola* (L.) P. Beauv.) has evolved to mimic domesticated rice (*Oryza sativa* L.) as a result of unintentional artificial selection by farmers and has become a noxious weed (*in* Ellstrand et al. 2010). Dlugosch and Parker (2008) studied variation and evolution in isolated populations of an introduced invasive ornamental shrub *Hypericum canariense* in California, compared to the native progenitors from the Canary Islands. They found that the introduced populations had lost about half the genetic variation of the original population, probably due to a founder effect. Nevertheless, the founding populations experienced rapid evolution of certain life history traits, like growth rate and date of first flowering. Dlugosch and Parker (2008) concluded that even populations that have experienced a genetic bottleneck and have lost a great deal of genetic diversity can still evolve to become invasive.

Pollen-mediated gene flow can lead to the introgression of crop alleles, if conditions are favorable. Unwanted traits may be incorporated into native populations, diluting the wild gene pool and conferring either increased or reduced fitness on hybrid offspring. Transgenes, in particular, are of concern because they provide traits such as insect resistance or herbicide tolerance that may confer a fitness or survival advantage (e.g., Knispel et al. 2008; Yang et al. 2011), which in turn could increase weediness or invasiveness. Transgene escape into wild populations has already been documented for some crops. Herbicide-resistant (HR) canola plants escaped cultivation in western Canada, resulting in widespread populations of HR canola volunteers throughout the region (Knispel et al. 2008). Corn volunteer plants with stacked glyphosate & insect-resistant traits have become a problem in rotational cropping systems across North America. Volunteer corn germinates in soybean fields, causing western corn rootworm larvae to persist between corn crop rotations (Krupke et al. 2009). In a noted case in Oregon, researchers discovered that the glyphosate-resistant transgene inserted in creeping bentgrass had escaped *via* pollen-mediated gene flow (Watrud et al. 2004), and pollen- and seed-mediated gene flow into non-agronomic areas several kilometers away (Reichman et al. 2006).

Seed-mediated gene flow may also be a concern if escaped crop genotypes with increased fitness become established and form feral populations or out-compete native plants. Two such examples are kudzu (*Pueraria Montana* Var. *lobata*), widely used for erosion control, and johnsongrass (*Sorghum halepense*), introduced as a forage crop.

Kudzu is a major pest in the southeast, and johnsongrass is listed as a noxious weed in 19 states (*in* Barney and DiTomaso 2008).

Volunteer plants typically emerge either in crop fields or along field edges, which decreases the distance that pollen or seeds must travel to reach wild populations. They can also increase exposure time through longer periods of overlapping flowering. However, there are not many documented accounts of volunteers acting as a conduit for gene flow from crops to true wild populations. In the case of second-generation biofuel crops like cultivated switchgrass, which may be on the verge of being mass-planted across much of the United States, it is increasingly important to investigate the potential for volunteers to facilitate gene flow. This is especially true if transgenes are used to improve switchgrass as a biofuel crop, but also because conventional breeding could allow cultivars to become taller and more vigorous. In places where ploidy levels match and there is overlap in flowering phenology, cultivated switchgrass, through the conduit of volunteer plants, could hybridize with native switchgrass genotypes and form feral populations.

Switchgrass is native to most of North America and is also cultivated for forage, and more recently, as a potential biofuel. Switchgrass plants occur mainly as either tetraploids or octaploids, and the two ploidy types do not successfully cross due to a post-zygotic mating barrier (Martinez-Reyna & Vogel 2002). Switchgrass also has two main phenotypes that correspond to specific ecotypes, expressed in traits such as height, rhizome morphology, and response to photoperiodism. Hultquist et al. (1996) tested several cultivars and discovered a chloroplast DNA restriction site that is present in lowland plants and absent in upland plants. Upland ecotypes generally occur at higher latitudes and higher on the landscape, while lowland types are usually found at lower latitudes and in wetter areas, but the two ecotypes can hybridize (Martinez-Reyna & Vogel 1998). Past research on ploidy level and ecotype has focused mainly on cultivated varieties; very few studies have documented either ploidy level or cytotype proportions of wild populations (but see Hopkins et al. 1996, and Hultquist et al. 1997), and only recently has anyone compared cytotype, phenotype, and ploidy levels of wild accessions (Zhang et al. 2011). To date, there have been no published reports about whether switchgrass cultivars produce volunteers in an agricultural setting.

To investigate volunteer recruitment from wild and cultivated switchgrass, I carried out surveys of common garden plots that had been established for other purposes (see Chapters 1 and 2). Two common gardens were started in 2008 and 2009 with cultivars and native Ohio switchgrass individuals that were made up of a mixture of ploidy type and ecotype. In 2010, a few volunteers were identified growing in the aisles between mature plants, and by 2011, the volunteer population had grown substantially. My goals were to investigate how closely volunteers resemble common garden biotypes and to examine the frequency with which volunteers occur. I examined ploidy level and ecotype, described certain morphological characteristics, and recorded flowering times. Morphology can provide some insight into which biotypes are producing successful offspring. Ploidy level and ecotype can shed light on which groups (e.g., tetraploid *vs*. octaploid, upland *vs*. lowland) may be contributing most to the volunteer population. I also estimated volunteer plant density. This work can provide an important baseline for future investigation into the spread of volunteers in managed areas.

METHODS

Common Garden Set-Up

In June of 2008, 16 individuals from each of 5 switchgrass biotypes (4 traditional forage cultivars and 1 Ohio native type) were planted in a common garden at the Ohio State University Agricultural Research Station in Columbus, Ohio. In June of 2009, the common garden site was expanded to include 20 individuals from each of 10 biotypes: 3 Ohio native populations, 4 traditional cultivars, and 3 advanced cultivars from the USDA (hereafter referred to as “cohort 1” and “cohort 2,” respectively) (Table 3.1). Both years, three-week old seedlings were transplanted from a greenhouse to a tilled field in a randomized complete block design, with one individual from each biotype represented in each block. They were spaced 2 m apart to avoid competition and were irrigated twice a week for four weeks, until they became established. After establishment, plants were never watered. In 2008 and 2009, weeds were controlled throughout the growing season by spraying aisles with glyphosate and hand-weeding around the base of each switchgrass plant. In 2010, a few volunteers were visible in the aisles of both common gardens, so glyphosate was not used, but plants were weeded around the base and 2,4-D, a dicot-specific herbicide, was used in the aisles. Toward the end of the 2010 season, mulch was suggested as a way to suppress weeds germinating from seed, and a few aisles were mulched. However, mulching was stopped in 2011 when it became apparent that there were a large number of switchgrass volunteers populating the aisles. Thus, in the 2011 growing season, 2,4-D was the only weed control used.

Sampling Design

Volunteers were sampled in a different way for the flowering survey and the ploidy level and ecotype analyses. To investigate flowering phenology, I selected 100 volunteers that were close to maturity or were already beginning to flower throughout both common gardens. To assess ploidy levels and ecotypes, I selected 50 individuals (25 from each common garden) at random, to compare their proportions with the existing common garden ploidy level and ecotype proportions. The design is outlined in Table 3.2 and described in detail below.

Abundance of Volunteers

In September of 2011, I marked ten 20-m transects – five transects in each common garden. I avoided areas that were heavily mulched. Transects ran parallel to each other in rows between or within research blocks and were placed 0.5 m to the right of center of each aisle. Every 4 meters, I placed a 0.5m x 0.5m quadrat on the ground so that the four corners of the quadrat were equidistant from the transect line (5 sampling plots per transect; 50 total plots). I counted individual switchgrass shoots, then counted switchgrass plants. I multiplied the total number of plants in each quadrat by four to obtain estimates of plant density per square meter. I then placed all the sampling plots into one of 6 categories: 0 volunteers present, or 1-10, 11-20, 21-30, 31-40, or 41-50 volunteers present. I used these parameters to construct Figure 3.1.

Investigating the Similarity to Common Garden Biotypes

Flowering Phenology

To compare the flowering phenology of volunteer plants with that of the mature plants in the common garden, I selected 100 volunteer plants that were approaching maturity or were already mature. I chose individuals that varied in height, shoot number, leaf color, and panicle size to represent the overall volunteer population in the common gardens, but selection was not random, since it was necessary to have volunteer plants that would flower. Each week beginning in mid-August of 2011, I recorded flowering phenology. At each survey, individuals were placed into one of five categories, as follows:

Early flowering	onset to 50% of shoots are flowering
Full flowering	50-100% of shoots are flowering
Late flowering	less than 50% of shoots are flowering
Done flowering	No shoots are flowering

The survey continued until the last plant had finished flowering in early October. When calculating the proportion of flowering plants for each biotype, I weighted the values by multiplying the mean number of flowering plants in each category thus:

Early flowering:	Proportion of plants x 0.25
Full flowering:	Proportion of plants x 1
Late flowering	Proportion of plants x 0.25

When I began the survey in mid-August, about half of the mature volunteer plants throughout the common garden were already flowering to some extent, so I estimated the number and amount that were likely flowering for three weeks leading up to the first survey date, based on the data that were collected and trends of past flowering surveys at

Waterman Farm. I pooled data from the 100 volunteers and compared it to flowering data collected in 2011 for extant biotypes in the 2009 common garden (flowering data for cohort 2 are reported in Chapter 1). To compare the volunteer flowering phenology to that of common garden plants, I first separated the mature, known biotypes into three categories: Ohio native biotypes (earliest flowering), Kanlow-type cultivars (latest flowering), and non-Kanlow type cultivars.

Ploidy level and Ecotype

At each of the 50 plots described above, I selected the switchgrass volunteer that was growing nearest to the upper left corner of the quadrat. In the event that there were no switchgrass volunteer plants growing in a particular quadrat, I selected the closest volunteer growing outside the quadrat. Random selection was necessary to compare ploidy and ecotype proportions with the existing proportions in the common garden. In October of 2011, I excavated rhizomes, wrapped them in wet paper towels, placed them in plastic bags and transported them to the OSU Biological Sciences greenhouse for each volunteer. Root parts were planted in untreated potting soil and placed under a mister. Pots were fertilized once per week with N-P-K treated water. By mid-November of 2011, 37 of the 50 sampled volunteers had grown large enough to test. Young leaf tissue was harvested and ploidy level was determined using the methods described in Chapter 1.

After ploidy was determined, plants were removed from pots and roots were washed and labeled with transect and plot number. Individuals were classified as being either upland or lowland ecotype according to rhizome morphology: upland plants have longer rhizomes with leaf scales, and new shoot growth occurs either from rhizome nodes or basal nodes of old shoots; lowland plants have much shorter rhizomes from which new shoots emerge (Beaty et al. 1978; Porter 1966) (Table 3.3, Figure 3.2). All but one of the 37 volunteers had mature roots that allowed for classification. The 36 plants were labeled with transect and plot number and categorized as belonging to either the upland or lowland ecotype, based on their rhizomes. Photographs were taken of each volunteer's roots. Two more people then viewed the photographs independently and categorized each volunteer; the three separate classifications were consistent (cytotype testing using cpDNA markers was not done because of time constraints).

Morphology

I utilized the two groups of volunteer plants (Table 3.2) to examine leaf color, since blue-green leaves are a characteristic of lowland ecotypes (blue-green leaves were not seen in any of the wild switchgrass populations). Also, I recorded any resemblance to Kanlow cultivar types (Kanlow and Advanced Kanlow). Kanlow types are the only lowland ecotypes in the common garden, and lowland types have a distinct morphology compared to upland plants (Parrish & Fike 2005) (Table 3.3).

Analysis:

Results were summarized in tables and figures. Chi-squared tests ($\alpha = 0.05$, df = 1) were used to compare ploidy and ecotype frequencies, and leaf color and resemblance to Kanlow, between volunteers and common garden plants. The null hypothesis for each test was that there was no difference in proportions between the two groups.

RESULTS

Flowering Phenology

All selected volunteers flowered during the course of the survey. Flowering times of the selected volunteer plants overlapped those of all other biotypes in the common garden to some degree. The greatest overlap occurred with cultivars, but there was also some overlap with Ohio native biotypes. Additionally, the estimated volunteer flowering times for the three weeks prior to August 12th (the first actual sampling date) are conservative, so overlap with native biotypes could be greater than what is shown here (Figure 3.3). Twenty volunteers did not initiate flowering until after August 28th, and of those, 10 were identified morphologically as likely to be Kanlow types.

Ploidy Level & Ecotype

Ploidy level and ecotype frequencies of the random volunteers, taken separately, were very similar to those of the mature plants in the common garden (Table 3.4). Results of the ploidy test showed 23 of the 37 volunteer plants that were tested were tetraploids, while 14 were octaploids. The three independent ecotype classifications revealed that 28 volunteers were upland types, while 8 were lowland plants, a ratio that was also consistent with that of the extant population (Table 3.4). A total of 20 volunteers, or 56%, had either a ploidy level or an ecotype that did not match those of the native plants, indicating that one or both parents were cultivars. In addition, two volunteers were classified as lowland octaploids (Table 3.5). Rhizome morphology closely resembled what Beatty et al. (1978) described (Figure 3.4.)

Morphology

Of the volunteers used for the flowering survey, 23% were classified as having blue-green leaves, but only 12% were characterized as resembling Kanlow types. For comparison, the extant population of mature switchgrass plants is made up of 20.2% Kanlow types. A chi-squared test revealed that the proportion of non-random volunteers with blue-green leaves was similar to that of the common garden plants, but there was a significant difference in the proportion of volunteer plants that looked like the Kanlow type, compared to the extant population ($X^2_{calc} = 11.39$, $\alpha = 0.05$, df = 1). In the random group of volunteer plants, only 3 of the 50 individuals were identified as having blue-green leaves (6%), which was significantly different from parent types ($X^2_{calc} = 34.10$, $\alpha = 0.05$, df = 1). Those plants were generally too immature to describe as resembling any particular known biotype.

Volunteer Abundance

Volunteer switchgrass plant abundance varied per quadrat, with the most densely covered areas located near the center of the older common garden (Figure 3.1). Shoot numbers per square-meter ranged from 0 to 436, with an overall mean of 95.1 (s.e. 14.84), and plant numbers per square-meter ranged from 0 to 48, with a mean of 10.3 (s.e. 1.67). Sixteen of the 50 plots (7 in the 2008 common garden and 9 in the 2009 common garden) had no switchgrass volunteers. In the plots with volunteers present, the mode for shoot number was 280 per m^2 (3 plots), and the mode for plant number was 4 per m^2 (10 plots). Means and standard errors by transect and by common garden are reported in Table 3.6.

DISCUSSION

Volunteer plants are of concern to the preservation of natural areas because they facilitate pollen- and seed-mediated gene flow between agricultural and wild relatives. Volunteers promote hybridization by acting as a genetic bridge between crops and wild counterparts. Successful hybridization events could potentially swamp local native gene pools, and the introduction of novel genes could confer unwanted traits on wild individuals. Volunteers also are evidence that crop plants are mobile through seed movement, and subsequent reproductive events could potentially move the cultivar “front” out of managed settings and into wild areas. Many volunteers in this study came from cultivated plants in the common garden, including some from the late-flowering Kanlow types. Evidence of cultivar parentage can be seen in the numbers of volunteers that did not match either the ploidy level or the ecotype of the Ohio native populations.

Flowering Phenology

Most volunteers flowered synchronously with parent plants. When comparing flowering times of volunteers with the three major groups of biotypes, volunteer flowering overlapped 22% with native biotypes and Summer on the date of greatest overlap, 55% with non-Kanlow cultivars, and 40% with Kanlow types. Switchgrass plants generally initiate flowering relatively late in an establishment year, so the volunteers could potentially initiate flowering earlier in their second season, which would create a greater overlap with native biotypes.

Ten volunteers identified as having Kanlow-type morphology initiated flowering after August 28th, which is consistent with the late flowering of Kanlow and Advanced Kanlow (Chapter 1). When looking at the bi-modal volunteer flowering distribution, early and late flowering might be a hereditary response to photoperiod and reflective of the volunteer plants' parentage. Response to photoperiod is highly heritable in grasses (Watson and McLean 1991, McLean and Watson 1992, Talbert et al. 1983). Photoperiod response can also vary within species that have a wide geographic distribution (Olmsted 1944, Van Esbroeck et al. 2003).

An additional 10 volunteers that flowered after August 28th did not have Kanlow-type morphology, which could be an indication of hybridization between Kanlow or Advanced Kanlow and another biotype. Hybrid offspring could be expressing the flowering phenology of the Kanlow-type parent, and leaf color/shoot morphology of the other parent. Thus, if flowering phenology is used to justify planting certain biofuels near wild populations (i.e., they are found to have non-overlapping flowering), hybrids that are expressing traits from both ecotypes could make it more difficult to predict the amount of overlap that could occur. Zhang et al. (2011) reported an intermediate phenotype for 11 of 21 putative ecotype-hybrids they identified through the use of STRUCTURE (Pritchard et al. 2000).

Ploidy Level & Ecotype

The majority of volunteers were tetraploids, like the parent plants. The similar ploidy ratio was expected, given the fact that plants with different ploidy levels typically don't produce viable seeds. It is not known whether the ploidy level ratio reflects an equal contribution from all the biotypes, or whether a few biotypes are overrepresented, because identifying parents was beyond the scope of this study. Nevertheless, even with a small sample size, 4x and 8x volunteers were growing in the common gardens, which shows that both ploidy levels can produce successful volunteers.

Ecotype proportions also closely matched those of the mature biotypes, but unlike different ploidy types, ecotypes can successfully cross. Martinez-Reyna & Vogel (1998) crossed lowland tetraploid Kanlow and upland tetraploid Summer by pollinating >2,200 florets and obtained an average 27% seed set. Switchgrass ecotypes are adapted to certain soil moisture conditions and particular daylengths. While no studies have been conducted to examine the phenotypes of ecotype hybrids in a natural setting, Martinez-Reyna and Vogel (1998) reported hybrids with at least one trait that was intermediate between the parents. Zhang et al. (2011) reported mixed results with regard to phenotypes of ecotype hybrids, with some hybrids resembling one or the other parent, and other hybrids having an intermediate phenotype. An interesting next step would be to measure the environmental response of ecotype hybrid plants of varying phenotypes, and compare them to those of parent genotypes.

Some of the volunteers represented two-biotype crosses resulting in hybrid offspring. For example, only half the plants identified as having blue-green leaves had Kanlow-type features (e.g., wider stems and bunched form). Additionally, none of the 6 volunteers identified as lowland tetraploids had blue-green leaves, even though the lowland ecotype is specific to Kanlow and Advanced Kanlow in the common garden. Interestingly, two of the plants identified as lowland ecotypes were also revealed to be octaploids. Past research has stated that octaploids are always of the upland ecotype, so, in order for a lowland octaploid to have arisen in the common garden, either hybridization between 4x and 8x plants, or chromosome doubling had to have occurred. Inter-ploidy crosses in switchgrass are reported as being inhibited by a post-fertilization barrier (Martinez-Reyna and Vogel 2002). Brink and Cooper (1947) explained that the main reason for failed crosses between ploidy levels is likely endosperm breakdown due to an abnormal maternal-to-paternal dosage. Past research has shown that when the maternal parent is the higher ploidy plant, and hence provides a higher than normal dosage to the endosperm, seeds are smaller but can still be viable (*in* Martinez-Reyna and Vogel 2002). Therefore, the lowland octaploids discovered in the volunteer population could have been the result of a maternal octaploid x paternal tetraploid cross, if the tetraploid gametes were unreduced (DNA tests to confirm parentage are outside the scope of this study). Because upland and lowland ecotypes typically occur in different environments and geographic regions, and because relatively few wild populations have been evaluated for ploidy level and ecotype, it is understandable that lowland octaploid plants have not been reported until very recently. In 2011, Zhang et al. sampled 480 switchgrass plants from 67 accessions and discovered 6 octaploids that had both the lowland cytotype and phenotype. Although this frequency is low, it shows that the lowland ecotype and cytotype are not necessarily indicative of tetraploids. Martinez-Reyna and Vogel (2002) obtained no normal seeds when they attempted 415 reciprocal crosses between 4x Kanlow plants and an 8x South Dakota accession, and they cited another study in which authors obtained only 4 normal seeds from > 6000 crossing attempts (*in* Martinez-Vogel 2002). Taliaferro (2002) used interploidy F_1 hybrids in a study to compare biomass production between hybrids and parent plants, but his hybrid plants were hexaploid (6x). Therefore, the origin of the lowland octaploid volunteers discovered in the common garden is ambiguous and requires further study.

Morphology

Leaf color and shoot morphology are useful for identifying lowland switchgrass ecotypes like Kanlow. Some volunteers identified as having blue-green leaves indicative of Kanlow types had other characteristics atypical of lowland plants. For instance, a few of the volunteers that were identified as having the appearance of Kanlow flowered much earlier than expected. A few others that flowered at the same time as Kanlow (and later than the other biotypes) had yellow-green leaves, suggesting the presence of hybrid offspring in the volunteer population. SSR markers can be difficult to use in switchgrass parentage analyses because it is challenging to identify alleles that are unique to a biotype. Nevertheless, tissue samples were collected from all the volunteers, and I hope to analyze the genetic structure of the sampled volunteers in the future to form a clearer picture of the proportion of hybrids *vs.* non-hybrids that make up the volunteer population in the common garden.

Volunteer Abundance

Switchgrass volunteer abundance varied among different areas of the common gardens. Volunteers were denser in cohort 1 than in cohort 2 and occurred more often in the center than at the edges of the common gardens. This makes sense, considering the greater age and larger size of the 2008 common garden plants. The older plants have more shoots and larger inflorescences than the younger plants (A. Stottlemyer, pers. obs.), and likely had a relatively heavier localized seed rain. Switchgrass plants produce abundant seeds (Chapter 2), and conditions in the common garden were conducive to seed germination and seedling establishment because the aisles were kept relatively clear of weeds. However, this study shows that, given suitable conditions, switchgrass volunteers will become established.

Overall Volunteer Assessment

Cultivars are likely to establish feral populations when conditions for establishment are suitable. Kanlow and Advanced Kanlow, which flowered much later, on average, than other biotypes and were probably pollen-limited, produced volunteer offspring and hybrid offspring with other biotypes, which shows that even a small overlap in flowering times can result in hybridization. Kanlow has a fitness advantage over native biotypes (Chapter 2), and would likely out-compete native populations over time, if the cultivar were to become established in natural areas.

Heritable differences in flowering times may prevent some gene flow from volunteers to wild populations. The degree of overlap will depend on the latitude of origin and the hardiness zone to which the cultivars and wild populations are adapted. There was overlap in flowering between cultivars and native genotypes that originated one hardiness zone from each other, and variation in overlap between years (Chapter 1) suggests that some years there may be more overlap than what was shown here. Also, once a volunteer population enters its second season of growth, flowering phenology is likely to shift to earlier initial and peak flowering times, since first-season switchgrass plants tend to flower later than normal.

Although this study was conducted on a single site, it shows that pollen- and seed-mediated gene flow between switchgrass cultivars and wild populations can occur under suitable conditions. Once volunteers become established, recurring gene flow could promote the establishment and persistence of feral cultivar populations in the wild. Hybridization events that lead to the introgression of crop genes could result in an increased overlap in flowering phenology and a positive feedback loop that shortens the time in which cultivated switchgrass displaces wild populations. Certain cultivars, like Kanlow or Advanced Kanlow, could become common in natural areas over time. Future research should focus on whether cultivars could displace native genotypes in natural settings, diluting native gene pools and displacing wild populations.

Biotype	Number of Plants 2008	2009	Origin	H.Z.
Shawnee	11	19	IL	6
Summer	14	20	NE	5
Trailblazer	12	20	Syn. EY & FF	5,6
Kanlow	13	20	OK & KS	6,7
Advanced Kanlow[1]	--	20	OK & KS	6,7
Advanced Octaploid 1[1]	--	19	Synthetic	5
Advanced Octaploid 2[1]	--	20	Syn. EY & FF	5,6
Local Native Restored[2]	13	--	OSU Marion	6
Ohio Native 1[2]	--	20	Green Camp RR	6
Ohio Native 2[2]	--	20	Daughmer BOS	6
Ohio Native 3[2]	--	19	Claridon RR	6

Biotype	Ploidy	Ecotype[3]	Use
Shawnee	8x	U	forage
Summer	4x	U	forage
Trailblazer	8x	U	forage
Kanlow	4x	L	forage
Advanced Kanlow	4x	L	yield/ winter survival
Advanced Octaploid 1	8x	U	yield/ ↓ lignin
Advanced Octaploid 2	8x	U	↓ lignin
Local Native Restored	4x	U	restoration
Ohio Native 1	4x	U	n/a
Ohio Native 2	4x	U	n/a
Ohio Native 3	4x	U	n/a

[1] Seeds of experimental strains were obtained from Dr. Kenneth Vogel, USDA-ARS, University of Nebraska.

[2] I determined the ploidy level (N = 10-20) for Ohio native biotypes using flow cytometry and the ecotype (N = 5) by examining rhizome morphology. See Chapters 1 & 3 for methods.

[3] L = lowland ecotype; U = upland ecotype

Table 3.1. Switchgrass biotypes planted in common gardens in 2008 and 2009. In 2008, N = 13-16 individuals per biotype. In 2009, N = 19-20 individuals per biotype.

Selection of Volunteers:

Non-Random	***Random***
N = 100 for flowering and leaf color	**N = 50** for ploidy, ecotype, and leaf color
	(-13 that were too small to test)
	= 37 for ploidy tests
	(-1 that was too immature to classify)
	= 36 for rhizome classification

Table 3.2. Sampling design for selection of volunteers from two common gardens planted in 2008 and 2009.

Characteristic	**Lowland Type**	**Upland Type**	**Source**
Leaf color	blue-green	green	*Casler 2005; Porter 1966*
Rhizomes	shorter, bunch-forming	longer, sod-producing	*Beaty et al. 1978*
Root internodes	shorter	longer	*Porter 1966*
Root nodes	larger	smaller	"
Height	taller	shorter	*Casler 2005; Porter 1966*
form	bunched	sod-producing	*Vogel 2000; Beaty et al. 1978*
stem	wider	narrower	*Casler 2005; Porter 1966*
Panicle	longer	shorter	"
Shoot growth	from rhizomes only	from either rhizomes or basal nodes of old shoots	"

Table 3.3. A list of some of the traits that have been ascribed to switchgrass plants of either the upland or lowland ecotype.

		Ploidy proportion		Ecotype proportion	
	n	**4x**	**8x**	**Lowland**	**Upland**
Cultivars	200	0.46	0.55	0.28	0.73
Ohio Native Biotypes	72	1.00	0.00	0.00	1.00
Combined Parent Proportion	**272**	**0.60**	**0.40**	**0.20**	**0.80**
Volunteer Proportion	**37,36[1]**	**0.62**	**0.38**	**0.22**	**0.78**
X² calculated value, df=1		**0.569**		**0.664**	

[1] N = 37 volunteers for the ploidy analysis and N = 36 volunteers for the ecotype analysis.

Table 3.4. Volunteer switchgrass ploidy level and ecotype proportions compared to 11 biotypes grown in a common garden in Columbus, Ohio in 2008 and 2009. A chi-squared test (α = 0.05, 1 df) revealed that volunteer proportions were not significantly different from those of parent biotypes.

	Lowland	**Upland**
4x	Kanlow Advanced Kanlow (Blue-green leaves, coarse thick shoots) **6 Volunteers:** Cultivar or cultivar-native hybrid parents	Ohio Native populations Summer **16 Volunteers** Native or cultivar-native hybrid parents
8x	No Cultivars or wild popualtions **2 Volunteers** Cultivar hybrid parents	Shawnee Trailblazer Advanced Octaploids 1 & 2 **12 Volunteers** Cultivar parents

Table 3.5. Volunteers were classified into one of four groups, based on ploidy level and ecotype analyses.

By Transect	Mean number of shoots/sq m	s.e.	Mean number of plants/sq m	s.e.
1	120.8	45.72	16.8	4.45
2	80.0	32.77	8.8	4.80
3	155.2	57.85	20.0	8.20
4	135.2	47.72	20.0	6.45
5	106.4	52.79	14.4	7.44
6	51.2	48.26	4.0	3.10
7	88.0	41.24	7.2	3.67
8	72.0	34.11	5.6	2.04
9	28.0	13.74	3.2	1.50
10	114.4	81.36	3.2	0.80
By Garden				
2008	119.5	20.32	16.0	2.76
2009	70.7	20.90	4.6	1.05

Table 3.6. Relative abundance of switchgrass volunteers in two adjacent common gardens. Transects 1-5 are located in the 2008 common garden, and transects 6-10 are located in the newer 2009 garden.

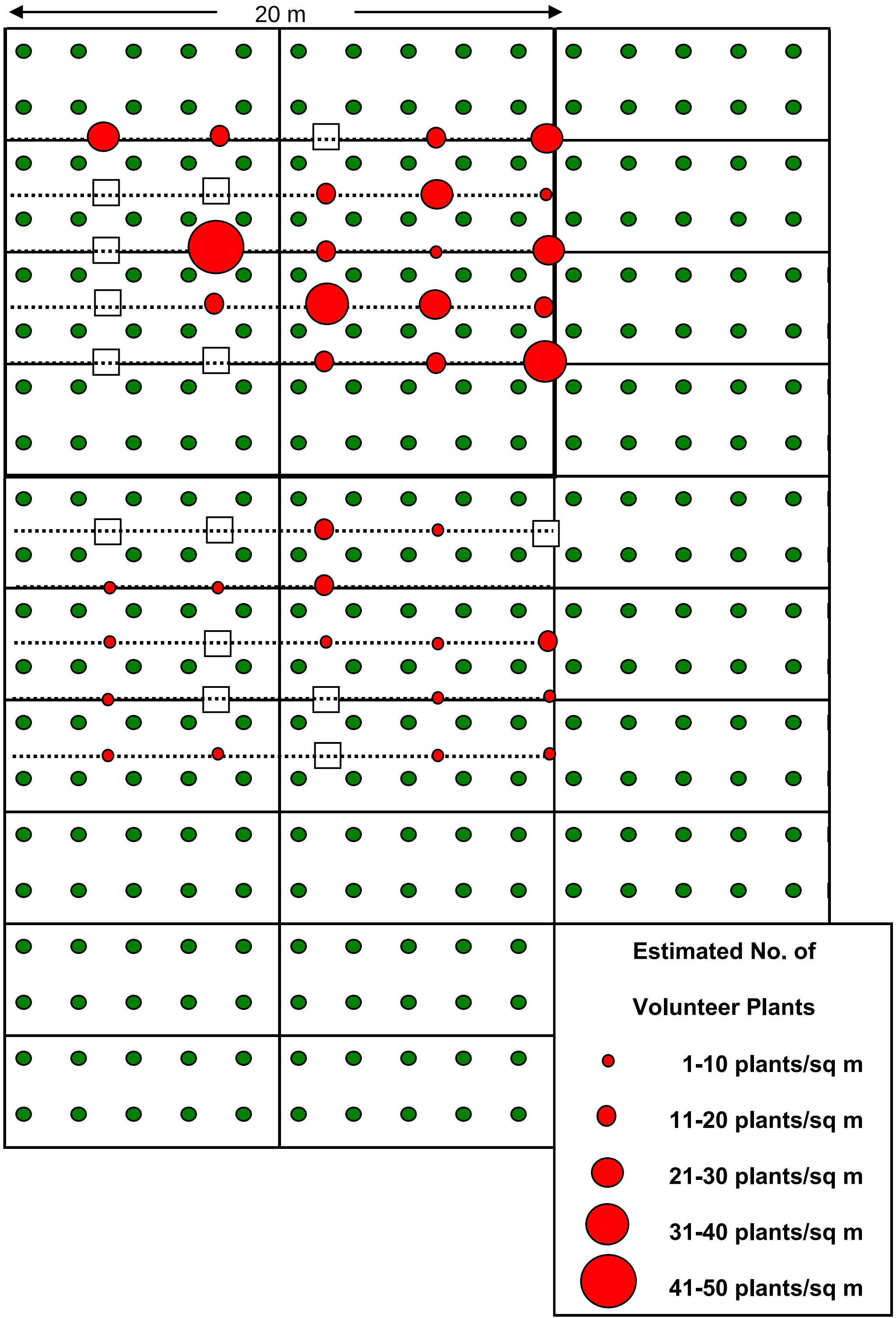


Figure 3.1. Visual depiction of the relative density of switchgrass volunteers in two common gardens. The 2008 common garden has a thick black border. Green-filled circles represent common garden switchgrass plants. Red-filled circles represent estimated volunteer switchgrass density. Translucent squares represent sampling plots with no switchgrass volunteers. N = 50 sampling plots.

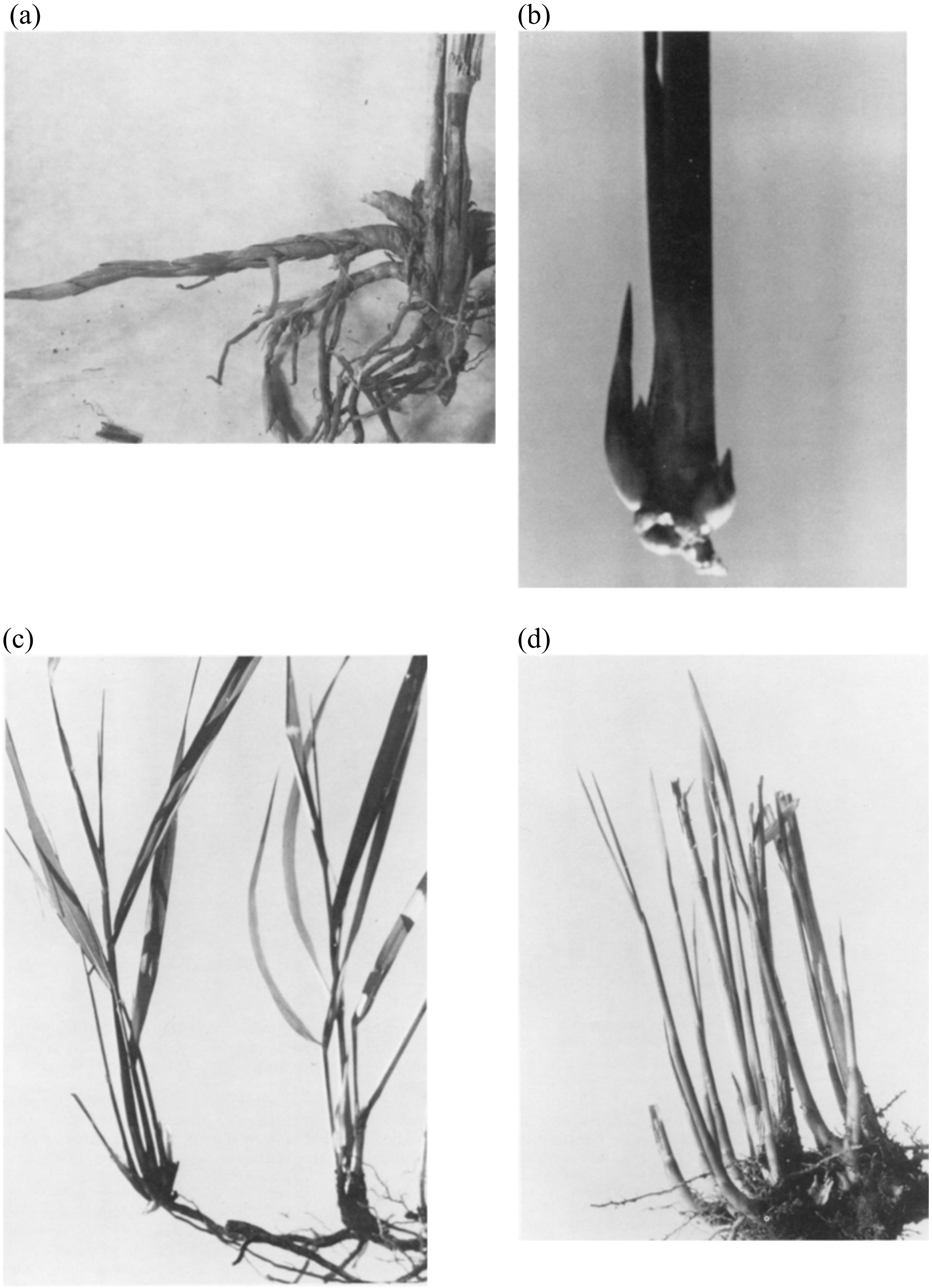


Figure 3.2. Photographs from Beaty et al. (1978) show rhizome morphology of upland and lowland switchgrass ecotypes. (a) Upland plants have longer rhizomes with leaf scales (b) Lowland plants have short rhizomes from which new shoots emerge. (c) Upland plants are typically sod-forming, while (d) lowland plants tend to grow in tight bunches.

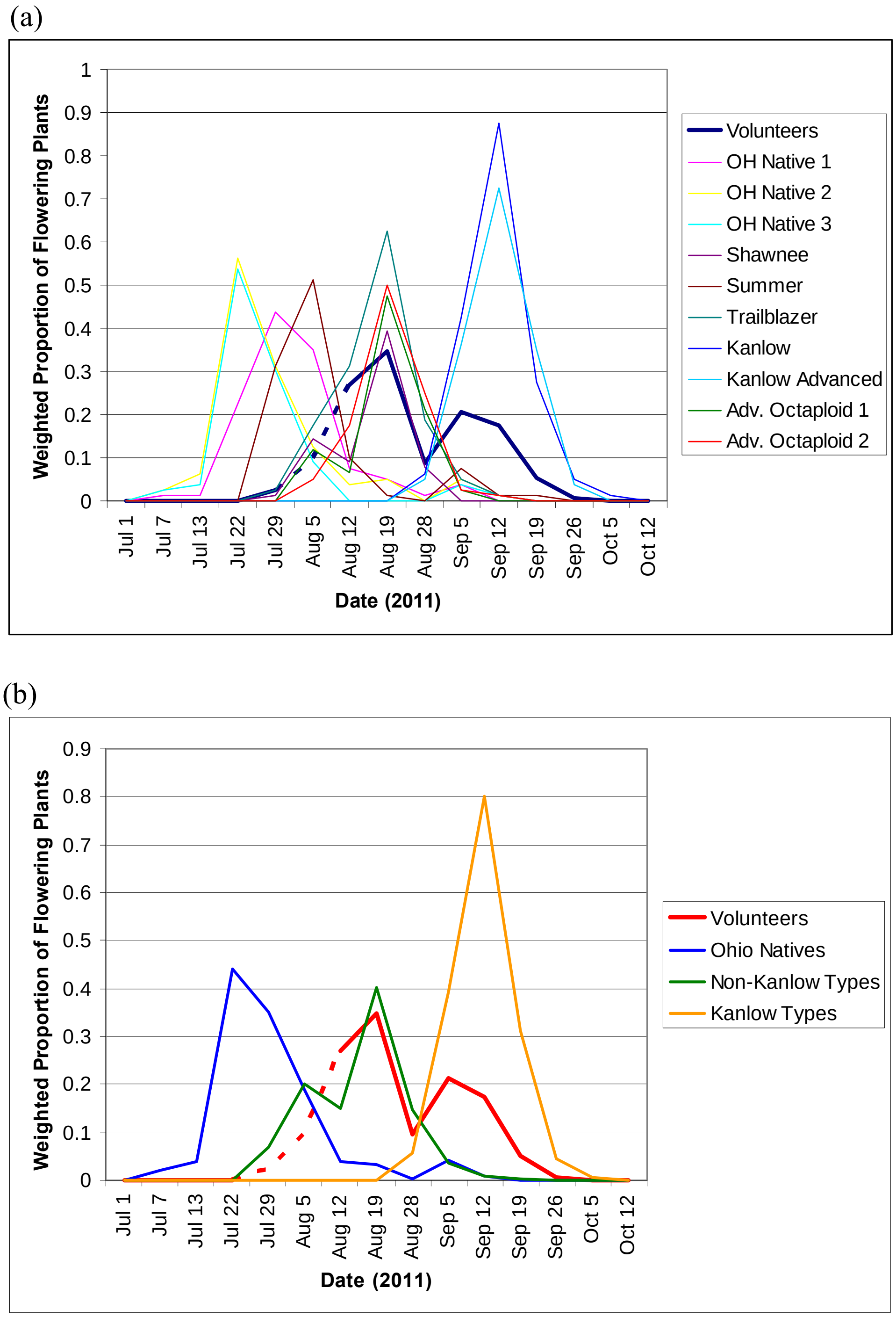


Figure 3.3. Flowering times of selected (non-random) volunteer switchgrass plants compared to established individuals in the common garden established in 2009. Dashed lines represent estimated proportion of flowering volunteers. N = 59 Ohio natives, 99 non-Kanlow types, 40 Kanlow types, and 100 volunteers. (a) Flowering of volunteers is shown with each biotype separately; (b) Flowering phenology is shown by group.

(a)

(b)

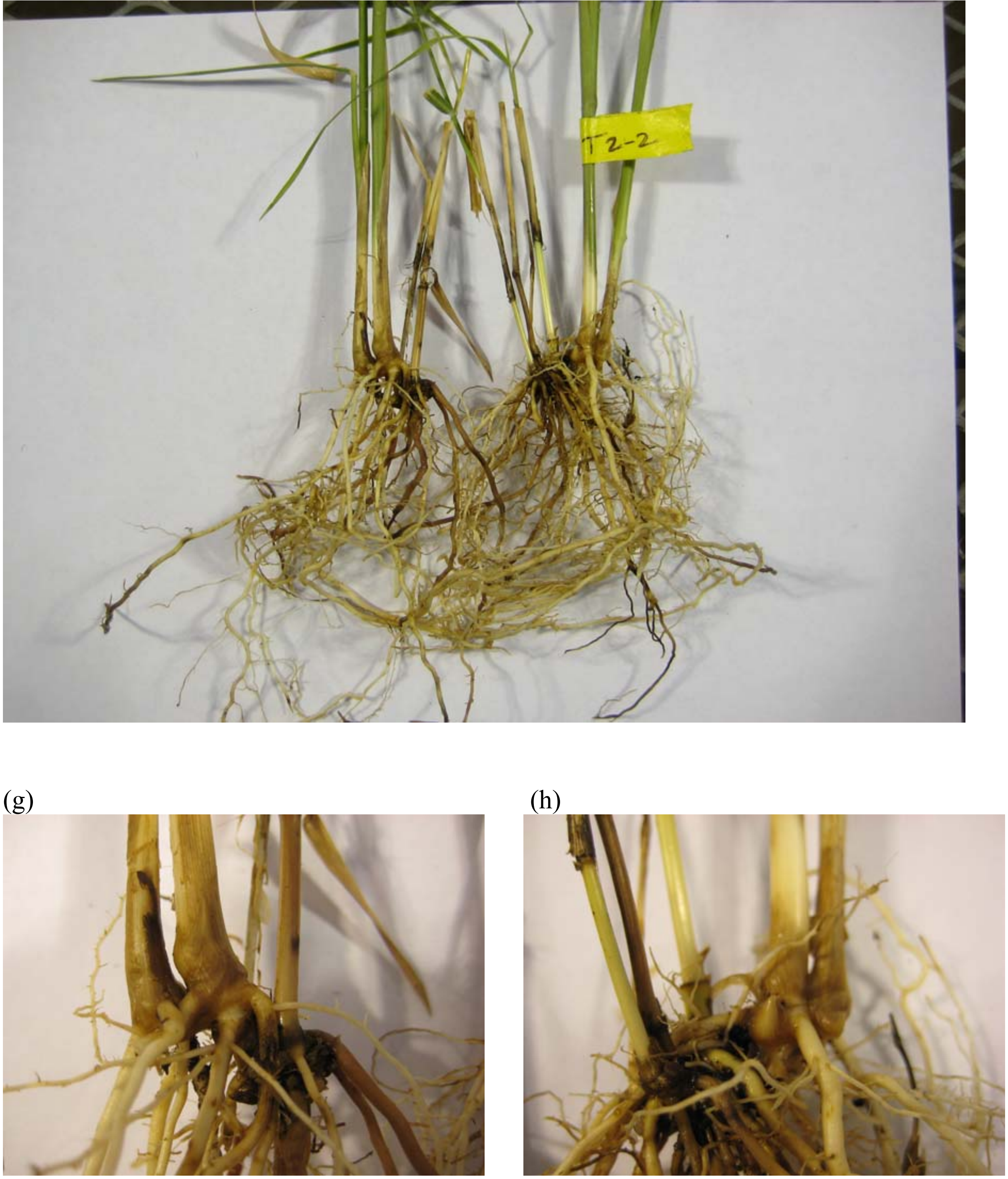
Figure 3.4. Photos of different root morphologies of switchgrass volunteers. (a) Lowland "bunch-forming" ecotype. (b) Upland "sod-forming" ecotype. (c), (d), (e) Photos show upland ecotype's longer rhizomes with leaf scales. (f), (g), (h) Photos show lowland ecotypes' shorter bud-like rhizomes, which lack leaf scales.

continued

Figure 3.4 continued

continued

Figure 3.4 continued



(f)

(g)

(h)

CHAPTER 4

IMPLICATIONS AND FUTURE DIRECTIONS

Since biofuel switchgrass cultivars may be planted on a large scale in the near future, we need ways to assess the risk to nearby wild populations. Ideally, field managers could use the knowledge gained from my study to make informed decisions about which cultivars to plant to minimize potential gene flow to wild populations, which could dilute the native gene pool, or minimize cultivar escape, which could displace native plants. The cultivars used in this study varied in ploidy levels, flowering phenology, and certain fitness-related traits, which means risk could be reduced by choosing certain cultivars over others to plant for biofuel use.

Based on my results, the lowland tetraploid Kanlow cultivar poses the highest risk for gene swamping and the establishment of feral populations in Ohio, given suitable environmental conditions. Kanlow had a much higher fecundity than the Ohio wild biotypes: Kanlow plants had significantly fewer shoots per plant, but still produced twice the seeds as Ohio plants, on average. Kanlow and Advanced Kanlow showed a potential for an even greater fecundity, as evidenced by the very high per-plant floret production. Pollen limitation or environmental constraints related to delayed flowering may have reduced seed production in the Kanlow types. If Kanlow plants were pollen-limited, it is possible that they could be released from those constraints when planted in large monotypic swards. Although there was much less overlap in flowering phenology between Kanlow-type plants and all other biotypes, the presence of volunteers with mixed Kanlow and non-Kanlow features is evidence that successful hybrid offspring can be produced. Thus, even a small overlap in flowering times can facilitate gene flow and hybridization between Kanlow and biotypes of matched ploidy levels. Added to this is the fact that some years may have greater flowering overlap than others, as shown by the variation in initial and peak flowering across the two years that data were collected. Finally, Kanlow was significantly taller than the Ohio populations, which could give feral Kanlow plants a competitive advantage over wild genotypes.

The upland tetraploid Summer cultivar poses the next highest risk to wild Ohio switchgrass populations. Although Summer plants produced fewer shoots, on average, seed production was very high. Summer plants had twice the number of seeds per plant as Ohio wild plants. In addition, Summer plants' initial and peak flowering times were very similar to the Ohio populations, leading to very heavy overlap in flowering phenology. Thus, there is a high potential for pollen and gene swamping between the Summer cultivar and wild populations, which is heightened by the fact that the majority of wild switchgrass plants in Ohio were discovered to be upland tetraploids, like Summer.

The upland octaploid cultivars Shawnee, Trailblazer, and Advanced octaploids 1 and 2 pose the lowest risk of the varieties used in this study. The upland octaploids were similar to wild plants in height, floret and seed production, and except for Advanced octaploid 2, were similar to wild plants in shoot production. There was overlap in flowering phenology between these cultivars and the Ohio wild plants, so the potential for gene flow is present. However, as stated earlier, most of the Ohio plants used in this study were tetraploids, and different ploidy levels typically do not successfully cross-pollinate. If care was taken to avoid planting octaploid cultivars in areas where known octaploids exist, like the Firelands region in northern Ohio, then the risk associated with gene flow can be minimized. Finally, although nothing is yet known about the relative competitive ability of the octaploid cultivars *vs*. Ohio wild plants, similar shoot numbers, height, and fecundity suggests upland octaploid cultivars may pose a smaller overall risk to wild populations, compared to cultivars that are tetraploid and/or are the lowland ecotype.

In summary, certain characteristics should be considered when planning which switchgrass cultivars to plant for biofuel production, in order to reduce the risk of gene flow between cultivated and wild switchgrass plants. It will be helpful to know the flowering phenology and the relative fitness of the cultivars *vs*. nearby wild populations. It will also be useful to know whether the cultivar can produce successful volunteers or hybrid volunteers, which facilitate gene flow and the establishment of feral populations. Since there is a high level of variation in the switchgrass genome, and populations may be locally adapted, it will be necessary to analyze risk on a case-by-case basis, at least in the short-term. Regional trends in wild populations with regard to these traits may emerge as more data are amassed, and as newly selected cultivars are developed as biofuel crops. Future research should continue to focus on ways to induce sterility, or ways to maintain biofuel switchgrass plants in a perpetual juvenile state. In addition, it is important to know more about the demography of wild *vs*. cultivated switchgrass, such that differences in seed germination, seed dormancy, seedling establishment, and other factors are taken into account in population projections. Meanwhile, my research provides important baseline information and a jumping-off point for further comparisons between switchgrass cultivars and their wild relatives.

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APPENDIX

DATA FOR 2008 COMMON GARDEN, SAMPLED IN 2009 AND 2010

(a)

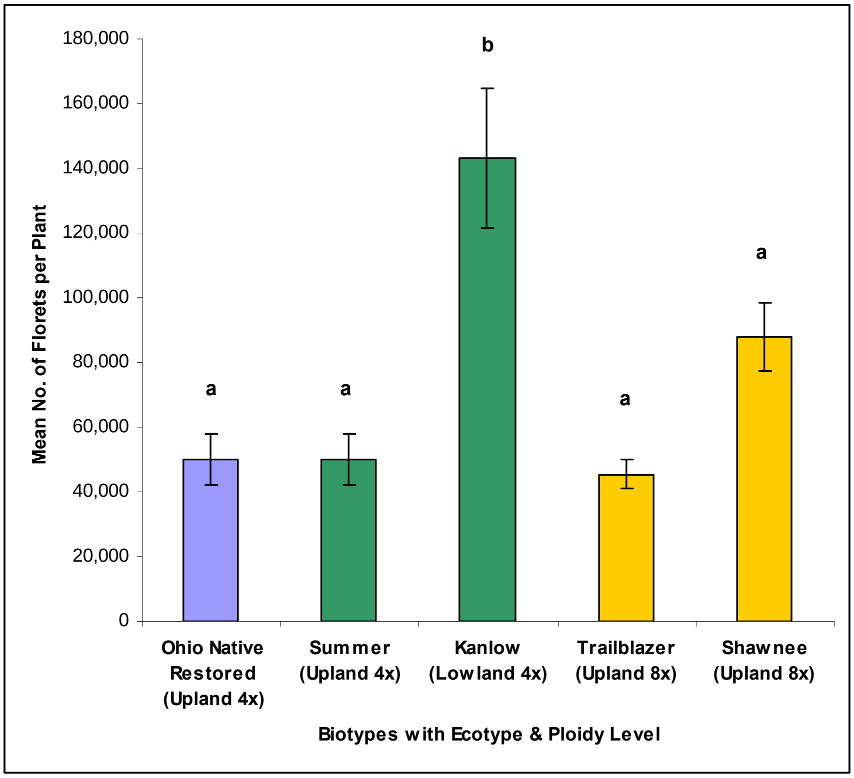
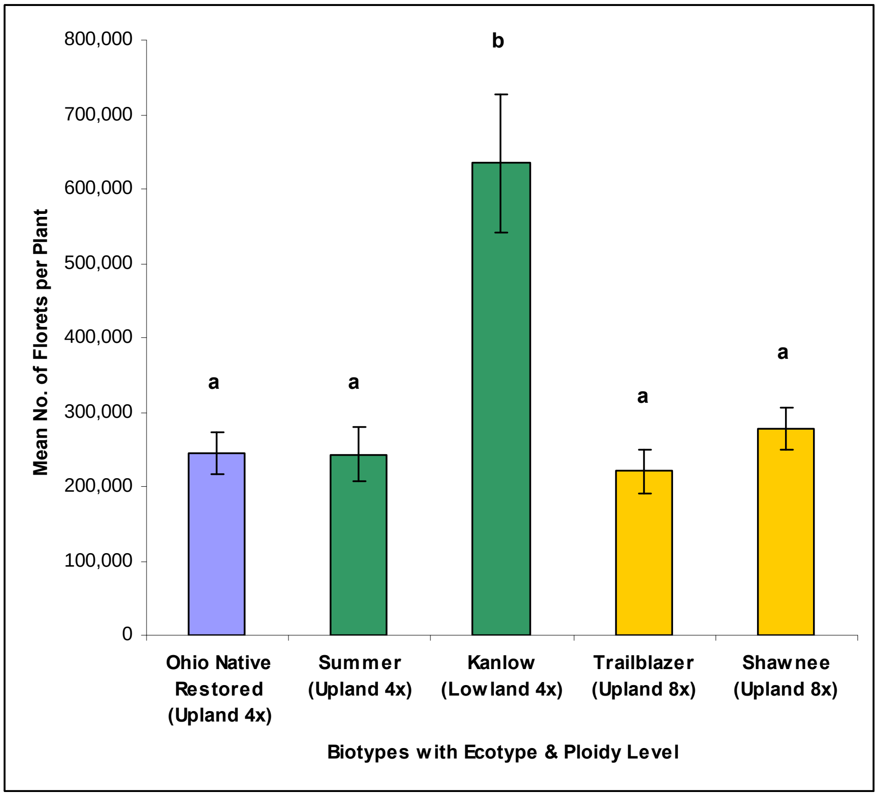


Figure A.1. Number of florets produced per biotype in a common garden planted in 2008 in Columbus, Ohio (means ± 1 SE). Floret number and floret weight were highly correlated ($P < 0.0001$), which is further evidenced by the similar results obtained by (a) counting florets in 2009, and (b) weighing florets in 2010 to estimate numbers. N = 11-14.

continued

Figure A.1 continued

(b)



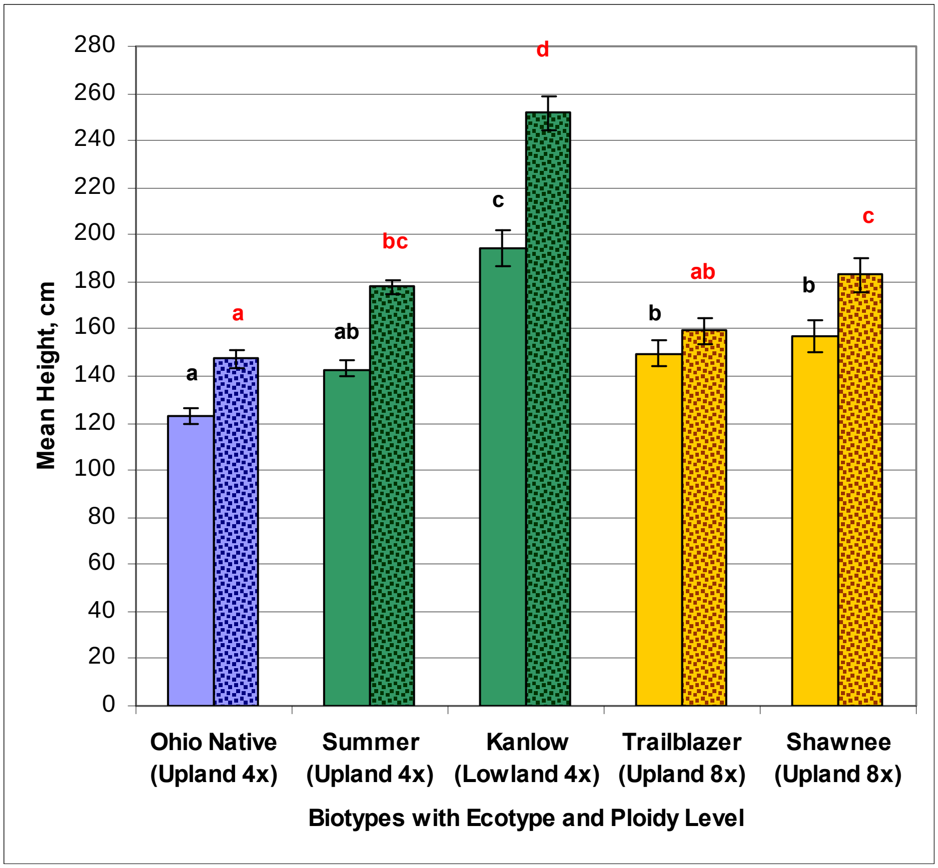


Figure A.2. Average height per plant of 5 biotypes grown in a common garden in 2008 in Columbus, Ohio. Left bars represent mean height in 2009 and right bars represent mean height in 2010. Differences were significant ($P < 0.0001$ for both years). Letters indicate significant differences among biotypes.

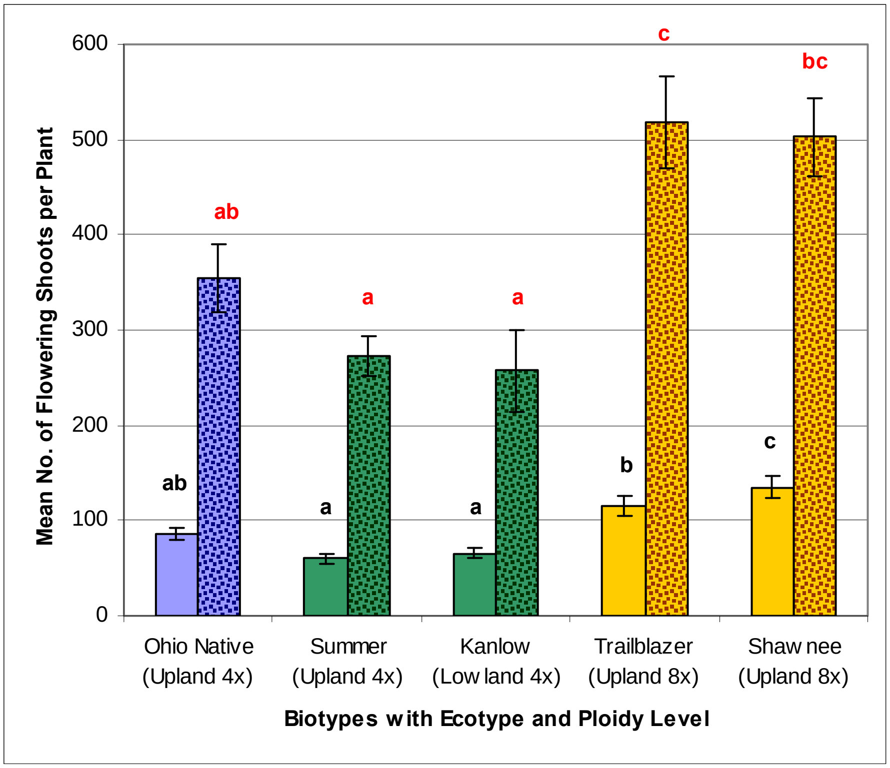


Figure A.3. Average numbers of flowering shoots produced per plant in a 2008 common garden. Left bars represent numbers of shoots produced in 2009 and right bars represent numbers of shoots produced in 2010. Differences were significant (*P* < 0.0001 both years). Letters indicate significant differences among biotypes.

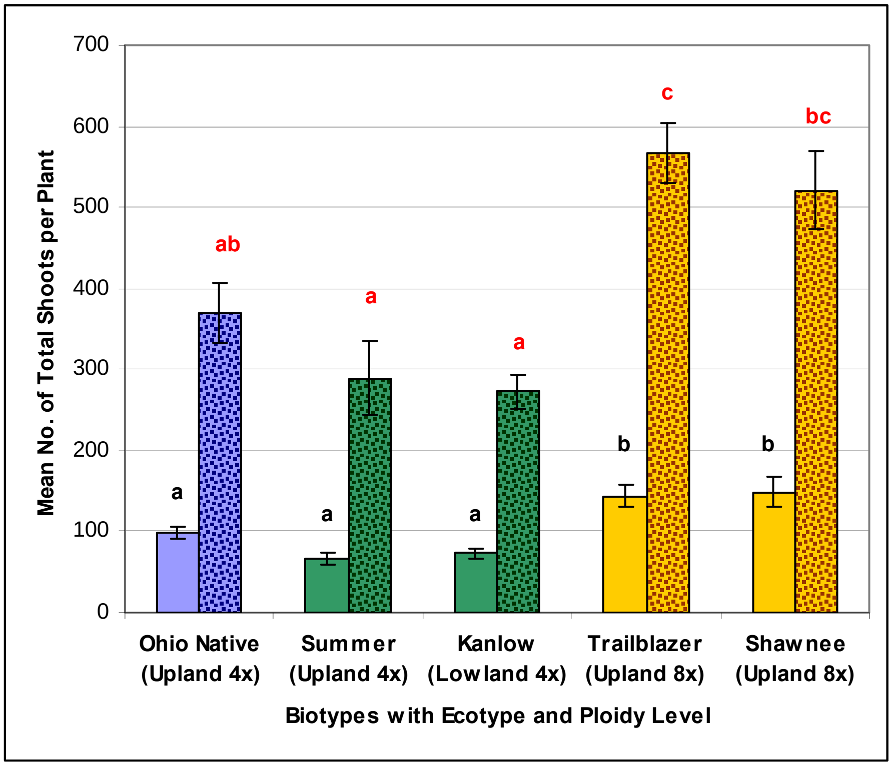


Figure A.4. Average numbers of total shoots produced per plant in a 2008 common garden. Left bars represent numbers of shoots produced in 2009 and right bars represent numbers of shoots produced in 2010. Differences were significant (*P* < 0.0001 both years). Letters indicate significant differences among biotypes.

2009

Biotype	n	Mean Height, cm	s.e.	Mean Total Shoot No.	s.e.
Ohio Native Restored	13	123.1	3.39	99.0	7.19
Summer	14	142.9	4.15	63.6	7.01
Kanlow	13	194.2	7.16	74.8	6.32
Trailblazer	12	149.4	5.93	141.8	13.00
Shawnee	11	157.4	6.26	164.5	17.69

Biotype	n	Mean Flowering Shoot No.	s.e.	Mean No. Florets	s.e.
Ohio Native Restored	13	85.5	5.84	49918.0	7785.85
Summer	14	57.8	6.11	49907.9	7966.92
Kanlow	13	67.8	5.82	150336.8	21865.47
Trailblazer	12	114.5	10.78	45484.5	4666.36
Shawnee	11	147.3	15.82	87926.8	10474.94

2010

Biotype	n	Mean Height, cm	s.e.	Mean Total Shoot No.	s.e.
Ohio Native Restored	13	147.3	3.75	369.8	36.81
Summer	14	177.8	2.97	288.9	45.49
Kanlow	13	251.6	7.14	272.6	21.01
Trailblazer	12	159.2	5.68	565.7	36.70
Shawnee	11	182.9	6.76	520.7	48.69

Biotype	n	Mean Flowering Shoot No.	s.e.	Mean No. Florets	s.e.
Ohio Native Restored	13	354.5	35.94	245242.9	27794.87
Summer	14	272.6	43.35	244212.6	36379.07
Kanlow	13	257.2	20.62	626641.2	84481.21
Trailblazer	12	517.8	35.38	219390.3	29557.85
Shawnee	11	503.3	47.76	271021.0	24835.32

Table A.1. Mean parameter values for one Ohio native biotype and four cultivars grown in a common garden in Columbus, Ohio in 2008. Data for 2009 and 2010 are reported.