INFLUENCE OF SWITCHGRASS ECOTYPE, CULTIVAR AND PLANTED STAND DIVERSITY ON HERBIVORES, NATURAL ENEMIES, AND BIOLOGICAL CONTROL IN BIOENERGY CROPPING SYSTEMS

By

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ABSTRACT

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Switchgrass is a perennial, C4 grass that has emerged as a model bioenergy crop for a large portion of the US. Because of the potential for switchgrass to occupy many acres, understanding how cultivar and planted stand diversity choices could impact insects and ecosystem services is key. To investigate how cultivars differed the preference of herbivores, fall armyworm was chosen to represent how a generalist, chewing herbivore. Two life stages were used to measure establishment, consumption levels, and life history traits on different cultivars of switchgrass. These experiments revealed that the lowland ecotype supported lower levels of feeding and tended to slow development. The second experiment investigated how herbivores, natural enemies, and biological control were impacted by different switchgrass cultivars and planted stand diversities. Switchgrass was established both as different cultivar monocultures and in mixtures with grasses and forbs. Sweep samples were used to collect arthropods and egg cards were used as sentinel prey to measure predation levels. While there were differences between sampling years, generally upland ecotypes supported a greater abundance of herbivores, natural enemies, and ultimately higher levels of biological control. The effect of planted stand diversity was more mixed. Diverse plots hosted more herbivores in both years, but more variable natural enemy numbers and no differences in biological control. Clearly, choices about which switchgrass cultivar and seeding diversity to establish can impact herbivores, arthropod communities, and potentially ecosystem services.
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KEY TO ABBREVIATIONS

AIC – Akaike information criterion
ALAM – Alamo
BLCK – Blackwell
BOMA – BoMaster
CIRA – Cave-in-Rock
E111 – EG 1101
E112 – EG 1102
EISA – Energy Independence and Security Act
FAW – Fall Armyworm
HIGH – High Tide
IVDMD - In-vitro dry matter digestibility
KANL - Kanlow
KLN1 – Kanlow N1 Syn 2
MWF – MI genotype
MSU – Michigan State University
NBSL – NE Summer Late Mat.
NRCS – Natural Resource Conservation Service
PERF – Performer
RFS – Renewable Fuel Standard
SHAW – Shawnee
SHAWGR – Shawnee with grasses
SHAWGRFO – Shawnee with grasses and forbs

SHEL – Shelter

SUMM – Summer

TRLB – Trailblazer

USDA- United States Department of Agriculture
CHAPTER 1: LITERATURE REVIEW
Abstract

A desire for reduced dependence on foreign energy sources has prompted US legislation that mandates increased production of transportation fuels from renewable, plant-based sources. Currently, most of these biofuels in the US are produced from corn grain (corn ethanol) or soybean (biodiesel), but legislation dictates an increase in fuel from dedicated bioenergy crops. One such potential crop is switchgrass (*Panicum virgatum* L.), a perennial C4 grass native to much of the US that is capable of producing high biomass yields and positive ecosystem effects with minimal agricultural inputs. However, the potential large-scale introduction of switchgrass production into agricultural landscapes could also have unexpected consequences. The two main choices made when planting switchgrass deal with switchgrass variety and stand diversity. There are a range of switchgrass cultivars available with different breeding histories, most conveniently divided by ecotype. Ecotypes and individual cultivars have different characteristics that may impact arthropods, both herbivores and natural enemies. The planted stand diversity (monoculture versus polyculture with grasses and forbs) may also alter these communities. Both cultivar and planted stand diversity could influence the insects present in switchgrass communities, and shape bioenergy cropping systems as they are implemented in the US.
Biofuels in the United States

Government Policies

The 2007 Energy Independence and Security Act (EISA) focused attention on the development of advanced biofuels as a part of US energy policy (US US Congress 2007). Broadly, the act aims to increase energy security in the US, aid the development and implementation of renewable fuels, and increase fuel efficiency in vehicles (EPA 2012). The legislation was prompted by a desire to reduce reliance on foreign oil sources, coupled with rising concern about long-term sustainability of petroleum, both from the perspective of oil depletion (Greene et al. 2004, Sorrell et al. 2010) and greenhouse gas emissions (Bang 2010). Coupled with the Renewable Fuel Standard (RFS), these policies mandate that 36 billion gallons of renewable fuel enter the national energy supply by 2022, of which 16 billion must be produced from cellulosic sources, with corn ethanol comprising a maximum of 15 billion gallons (Harrison 2009). While progress toward RFS aims have been made, goals for cellulosic fuel use have been continually amended due to lower outputs than predicted (McKee 2013).

Current Sources: First-Generation Biofuels

Two major types of biofuel are currently produced in the US – corn ethanol and soybean biodiesel. These two fuels are known as first-generation biofuels, as they utilize starch and vegetable oils and conventional technology. The largest source of biofuels in the US is ethanol derived from corn (Zea mays L.) grain, and an estimated 13.3 billion gallons were produced in 2014 (Renewable Fuels Association, 2014). Corn production is at a record high (USDA-
National Agricultural Statistics Service 2014), and approximately 38% of US corn production is devoted to creating corn ethanol (Capehart 2013).

Often grown in rotation with corn, soybean (*Glycine max* L.) is a major crop throughout the world that has uses as both food and biodiesel. Soybeans are composed of 20% oil (Qiu et al. 2010), which can be extracted and used to power diesel engines (Jones and Peterson 2002). In 2013, 1.4 billion gallons of biodiesel were produced (US Energy Information Administration 2014). Converting all soy into biodiesel is not feasible as soy is a major dietary component (Hay). Additionally, devoting all US soybean production to biofuels could only meet a small portion of US diesel demands (Hill et al. 2006).

Beyond government legislation, intrinsic problems with corn ethanol and soy biodiesel have highlighted the need for dedicated biofuel crops. A concern for many people is the “fuel versus food” debate, which questions how appropriate it is to produce fuel from food sources (Timilsina 2013). In 2006 (pre-EISA), approximately 20% of the corn produced in the US was going to the production of ethanol (Capehart 2015), and in the decade after EISA nearly 40% of corn was being used for ethanol (Carter and Miller 2012). A majority of soybeans grown in the US are used for food, with approximately 15% being used for biodiesel (Wisner 2014). Global population growth is higher than ever (Fedoroff and Cohen 1999, United States Census Bureau 2015), and food security is an issue in many areas of the world. As demand for both food and fuel will likely increase in coming years (Hill et al. 2006), some have asked if is it appropriate or wise to use food sources for fuel (Elobeid and Hart 2007, Srinivasan 2009, Timilsina 2013).

There are also negative environmental effects associated with the increased production of crops for biofuels, especially increased corn production. Greater demand for corn has led to more acreage devoted to production of this crop, which has negative ecosystem impacts (USDA-
In 2013, 95.3 million acres were planted with corn, up from the 71.1 million acres of corn planted in 2003 (USDA- Economic Research Service 2004, USDA- National Agricultural Statistics Service 2014). In 2014 88.9 million acres were planted (USDA- National Agricultural Statistics Service 2015). This increased acreage has led to concerns about soil health, including increased erosion (Pimentel et al. 1995, Schnepf and Yacobucci 2013) and loss of sequestered carbon in the soil (Paustian et al. 1997, Lal 2004). There are also concerns about water quality, as the fertilizers (nitrogen and phosphorus) and pesticides used in corn can lead to increased water pollution (Hill et al. 2006). Increased corn acreage has also been tied to land use changes that lead to an increase in greenhouse gas emissions (Searchinger et al. 2008, Ogle et al. 2014).

A concern for all energy sources is the energy balance, which compares the energy obtained from a product to the (often nonrenewable) energy used to produce the product. For example, in corn ethanol the net energy balance is the ratio between the energy inputs used in growing and converting corn to ethanol and the resulting energy in the fuel (Chambers et al. 1979, Shapouri et al. 2002, Pimentel 2003). Some studies suggest that the energy balance of corn ethanol is negative (Pimentel 1991, 2001, 2003, Wang et al. 2011), while others suggest that it is modestly positive (Marland and Turhollow 1991, Lorenz and Morris 1995, Shapouri et al. 1995, Wang et al. 1999, Shapouri et al. 2002, Hill et al. 2006). For soybean biodiesel, the net energy usage is more favorable, with biodiesel producing 93% more energy than goes into its production (Hill et al. 2006).
Second-Generation Biofuels

With all of the issues linked to the current sources of biofuel, a great deal of effort has been put into discovering alternate energy derived from plants, leading to the rise of so-called second-generation biofuel sources. Second-generation biofuels are produced from renewable, non-food plant biomass, utilizing lignocellulose instead of starch; because of this, they are often referred to as cellulosic biofuel.

The potential biomass resources for second-generation biofuels were estimated in the US Department of Energy’s 2005 Billion-Ton Study, which estimated how many tons of dry biomass could be produced in the US as a way of estimating cellulosic biofuel capacity. The original report provided an estimate of available quantities of different biomass sources, predicting there were between 1.1 and 1.6 billion tons biomass that could be made available for processing by 2030, and that this could represent 30% of the country’s fuel supply (Perlack et al. 2005, Downing et al. 2011). The 2011 update expanded this initial finding by taking more factors, such as land-use changes and more localized estimates, into account (Downing et al. 2011, ORNL-Center for Bioenergy Sustainability 2011, Perlack et al. 2011). Both studies took many kinds of potential biomass into consideration, including crop and forest residues, as well as dedicated bioenergy crops, such as switchgrass (Downing et al. 2011, Perlack et al. 2011).

Advantages

Besides the potentially large availability of cellulosic biomass in the US, there are other advantages to second-generation biofuel sources. A key advantage is in the potential to use perennial instead of annual crops as biomass sources. It has been argued that perennial crops are likely to exceed annual crops in regards to providing consistent yields, high-quality feedstock,
minimal inputs, and soil protection (Fike et al. 2007). Because perennials are present on the landscape longer, they have potential to increase carbon sequestration in the soil (Ma et al. 2000), reduce erosion (McLaughlin and Walsh 1998), provide habitats for many beneficial organisms (Werling et al. 2014), and increase ecosystem services such as pollination and pest suppression (Landis et al. 2000, Werling et al. 2014).

An additional benefit of many second-generation biofuel crops is their ability to grow on marginal lands. Marginal lands are defined as being suboptimal for food plant agriculture, due to limitations imposed by soil quality, slope, or soil moisture. Research has shown that on marginal lands, switchgrass had equal or greater potential biofuel yield than fields where both corn grain and corn stover were harvested (Varvel et al. 2008). Another benefit of production on marginal lands is limited competition with land suitable for food production (Valentine et al. 2012). Many hectares of marginal lands in the US are currently in the Conservation Reserve Program, a program that aims to remove marginal croplands from production to help protect soil, provide wildlife habitat, and improve water quality (Sanderson and Adler 2008). Some reports have suggested these lands, up to 6.8 million ha, could potentially be used to produce biomass (Ugarte 2000), a part of the predicted 100 million ha needed to meet the US’s energy needs (Perlack et al. 2005, Graham et al. 2007, Schmer et al. 2008).

**Potential Biomass Sources in the Upper Midwest**

In the US Midwest, a combination of agricultural residues and dedicated biomass crops are likely to form the primary biomass feedstock for biofuel production. Corn stover, the main residue available in the upper Midwest, made up nearly half of agriculture’s contribution in the Billion Ton Report (Perlack et al. 2005). An advantage of corn stover is that it is already being
produced. However, the removal of residue from corn fields could increase risk of soil erosion (Fike et al. 2007) as the soil is more exposed through the winter and spring. The loss of soil carbon is also a concern, although there is potential that this loss could be addressed with cover crops (Fronning et al. 2008). The removal of corn stover may also reduce corn grain yields (Varvel et al. 2008). Despite stover’s more attractive features, it is unlikely that crop residues alone can produce enough biomass to meet US energy goals (Lynd et al. 1991, McLaughlin et al. 1999).

Perennial Crops as Dedicated Biomass Crops

A possible energy system that could provide a variety of ecosystem services is one based on the tallgrass prairies that once occupied much of the land in the central US (Sanderson and Adler 2008, Werling et al. 2011a). Studies comparing yields of high diversity systems and monocultures have found higher (Tilman et al. 2006), lower (Zilverberg et al. 2014, Dickson and Gross 2015) or comparable (Jungers et al. 2015) yields. Regardless, a multitude of ecosystem services are provided, such as increased biological control of pest insects (Gardiner et al. 2009, Werling et al. 2011a, Werling et al. 2014). A recent economic study revealed that the inclusion of ecosystem services in economic models of biomass crops could increase their adoption in the US Midwest (Skevas et al. 2014). The addition of perennial grasslands can also increase bee abundance, supporting pollination services at the landscape level (Bennett and Isaacs 2014), and butterfly species richness (Meehan et al. 2013). Perennial grasses can also act as a filter in riparian zones, reducing nutrient load in watersheds (Meehan et al. 2013, Zhou et al. 2014, Gu et al. 2015). In addition, grasslands can potentially provide habitat for birds, including those of
Switchgrass

Basic Information

One bioenergy crop that has received attention for its dual capacity to produce high biomass yields and provide ecosystem services is switchgrass (*Panicum virgatum* L.). Switchgrass is a C4 grass native to the tallgrass prairie in the US. It was selected as the model bioenergy crop in the US by the Department of Energy in the early 1990’s based on factors such as drought resistance, erosion prevention, widespread range, and high potential yield (Wright and Turhollow 2010). Its native range extends from Northern Mexico to Southern Canada, east from the Rocky Mountains to the southern Atlantic seaboard (Casler 2012). Studies have suggested varying estimates for both the amount of energy produced by switchgrass as well as the sustainability of switchgrass as a monoculture.

Switchgrass was originally selected as a potential biofuel crop based on work conducted at the Oak Ridge National Laboratory, earning the designation of model bioenergy crop (Wright et al. 1993). It grows from 0.5 to 3m tall (Vogel 2004) with roots that extend up to 3m into the soil (Weaver 1954). The grass can be identified by its inflorescence which forms large, diffuse panicles, and by its hairy ligules (Vogel 2004). Switchgrass has been noted for providing consistently high yields in adverse conditions, such as drought (Parrish and Fike 2005), or when planted on marginal lands (Sanderson and Adler 2008). Another positive aspect of switchgrass is the high adaptability of the species, which is likely linked to its large geographic distribution. This has led to great genotypic, and thus phenotypic, diversity (Parrish and Fike 2005). Switchgrass makes efficient use of nutrients (McLaughlin and Walsh 1998) and can act as a carbon sink (Garten and Wullschleger 2000). It also has positive environmental effects, including benefits to soil, habitat for wildlife, and relatively low needs for agricultural inputs.
such as water and fertilizer (McLaughlin and Walsh 1998). Another positive aspect was its history as a forage crop, meaning that growers already have insight and equipment appropriate for planting and harvesting switchgrass (Sanderson and Adler 2008). An example of this is the ability for stands to be planted with a regular no-till drill (Wolf and Fiske 2009). The perennial nature of switchgrass and its deep rooting system are linked to decreased erosion, more favorable soil structure, stable and resilient microbial communities, and improved nutrient retention (McLaughlin and Walsh 1998, Orr et al. 2015). Switchgrass can also be harvested for up to ten years after planting; some suggest there is no reduction in yields, while other say yields peak at year five (McLaughlin and Walsh 1998, Fike et al. 2006a, Arundale et al. 2014).

Schmer et al. (2008) established switchgrass monocultures on ten marginal cropland sites in North and South Dakota and Nebraska, and obtained average yield between 5.2 and 11.1 Mg·ha⁻¹. They also found a positive energy balance, with switchgrass producing 540% more renewable energy than the nonrenewable energy used to produce it (Schmer et al. 2008).

Switchgrass still has drawbacks, many of which are related to establishment. Seed dormancy is a major issue, as it is common in seed lots that only 5% of harvested seeds are germinable (Parrish and Fike 2005). Weed control during establishment is critical (Evers et al. 2000, Mitchell and Britton 2000) and frequently requires herbicide use (Mitchell and Britton 2000, Parrish and Fike 2005). Switchgrass does not reach full productivity for several years, and first year yields are often low (Sanderson and Adler 2008). Finally, efforts continue to increase the efficiency of converting switchgrass biomass into usable fuel. There are at least six major pathways for conversion being investigated (Brown and Brown 2013), all of which aim to access sugars stored in plant cells. Currently, no pathway has been perfected, and conversion efficiency
prevents switchgrass from reaching its predicted energy outputs and greenhouse gas emission goals.

*Breeding Efforts, Cultivar Varieties, and Production*

Because of the large gene pool for switchgrass breeders to draw from, many different cultivars of switchgrass have been produced. Traditionally, switchgrass breeding consisted of collecting seeds from a specific area (an accession), screening these for traits, then picking some of the accessions to test more formally. After more testing, the accession that was most successful at producing the desired trait was selected as a cultivar (Vogel 2004). Other breeding techniques involve crossing different accessions (Casler and Vogel 2014).

In the past, switchgrass has been utilized as a forage crop (McLaughlin and Walsh 1998, Parrish and Fike 2005, Sanderson and Adler 2008) and older cultivars were bred for that purpose (Table 1). Switchgrass has also been used for soil retention (Wolf and Fiske 2009) and habitat reconstruction (Wolf and Fiske 2009, 2015). More recently, switchgrass has been bred for high biomass yield (Vogel et al. 2014), as well as other characteristics that could help switchgrass become a viable biofuel crop (Table 2). Molecular tools have allowed for more advanced breeding (McLaughlin et al. 1999, Vogel 2004), although hurdles still exist as switchgrass cannot self-fertilize (Martínez-Reyna and Vogel 2002).

The most important designation linked to different cultivars of switchgrass is its ecotype, a concept of differentiation within a species based on habitat (Gregor 1944, Turrill 1946). For switchgrass two major ecotypes exist – lowland and upland (Porter Jr 1966, Newell 1968, Brunken and Estes 1975, Parrish and Fike 2009). In this review and the following studies I am focusing on so-called southern lowlands and northern uplands (Lowry et al. 2014). Lowland
ecotypes are generally found in southern regions (Casler 2012), are adapted to flood plains, produce fewer, thicker, tillers, and are generally considered both coarser and taller than upland ecotypes (Vogel 2004, Parrish and Fike 2009, Casler 2012). Upland ecotypes are better adapted to higher latitudes and tall-grass prairies, produce more tillers which are often slimmer, and are generally shorter (Brunken and Estes 1975, Parrish and Fike 2009, Casler 2012). Genetically, lowland ecotypes are often tetraploid, while upland ecotypes are usually octaploid (though they can be tetraploid) (Brunken and Estes 1975, Martinez-Reyna et al. 2001, Parrish and Fike 2009, Zhang et al. 2011). Many breeding efforts work to mix these two ecotypes together, which often proves difficult as upland ecotypes may flower as much as a month earlier than lowland ecotypes, which makes crossbreeding difficult (Taliaferro and Das 2002). Hybridization is possible and is sometimes seen in areas where upland and lowland switchgrass grow in close proximity to each other (Martinez-Reyna et al. 2001, Zhang et al. 2011).

Grower decisions can also help maximize switchgrass yields. One of these decisions is harvesting date. Final harvests are generally recommended after the first frost to assure the maximum amount of nutrient movement to the roots (Vogel et al. 2002, McLaughlin and Adams Kszos 2005, Parrish and Fike 2009). Another method of maximizing yield is delaying flowering, as once flowering occurs biomass accumulation slows. Switchgrass utilizes photoperiod to cue its flowering (Benedict 1940), which is often tied to where cultivars originate, thus allowing cultivars with southern origins to produce more biomass when moved northward as they remain vegetative for longer (Newell 1968, Vogel 2004). While each cultivar behaves differently, it is a general rule that cultivars are best not moved more than one USDA Plant Hardiness Zone north of the area where it originated (Vogel 2004). It is likely that selecting an appropriate cultivar for both the region and the specific area will be key in increasing switchgrass yield (Vogel et al. 2002, McLaughlin and Adams Kszos 2005, Parrish and Fike 2009).
2000, Parrish and Fike 2005, Parrish and Fike 2009). For example, in a modelling experiment, while lowland cultivar Alamo had higher and more stable yields in the Southern US, upland cultivar Cave-In-Rock had higher and more stable yields in northern regions (Song et al. 2015).

Selective breeding will increase yields, but it will possibly also increase susceptibility to pests and disease in switchgrass. In experiments on five commercially available cultivars representing a range of breeding history, it was found that highly-selected, productive cultivars were both preferred hosts for aphids and more likely to contract viruses spread by aphids (Schrotenboer et al. 2011). The exact traits linked to this are being investigated, with biomass accumulation rate was correlated with virus susceptibility while foliar digestibility is not (Schrotenboer et al. 2011).

Pests of switchgrass may also respond differentially to cultivars with different ecotypes and breeding histories. Studies on consumption rates and the potential for significant damage have been conducted on a few herbivores. Work conducted on a common grassland grasshopper, the red-legged grasshopper (*Melanophus femurrubrum* De Geer), in Nebraska found that grasshoppers consumed six times more soybean on average than switchgrass, and it was concluded that only incredibly high grasshopper numbers could lead to yield reductions (Mustafa 2013). Feeding trials comparing corn and switchgrass consumption by armyworms (*Mythimna unipuncta* Haworth) suggested that the Kanlow cultivar of switchgrass had some chemical defenses that protected it against feeding, but concluded switchgrass could be an important alternate host for armyworm in the spring, allowing it to then move onto annual crops (Prasifka et al. 2011a). Another lepidopteran pest capable of feeding on switchgrass is the fall armyworm (*Spodoptera frugiperda* JE Smith). Initial tests found that fall armyworm was capable of surviving through pupation on switchgrass, with survivorship rates being very similar to those
seen on alternate hosts already available, such as Bermuda grass (*Cynodon dactylon* L.) (Prasifka et al. 2009). Corn is preferred over switchgrass by the fall armyworm (Prasifka et al. 2009, Nabity et al. 2011), and the difference in preference and the lower larval growth rates observed have been tied to the toughness of switchgrass leaves (Nabity et al. 2011). Another element of switchgrass linked to fall armyworm growth rates is leaf nitrogen levels, as it is seen as a limiting factor in growth (Nabity et al. 2011, Nabity et al. 2012). Studies where nitrogen levels are increased in switchgrass resulted in increased feeding and conversion efficiency in fall armyworm, suggesting that both breeding and management practices could make switchgrass more palatable (Nabity et al. 2012). Another element of breeding that could increase fall armyworm feeding is silica content, as silica makes conversion of biomass to fuel more difficult, though lower silica levels also makes it easier for caterpillars to assimilate plant material (Nabity et al. 2012). The effects of breeding have already been between cultivars, as Dowd and Johnson (2009) found different feeding rates by fall armyworm in six cultivars bred for forage. Feeding tests on plant clones have suggested lignin could be an important defense, but also noted that switchgrass with low lignin levels still avoided herbivory (Dowd et al. 2013). A small-scale experiment which examined fall armyworm survivorship on wild switchgrass versus switchgrass cultivars found mortality rates were variable (and occasionally high) on wild type switchgrass (Dowd and Johnson 2009).
**Herbivores and Natural Enemies in Switchgrass**

Monocultures of grasses with low genetic diversity are frequently more susceptible to pest outbreaks (Tscharntke and Greiler 1995), and switchgrass is likely to be no exception. Both specialists and generalist herbivores are known to attack switchgrass. Yield reductions from these pests may be a result of direct herbivory or via vectoring of plant disease.

**Switchgrass-Specific Pests**

As switchgrass production has become more widespread in the US, several switchgrass-specific pests have emerged. Stem-boring caterpillars (Lepidoptera) from various families are capable of feeding on switchgrass (Prasifka et al. 2011b). The biggest species of concern is the switchgrass moth (*Blastobasis repartella* Dietz), which is capable of causing tiller death in the US (Prasifka et al. 2010, Calles Torrez et al. 2013). In one study, *B. repartella* was common in switchgrass stands older than five years (Prasifka et al. 2011b), while other work suggests it infests different cultivars at the same level (Calles Torrez et al. 2013). In the Northern Great Plains its lifecycle is becoming better understood (Calles Torrez et al. 2014). Boe and Gagné (2011) identified the switchgrass gall midge (*Chilophaga virgate* Gagné) as a pest that is capable of infesting switchgrass tillers and reducing seed set and yield. It is most active in the early summer and infestation rates appear to differ based on phenology (Calles Torrez et al. 2014). The midge has been observed in South Dakota, Minnesota, Nebraska, Illinois, Oklahoma, New York, and New Jersey, though the potential full range is unknown (Calles Torrez et al. 2014).
Generalist Herbivores

A variety of generalist herbivores have the potential to feed on switchgrass, and thus reduce biomass yields. Potential pests of switchgrass include grasshoppers (Orthoptera: Acrididae) and Japanese beetle (*Popillia japonica* Newman) (Vogel 2004, Malmstrom et al. 2011). Japanese beetles are a problem for many crops (Potter and Held 2002, Szendrei and Isaacs 2006), and switchgrass is likely susceptible to both root and leaf herbivory (Malmstrom et al. 2011). Sweep samples taken over three years in South Carolina found high numbers of leafhoppers (Cicadellidae), seed bugs (Lygaeidae), and katydids (Tettigonidae), which led researchers to suggests that these herbivorous families could represent pests (Holguin et al. 2010). Work in Germany during summer months found thrips to be a problem under warm and dry conditions (Gottwald and Adam 1998). A two-year study of insects in both the soil and switchgrass canopy found high numbers of leafhoppers, grasshoppers, and thrips, and suggested grass flies (Diptera: Chloropidae) could be an important specialized pest (Schaeffer et al. 2011).

Other generalist pest species of switchgrass are a concern for their ability to vector disease. These include aphids (Hemiptera: Aphididae) and leafhoppers (Homoptera: Cicadellidae). Burd *et al.* (2012) found corn leaf aphid (*Rhopalosiphum maidis* Fitch), yellow sugarcane aphid (*Sipha flava* SA Forbes), and to a lesser extent the bird cherry oat aphid (*Rhopalosiphum padi* L.) as capable of establishing on some cultivars of switchgrass. The first two are of special concern as they are capable of spreading strains of the *Barley yellow dwarf viruses*, a group of Luteoviruses that manifest themselves in the phloem of the plant (Miller and and Rasochová 1997). The leafhopper *Haplaxius ovatus* (Ball) demonstrates how new species may emerge in higher numbers as switchgrass becomes more widespread (Wheeler and Wilson 2014), while *Graminella aureovittata* (Sanders & DeLong) has emerged as a vector of a
novel switchgrass virus, *Switchgrass Mosaic Virus*, which is capable of infecting switchgrass stands at high rates (Agindotan et al. 2013). The presence of vectors of debilitating viruses raises landscape-scale concerns as switchgrass could act as a virus reserve in the landscape, allowing for persistent infection of other cereal crops, such as wheat (Malmstrom et al. 2011, Burd et al. 2012).

*Natural Enemies*

A variety of natural enemies are likely to colonize switchgrass and potentially contribute to pest suppression. These include taxa that are common in soil and litter as well as foliar foraging natural enemies. Ground beetles (Coleoptera: Carabidae) are a diverse group of generally predacious beetles that are often numerous in grassland and agricultural settings (Holland 2002). In a study comparing sweetgrass, corn, and switchgrass, ground beetle captures were higher in switchgrass and corn, though carabid species richness was highest in the switchgrass (Ward and Ward 2001). Other groups of importance are staphylinids (Coleoptera: Staphylinidae) and ground-dwelling spiders (Aranae). Herbivore suppression in the switchgrass canopy is likely to come from the pool of natural enemies commonly seen in cereal crops. In the US, these include a variety of ladybird beetle species (Coleoptera: Coccinelidae), hemipterans from the families Nabidae and Anthocoridae, as well as the larva of lacewings (Neuroptera: Chrysopidae and Neuroptera: Hemerobiidae) and syrphid flies (Diptera: Syrphidae) (reviewed in Brewer and Elliott 2003). Parasitoids also exist for a variety of pests, such as aphids and leafhoppers (Waloff 1975, Tscharntke and Greiler 1995, Schmidt et al. 2003). Parasitoids have also been discovered for some of the switchgrass-specific pests, such as the switchgrass gall
midge (Calles Torrez et al. 2014). A variety of web-building spiders could also play an important role (Riechert and Lockley 1984).

Factors Impacting Pest and Natural Enemy Communities

An increase in perennial grasslands on the landscape could lead to increased landscape diversity, and could support biocontrol both within switchgrass and potentially in neighboring crops (Landis and Werling 2010). In a study that compared natural enemies (Coccinelidae, Anthocoridae, Syrphidae, and Chrysopidae) in three bioenergy crops – corn, switchgrass, and mixed prairie – it was suggested that perennial grasslands had the potential to host natural enemies that were less common in corn, though it is important to note different families responded to different landscape factors (Werling et al. 2011a). Studies on pollinators have suggested that an increase of perennial grasslands in the landscape will lead to higher bee abundance and a more diverse community of bees, allowing for stable pollination services (Bennett and Isaacs 2014).

The addition of switchgrass to the landscape will also increase landscape diversity, which has been associated with benefits for many beneficial insect groups. More complex landscapes are often associated with increased natural enemy abundance and diversity as well as increased pest suppression (Bianchi et al. 2006, Veres et al. 2013). Generalist natural enemies tend to respond to various scales of landscape complexity, while specialists respond at much smaller scales (Chaplin-Kramer et al. 2011).

How switchgrass is planted in regards to stand diversity will also affect the level of insect-mediated services at the landscape scale. Switchgrass could be established as a monoculture, or as a polyculture with other native grasses and forbs. A meta-analysis of
pollinators and natural enemies at different levels found that plant diversity at the local level had positive effects on both pollinators and natural enemies and that the more complex landscape was also positive for both groups (Shackelford et al. 2013). Individual studies have noted that natural enemies are more common in switchgrass and mixed prairie than in corn (Werling et al. 2011a). Other studies that examined biomass as a measure of arthropod abundance as well as diversity have found both measures to be the highest in mixed prairie, with intermediate levels in switchgrass, and the lowest levels in corn (Robertson et al. 2012a). Studies of high diversity polycultures grown with realistic management techniques for wide-scale production did not find that biomass production was higher than that of switchgrass monocultures (Dickson and Gross 2015), meaning that the value of these ecosystem services would need to be dramatic to make implementation practical. More simple mixtures are capable of maintaining or increasing yields when placed strategically (Zilverberg et al. 2014).

Whether switchgrass is established as a mono- or polyculture, it will be a new landscape component with the potential to change ecosystem services across the landscape. Different natural enemies respond differently to surrounding landscape factors (Batáry et al. 2010, Werling et al. 2011a, Woltz et al. 2012), though more biocontrol is often associated with increased diversity in the landscape (Gardiner et al. 2009). This diversity will have spillover effects into other systems and likely be more sustainable over time (Tscharntke et al. 2007). The landscape will also likely impact the yield and its variability, making understanding the potential relationship important (Zilverberg et al. 2014).
**Thesis Objectives**

As dedicated bioenergy crops will likely be implemented across the US, it is important to investigate these systems to understand their impacts in agricultural landscapes. Switchgrass is currently considered a promising biomass crop for the Midwestern US. Both the cultivars selected for production and the planted stand diversity will likely impact switchgrass susceptibility to herbivory and potential for natural enemies to provide biological control. The overall goal of this research is to better understand how switchgrass cultivar, especially in regards to ecotype, influences insect herbivores, then to expand to consider cultivar, ecotype, and planted stand diversity in regards to herbivore and natural enemy communities, and ultimately biological control, in the field. This information can inform best practices for establishing switchgrass to maximize ecosystem services. To accomplish this, I have two investigations:

1. Investigate generalist herbivore fall armyworm (*Spodoptera frugiperda*) establishment, consumption, and life history traits when fed diets of different switchgrass ecotypes and cultivars

2. Characterize the community response of both herbivores and natural enemies to different switchgrass cultivar, ecotype, and cropping system, and link this to a measure of biological control services in the field

This work is part of a USDA NIFA-funded project “Control and Mitigation of Generalist Pests in Perennial Grass-Dominated Bioenergy Landscapes.”
CHAPTER 2: EFFECT OF SWITCHGRASS ECOTYPE AND CULTIVAR ON ESTABLISHMENT, CONSUMPTION LEVEL, AND DEVELOPMENT OF A GENERALIST HERBIVORE
Abstract

As US energy policy increases interest in second-generation biofuels, perennial crops are likely to be called upon to help meet demands. Switchgrass, a native perennial grass, is a strong candidate as it provides both high biomass yields and many ecosystem services. Currently, switchgrass has only minor pest problems, but as it is bred more intensely for biofuel production and comes to occupy more acres, this may shift. To investigate how pest preference of generalist insects may vary by switchgrass ecotype and cultivar, I performed feeding trials with both neonate and late instar fall armyworm larvae to investigate how switchgrass affects establishment, consumption levels, and life history traits. In neonates, switchgrass did not increase mortality, though it was fed upon less than corn, and lowland ecotypes were fed upon less than upland ecotypes. In late instar larvae, which do a majority of the larval feeding, ecotype and cultivar differences emerged, with lowland ecotypes having lower feeding and trend toward increased time to pupation and ultimately lighter pupae. The outlier was Trailblazer, an upland cultivar that was fed upon less than other upland cultivars. These results suggested that there are differences in pest susceptibility between cultivars and ecotypes, which can be used in breeding and planting decisions.
Introduction

Shifts in US government energy policy in the last decade have led to a rapid increase in biofuel production (Bang 2010). This increase was driven in large part by the 2005 Renewable Fuel Standard (RFS), which called for increasing levels of renewable sources of domestic transportation fuels (Schnepf and Yacobucci 2013). Under the RFS, renewable fuels initially came from corn grain ethanol and biodiesel from soybean. While these sources were high-yielding, alone they are likely unsustainable and unable to fully meet fuel demands (Wang et al. 2011, Timilsina 2013). This reasoning led to interest in “second-generation” biofuels, derived from the sugars present in lignocellulosic feedstocks including crop residues (e.g. corn stover or wheat straw) or dedicated biomass crops (e.g. poplar and switchgrass) (reviewed in Simmons et al. 2008). While the conversion technology is still being developed, these sources are appealing as they can grow in a variety of circumstances (McKendry 2002, Varvel et al. 2008) and provide numerous ecosystem services (McLaughlin and Walsh 1998, Fike et al. 2007).

Switchgrass (*Panicum virgatum* L.) is a C4 grass native to central and eastern portions of the United States that has the potential to be grown as a biomass crop across large areas (Casler 2012). Switchgrass is an ecologically important prairie species that has also been developed as a forage crop, and later was designated as a model bioenergy crop (Wright et al. 1993). The positive attributes of switchgrass include a large native range (Parrish and Fike 2005), effective use of nutrients (McLaughlin and Walsh 1998), and high biomass yields across many years, even on marginal lands (Fike et al. 2006a, Schmer et al. 2008). Another benefit is that switchgrass is generally resistant to pests and herbivory, although when planted as monocultures grasses may become more susceptible to pest outbreaks (Tscharntke and Greiler 1995). Indeed, as more switchgrass has been planted as part of breeding efforts, novel pests have emerged (Prasifka et
al. 2010, Calles Torrez et al. 2013, Calles Torrez et al. 2014). As switchgrass undergoes selection to develop high-yielding varieties, selection for increased growth rate may lead to fewer plant defenses (Herms & Mattson, 1992). Other insects which may become pests of switchgrass include thrips (Thysanura) (Gottwald and Adam 1998), grasshoppers (Orthoptera) (Casler et al. 2004), aphids (Aphididae) (Bradshaw et al. 2010, Schrotenboer et al. 2011, Burd et al. 2012), leafhoppers (Cicadellidae) (Holguin et al. 2010), and lepidopteran larvae (Prasifka et al. 2009, Prasifka et al. 2011b, Calles Torrez et al. 2013).

An important factor tied to switchgrass growth rates is ecotype, a term used to differentiate between forms in a species adapted to different environmental conditions (Gregor 1944, Turrill 1946). In switchgrass, two major ecotypes exist: upland and lowland (Porter Jr 1966, Newell 1968, Brunken and Estes 1975, Parrish and Fike 2009). Switchgrass ecotypes vary in appearance, with upland ecotypes having thinner, more numerous shoots and thin green leaves, while lowland varieties typically have a blue-hue throughout, rough leaves and fewer, but thicker, tillers (Porter Jr 1966, Cortese et al. 2010, Zhang et al. 2011). Generally, upland switchgrass is tetraploid and lowlands are octoploid, although there are exceptions (Brunken and Estes 1975, Martinez-Reyna et al. 2001, Zhang et al. 2011). Lowland ecotypes are generally higher yielding but have poor winter survival in northern regions (Lemus et al. 2002, Alexopoulou et al. 2008, Wullschleger et al. 2010). Uplands types are adapted to dry areas in higher latitudes (Casler et al. 2004). How insect pest activity and damage may interact with ecotype is poorly known as previous work on herbivory in switchgrass has focused on one ecotype at a time (Dowd and Johnson 2009, Prasifka et al. 2009, Prasifka et al. 2011a).

One potential pest of switchgrass is the fall armyworm (FAW) (*Spodoptera frugiperda* J.E.Smith), a generalist noctuid pest whose larvae feed on over 60 plant species, with a noted
preference for grasses (Poaceae) (Luginbill, 1928). In the US, FAW overwinters at southern latitudes and adults move northward over the growing season aided by air currents and storms (Alton 1979, Flanders 2011). In the northern US, FAW usually has low population numbers in the late summer, and is an occasional sweetcorn pest (Flanders 2011, Bohnenblust and Tooker 2012). As FAW is a generalist pest with previous work conducted on cultivar preferences in multiple systems, including switchgrass, it is an ideal model to investigate how cultivar may impact herbivores. Additionally, widespread planting may make this crop susceptible to this pest.

The effect of different cultivars of host plants on FAW has been investigated for some hosts. For example, larvae fed exclusively on non-preferred cultivars of peanuts over subsequent generations showed extended larval development time, with increased mortality in larval and pupal stages (Leuck and Skinner 1971). In an experiment that measured FAW consumption and development in wild grasses, both measures varied widely across species (Pencoe and Martin 1981). In Bermuda grass, feeding on less-susceptible varieties led to increased FAW pupal mortality, which increased in the following generations fed on the same hosts (Leuck and Skinner 1970). However, larval development and leaf area consumption in newer, heat-resistant cultivars of creeping bentgrass and Bermuda grass did not vary (Hong et al., 2015). Cultivar effects of switchgrass on FAW are less well known. One study has shown that FAW can survive through pupation on switchgrass at rates similar to that of Bermuda grass (Prasifka et al. 2009), a host on which FAW is a pest (Leuck et al. 1968, Prasifka et al. 2009). In another study, Dowd and Johnson (2009) showed that across six upland cultivars neonate FAW mortality was minimal and leaf consumption levels varied by cultivar.

Here I use the FAW as a model to investigate how a potential generalist chewing herbivore may respond to different switchgrass cultivars. In particular, I was interested in how
switchgrass ecotype may impact herbivory levels and life history. To test this I selected 16 cultivars representing a range of upland and lowland ecotypes and breeding histories to determine rates of neonate establishment and herbivory in contrast to corn (*Zea mays* L.), a known host plant. I then selected a subset of eight cultivars to test the developmental rates and survival of late instar FAWs. All feeding trials were conducted in the lab using field grown switchgrass leaves collected from established (second year) plants. My hypothesis was that lowland ecotypes would be less favorable hosts due to their leaf characteristics, and my prediction was that the characteristics of lowland ecotypes would decrease feeding, thus increasing mortality and slowing larval development.
Materials and Methods

Plants

Switchgrass plants for all tests (Table 3) were established from seed and transplanted into the field in spring of 2014. Seeds were cold-stratified in distilled water at 4°C and germinated in X-72PS cell plug trays (Landmark Plastic Corp, Akron, OH) grown under high light conditions (16:8 day length) in a 26°C plant growth chamber for two weeks. Trays were then moved to a greenhouse until seedlings were approximately 0.3m tall, and were then moved into an outdoor courtyard to acclimate to outdoor conditions for one week. In mid-June, 2014 plants were transplanted to a field at the Michigan State Entomology Farm (East Lansing, MI) with a fine Marlette sandy loam soil type. The field was arranged as randomized complete block design with each cultivar represented by two plants in 1m² plots in each of four blocks. Plots were separated by 1m in all directions by a buffer of mown turf grass. Within all plots, weeds were controlled by a combination of hand weeding and hand-wand applied herbicide (glyphosate). Plots were fertilized each spring at a rate of 50kg/ha of nitrogen (Thelan and Pennington, personal communication). Corn, a known host of FAW, was used as the control; I used the sweet corn variety “Golden Bantem” (Burpee Seeds, Warminster, PA). Corn plants were established in DSQVP45PFD pots (Dillen Products, Middlefield, OH) in plotting soil in the greenhouse in May 2015; they were then transplanted into Classic 800s Pots (Nursery Supplies Inc., Chambersburg, PA) and moved into an outdoor courtyard where they were watered daily. Leaf material was harvested from all plants on the morning of each trial and subsequently as needed to replenish feeding dishes.
Insects

FAW eggs (corn strain) were obtained from French Agricultural Research Inc. (Lamberton, MN) and reared on FAW diet (Southland Products Incorporated. Lake Village, AR). All rearing and experiments took place in an insect rearing room with 14:10 L:D day length and an average temperature of 25°C.

Neonate Establishment and Feeding Trial

The establishment of FAW neonates on switchgrass and corn was tested in a 48h lab no-choice trial, based on the methods of Dowd and Johnson (2009). Sixteen switchgrass cultivars were tested in this trial (Table 3) against corn as a control. The uppermost, fully-expanded leaf from a healthy tiller was clipped in the field and transported to the lab in a cooler. In the lab, 2cm segments were cut from the center of the leaves and placed in 10cm Petri dishes with filter paper moistened with 30ml of DI water. Ten neonates were randomly selected from the over 30 concurrently hatching egg masses and transferred into Petri dishes using an artist’s paintbrush. The sides of the dishes were then individually wrapped with parafilm and secured with a rubber band to prevent leaf desiccation and neonate escape. There were 15 replicates (dishes) per cultivar, blocked by the time at which neonates were added. At 24 and 48h, dishes were assessed for neonate mortality, with neonates categorized as alive/feeding (on leaf and responsive to a touch stimulus), moribund (alive but moving erratically, or slow to respond to stimulus), wandering (on filter paper or Petri dish lid and responsive to stimulus), dead (discolored and unresponsive to stimulus), or vanished (potentially escaped or cannibalized). At 48h, all leaves were removed and leaf area consumed was determined (see details below).
Late Instar Feeding Trial

A majority of food consumption by FAW larvae occurs in the final two instars (Alton 1979, Flanders 2011), and a non-preferred diet can slow development (Leuck and Skinner 1970, Leuck and Skinner 1971). To test how different switchgrass cultivars influenced these factors in FAW, a feeding trial with late instar larva was performed based on the methods of Prasifka et al. (2011a) and Nabity et al. (2011). Eight switchgrass cultivars were tested against a standard FAW diet and corn as controls (Table 3, highlighted). Leaves were harvested from healthy plants as described in neonate methods. Leaves were cut into 5cm segments, with four to five segments added to each dish. An equal amount of corn or FAW diet was added to control dishes. Larvae for this trial were previously grown from neonates for nine days in 60ml plastic cups containing 1cm of diet. After nine days, larva were moved into empty 60ml cups and starved for 24h, removed, and individually weighed. Based on a visual assessment of head capsule width, larvae ranged from late 3rd to early 5th instar and weights ranged from 0.001g to 0.005g. Larvae were blocked by starting weight and individually placed into dishes described above. Leaves were replaced ad libitum, with all leaves changed every two to three days. As larvae entered the non-feeding pre-pupal stage (Luginbill, 1928), leaf replacement halted. After a larva molted to a pupa, it was individually weighed, placed in a 60ml cup with a small amount of diet to provide moisture, and held in the growth room and checked daily for survival and adult emergence. Voucher specimens of arthropods collected in this study are kept at Michigan State University’s A.J. Cook Arthropod Research Collection.
**Image Processing**

All leaves removed were scanned to determine leaf area consumed. Leaf consumption was measured using scanned images analyzed with ImageJ, modified from O'Neal et al. (2002). Leaves were scanned in 24-bit color and 300dpi, with a ruler included to set the scale of the image (~114 pixels/cm) using an Epson Perfection V39 scanner. Images were edited with the ImageJ’s paintbrush tool to create clear borders between remaining leaf area and any consumed areas. The threshold tool was then used and adjusted until the unconsumed leaf area was highlighted, then the magic wand tool was used to highlight these areas. The measurement function was then used to obtain the leaf or consumed area in cm². For the neonate trial, herbivory was minimal, so the leaf borders and herbivory could easily be measured from one image. For the late instar trial, the scan of the area before consumption was subtracted from the area of the leaf after feeding. For one time period of two dishes (Kanlow N1 Syn 2 block 14, Performer block 9) a final image was not taken, so an estimation based on photographs from the time period were used.

**Statistical Analysis**

All response variables were modelled using linear or generalized linear mixed models (R 3.0.3, R Development Core Team 2014), followed by chi-square analyses of deviance to determine differences between treatments. Count data was modeled using a Poisson error structure (neonate mortality, days to pupation); all other measures met normality assumptions, so a Gaussian error structure was used. Final model structure was determined using AIC (Burnham and Anderson 2004). For the older larvae, start weight was used either as a model factor (days to pupation, total consumption, and days to pupation) or a model offset (average consumption per
day), capturing variance represented by starting with larvae of different weights. Start weight varied among larvae, and this variation was accounted for in the model when performing the analysis of deviance and in post-hoc tests by using residual data sets that removed variation due to start weight. In both experiments, including the blocking variable in the model structure did not substantially improve model fit according to AIC, so it was not used in analysis. Across all measures, analyses were performed on ecotype and cultivar, as both were of interest and captured variation in different ways. The ecotype model included upland and lowland ecotypes, as well as the corn and/or diet control category. When significant deviances were detected, I compared treatments using paired t-tests that had been Holms-adjusted for multiple comparisons (Aickin and Gensler 1996).
Results

Neonates

Mortality of neonate larvae was minimal, with an average of 1 neonate dead per dish (Table 4). There were no significant differences by ecotype (lowland, upland, and control) ($\chi^2=104.03$, df=2, $p=0.85$), or cultivar ($\chi^2=98.74$, df=16, $p=0.991$), although in both models mortality was significantly higher at 48 than 24h ($p<0.001$). Cannibalism was directly observed, but it could not be quantified as some neonates were observed to escape dishes and the presence of head capsules could not be reliably attributed to cannibalism or molting. In some non-preferred cultivars, extensive neonate wandering was evidenced by trails in the condensation which occurred on the Petri lid.

Overall, neonates consumed only small amounts of switchgrass, with total consumption ranging from 0.006cm$^2$ (in Nebraska Summer Late Maturation germplasm) to 3.13cm$^2$ (in corn). There was a significant effect of ecotype or control treatments on consumption levels ($\chi^2=10.91$, df=2, $p<0.001$), with lowland ecotypes less consumed than upland ecotypes ($p<0.001$) and corn ($p<0.001$), and upland ecotypes having lower levels than corn ($p<0.001$) (Figure 1). Consumption levels across individual cultivars varied significantly ($\chi^2=9.36$, df=16, $p<0.001$), with all switchgrass cultivars fed on less than corn ($p<0.05$) and some cultivars varying from each other. Lowland cultivars (BoMaster, EG 1102, Kanlow, Kanlow N1 Syn 2, Alamo, EG 1101) and upland cultivar Trailblazer were fed on less than Cave-in-Rock ($p<0.05$) (Figure 1).
**Late Instar Larvae**

Overall, there were no significant differences in the mortality of older larvae by ecotype ($\chi^2=0.13$, $df=2$, $p=0.92$), with mortality in cultivars ranging from 0.06% in Cave-in-Rock and Summer to 0.26% in Kanlow. The amount of larval mortality was similar in the control and experimental treatments (Table 4).

Minimum days to pupation for individuals in this experiment was 19 (Shawnee), while maximum days required was 38 (Trailblazer). Analysis of deviance on the ecotype model revealed no differences by ecotype ($\chi^2=40.73$, $df=2$, $p=0.054$) (Figure 2), though there were differences among starting weights ($\chi^2=26.50$, $df=1$, $p=0.002$). In the cultivar model, differences were not present by cultivar ($\chi^2=36.10$, $df=9$, $p=0.315$) (Figure 2), and again starting weights varied ($\chi^2=20.917$, $df=1$, $p<0.001$). There was a tendency towards switchgrass slowing development when compared to corn and diet treatments, and generally larvae fed Trailblazer took the longest to reach pupation (Figure 2).

Overall, late instar FAW larvae consumed a minimum of 56cm$^2$ (corn) and a maximum of 240cm$^2$ (Shawnee) before reaching pupation, with mean leaf area consumption across all treatments at 137.3cm$^2$. There were significant differences detected between both ecotype ($\chi^2=100077$, $df=2$, $p=0.001$) and start weight ($\chi^2=96400$, $df=1$, $p=0.04$). Post-hoc tests revealed differences in total leaf area (cm$^2$) consumed between lowland and upland ecotype ($p=0.002$) but no differences between either lowland or upland switchgrass and the corn control ($p=0.17$ and $p=0.44$ respectively) (Figure 2). Lowland switchgrass was fed upon at lower levels than upland ecotypes (Figure 2).

When total leaf consumption was examined by cultivar, there were significant differences present among treatments ($\chi^2=87941$, $df=8$, $p<0.001$) and with starting weight ($\chi^2=84573$, $df=1$, $p=0.04$).
Post-hoc analyses revealed differences between some of lowland and upland cultivars, with Kanlow consumed less than Kanlow and BoMaster consumed less than Cave-In-Rock and Shawnee, which were comparable to corn (Figure 3). At the start of the study, larvae generally did not consume the leaf midrib, although as larvae entered the sixth instar this portion of the leaf was commonly consumed. Smaller larvae did not typically eat through the leaf, instead scraping its upper tissues while leaving the epidermis on the opposite surface.

The range of average leaf area consumed per day until pupation was 2.2cm² (corn) to 9.4cm² (Cave-in-Rock). In models that used ecotype and starting weight as an offset, there were differences in average daily consumption by ecotype ($\chi^2=201.04$, $df=2$, $p<0.001$). Post-hoc tests reveal differences between lowland ecotypes and both corn ($p=0.002$) and upland ecotypes ($p=0.003$), while similar amounts of corn and upland ecotypes were eaten ($p=0.10$) (Figure 4). When examining variation by individual cultivar, analysis of deviance found significant differences ($\chi^2=166.05$, $df=8$, $p<0.001$). BoMaster, Trailblazer, Kanlow, and Performer experienced lower daily consumption than corn, Shawnee, and Cave-in-Rock ($\alpha=0.05$) (Figure 4).

Individual pupal weights ranged from 0.07g (Performer) to 0.31g (diet). Pupal weights varied by both ecotype ($\chi^2=0.12$, $df=2$, $p<0.001$) and cultivar ($\chi^2=0.10$, $df=9$, $p<0.001$). Both upland ($p<0.001$) and lowland ecotypes ($p<0.001$) had lower pupal weights than the control treatments, and FAW fed lowland ecotypes pupated at a lower weight than those fed upland ecotypes ($p=0.038$) (Figure 5). Analysis of deviance found differences in starting weight ($\chi^2=0.10$, $df=1$, $p<0.001$). By cultivar, pairwise t-tests suggested that all larvae fed switchgrass cultivars had lower pupal weights than those fed on diet, and Trailblazer, Kanlow N1 Syn 2, Performer, and BoMaster all had pupal weights significantly less than corn ($p<0.05$) (Figure 5).
Again, analysis of deviance results suggested starting weight varied by cultivar ($\chi^2=0.09$, $df=1$, $p<0.001$). Nearly all pupae successfully emerged as viable adults, with the exception of two fed on Performer and one fed on Kanlow N1 Syn 2.
**Discussion**

As switchgrass occupies more land area in the US, understanding how cultivars may vary with respect to the feeding and development of pest herbivores is important. In this study I used a generalist pest with a preference for grasses to investigate which cultivars are fed upon more to gain insight as to which may have pest problems. Overall, corn was preferred to switchgrass, and lowland switchgrass experienced reduced feeding and a trend towards slowed development when compared to upland ecotypes. The exception to this general rule was upland cultivar Trailblazer, where FAW preference and performance parameters exhibited response patterns more closely resembling lowland varieties.

**Neonates**

Neonates exhibited low rates of both mortality and feeding on switchgrass. In a prior study using a similar experimental design, Dowd and Johnson (2009) also saw low levels of mortality neonate on six upland cultivars (including Blackwell, Cave-in-Rock, Trailblazer), though they did note increased mortality on wild type switchgrass collected from roadways. My study included two “wild type” cultivars, High Tide, a germplasm release drawn from switchgrass population on the eastern seaboard, and a Michigan native switchgrass germplasm purchased from Michigan Wildflower Farms (Portland, MI). At 48 h, mortality on these varieties was comparable to all other varieties (0.10 ± 0.03 in High Tide and 0.12 ± 0.03 in Michigan Wildflower Farms). Although this study was by no means exhaustive, its results suggest that, at least among varieties tested, breeding efforts have not negatively impacted plant defenses.
A factor impacting mortality which I was unable to control for was cannibalism. Cannibalism occurs in FAW when neonates are present at high densities (Andow et al. 2015) or are limited to a non-preferred diet (Raffa 1987). I observed FAW neonates in the act of cannibalism only in switchgrass treatments, with multiple observations in Trailblazer. Cannibalism was not observed in corn treatments. These observations align with prior work on diet quality in FAW, which often see the highest growth rate on a preferred diet (here corn), and that cannibalism can support a higher growth rate than feeding on a non-preferred diet (Raffa 1987).

In regards to leaf consumption, neonate larvae preferred corn over switchgrass, and fed less on lowland ecotypes when compared to upland ecotypes. Neonates prefer the most tender parts of a leaf (Luginbill 1928), which was controlled for in this study by selecting the middle 2 cm of the uppermost fully expanded leaf, though the tenderness of the leaf could vary between cultivar. Different trends may be observed if this study was repeated to examine feeding closer to the whorl, as this is a preferred area when FAW feeds on corn (Capinera 2014). When comparing amounts eaten by neonates of older switchgrass plants, Dowd and Johnson (2009), they observed a range from approximately 2.98 cm² (Blackwell) to 4.54 cm² (Cave-in-Rock). This is a higher consumption level than in my study, though their study used first-year, greenhouse-grown plants, so the growth conditions of switchgrass likely contributed to part of this difference.

In FAW the first three instars generally cause less than 2% of total consumption (Alton 1979), so my neonate results reflect the establishment phase rather than direct economic damage. These results, when taken together, suggests that neonate FAW can survive on switchgrass, but their escape from sealed Petri dishes, cannibalism, and general neonate behavior of increased
movement and searching on less preferred diets (Eigenbrode et al. 1991, Zalucki et al. 2002), suggests that FAW are unlikely to stay on switchgrass if a more preferred host is available (Zalucki et al. 2002, Nabity et al. 2011). As of writing, it is unknown if FAW adults will oviposit on switchgrass, especially in a field settings, as non-preferred plant species FAW feed on in the lab may be ignored by ovipositing adults (Meagher et al. 2004). If switchgrass becomes a major crop at the landscape level, these behaviors may change, thus future work on oviposition and neonate behavior on plants in the field is warranted.

**Late Instar Larvae**

Increased development time and lower pupal weight observed for FAW on switchgrass is consistent with other studies addressing development on non-preferred diets (Ali et al. 1990) and to studies comparing development on switchgrass and corn (Nabity et al. 2011). Pupal weight in FAW has been linked to adult fecundity (Leuck and Perkins 1972). This suggests that regardless of cultivar, continuous feeding on switchgrass could have increased negative impacts across subsequent generations, which has been observed in other Lepidoptera fed non-preferred diets (Rossiter 1991, 1996). The tendency for slower development on switchgrass could also lead to an increase in predation and parasitism (Moran and Hamilton 1980, Price et al. 1980). For example, the increased movement elicited by non-preferred diets has been suggested to increase the exposure of FAW to potential parasites (Pair et al. 1986).

The amount of feeding, both average daily and total consumption, is consistent with other work on lepidopteran larvae on non-preferred diets. FAW fed diluted diet (food source with decreased nutritional value) have been observed to eat more to compensate for the lack of nutrients (Wheeler and Slansky 1991). This could explain the high amounts of feeding in Cave-
in-Rock and Shawnee, as these cultivars may be palatable but of decreased nutrient quality, causing the larvae to eat more to compensate. Other studies in Noctuid moths have suggested that increased feeding could lead to a buildup of toxic chemicals, though the similar levels of mortality across treatments suggest this was not a factor in this study (Slansky and Wheeler 1992). The feeding pattern is consistent with prior work. In an eudicot (*Castanopsis fissa*), the mid-rib is the toughest part of the leaf and the lepidopteran larvae avoided feeding on it (Choong 1996). This finding is in keeping with the observation that older larvae ate that part of the leaf last, and usually only if a majority of the other leaves had been consumed.

*Plant Factors Relevant to Both Larval Ages*

The most readily observable difference between upland and lowland ecotypes of switchgrass is leaf coarseness, with lowland ecotypes being generally tougher than uplands (Cornelius and Johnston 1941). In other insect species, young and tender leaves were fed upon more readily than tougher, older leaves, with continued feeding on tough leaves increasing wear on the insect mandibles, which can lead to fewer leaves consumed daily (Raupp 1985). A foliar factor linked to leaf toughness in grasses is silica content, which may confer plant defense against herbivores (reviewed in Reynolds 2009). Massey et al. (2006) compared feeding by two generalist herbivores (including one from the genus *Spodoptera*) across five grass species manipulated for high and low silica content. They found that silica made leaves more abrasive, which they linked to non-preference, slowed growth rates, and less efficient digestion in both insects. In a study that looked specifically at switchgrass grown in soils with varying levels of silica, it was found that the amount of silica in switchgrass leaves increased when grown in there was higher amounts of silica in the soil, and subsequently feeding by FAW slowed their growth.
yet this did not influence FAW consumption rates. Unfortunately, the cultivar under study was not reported, though location and information provided on the website their of seed source suggests an upland variety (Nabity et al. 2012). Silica levels are determined by abiotic factors (i.e. soil type, location of planting), though switchgrass variety may also have an impact. In a study that compared mineral concentrations in four upland (including Cave-in-Rock, Blackwell, and Trailblazer) and two lowland cultivars (including Kanlow), interactions between the population and location explained a majority of the variance in silicon (the main component of silica), though there was some evidence of ecotype and cultivar influence. In a majority of the tested locations, upland cultivars counterintuitively contained more silicon, though Kanlow also contained high levels, while the other lowland cultivar often contained the least. One goal of switchgrass breeding is to reduce silica content (Samson et al. 2005), as it can create ash (Baxter 1993), making it less favorable as a bioenergy feedstock. This may make switchgrass more susceptible to herbivory.

Another plant defense that has drawn attention is lignin, especially as a goal of switchgrass breeding is reducing lignin levels for ease of biofuel synthesis (Vogel and Jung 2001, Sarath et al. 2011). Prior work in switchgrass and fall armyworm suggested that lignin was an important defense for young plants, though this can be variable (Dowd et al. 2013). While I took no measurement of lignin, in comparing my herbivory results to studies of field grown switchgrass populations with estimates of lignin content via an acid detergent (Goering and Van Soest 1970). Lemus et al. (2002) found a range of lignin values in field grown plants in Iowa, though cultivars with high lignin in their study, such as Cave-in-Rock, were more preferred by FAW than cultivars with more middling lignin levels, such as Trailblazer and
Kanlow. This comparison is in keeping with the conclusions of Dowd et al. (2013), who proposed that lignin is likely only one of many plant defenses in switchgrass.

Another characteristic switchgrass has been bred for in the past is in-vitro dry matter digestibility (IVDMD) (Casler et al. 2002). This parameter estimates how digestible forage is to ruminant animals, with a higher value indicating higher digestibility (Tilley and Terry 1963). Cultivars in this study bred for higher IVDMD included BoMaster, Performer, Shawnee, and Trailblazer (Casler 2012). Trailblazer specifically was a cultivar bred for high IVDMD, utilizing tests on both sheep and cattle in its cultivar selection (Vogel et al. 1991), and it’s digestibility has (Anderson et al. 1988). Counter-intuitively, BoMaster, Performer, and Trailblazer were often consumed at lower levels (Figure 3, 4), though Shawnee was often more preferred according to these measures, with one of the highest mean consumption by older FAW larvae (Figure 4). While ruminant animals and insects have wildly different digestive systems, the compounds eliminated when measuring IVDMD would likely need to be broken down by both groups of organisms. These results suggest the IVDMD may not have a clear impact on insects and is a poor character for predicting insect pest preference.

Other researchers have suggested that switchgrass cultivars with increased chromosome number have increased plant defenses (Dowd and Johnson 2009). The present experiment included a wider range of cultivars than previously tested, representing both tetraploid and octoploid varieties, and no evidence supporting this assertion was found, as octoploid upland cultivars were more preferred and tetraploid lowland cultivars were generally less preferred across all measures. Summer, an upland tetraploid, was a cultivar that was fed upon more readily than many octoploid cultivars, further suggesting that something other than ploidy alone drives the different responses seen between ecotypes.
This study considered an expanded selection of cultivars that allowed for ecotype comparisons, which may be more useful for recommendations due to the amount of genetic variability within cultivars. Despite multiple rounds of selection, switchgrass cultivars still have high levels of genetic variation (Vogel et al. 2000, Casler et al. 2007), with one study finding that switchgrass cultivars and wild populations had comparable levels of genetic variation (Mutegi et al. 2014). This study highlighted that Blackwell, Kanlow, Summer, and Trailblazer were different than wild switchgrass populations (Mutegi et al. 2014), though other studies have found that the use of certain genetic markers cannot differentiate between wild switchgrass and domestic cultivars (including Blackwell, Cave-in-Rock, Shawnee, Summer, and Trailblazer) (Casler et al. 2007). This genetic variability inherent in switchgrass will also vary with the local environment (Parrish and Fike 2005). In my study, feeding the late instar FAW leaves from different plants was designed to examine a more cultivar-wide response as opposed to a plant specific response, hopefully leading to more broadly applicable results.

Future work should continue to investigate how breeding can be linked to pest preference, especially in regards to balancing plant defenses and characteristics desired for high biomass yields and easy conversion to biofuel. Major FAW pest concerns are likely only a reality in the southernmost regions of the US (Luginbill 1928, Alton 1979), so studies similar to this one should be tested on cultivars selected for southern regions (i.e. “Miami”) (USDA-NRCS Brooksville Plant Materials Center 2006). The reality of FAW and other pest invasion may also depend on switchgrass planting time and age of stand, as cultural practices for fall armyworm control involve early planting dates and fast maturing varieties (Capinera 2014), two practices not applicable in a C4 perennial biomass crop. The herbivory levels present in my study were low and likely not economically damaging, so studies similar to this should be conducted on
other chewing pests, as well as pests such as aphids, whose potential as disease vectors may make them the pest of highest concern.
Conclusions

The generalist herbivore FAW performed poorly on switchgrass regardless of instar according to multiple measures, suggesting that switchgrass overall is unlikely to experience major biomass reduction as a result of FAW damage. Within switchgrass, lowland ecotypes were consumed at lower rates and tended to reduce or slow FAW developmental measures. Lowland ecotypes are also often higher yielding, and may be favored if plant breeders are able to overcome the problems associated with moving into northern latitudes. Among ecotypes, there was variability in how individual cultivars performed. Trailblazer was an outlier among uplands, as across measures it seemingly performed like lowland ecotypes. As switchgrass breeding continues, work should be done to better understand the physiological differences among cultivars, so recommendations to growers can be based on an understanding of how plant characteristics will influence multiple measures, including pest preference.
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CHAPTER 3: INFLUENCE OF SWITCHGRASS CULTIVAR, ECOTYPE, AND PLANTED STAND DIVERSITY ON INSECT COMMUNITIES AND BIOLOGICAL CONTROL SERVICES
Abstract

Switchgrass (*Panicum virgatum* L.) is a perennial C4 grass that has been studied as a bioenergy crop. It is capable of producing high yields while supporting other ecosystem services, such as the biological control of pests by providing perennial habitat for natural enemies. To investigate the role of switchgrass ecotype and planted stand diversity on herbivores, natural enemies, and biocontrol services, a network of plots of switchgrass, alone and in combination with other grasses and forbs, was established across southern Michigan in 2012. In 2014 and 2015 the insect community of each plot was monitored using sweep net samples, and sentinel prey were used to measure the biological control service at corresponding times. While herbivore and natural enemy abundance varied by years, herbivores were generally more abundant in upland ecotypes and high diversity plantings of switchgrass. In 2014, natural enemies mirrored these trends in regards to abundance and Shannon diversity, though in 2015 only high diversity polycultures supported significantly higher abundance and diversity. The proportion of sentinel prey removed was more stable across years, with biological control consistently increasing over the season, and greater sentinel prey removal in upland ecotypes than lowland ecotypes. Overall, this study suggests that switchgrass production systems based on different cultivars, ecotypes, and planted diversity can alter insect communities and ecosystem services, and can inform the design of emerging cellulosic biomass cropping systems.
Introduction

The advent of cellulosic biofuels, due in large part to US government policy (US Congress 2007), has led to interest in how novel biomass crops may change agricultural landscapes. One crop with the potential to be used as biofuel feedstock is switchgrass (*Panicum virgatum* L.), a warm season grass that has emerged as a model bioenergy crop for large portions of the US (Wright and Turhollow 2010). Native from east of the Rocky Mountains to the Atlantic seaboard (Casler 2012), switchgrass has a large range of adaptation that provides significant genotypic and phenotypic diversity for breeders (Parrish and Fike 2009). Switchgrass balances high biomass yield across multiple years (Fike et al. 2006a), even on marginal lands (Schmer et al. 2008), with a variety other benefits, including the ability to withstand poor growing conditions (Parrish and Fike 2005), low requirements for agricultural inputs, and provision of wildlife habitat (McLaughlin and Walsh 1998, Meehan et al. 2010, Werling et al. 2014). These ecosystem services, especially if incentivized (Prager et al. 2012), are likely to aide in the long-term sustainability and profitability of switchgrass.

Among the many ecosystem services provided by switchgrass is biological control of herbivorous insects, a service estimated to be worth over four billion dollars annually in the US (Losey and Vaughan 2006). Switchgrass, as a native perennial grass, is likely to provide habitat for a large suite of natural enemies, and although there are currently relatively few key pests of switchgrass (Prasifka et al. 2010, Calles Torrez et al. 2014), this could change as switchgrass comes to occupy more acres. Biological control in switchgrass may help reduce herbivore abundance and damage, and may have the potential to increase or stabilize yields under increased pest pressure (Altieri et al. 2012, Bommarco et al. 2013). Biological control may also
help slow the spread of arthropod-vectored viruses both within switchgrass and between
switchgrass and important annual grasses, such as wheat (Schrotenboer et al. 2011, Burd et al.
2012, Stewart et al. 2015).

The biological control level mediated by arthropods will be determined by a variety of
factors. At the most basic level, biological control will be a function of the insect community,
both its herbivores, some of which are pests, and natural enemies (Kremen 2005, Bianchi et al.
2006). Switchgrass supports a variety of generalist herbivores, including grasshoppers
(Orthoptera: Acrididae) (Casler et al. 2004), aphids (Hemiptera: Aphididae) (Bradshaw et al.
2010, Burd et al. 2012), thrips (Thysanura) (Gottwald and Adam 1998), and leafhoppers
(Hemiptera: Cicadellidae) (Holguin et al. 2010). Some switchgrass specialists have also been
singled out for their potential to impact yields (Schaeffer et al. 2011, Calles Torrez et al. 2013,
Calles Torrez et al. 2014). Natural enemies present in switchgrass consist of the major groups
common in agro-ecosystems, including Coccinellidae (Werling et al. 2011a), Anthocoridae
(Hemiptera), Nabidae (Hemiptera), and the larvae of Syrphidae (Diptera) and lacewings
( Neuroptera) (reviewed in Brewer and Elliot 2003).

The mix of herbivores and predators in switchgrass stands will also be a function of both
landscape and within-field factors. In systems that incorporate bioenergy crops, increased
landscape diversity surrounding bioenergy crops has been associated with increased natural
enemy numbers (Werling et al. 2011a) and pest suppression (Werling et al. 2011b, Meehan et al.
2012). These landscape-level factors are often not under an individual grower’s control and may
shift as biofuel demands change the agricultural landscape. In-field factors can be partially
controlled by growers, and will consist of decisions on which switchgrass variety to plant and
whether to establish in monoculture or polyculture. Increased plant diversity within fields has
been associated with fewer herbivores, more natural enemies, and suppression of crop damage (Letourneau et al. 2011).

Switchgrass has two widely recognized ecotypes in the US, lowland and upland (Porter Jr 1966, Newell 1968, Brunken and Estes 1975, Parrish and Fike 2009, Lowry et al. 2014) (Figure 6A-B). Lowland ecotypes typically originate from southern latitudes and are well adapted to floodplains (Parrish and Fike 2005). They often have a blue tint, fewer stems, coarse leaves, and are generally high-yielding (Porter Jr 1966, Stroup et al. 2003, Vogel 2004, Cortese et al. 2010, Wullschleger et al. 2010, Lowry et al. 2014, Vogel et al. 2014); however winter kill is often a problem as lowland cultivars are moved north of their area of adaptation (Casler et al. 2004, Vogel 2004, Parrish and Fike 2005). Upland cultivars are adapted to somewhat dryer soils and northern latitudes. They typically produce a multitude of green, thin stems and leaves (Porter Jr 1966, Casler et al. 2012, Lowry et al. 2014). This ecotype is generally associated with the tallgrass prairies of the North Central US (Parrish and Fike 2009). Differences in switchgrass leaf characteristics (Cornelius and Johnston 1941) and growth form (Lemus et al. 2002) will likely have different impacts on different herbivores, as chewing herbivores and sucking herbivores respond differently to plant characters (Massey et al. 2006), and how these may influence natural enemies is not known. How individual cultivars of each ecotype will themselves influence arthropods is even less understood.

Beyond ecotype and cultivar, switchgrass stand diversity (i.e. established as monoculture or in polycultures) will also impact arthropod communities. Meta-analyses have suggested that insects respond positively to increased plant diversity (Letourneau et al. 2011, Shackelford et al. 2013), and several studies have shown that polycultures can increase natural enemy abundance, biological control, and ultimately yield (Altieri 1999, Tilman et al. 2006, Poveda et al. 2008).
However, other studies have questioned the practicality of diverse polycultures as many species are difficult to establish (Russelle et al. 2007, Zilverberg et al. 2014) and yield of the main crop may decline if its density is reduced in polycultures (Letourneau et al. 2011). The potential to increase multiple ecosystem services, including biological control, pollination, and wildlife habitat, may help justify the use of these polyculture systems to bioenergy production (Werling et al. 2014).

As biological control of herbivores may enhance the long-term sustainability and profitability of switchgrass, understanding how different factors impact this ecosystem service is important in deciding how to best implement large-scale switchgrass production. As part of a larger project, I tested how different switchgrass cultivars and stand diversity influenced natural enemy communities and the biological control services they may provide. I predicted that natural enemy communities and biological control would vary by ecotype given the aforementioned differences in form and physical characteristics between upland and lowland ecotypes. I also predicted that increased plant diversity would result in increased natural enemy diversity and abundance, leading to increased rates of biological control.
Materials and Methods

This study was conducted at 12 field sites across southern Michigan, selected to represent a variety of soil types and landscapes in which switchgrass may be produced (Figure 6A, Table 6). Each site was a minimum of 0.71 hectares converted from soybean cultivation and planted in the spring of 2012 to one of five switchgrass cultivars, selected to represent a range of switchgrass breeding histories, with both upland and lowland ecotypes represented (Table 7). Treatments consisted of each cultivar alone (monoculture), or one cultivar (Shawnee) seeded in combination with other grasses (low diversity polyculture) or grasses and forbs (high diversity polyculture) (Figure 7C, Table 8). Each site contained ten 20m x 20m plots (Figure 7B-C), with 3m or 6m aisles between plots. Three plots at each site were treatments not considered in this study (Figure 7C).

During the summers of 2014 and 2015, insect samples were collected from all plots in June, July, and August using sweep nets. A sample consisted of the insects collected in 30 total sweeps in the top 1/3 of the canopy, with one half of the sweeps taken along a transect 1/3 and 2/3 of the way through the plot in a North or South direction (Figure 7B). The samples were sorted and potential switchgrass herbivores and natural enemies were counted and identified with the level of taxonomic precision varying from order to species depending on the relevance of the taxa in question and the level of taxonomic identification possible (Table 9). Voucher specimens of arthropods collected in this study are retained at the A.J. Cook Arthropod Research Collection at Michigan State University, with the exceptions of hoppers and aphids, as they were all given to a collaborator for virus extraction.

To quantify levels of predation occurring in each treatment, sentinel egg cards were used (Werling et al. 2011b). Egg cards consisted of approximately 25 (2014) or 75-100 (2015) frozen
corn earworm (*Helicoverpa zea* Boddie) eggs glued to a 3cm x 5cm slip of cardstock. Initial egg number per card were counted and recorded on the cardstock and cards were paired based on similar initial egg numbers. Egg cards were deployed monthly in each plot during the summer season simultaneously as sweeps were performed (Figure 7B). Paired egg cards were deployed on the underside of 30.5cm² pieces of neutrally colored, corrugated plastic cardboard (Figure 8A). Egg cards were attached to the cardboard on opposite corners using pushpins (Figure 8A). One card was left exposed to natural enemies, while the other was protected by a medium sized Petri dish (60cm diameter) with a small (~2.54cm diameter) mesh window over the eggs. The boards were supported by a 1.2 m tall step-in fence post, with the board set at canopy height early in the season and at maximum height later in the season when the switchgrass was taller (Figure 8B). Egg cards were left in the field for 48h. In the lab, the cards were processed, noting the original number of eggs, number of eggs damaged, and final egg number.

All response variables were modelled using generalized linear models (R 3.0.3R Development Core Team 2014), followed by a Chi-square analysis of deviance which detected differences between factor levels. A binomial structure was used for models with proportional responses (egg cards), a Poisson structure for counts (abundances), and a Gaussian (normal) structure for Shannon diversity of natural enemy communities.

The model structure used to test all factors was constructed similarly, with the response variable $\mu$ modelled as the results of year (only in the case of egg cards), month, site, and factor of interest (treatment, ecotype, or planted stand diversity).

$$F(\mu) \sim \text{month} + \text{site} + \{ \text{Treatment, Ecotype, or Planted Stand Diversity} \}$$

$F(\mu)$ varied with model structure as described above (Jackman et al. 2012). Each model was subjected to an analysis of deviance to detect differences between levels for each model factor.
When the analysis of deviance detected significant differences among treatment levels, treatment differences were compared using t-tests that had been Holm Adjusted for multiple comparisons (Aickin and Gensler 1996). Because site and month effects were strong and had the potential to mask treatment differences, this post-hoc test was applied to a residual dataset that had been adjusted for variation due to site and month. For the herbivore abundance, three outliers were dropped from the BoMaster treatment for having extremely high aphid numbers that skewed distributions (i.e. greater than 2000 aphids captured), and all plots excluded from herbivore analysis were also excluded from natural enemy analysis. For egg cards, both 2014 and 2015 followed similar patterns across months and thus were analyzed together, so year was another factor included in the model.

Preliminary correlations were conducted between major natural enemy groups and the proportion of eggs removed in a site, and when no significant correlations were found the investigation was discontinued.

As part of the larger experiment a variety of other metrics were collected from each site. These included site characters, such as percent landscape cover, alate aphid arrivals (collected via suction trap), and soil type. In 2014, percent cover and species richness was measured for all plots, and estimates of yield were also made. This data will be incorporated into further analyses; for more information on this data contact project head Carolyn Malmstrom.
Results

Herbivores

A total of 11,542 herbivorous insects representing five taxa were collected in this study, with 8,984 collected in 2014, and 2,558 collected in 2015. Herbivore abundance was higher in 2014 than in 2015 (Table 10), and the taxa driving this abundance differed, with 2014 abundances driven by high numbers of grasshoppers, aphids, and hoppers, while in 2015 hoppers represented a large majority of the herbivores (Table 10). There were similar within-season patterns in herbivore abundance across years, with July having the lowest herbivore numbers and August having the highest (Table 10).

Because of the extreme compositional difference between the insect communities across years, I repeated the analysis on each of the years separately. Analysis of deviance results for all model factors are reported in Table 11. Herbivore abundance varied among treatments in 2014 by analysis of deviance, \( \chi^2 = 787.5, df = 6, p < 0.001 \); however no clear pattern emerged among treatments in post-hoc tests when variability due to month and site were accounted for (Figure 9). In 2015, abundance again varied among treatments \( \chi^2 = 1594.4, df = 6, p < 0.001 \), though as above, post-hoc analysis did not reveal clear patterns between treatments (Figure 9).

Across both years, upland ecotypes supported more herbivores than lowland ecotypes. Analysis of deviance detected that herbivore abundance differed by ecotype in 2014 \( \chi^2 = 3801.5, df = 1, p < 0.001 \), with lowland ecotypes supporting lower herbivore abundance than uplands (Figure 10). In 2015, patterns were similar: herbivore abundance varied among ecotypes \( \chi^2 = 1201.6, df = 1, p < 0.001 \), and again lowland ecotypes supported fewer herbivores than upland (Figure 10).
Finally, when comparing herbivore abundance by planted stand diversity, both 2014 and 2015 had differences emerge among levels of all model factors (Table 11). In 2014, there was variance among treatment levels of planted stand diversity ($\chi^2=2466.7$, $df=2$, $p<0.001$); however, post hoc tests revealed no significant differences among the three levels of diversity ($p>0.05$) (Figure 11). In 2015, planted stand diversity again had significant differences among levels ($\chi^2=633.97$, $df=2$, $p<0.001$), and post hoc tests revealed that the high diversity planting had significantly higher herbivore numbers than the monoculture ($p=0.011$) and low diversity polyculture ($p=0.011$) (Figure 11).

**Natural Enemy Abundance and Diversity**

A total of 3,270 natural enemies representing 20 taxa were collected (Table 5). Total natural enemy number varied across years, with 2,460 collected in 2014 and 810 in 2015. The most commonly observed taxa were parasitoid wasps, with 1,279 collected in 2014 and 247 collected in 2015. The only taxa whose numbers increased between the two years was syrphid flies, with 155 collected in 2014 compared to 199 in 2015. Overall, natural enemy abundance was much lower in 2015 than in 2014, with a mean of 10.60±0.73 natural enemies per sample in 2014 compared to 3.45±0.23 in 2015. Patterns of abundance across each season also differed. In 2014, the peak number of natural enemies occurred in August (14.38±1.76) with lower numbers in June (9.53±0.83) and July (7.71±0.73), while in 2015 I observed much lower numbers throughout the season, with slightly higher numbers in June (4.63±0.35) compared to July (2.60±0.32) and August (3.14±0.48) (Table 5). Because of this variability, years were analyzed separately.
Natural enemy abundance varied by treatment in 2014 and 2015, with many model factors containing significant levels of variance (Table 11). At the treatment level, in 2014 natural enemy abundance varied by treatment according to an analysis of deviance ($\chi^2 = 1471.8$, $df = 6$, $p < 0.001$). Post hoc tests detected differences between treatment levels, with Kanlow supporting fewer natural enemies than Shawnee with grasses ($p = 0.004$), Shawnee with grasses and forbs ($p = 0.024$), and Summer ($p = 0.018$) (Figure 12). In 2015 differences again emerged by treatment ($\chi^2 = 522.99$, $df = 6$, $p < 0.001$), with Shawnee with grasses and forbs supporting more natural enemies than Kanlow ($p < 0.001$), Shawnee ($p > 0.001$), and Cave-in-Rock ($p = 0.002$) (Figure 12).

Natural enemy abundance also varied by ecotype, with differences detected in 2014 but not 2015 (Table 11). In 2014, difference occurred by ecotype ($\chi^2 = 1021.8$, $df = 1$, $p < 0.001$), with lowland ecotypes supporting fewer natural enemies than upland ecotypes (Figure 13). In 2015, ecotype ($\chi^2 = 349.49$, $df = 1$, $p = 0.76$) did not significantly impact natural enemy abundance (Figure 13).

Natural enemy abundance varied with planted stand diversity, and other model factors contained variance (Table 11). In 2014, significant variance in natural enemy abundance was detected by an analysis of deviance ($\chi^2 = 529.46$, $df = 2$, $p < 0.001$), and post hoc tests revealed that low diversity polycultures had a greater natural enemy abundance than the Shawnee monoculture ($p = 0.038$) (Figure 14). In 2015, differences were again found ($\chi^2 = 249.99$, $df = 2$, $p < 0.001$), with post hoc tests revealing that the high diversity polyculture had significantly higher natural enemy abundance than either the low diversity planted stand ($p = 0.039$) and the Shawnee seeded monoculture ($p < 0.001$), and the low diversity polyculture had higher natural enemy abundance than the monoculture ($p = 0.039$) (Figure 14).
Across years, overall Shannon diversity of the natural enemy community differed, with 2014 having an average diversity of 0.97±0.03 and 2015 one of 0.49±0.03. In the model created for the study’s first year, analysis of deviance detected significant differences in natural enemy diversity by treatment ($\chi^2=49.38$, $df=6$, $p=0.013$), as well as some of the other model factors (Table 11). The only differences between treatments came as SHAWGRFO had significantly higher Shannon diversity of natural enemies than KANL ($p=0.039$) (Figure 15). In 2015, natural enemy diversity varied by treatment ($\chi^2=51.164$, $df=6$, $p=0.001$), with post hoc tests revealing that CIRA, KANL, and SHAW all had significantly lower Shannon diversity than SHAWGRFO ($p=0.048$, $p=0.023$, and $p=0.033$, respectively) (Figure 15).

When comparing Shannon diversity by ecotype, differences emerge in 2014 but not 2015. Analysis of deviance revealed variance in multiple model factors (Table 11), including ecotype ($\chi^2=34.63$, $df=1$, $p=0.001$). Lowland ecotypes supported lower natural enemy diversity than uplands (Figure 16). In 2015, differences were detected in some model factors (Table 11), but not within ecotype ($\chi^2=39.684$, $df=1$, $p=0.61$) (Figure 16).

Finally, in 2014, planted stand diversity had no effect on natural enemy diversity, while in 2015 the addition of forbs increased natural enemy diversity. In 2014, analysis of deviance detected no differences within model factors (Table 11), including planted stand diversity ($\chi^2=18.702$, $df=2$, $p=0.3313$) (Figure 17). By contrast, in 2015, natural enemy diversity varied by planted stand diversity ($\chi^2=21.208$, $df=2$, $p=0.008$), with post hoc tests revealing the high diversity planting to have higher natural enemy Shannon diversity than either the monoculture ($p=0.009$) or the low diversity polyculture ($p=0.02$) (Figure 17).
Investigatory correlations between herbivore and natural enemy communities, as well as between natural enemy and pest groups, was conducted, and only modest correlations were found (see appendix C).

**Sentinel Prey Removal**

Overall, an average of 68% of the sentinel prey were removed from the open egg cards, with an average of 64% removed in 2014 and 72% removed in 2015 (Figure 17A). Sentinel egg removal varied by year (Figure 17A), and the proportion of eggs consumed was greater in 2015 than 2014 in some models (Table 11). The level of biocontrol also varied among the months (Table 11), with a tendency to increase throughout the season (Figure 17B).

No differences in sentinel prey removal were detected by treatment ($\chi^2=297.69, df=6, p=0.25$) (Figure 19A), though some other factors contained differences between levels (such as year) (Table 11). In the model that considered ecotype, again different model factors contained variance according to analysis of deviance (Table 11), including ecotype ($\chi^2=220.60, df=1, p=0.032$). Upland ecotypes had higher levels of sentinel prey removal than lowlands ($p=0.032$) (Figure 19B). In the examination of planted stand diversity, it was not found to impact egg removal ($\chi^2=105.5, df=2, p=0.398$) (Figure 19C).

Correlations between proportion of eggs remaining and natural enemy abundance and Shannon diversity, as well common natural enemy groups, were performed and when no significant correlations were revealed this investigation was discontinued (see appendix C).
Discussion

This study compared herbivore and natural enemy abundance and diversity across different switchgrass treatments representing a variety of cultivars and planted stand diversity. Arthropods were generally more abundant in 2014 than in 2015, and both herbivores and natural enemies generally responded to ecotype, with upland ecotypes harboring higher arthropod numbers than lowland ecotypes. The impact of increasing stand diversity varied by year, though generally the addition of forbs increased both herbivore and natural enemy abundance. Sentinel egg removal rates remained comparatively stable across years, but were higher in upland ecotypes.

Herbivore Abundance

Herbivore abundance was higher in 2014 than in 2015, and the groups driving this abundance differed in the two years. In 2014 hoppers, thrips (primarily in June), aphids (especially in August), and grasshoppers drove the patterns of abundance. In 2015, hoppers appeared to be the main driver of herbivore abundance (Table 10). While differences across all treatments could not be determined, when breaking down switchgrass monocultures by ecotype, or focusing on the gradient of planted stand diversity, differences in herbivore abundance were detected in both years.

Upland ecotypes supported more herbivores than lowland ecotypes (Figure 10). This was partly driven by high herbivore numbers in Summer (Figure 9). Summer is a leafy cultivar of switchgrass, and this greater leaf area could potentially support increased herbivore abundance. Upland ecotypes also are generally thought to be less coarse than lowland ecotypes (Casler et al. 2004), which could impact chewing herbivores, though it may not impact the sucking herbivores.
that constituted the majority of the herbivores captured in this study (Massey et al. 2006). Herbivores could also be responding to the general leafiness in upland ecotypes, and as seen in other grass systems (Stinson and Brown 1983), leafhopper species richness and abundance were positively related to complexity as well as height.

Aphid abundance varied within and between years and is likely driven in part by other plants in the stands. For example, lowland ecotypes more frequently experience winter kill (Casler et al. 2004), which may allow weedy plants that can support high aphids numbers to occur in the stand. For example, a common weed in many plots was annual sow thistle (*Sonchus oleraceus* L.), which is known to support aphid populations (Hutchinson et al. 1984). Further analysis on aphid identity can help illuminate which aphids present in switchgrass monocultures were likely feeding on non-switchgrass host plants. Similarly, the herbivore abundance in the high diversity plots may not be attributable to switchgrass herbivores, as some of the native plants in our high diversity treatment, such as wild bergamot (Table 8), support native aphids (Wyckhuys et al. 2008). This higher number is likely related to the increased species richness in these plots (Tilman et al. 1997) and these herbivores may not negatively impact yield (Tilman et al. 2006).

The high number of hoppers and aphids captured in this study may be of concern from the perspective of plant viruses. Both aphids and leafhoppers (a subset of our hopper category) are capable of spreading viruses between plants. Many of the aphid-vectored viruses are well understood, though there potential for movement into annuals such as wheat if switchgrass acts as a reserve for virus populations, as may be in the case for aphids such at the bird cherry-oat aphid (*Rhopalosiphum padi* L.) and *Barley yellow dwarf virus* (Burd et al. 2012). Leafhoppers are believed to vector novel groups of viruses that are poorly understood (Agindotan et al.
2013), though could become a problem if switchgrass becomes a major commodity and landscape element. Natural enemies could potentially decrease or increase the spread of viruses, as the threat is eliminated if the herbivore/vector is consumed (Landis and Werf), but is likely to be spread to new plants if the vector moves to a new plant to avoid consumption (M E Irwin and Thresh 1990, Preisser and Bolnick 2008).

**Natural Enemy Communities**

Natural enemy abundance and diversity responded to treatments at many different levels. While there were differences between 2014 and 2015, Kanlow generally supported lower natural enemy numbers, while Summer, Shawnee with grasses, and Shawnee with grasses and forbs tended to support higher natural enemy numbers (Figure 13). Many of these differences may be explained by ecotype and planted stand diversity differences. For example, Kanlow, which consistently supports lower natural enemy abundance, likely drove some of the differences seen between ecotypes in 2014. Natural enemies may be negatively influenced by the lack of prey in lowland cultivars. Another factor may be plant structure, which can impact the interactions of herbivore and natural enemies (Denno et al. 2002), and comparisons of leaf to stem ratios in harvested switchgrass (Lemus et al. 2002) have suggested that there are general structural differences between cultivars. Studies in other systems have suggested natural enemies follow herbivores, and likely also respond to habitats with varied structure as well as the presence of alternative prey (Denno et al. 2002).

Natural enemy abundance tended to increase with plant diversity in both years (Figure 14). This response is likely driven in part by the presence of floral resources, which provide alternate food sources in the form of pollen and nectar (reviewed in Isaacs et al. 2008), which
can lead to increases in overall arthropod diversity as well as general predator and parasitoid numbers (Robertson et al. 2012a). Diversity in insects is also linked to diversity in plants (Murdoch et al. 1972). The combination of these floral resources, as well as increased herbivore number, likely supported increased abundance in the high diversity treatments.

In this experiment I only looked at the planted stand diversity, and these results do not take actualized stand diversity into account. The success of flowering plants in establishing and the resulting richness and diversity of these stands likely varies among the diversity treatments, so while these results may represent the range of what a grower who plants but does not maintenance for plant diversity might expect, differences between monocultures and polycultures may emerge if plant cover and floral area are taken into account.

The diversity of natural enemy communities mirrored many of the trends in natural enemy abundance. Natural enemy diversity was higher in 2014, with diversity decreasing by 50% in some treatments in 2015 (Figure 15). Upland ecotypes supported higher natural enemy diversity in 2014 but not 2015, and natural enemy diversity increased with planted stand diversity in 2015 but not 2014. This may suggest that in the year where prey insects were less available (2015), the floral resources in the SHAWGRFO plots become more important in supporting natural enemy diversity. This is in line with that known impact of floral resources on (reviewed in Issacs et al. 2008).

The size of our plots may have also influenced natural enemy communities. Ladybeetle diversity has previously been associated with field size in switchgrass bioenergy systems (Gardiner et al. 2010), though modelling work has suggested predator biomass actually decreases in large switchgrass fields (Robertson et al. 2012a). There are various ways abundances and
diversity could change as switchgrass production is scaled up, as increasing field size will interact with landscape-level changes.

Sentinel Egg Removal

Egg removal rates and patterns remained similar across years (Figure 17A), with removal rates increasing over the sampling season in 2014 and 2015 (Figure 17B). Egg removal was significantly different by ecotype (Figure 19B), likely driven by the high amounts of consumption in upland cultivars Cave-in-Rock and Summer (Figure 19A). These ecotypes supported higher herbivore numbers, and higher natural enemy number depending on the year, suggesting a mechanism for differences seen in sentinel eggs removal.

The consistent pattern did not match the trends in the abundance of any major predator groups except parasitoid wasps, although this taxa is unlikely to parasitize freeze-killed eggs (Vinson 1976) and would not produce the type of damage quantified in this study. However, these organisms require similar resources to other natural enemies and may represent a measure of habitat quality for natural enemies. Another explanation of the similar egg removal rates and patterns across may be that predation is about diversity of natural enemies rather than their abundance. The average Shannon diversity of natural enemies did decrease between the two years, but this level of diversity may have still been adequate to support sentinel prey consumption (reviewed in Symondson et al. 2002). Other work has suggested that natural enemy communities, as opposed to specialist predators, are key to biological control in native plant systems (Hawkins et al. 1999), suggesting that identifying traits within the natural enemy community may be key to fully understanding and utilizing biological control services in the switchgrass system.
Prior studies have utilized video equipment to record the predators visiting egg cards, and the arthropods measuring the cards validated this method as a measure of arthropod-mediated biological control. Although these studies have looked at egg cards at the ground level, and my egg cards were placed in the canopy, studies using a variety of prey types (i.e. coccinellid eggs, lepidopteran eggs, lepidopteran larvae) in multiple systems (i.e. blueberry fields, grasslands) have recorded ants, crickets, slugs, and opiliones (Grieshop et al. 2012, Smith and Gardiner 2013). While these same predators may not be the most active in the switchgrass canopy, I did collect opiliones and ants in our experiment (Table 10).

The video evidence from other studies, as well as observations made in the field here, suggest that one egg consumer who was not measured (and not likely a natural enemy) was slugs. Slugs are usually not collected in sweeps but were observed near the sentinel eggs cards and their presence could be inferred by certain types of egg card damage and the depositing of a shiny, white mucus. Grieshop et al. (2012) recorded video of slugs consuming sentinel waxworm larvae, backing up the notions that slugs are opportunistic feeders (Pallant 1972). Slugs have been observed to eat aphids and moth eggs in lab conditions (Fox and Landis 1973), and in field conditions slugs have been seen eating dead and moribund mites and collembola (Pallant 1972). Generally, it seems slugs prefer to feed on plant material (Pallant 1972), and in other grass systems slugs are pests (Barratt et al. 1994). While little is known about the reality of slugs as predators, it seems unlikely they could provide biological control against more agile herbivores such as grasshopper and leafhoppers. The ability of slugs to consume eggs likely impacted the final proportions, making inferences about biological control from this measure more difficult.
Conclusions

This study shows that choice of switchgrass ecotype, cultivar, and planted stand diversities can influence arthropod communities in a bioenergy crop setting, and how these factors influence herbivore and natural enemy communities, and biological control services. The study also suggested ways to improve the development and implementation of biomass cropping systems.

In this study, switchgrass ecotype influenced both herbivore and natural enemy abundance and diversity, with lowland ecotypes generally supporting a less abundant and less diverse arthropod communities. Previous work has shown that lowland ecotypes can be higher yielding (Lemus et al. 2002, Fike et al. 2006b) and their traits, such as increased coarseness (Vogel 2004), waxiness (Casler 2012), or general structure (Cortese et al. 2010, Casler 2012), may make them less preferred by herbivores. While lowland ecotypes are not well-adapted to all areas, these characteristics suggest that this may be a preferable ecotype to further develop into bioenergy crops. Further work is needed to identify traits or stand characteristics that contribute to both ecotype differences and altered insect responses.

Beyond ecotype, individual cultivars also show variation in their ability to support herbivore and natural enemy communities. In this study, lowland cultivar Kanlow generally supported the lowest abundance and diversity of arthropods, though this tended to not be different than the other lowland cultivar included in this study. On the opposite end of the spectrum was SUMM, an upland cultivar that frequently supported higher herbivore and natural enemy abundance and diversity than the other upland cultivars (though this difference was not always significant). These two cultivars represent two extremes in arthropod response and may be good candidates into further investigation for cultivar traits and how they promote or preclude
insects, which can improve our understanding of switchgrass and aide the development of herbivore resistant switchgrass cultivars.

In this study, planted stand diversity also influenced arthropod communities, though the patterns were more complex than in other measures. Often, but not always, arthropod communities increased in abundance and diversity increased as planted stand diversity increased. This pattern is consistent with much of the literature (reviewed in Letourneau et al. 2011) and could contribute to multiple ecosystem services in bioenergy systems (Werling et al. 2014). However, these results also raise concerns that more diverse stands could have increased susceptibility to herbivore feeding or virus transmission. Additional work aimed at addressing the virus transmission questions are currently being investigated as part of the larger project.

Finally, sentinel prey eggs were used to measure the biological control services provided by the overall natural enemy community. Overall, egg removal was high in all systems and did not mirror trends seen in herbivore and natural enemies, suggesting that other ways of measuring this service are required.

Clearly, the choice of switchgrass ecotype, cultivar, and planted stand diversity can play an important role in shaping arthropod communities and the resultant ecosystem services they provide and should inform decisions when establishing switchgrass stands. These results also highlight that as dedicated cellulosic bioenergy cropping systems continue to be developed, crop breeders, agronomists, and pest management specialists should cooperate to develop the most sustainable and resilient systems.
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APPENDICES
APPENDIX A:

TABLES
Table 1. Examples of upland and lowland switchgrass cultivars with minimal breeding and selection, demonstrating the range of release years and geographic origin (Casler 2012).

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Ecotype</th>
<th>Release Year</th>
<th>Geographic Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alamo</td>
<td>Lowland</td>
<td>1978</td>
<td>Southern Texas</td>
</tr>
<tr>
<td>Kanlow</td>
<td>Lowland</td>
<td>1963</td>
<td>Northern Oklahoma</td>
</tr>
<tr>
<td>Dacotah</td>
<td>Upland</td>
<td>1989</td>
<td>Southern North Dakota</td>
</tr>
<tr>
<td>Blackwell</td>
<td>Upland</td>
<td>1944</td>
<td>Northern Oklahoma</td>
</tr>
<tr>
<td>Cave-In-Rock</td>
<td>Upland</td>
<td>1973</td>
<td>Southern Illinois</td>
</tr>
<tr>
<td>Forestburg</td>
<td>Upland</td>
<td>1987</td>
<td>East South Dakota</td>
</tr>
<tr>
<td>Nebraska 28</td>
<td>Upland</td>
<td>1949</td>
<td>Northeast Nebraska</td>
</tr>
<tr>
<td>Shelter</td>
<td>Upland</td>
<td>1986</td>
<td>Central West Virginia</td>
</tr>
<tr>
<td>Southlow</td>
<td>Upland</td>
<td>2003</td>
<td>Southern Michigan</td>
</tr>
</tbody>
</table>
Table 2. Examples of selectively bred and improved cultivars of switchgrass, highlighting release year and selection criteria (Casler 2012)

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Ecotype</th>
<th>Release Year</th>
<th>Selection Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>BoMaster</td>
<td>Lowland</td>
<td>2006</td>
<td>Biomass yield, in vitro dry matter digestibility</td>
</tr>
<tr>
<td>Performer</td>
<td>Lowland</td>
<td>2006</td>
<td>Biomass yield, in vitro dry matter digestibility</td>
</tr>
<tr>
<td>Pathfinder</td>
<td>Upland</td>
<td>1967</td>
<td>Biomass yield, vigor</td>
</tr>
<tr>
<td>Shawnee</td>
<td>Upland</td>
<td>1996</td>
<td>Biomass yield, in vitro dry matter digestibility</td>
</tr>
<tr>
<td>Sunburst</td>
<td>Upland</td>
<td>1998</td>
<td>Large seed size and mass</td>
</tr>
<tr>
<td>Trailblazer</td>
<td>Upland</td>
<td>1984</td>
<td>Biomass yield, in vitro dry matter digestibility</td>
</tr>
<tr>
<td>Summer</td>
<td>Upland</td>
<td>1963</td>
<td>Earliness, rust resistance</td>
</tr>
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</table>
Table 3. Switchgrass cultivars used in this study. Highlighted cultivars were included in both the fall armyworm neonate and late instar larvae experiments. Ploidy levels are unknown for wild type switchgrass varieties. State of origin refers to area where germplasm was collected.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Code</th>
<th>Release Year</th>
<th>Ecotype</th>
<th>Ploidy</th>
<th>State of Origin</th>
<th>Seed Source</th>
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<tr>
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<td>Texas</td>
<td>Ernst Seed</td>
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<tr>
<td>Blackwell</td>
<td>BLCK</td>
<td>1973</td>
<td>Upland</td>
<td>8x</td>
<td>Oklahoma</td>
<td>Ernst Seed</td>
</tr>
<tr>
<td>BoMaster</td>
<td>BOMA</td>
<td>2006</td>
<td>Lowland</td>
<td>4x</td>
<td></td>
<td>Ernst Seed</td>
</tr>
<tr>
<td>Cave-in-Rock</td>
<td>CIRA</td>
<td>1973</td>
<td>Upland</td>
<td>8x</td>
<td>Illinois</td>
<td>Star Seed</td>
</tr>
<tr>
<td>EG 1101</td>
<td>E111</td>
<td>2010</td>
<td>Lowland</td>
<td>4x</td>
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<td>Ceres, Inc.</td>
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<tr>
<td>EG 1102</td>
<td>E112</td>
<td>2012</td>
<td>Lowland</td>
<td>4x</td>
<td></td>
<td>Ceres, Inc.</td>
</tr>
<tr>
<td>High Tide</td>
<td>HIGH</td>
<td>2012</td>
<td>Upland</td>
<td>NA</td>
<td>Maryland</td>
<td>Cape May Plant Materials Center</td>
</tr>
<tr>
<td>Kanlow</td>
<td>KANL</td>
<td>1963</td>
<td>Lowland</td>
<td>4x</td>
<td>Oklahoma</td>
<td>Ernst Seed</td>
</tr>
<tr>
<td>Kanlow N1 Syn 2</td>
<td>KLN1</td>
<td>Unreleased</td>
<td>Lowland</td>
<td>4x</td>
<td></td>
<td>Ken Vogel, USDA</td>
</tr>
<tr>
<td>MI genotype</td>
<td>MWF</td>
<td>Unreleased</td>
<td>Upland</td>
<td>NA</td>
<td>Michigan</td>
<td>Michigan Wildflower Farms</td>
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<td>NE Summer Late Mat.</td>
<td>NBSL</td>
<td>Unreleased</td>
<td>Upland</td>
<td>4x</td>
<td></td>
<td>Ken Vogel, USDA</td>
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<td>Performer</td>
<td>PERF</td>
<td>2006</td>
<td>Lowland</td>
<td>4x</td>
<td></td>
<td>Ceres, Inc.</td>
</tr>
<tr>
<td>Shawnee</td>
<td>SHAW</td>
<td>1996</td>
<td>Upland</td>
<td>8x</td>
<td></td>
<td>Ernst Seed</td>
</tr>
<tr>
<td>Shelter</td>
<td>SHEL</td>
<td>1986</td>
<td>Upland</td>
<td>8x</td>
<td>West Virginia</td>
<td>Ernst Seed</td>
</tr>
<tr>
<td>Summer</td>
<td>SUMM</td>
<td>1963</td>
<td>Upland</td>
<td>4x</td>
<td>Nebraska</td>
<td>Ernst Seed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nebraska and Kansas</td>
<td></td>
</tr>
<tr>
<td>Trailblazer</td>
<td>TRLB</td>
<td>1984</td>
<td>Upland</td>
<td>8x</td>
<td></td>
<td>Sharp Brothers Seed</td>
</tr>
</tbody>
</table>
Table 4. Average fall armyworm mortality (dead larvae per dish) at 24 and 48 h across all treatments. Control treatment was sweet corn. A $\chi^2$ analysis of deviance was performed using Holms adjusted T-test for treatment comparisons. Means sharing a letter are not significantly different ($\alpha=0.05$). There were no differences by cultivar or ecotype, though differences were present by time ($\alpha=0.05$).

<table>
<thead>
<tr>
<th>Hours</th>
<th>Ecotype</th>
<th>n</th>
<th>Mean Mortality ($\pm$sem)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>Control</td>
<td>15</td>
<td>0.072 ± 0.031 a</td>
</tr>
<tr>
<td></td>
<td>Lowland</td>
<td>104</td>
<td>0.050 ± 0.008 a</td>
</tr>
<tr>
<td></td>
<td>Upland</td>
<td>135</td>
<td>0.046 ± 0.009 a</td>
</tr>
<tr>
<td>48</td>
<td>Control</td>
<td>15</td>
<td>0.098 ± 0.037 b</td>
</tr>
<tr>
<td></td>
<td>Lowland</td>
<td>104</td>
<td>0.134 ± 0.014 b</td>
</tr>
<tr>
<td></td>
<td>Upland</td>
<td>134</td>
<td>0.109 ± 0.014 b</td>
</tr>
</tbody>
</table>
Table 5. Mean (±sem) number of older fall armyworm larval morality (larvae dead) of older larvae dead before pupation by ecotype. Control treatments are corn and a diet formulated for fall armyworm. A $\chi^2$ analysis of deviance detected no differences in mortality between treatments.

<table>
<thead>
<tr>
<th>Ecotype</th>
<th>n</th>
<th>Mean Mortality (±sem)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30</td>
<td>3.00 ± 0.00</td>
</tr>
<tr>
<td>Lowland</td>
<td>60</td>
<td>3.25 ± 0.25</td>
</tr>
<tr>
<td>Upland</td>
<td>60</td>
<td>1.75 ± 1.48</td>
</tr>
</tbody>
</table>
Table 6. Location of 12 study sites across southern Michigan.

<table>
<thead>
<tr>
<th>Site</th>
<th>County</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF05</td>
<td>St. Joseph</td>
<td>41.985399</td>
<td>-85.517841</td>
</tr>
<tr>
<td>NF06</td>
<td>Cass</td>
<td>42.020225</td>
<td>-86.026182</td>
</tr>
<tr>
<td>NF09</td>
<td>Kalamazoo</td>
<td>42.419190</td>
<td>-85.370391</td>
</tr>
<tr>
<td>NF07</td>
<td>Allegan</td>
<td>42.603549</td>
<td>-85.787700</td>
</tr>
<tr>
<td>NF10</td>
<td>Ingham</td>
<td>42.603681</td>
<td>-84.554662</td>
</tr>
<tr>
<td>NF13</td>
<td>Barry</td>
<td>42.652241</td>
<td>-85.498632</td>
</tr>
<tr>
<td>NF01</td>
<td>Ingham</td>
<td>42.690963</td>
<td>-84.490441</td>
</tr>
<tr>
<td>NF03</td>
<td>Eaton</td>
<td>42.702020</td>
<td>-84.651777</td>
</tr>
<tr>
<td>NF15</td>
<td>Eaton</td>
<td>42.734763</td>
<td>-84.870654</td>
</tr>
<tr>
<td>NF14</td>
<td>Barry</td>
<td>42.738953</td>
<td>-85.516516</td>
</tr>
<tr>
<td>NF02</td>
<td>Clinton</td>
<td>43.055431</td>
<td>-84.535849</td>
</tr>
<tr>
<td>NF12</td>
<td>Ottawa</td>
<td>43.060256</td>
<td>-85.971143</td>
</tr>
</tbody>
</table>
Table 7. Switchgrass cultivars and stand diversity combinations (treatments) present at each site.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Code</th>
<th>Release Date</th>
<th>Ecotype</th>
<th>Ploidy</th>
<th>State of Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>BoMaster</td>
<td>BOMA</td>
<td>2006</td>
<td>Lowland</td>
<td>4x</td>
<td></td>
</tr>
<tr>
<td>Cave-in-Rock</td>
<td>CIRA</td>
<td>1973</td>
<td>Upland</td>
<td>8x</td>
<td>Illinois</td>
</tr>
<tr>
<td>Kanlow</td>
<td>KANL</td>
<td>1963</td>
<td>Lowland</td>
<td>4x</td>
<td>Oklahoma</td>
</tr>
<tr>
<td>Shawnee</td>
<td>SHAW</td>
<td>1996</td>
<td>Upland</td>
<td>8x</td>
<td></td>
</tr>
<tr>
<td>Low Diversity</td>
<td>SHAWGR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Diversity</td>
<td>SHAWGRFO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>SUMM</td>
<td>1963</td>
<td>Upland</td>
<td>4x</td>
<td>Nebraska</td>
</tr>
</tbody>
</table>
Table 8. Plant species planted with Shawnee switchgrass in the low (SHAWGR) and high (SHAWGRFO) diversity treatments.

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Common name</th>
<th>SHAWGR</th>
<th>SHAWGRFO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panicum virgatum</td>
<td>Shawnee switchgrass</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Andropogon gerardii</td>
<td>Big bluestem</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Elymus canadensis</td>
<td>Canada wild rye</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Sorghastrum nutans</td>
<td>Indiangrass</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Anemone cylindrica</td>
<td>Thimbleweed</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Asclepias tuberosa</td>
<td>Butterfly weed</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Rudbeckia hirta</td>
<td>Black-eyed-susan</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Monarda fistulosa</td>
<td>Wild bergamot</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Ratibia pinnata</td>
<td>Gray-headed coneflower</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Silphium perfoliatum</td>
<td>Cup plant</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Symphyotrichum (formerly Aster novae-angliae)</td>
<td>New England aster</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solidago rigida</td>
<td>Stiff goldenrod</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Solidago speciosa</td>
<td>Showy goldenrod</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Baptisia lactea</td>
<td>White false indigo</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Desmodium canadense</td>
<td>Showy tick-trefoil</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Lespedeza capitata</td>
<td>Roundhead bushclover</td>
<td></td>
<td>x</td>
</tr>
</tbody>
</table>
Table 9. Insects and spiders evaluated from sweep samples, representing herbivores with pest potential and natural enemies commonly found in grass and prairie systems.

<table>
<thead>
<tr>
<th>Order: Family, or Species</th>
<th>Common Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Herbivores</strong></td>
<td></td>
</tr>
<tr>
<td>Hemiptera: Aphididae</td>
<td>Aphids</td>
</tr>
<tr>
<td>Hemiptera: Cicadellidae, Fulgoroidea, Cercopoidea</td>
<td>Hoppers</td>
</tr>
<tr>
<td>Lepidoptera</td>
<td>Lepidoptera Larvae</td>
</tr>
<tr>
<td>Orthoptera</td>
<td>Grasshoppers</td>
</tr>
<tr>
<td>Thysanoptera</td>
<td>Thrips</td>
</tr>
<tr>
<td><strong>Natural Enemies</strong></td>
<td></td>
</tr>
<tr>
<td>Coleoptera: Cantharidae</td>
<td>Soldier Beetles</td>
</tr>
<tr>
<td>Coleoptera: Carabidae</td>
<td>Ground Beetles</td>
</tr>
<tr>
<td>Coleoptera: Coccinellidae</td>
<td>Ladybeetle</td>
</tr>
<tr>
<td>Coleoptera: Coccinellidae</td>
<td>Ladybeetle larvae</td>
</tr>
<tr>
<td>Coleoptera: Lampyridae</td>
<td>Firefly</td>
</tr>
<tr>
<td>Diptera: Dolichopididae</td>
<td>Long-Legged Flies</td>
</tr>
<tr>
<td>Diptera: Pipunculidae</td>
<td>Big-Headed Flies</td>
</tr>
<tr>
<td>Diptera: Syrphidae</td>
<td>Syrphids</td>
</tr>
<tr>
<td>Hemiptera: Anthocoridae</td>
<td>Minute Pirate Bugs</td>
</tr>
<tr>
<td>Hemiptera: Nabidae</td>
<td>Damsel Bugs</td>
</tr>
<tr>
<td>Hymenoptera (Apocrita)</td>
<td>Parasitoid Wasp</td>
</tr>
<tr>
<td>Hymenoptera: Formicidae</td>
<td>Ants</td>
</tr>
<tr>
<td>Odonata</td>
<td>Damsel flies</td>
</tr>
<tr>
<td>Neuroptera</td>
<td>Lacewing larvae</td>
</tr>
<tr>
<td>Neuroptera: Chrysopidae</td>
<td>Green Lacewing</td>
</tr>
<tr>
<td>Neuroptera: Hemerobiidae</td>
<td>Brown Lacewing</td>
</tr>
<tr>
<td>Araneae: Salticidae</td>
<td>Jumping Spider</td>
</tr>
<tr>
<td>Araneae: Thomisidae</td>
<td>Crab Spider</td>
</tr>
<tr>
<td>Araneae</td>
<td>Other Spider</td>
</tr>
<tr>
<td>Opiliones</td>
<td>Harvestmen</td>
</tr>
</tbody>
</table>
Table 10. Average abundance per sample (30 total sweeps) of herbivores and natural enemies by year and month.

<table>
<thead>
<tr>
<th>Herbivore Taxa</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>June</th>
<th>July</th>
<th>August</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrididae</td>
<td>1.68</td>
<td>2.20</td>
<td>0.62</td>
<td>0.39</td>
<td>0.53</td>
<td>0.47</td>
</tr>
<tr>
<td>Aphididae</td>
<td>3.01</td>
<td>1.96</td>
<td>26.80</td>
<td>0.01</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Hoppers</td>
<td>11.83</td>
<td>15.33</td>
<td>26.51</td>
<td>7.61</td>
<td>6.08</td>
<td>17.90</td>
</tr>
<tr>
<td>Lepidoptera Larvae</td>
<td>0.27</td>
<td>0.20</td>
<td>0.26</td>
<td>0.14</td>
<td>0.10</td>
<td>0.32</td>
</tr>
<tr>
<td>Thripidae</td>
<td>16.97</td>
<td>2.79</td>
<td>1.39</td>
<td>0.27</td>
<td>0.09</td>
<td>0.17</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td>33.76</td>
<td>22.48</td>
<td>55.58</td>
<td>8.42</td>
<td>6.83</td>
<td>18.89</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Natural Enemy Taxa</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Coleoptera</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lampyridae</td>
<td>0.01</td>
<td>0.05</td>
<td>0.00</td>
<td>0.13</td>
<td>0.05</td>
<td>0.00</td>
</tr>
<tr>
<td>Cantharidae</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.04</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>Carabidae</td>
<td>0.05</td>
<td>0.00</td>
<td>0.05</td>
<td>0.00</td>
<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>Coccinellidae Larva</td>
<td>0.56</td>
<td>0.03</td>
<td>0.34</td>
<td>0.04</td>
<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>Coccinellidae</td>
<td>0.64</td>
<td>0.20</td>
<td>0.40</td>
<td>0.03</td>
<td>0.14</td>
<td>0.06</td>
</tr>
</tbody>
</table>

| Diptera                 |      |      |        |      |      |        |
| Syrphidae               | 0.79 | 0.45 | 0.67   | 2.05 | 0.24 | 0.28   |
| Dolichopodidae          | 1.20 | 0.99 | 0.35   | 0.27 | 0.11 | 0.04   |
| Pipunculidae            | 0.03 | 0.11 | 0.05   | 0.00 | 0.08 | 0.03   |

| Hemiptera               |      |      |        |      |      |        |
| Anthocoridae            | 0.57 | 0.24 | 0.26   | 0.06 | 0.09 | 0.12   |
| Nabidae                 | 0.40 | 0.20 | 0.29   | 0.26 | 0.14 | 0.18   |
| Hymenoptera             |      |      |        |      |      |        |
| Formicidae              | 0.12 | 0.13 | 0.09   | 0.13 | 0.04 | 0.05   |
| Parasitoids             | 4.05 | 3.01 | 8.14   | 0.91 | 0.76 | 1.49   |

| Neuroptera              |      |      |        |      |      |        |
| Chrysopidae             | 0.03 | 0.03 | 0.05   | 0.06 | 0.03 | 0.00   |
| Hemerobiidae            | 0.00 | 0.01 | 0.01   | 0.00 | 0.00 | 0.00   |
| Lacewing Larvae         | 0.03 | 0.05 | 0.11   | 0.01 | 0.00 | 0.00   |

| Odonata                 |      |      |        |      |      |        |
| Zygoptera               | 0.04 | 0.12 | 0.03   | 0.05 | 0.04 | 0.00   |

| Other Arthropods        |      |      |        |      |      |        |
| Araneae (Other)         | 0.88 | 1.05 | 0.77   | 0.51 | 0.36 | 0.40   |
| Opiliones               | 0.01 | 0.01 | 0.00   | 0.01 | 0.00 | 0.00   |
| Salticidae              | 0.07 | 0.19 | 0.29   | 0.05 | 0.03 | 0.04   |
| Thomisidae              | 0.17 | 0.93 | 0.58   | 0.01 | 0.41 | 0.32   |

| **Total:**              | 9.65 | 7.8  | 12.48  | 4.62 | 2.63 | 3.15   |
Table 11. The model factors included in model for all response variables. Analysis of deviance results for all model factors, with site $df=11$, month $df=2$, and year $df=1$.

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Main Effect</th>
<th>Site</th>
<th>Month</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\chi^2$</td>
<td>df</td>
<td>$p$</td>
<td>$\chi^2$</td>
</tr>
<tr>
<td>Herbivore Abundance 2014</td>
<td>Treatment</td>
<td>787.5</td>
<td>6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Ecotype</td>
<td>9707.2</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Planted Stand Diversity</td>
<td>633.93</td>
<td>2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Herbivore Abundance 2015</td>
<td>Treatment</td>
<td>1594.4</td>
<td>6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Ecotype</td>
<td>1201.6</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Planted Stand Diversity</td>
<td>633.97</td>
<td>2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Natural Enemy Abundance 2014</td>
<td>Treatment</td>
<td>1471.8</td>
<td>6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Ecotype</td>
<td>1021.8</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Planted Stand Diversity</td>
<td>529.46</td>
<td>2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Natural Enemy Abundance 2015</td>
<td>Treatment</td>
<td>522.99</td>
<td>6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Ecotype</td>
<td>349.49</td>
<td>1</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>Planted Stand Diversity</td>
<td>249.99</td>
<td>2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Natural Enemy Shannon Diversity 2014</td>
<td>Treatment</td>
<td>49.38</td>
<td>6</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>Ecotype</td>
<td>34.63</td>
<td>1</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Planted Stand Diversity</td>
<td>18.702</td>
<td>2</td>
<td>0.331</td>
</tr>
<tr>
<td>Response Variable</td>
<td>Main Effect</td>
<td>Site $\chi^2$</td>
<td>df</td>
<td>p</td>
</tr>
<tr>
<td>----------------------------</td>
<td>------------------</td>
<td>---------------</td>
<td>-----</td>
<td>------</td>
</tr>
<tr>
<td>Natural Enemy Shannon</td>
<td>Treatment</td>
<td>51.164</td>
<td>6</td>
<td>0.001</td>
</tr>
<tr>
<td>Ecotype</td>
<td>39.684</td>
<td>1</td>
<td>0.61</td>
<td>39.752</td>
</tr>
<tr>
<td>2015 Planted Stand Diversity</td>
<td>21.208</td>
<td>2</td>
<td>0.008</td>
<td>23.519</td>
</tr>
<tr>
<td>Proportion of Eggs Remaining</td>
<td>Treatment</td>
<td>297.69</td>
<td>6</td>
<td>0.25</td>
</tr>
<tr>
<td>Ecotype</td>
<td>220.6</td>
<td>1</td>
<td>0.032</td>
<td>225.19</td>
</tr>
<tr>
<td>Planted Stand Diversity</td>
<td>105.5</td>
<td>2</td>
<td>0.398</td>
<td>107.34</td>
</tr>
</tbody>
</table>
APPENDIX B:

FIGURES
Figure 1. Mean (±sem) cm² of leaf consumed in 48h by neonate fall armyworm by ecotype (A) and cultivar (B). A $\chi^2$ analysis of deviance was performed followed by pairwise t-tests that had been using Holms Adjusted for multiple comparisons. Significant differences between treatments were found between ecotypes but not cultivars ($\alpha=0.05$).
Figure 2. Mean (±sem) days to pupation of late instar fall armyworm fed different diets, by ecotype (A) and treatment (B). No significant differences between treatments were detected by a $\chi^2$ analysis of deviance.
Figure 3. Mean (±sem) cm² of leaf consumed prior to pupation by late instar FAW larvae. A $\chi^2$ analysis of deviance was performed followed by pairwise t-tests that had been using Holms Adjusted for multiple comparisons on residual data sets. Means sharing a letter are not significantly different ($\alpha=0.05$).
Figure 4. Mean (±sem) cm² of leaf consumed per day until pupation by late instar FAW. A $\chi^2$ analysis of deviance was performed followed by pairwise t-tests that had been using Holms Adjusted for multiple comparisons on residual data sets. Means sharing a letter are not significantly different ($\alpha=0.05$).
Figure 5. Mean (±sem) FAW pupal weight by ecotype (A) and treatment (B). A χ² analysis of deviance was performed followed by pairwise t-tests that had been using Holms Adjusted for multiple comparisons on residual data sets. Means sharing a letter are not significantly different (α=0.05).
Figure 6. (A) Lowland and (B) upland switchgrass in the field.
Figure 7. (A) Location of the 12 study sites across southern Michigan, (B) generalized location of sampling within plots, and (C) generalized plot layout at each site. Location of sweep transects in grey, with location of egg card sampling station denoted by the black square (B). Plots are randomized at each location, with cream colored plots representing lowland varieties, green representing upland varieties, teal a low diversity polyculture, and blue a high diversity polyculture. Plots in grey were treatments not considered in this study (C).
**Figure 8.** Set up of egg card in the field. (A) the underside of the plastic corrugated cardboard with the covered and exposed egg card. (B) The setting of the board on a step-in fence post at canopy height.
Figure 9. Mean (±sem) herbivore abundance by treatment in 2014 and 2015. Analysis of deviance detected differences between treatment levels, but post-hoc test variability and inconsistency prevented firm conclusions.
Figure 10. Mean (±sem) herbivore abundance by ecotype in 2014 and 2015. A $\chi^2$ analysis of deviance was performed followed by pairwise t-tests that were Holms Adjusted for multiple comparisons. A residual dataset was used to minimize variance due to other model factors (month, site), so differences may not appear intuitive with raw means presented on the graph. Means sharing a letter are not significantly different ($\alpha=0.05$).
Figure 11. Mean (±sem) herbivore abundance by planted stand diversity in 2014 and 2015. A χ² analysis of deviance was performed followed by pairwise t-tests that had been Holms Adjusted for multiple comparisons, which were performed on residual datasets to minimize influence of other factors. Means sharing a letter are not significantly different (α=0.05)
Figure 12. Mean (±sem) natural enemy abundance in 2014 and 2015 by treatment. A $\chi^2$ analysis of deviance was performed followed by pairwise t-tests that had been Holms Adjusted for multiple comparisons, which were performed on residual datasets to minimize influence of other factors. Means sharing a letter are not significantly different ($\alpha=0.05$).
Figure 13. Mean (±sem) natural enemy abundance in 2014 and 2015 by ecotype. A $\chi^2$ analysis of deviance was performed followed by pairwise t-tests that had been Holms Adjusted for multiple comparisons, which were performed on residual datasets to minimize influence of other factors. Means sharing a letter are not significantly different ($\alpha=0.05$).
Figure 14. Mean (±sem) natural enemy abundance in 2014 and 2015 by planted stand diversity system. A χ² analysis of deviance was performed followed by pairwise t-tests that had been Holms Adjusted for multiple comparisons, which were performed on residual datasets to minimize influence of other factors. Means sharing a letter are not significantly different (α=0.05).
**Figure 15.** Mean (±sem) natural enemy Shannon diversity in 2014 and 2015 by treatment. A $\chi^2$ analysis of deviance was performed followed by pairwise t-tests that had been Holms Adjusted for multiple comparisons, which were performed on residual datasets to minimize influence of other factors. Means sharing a letter are not significantly different ($\alpha=0.05$).
Figure 16. Mean (±sem) natural enemy Shannon diversity in 2014 and 2015 by ecotype. A $\chi^2$ analysis of deviance was performed followed by pairwise t-tests that had been Holm's Adjusted for multiple comparisons, which were performed on residual datasets to minimize influence of other factors. Means sharing a letter are not significantly different ($\alpha=0.05$).
Figure 17. Mean (±sem) natural enemy Shannon diversity in 2014 and 2015 by planted stand diversity. A χ² analysis of deviance was performed followed by pairwise t-tests that had been Holms Adjusted for multiple comparisons, which were performed on residual datasets to minimize influence of other factors. Means sharing a letter are not significantly different.
Figure 18. Mean (±sem) proportion of eggs removed by year (A) and month (B). A higher proportion of eggs were consumed in 2014 and as the summer progressed (averaged over years). A $\chi^2$ analysis of deviance was performed followed by pairwise t-tests that had been Holms Adjusted for multiple comparisons, which were performed on residual datasets to minimize influence of other factors. Means sharing a letter are not significantly different ($\alpha$=0.05).
Figure 19. Mean (±sem) proportion of eggs consumed in 2014 and 2015 by treatment. A χ² analysis of deviance was performed followed by pairwise t-tests that had been Holm Adjusted for multiple comparisons, which were performed on residual datasets to minimize influence of other factors. Means sharing a letter are not significantly different (α=0.05).
APPENDIX C:

CORRELATIONS BETWEEN HERBIVORES, NATURAL ENEMIES, AND BIOLOGICAL CONTROL
Spearman’s rank correlations were performed in R 3.0.3 (R Development Core Team 2014) on herbivore abundance and natural enemy abundance and diversity across both years, as well as between all natural enemy and pest groups across all years. The correlation coefficient between herbivore and natural enemy abundances as 0.53, and the correlation coefficient between herbivore abundance and natural enemy diversity was 0.43. Both of these are modestly positive correlations.

Correlations coefficients between pest and natural enemy groups were generally small, indicating no or weak correlation. The highest correlation was between aphids (aphididae) and parasitoid wasps (0.47) (Table 12). To further investigate the link between natural enemies and pests, a more in-depth analysis was conducted on a two groups with a well-established relationship, aphids and coccinellid beetles. The correlation coefficient across years is 0.27, and when this is broken down by year, in 2014 the correlation coefficient for the relationship between coccinellids and aphids was 0.18 and 0.08 in 2015. The weak correlation coefficients for two taxa of known relationship suggested that additional temporal factors may be at play, i.e. density dependence.

Spearman rank correlations between proportion of sentinel prey removed and natural enemy abundance were also conducted. The correlation coefficient of -0.02 suggests minimal relationship between the two.
**Table 12.** Spearman’s rank correlation coefficients for correlations between natural enemy and pest groups across all months and years. The closer a coefficient is to one, the more correlated the groups are to each other.

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<th>Lepidoptera Larvae</th>
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APPENDIX D:

APHID EXPERIMENT
In 2014, an experiment was devised to test if corn leaf aphid (*Rhopalosiphum maidis* Fitch) has different establishment successes on various cultivars of switchgrass in the field and if natural enemies could control these aphids in a common garden experiment. This experiment utilized the switchgrass plants that were eventually used in that fall armyworm experiments discussed in chapter 2 of this thesis. These plants are established as a randomized complete block design, with four blocks consisting of 24 cultivars in 1m² plots. At opposite corners of the plot are plans of the cultivar. For this experiment, 20 aphids of mixed age class were inoculated on a healthy tiller of each first-year switchgrass plant, with 18 of the 24 cultivars tested. Aphids were moved from colonies into microcentrifuge tubes using an artist’s paintbrush, and then transported to the field. Aphids were established in cages designed specifically for this experiment. Cages consisted of a 30.5cm mesh tube held to the plant by two 5.4cm diameter foam circles; the aphids were established by placing the microcentrifuge tube in the bottom piece of foam near the base of a switchgrass leaf. The cage was then assembled around the tube and once it was completed the tube was opened and aphids were allowed to establish on the leaf. At 24h the cage was removed from one plant in each plot, allowing for natural enemies to potentially consume these aphids. While observations at 24h and initial aphid counts 48h after inoculation suggested aphids had successfully moved onto the switchgrass plants, formal aphid counts in the field after one week and two weeks found very low aphid numbers, with a total of 11 aphids found at the experiments end across all plants and treatments. This low aphid establishment is suspected to be the result of low temperature (overnight lows were between 4°C and 10° during the trial’s first two nights) and rainfall during the initial nights of the experiment. Due to the unpredictable establishment of aphids on switchgrass, aphids were discontinued as a study organism.
APPENDIX E:

RECORD OF DEPOSITION OF VOUCHER SPECIMENS
RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2016-02

Author and Title of thesis:
Influence of switchgrass ecotype, cultivar and planted stand diversity on herbivores, natural enemies, and biological control in bioenergy cropping systems
By Marissa K. Schuh

Museum(s) where deposited:
Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)
Table 13. Voucher specimens deposited at the Albert J. Cook Arthropod Research Collection (Michigan State University). Aphids and hoppers collected in this experiment were used by a collaborator for viral DNA extraction, so no vouchers were collected.

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LITERATURE CITED
LITERATURE CITED


Capehart, T. 2015. Background, Corn. USDA- ERS.

Capinera, J. L. 2014. Fall Armyworm, Spodoptera frugiperda (J.E. Smith)(Insecta: Lepidoptera: Noctuidae). In University of Florida Institute of Food and Agricultural Sciences [ed.]. University of Florida Institute of Food and Agricultural Sciences,, Online.


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