Insect Pest Communities in Potential Biofuel Grasses of Upper Midwest USA

Vanessa Klimowicz
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Abstract

With the increasing concern about the use of fossil fuels, cellulosic biofuel crops are gaining additional attention. Two of these potential bioenergy crops are switchgrass and miscanthus, and the majority of research concerning these grasses has concentrated on expected yields, physiology, and cultivation practices. Little attention has been given to the insect pests that may develop on these fast growing crops. To address this issue, a series of experiments were designed to test the host preference of major insect pests as well as the feasibility of current management strategies in biofuel agriculture. Based on observations in other agricultural cropping systems, it was hypothesized that communities of aphids and thrips have the capacity to infest miscanthus (Miscanthus x giganteus) and switchgrass (Panicum virgatum). Weekly trap counts indicated the population of thrips peaked between late May and early June on miscanthus and switchgrass. Both aphids and thrips were observed to demonstrate certain host preferences when introduced to various biofuel grasses. In addition, higher levels of nitrogen fertilization have the capacity to increase the level of insect damage on switchgrass and other bioenergy crops. This information can provide further insight into the ways in which insect pest communities impact biofuel cropping systems. Research on the pests of potential biofuel crops can then aid in the development of sufficient management strategies and practices for bioenergy agriculture.

Introduction

Investments in potential biofuels are becoming more prominent in today’s society as fossil fuel deposits continue to be depleted. Faced with the loss of these raw materials in the near future, governments around the world are pushing for the development of renewable energy sources and the possibility of “growing fuel” is appealing to agricultural stakeholders and governmental officials alike. In 2007, the Bush Administration passed the Energy Independence and Security Act that strengthened the 2005 Energy Policy Act by increasing the renewable fuel standard to 36 billion gallons by the year 2022 (Somma et al. 2010). The U.S. generated over ten billion gallons of ethanol in 2009, of which the majority was produced from corn grain (Renewable Fuels Association 2010). Biodiesel, generated from vegetable oils like soybean and canola, also accounted for 700,000,000 gallons of biofuel produced in the country in 2008 (National Biodiesel Board 2010). Ethanol and biodiesel both convert plant material (biomass) into fuel and are known as biofuels (Somma et al. 2010). These first generation biofuels have advanced with the use of current agricultural technologies that use edible food parts of crops in
the conversion to bioenergy (Landis & Werling 2010). However, growing food crops specifically for biofuel production has been met with controversy such as limited agricultural land (Robertson et al. 2008). A solution to the “food vs. fuel” debate is second-generation biofuels that are produced from the cellulose contained within non-food plants and inedible remnants of food crops (Shubert 2006). These are known as cellulosic biofuels and are generated from various plant materials that range from herbaceous vegetation and forests to dedicated bioenergy crops (Perlack et al. 2005). U.S. dependence on foreign oil, rising gasoline prices, and mounting scientific consensus on climate change all have led to recent increases in bio-ethanol production and policies concerning other promising biofuels (Khanna et al. 2008; Somma et al. 2010).

To meet federal demands for biofuel production increase, approximately 100,000,000 hectares of U.S. forest and croplands may be devoted to biomass agriculture (Landis & Werling 2010). Modifying the landscape on such a large-scale could drastically alter ecosystems and the inhabiting wildlife. Arthropods, in particular, are vital in ecosystems functions, and the ways in which humans designate bioenergy crops for agricultural production systems have massive implications. One aspect for consideration will be herbivorous arthropods acting as biofuel crop pests that have the capacity to decrease productivity and quality of the plant biomass. Furthermore, arthropods also provide beneficial services such as pollination, predation, and decomposition which will be advantageous to biofuel agriculture. Distribution of ecosystem services mediated through arthropods may change as landscapes are modified in the adoption of biofuel cropping systems (Landis & Werling 2010). By concentrating research efforts on factors that produce high yield, we may fail to notice adverse ecological effects that arise in modifying natural landscapes and agricultural lands to novel biomass production systems. Extensive changes in land use could impact arthropod communities and result in the emergence of insect pests. Potential biofuel crops will be highly vulnerable to insect damage and will require improved agricultural practices and management strategies to mitigate these issues.

**Literature Review**

In the U.S., the most popular biofuel on the market is corn ethanol, made from the food part (kernels) of the plant. Corn ethanol is also a grain-based biofuel that is generated from starch contained within the corn grain. However, using such a prominent food crop as an energy source has created widespread controversy leading to the food vs. fuel debate. Using only grain
feedstocks to produce biofuels can cause complex issues in deciding what and how much land should be dedicated to producing food vs. energy crops.

Cellulosic ethanol is also available as a possible renewable energy source due to recent achievements in biotechnology. Cellulose is the most abundant material on this planet and is converted to ethanol from plant biomass – leaves, stems, and other aboveground vegetation that does not include grain - that contains high quantities of cellulose and hemicelluloses (complex sugars) (Robertson et al. 2008). Sugar polymers from the cellulosic biomass are converted into ethanol through the process of fermentation (USDOE 2010b). Plants grown for the production of cellulosic ethanol are referred to as “cellulosic biofuel crops or dedicated energy crops” (KBS 2010). Excess crop residues like corn stover and high output herbaceous perennial grasses have been investigated so that land competition may be alleviated and higher yields per acre can be achieved than what is currently being accomplished with corn-grain based ethanol (Khanna 2008). The potential of utilizing annual and perennial plant species is also being researched. Annual crops (corn for instance) require planting every year due to a one year life cycle. Perennial crops (switchgrass for instance) are able to survive over several years after the initial planting. In addition, poplar trees, native prairie grass species, mixed field, as well as rhizomatous grasses such as miscanthus are being studied for their viability as cellulosic biofuels.

Biofuels developed through cellulosic biomass have the capacity to reduce competition for land because they are able to grow on marginal lands - those not suitable for food crops - and are able to be entered directly into ethanol production in the case of food crop wastes (Robertson et al. 2008). Perennial crops decrease soil erosion and accumulate carbon, that conserve nitrogen in the soil to their extensive rooting system. Growing perennial crops consequently require less management and care following establishment. Moreover, growing diverse cellulosic plant systems like prairies can maintain a high level of biodiversity in a landscape (Robertson et al. 2008). Higher plant diversity can lead to an increased diversity of other organisms – plants, birds, and insects – inhabiting the area (Khanna 2008). Crucial ecosystem services can then be provided by this complex of diversity. Ecosystem services are benefits that humans receive from the environment such as fuel, clean water and air, fiber materials, pollination, as well as carbon sequestration (KBS 2010).
Cellulosic biofuels are energy efficient and are chemically identical to grain-based ethanol generated from corn but produces triple the net energy content of corn ethanol. Also, they release lower levels of greenhouse gas emissions into the atmosphere than corn ethanol (Greer 2005; USDOE 2006). Both grain-based and cellulosic biofuels show promising results as potential alternative energies. However, cellulosic biofuels have the ability to offset more gasoline consumption than do grain-based biofuels with added environmental co-benefits (Robertson et al. 2008; USDOE 2006).

According to BP's Statistical Review of World Energy, published June 2007, known world oil deposits will last approximately 40 years at current rates of consumption (Howden 2007). Cellulosic biofuel research is tremendously significant because it has the capability of reducing our dependence on gasoline and other fossil fuels. This potential has been recognized by key companies and research facilities around the world. A statement released from Shell Oil has announced that “the global market for biofuels such as cellulosic ethanol will grow to exceed $10 billion by 2012” (Greer 2005). Furthermore, the Energy Foundation and the National Commission on Energy Policy conducted a recent study that determined “biofuels coupled with vehicle efficiency and smart growth could reduce the oil dependency of our transportation sector by two-thirds by 2050 in a sustainable way” (Greer 2005). By replacing fossil fuels with ethanol derived from biomass, the effects of climate change may be minimized. Though all fuel combustion, including biofuel production, emits atmospheric carbon dioxide, plants require carbon dioxide for photosynthesis in order for growth to occur and therefore the carbon dioxide released during biofuel combustion is offset by the amount that was absorbed during the life cycle of the plant (US DOE 2010). In contrast, carbon dioxide emitted from the combustion of fossil fuels had been sequestered thousands and thousands of years ago. By continuing to research biofuels, in particular cellulosic biofuels, the hope of minimizing our dependence on fossil fuels and decreasing global carbon dioxide emissions may be achieved in the foreseeable future.

**Grain-based and Cellulosic Ethanol Production**

Conventional ethanol derived from corn and wheat grain crops is chemically identical to cellulosic ethanol derived from cellulosic biomass, but the two differ in how they are processed (Greer 2005; Somma et al. 2010). Each type generates ethanol through the utilization of different feedstocks and processes (Greer 2005). Two main technologies are used to convert biomass to
ethanol (Solomon et al. 2007). The first widely used, grain-based process involves converting the starch (contained within the corn kernel or similar food crops) to fuel through several steps: “milling, liquefaction, saccharification, fermentation, distillation, dehydration, and denaturing” (Solomon et al. 2007); Figure 1 demonstrates the key steps of the corn grain ethanol production process.

![Dry Mill Ethanol Process](image)

**Figure 1.** Corn Grain Ethanol Production Process (Dry Mill) (USDOE 2010a)

To begin, milling grinds the grain kernels into a loose powder referred to as “meal.” Grain-based ethanol production can either entail wet or dry milling processes. Wet milling first soaks the grain in water or diluted acid in order to break apart the grain into its component parts (e.g. starch and protein). The starch then can be converted to sugars that will be fermented by yeast or bacteria to produce ethanol. Dry milling grinds the kernels into a fine powder without separating the grain into its individual parts. In the U.S., most milling facilities currently use the dry-mill process. Next, the liquefaction step involves creating a mash by adding a liquid to the meal and increasing the temperature to remove present bacteria and to transform the starch into a liquid solution. Enzymes are then introduced in the saccharification/hydrolysis step in order to break down the complex carbohydrate structure that forms starch into simple six-carbon sugars of glucose. Following hydrolysis, the glucose is converted to carbon dioxide and ethanol by transporting the hydrolyzed mash to a tank where fermentation occurs through yeast. A dilute
(10-12%) ethanol solution is produced from the fermentation step. This “broth” contains both mash solids and yeast that are then pumped through distillation chamber columns to obtain only ethanol from the mixture. After the distillation process, the resulting ethanol is 96% pure. The dehydration step then removes the existing water contained within the distilled ethanol by molecular sieves; this process produces pure ethanol (200 proof). Lastly, gasoline is added to the ethanol before entering gasoline distribution (USDOE 2010a).

The other less commercially utilized process involves converting plants cellulosics (grasses, woody parts of trees, and plant residues) to simpler sugars that can be fermented in order to produce ethanol (Solomon et al. 2007); Figure 2 demonstrates the key steps of the cellulosic ethanol production process.

**Schematic of a Biochemical Cellulosic Ethanol Production Process**

![Diagram](image)

The first step of converting cellulose to ethanol involves biomass handling, where unwanted debris and soil are detached from received biomass. The biomass is then shred into small pieces and entered into pretreatment. The goal of this process is to make the cellulose more susceptible to hydrolysis (enzymatic breakdown) and to make hemicellulose polysaccharides (sugars) soluble. Biomass pretreatment applies acid, pressure, or heat to isolate cellulose from other polymers present in the plant matrix (lignin and hemicellulose) prior to hydrolysis; lignin and hemicellulose both aid in structural support in plant cells. Hydrolysis then degrades cellulose into basic sugar molecules using enzymes, such as cellulase, that are produced by fungi and bacteria. This step contributes to the reason why cellulosic ethanol is not yet a commercial industry; current methods are expensive and the enzymes used are not very efficient. However, research is being conducted to mass produce efficient enzymes for the breakdown of cellulose. Next, microbial fermentation occurs by allowing yeast or other microorganisms to consume glucose.
molecules, which produces ethanol and carbon dioxide (USDOE 2006). The generated ethanol is then separated out of the mixture through purification and distillation processes (USDOE 2010b).

Though there are obstacles encountered in the conversion of biomass to ethanol, cellulosic biofuels are ideal renewable energy sources. In comparison with cornstarch ethanol production, synthesizing cellulosic ethanol is more expensive and difficult to process into ethanol; cellulosic biomass is structurally more complex than corn grain, which is why more effort and expenses are required (USDOE 2010b). According to a study conducted by McAlloon et al. (2000), the additional steps required to degrade cellulose into fermentable sugars causes approximately a 70% increase in cellulosic ethanol production costs compared to corn ethanol production. However, cellulose is found more in abundance and is less costly than corn grain feedstock (USDOE 2006). Cellulosic feedstocks contain higher amounts of energy than corn feedstocks and require less land to produce the same amount of energy (Righelato & Spracklen 2007); observations of decreased soil erosion and improved soil fertility also ensue (USDOE 2006). Despite the huge increase in the corn ethanol industry, a very small section in the U.S. produces it; approximately 75% of ethanol is generated in Iowa, Minnesota, Illinois, South Dakota, and Nebraska (Yacobucci 2007). Urban areas of the Midwest consequently use the majority of corn ethanol produced in the top-growing states region, proximate to the feedstock supply (Somma et al. 2010). A pipeline system designed for corn ethanol is non-existent and is corrosive to existing oil infrastructures (Somma et al. 2010). These factors have led to additional research and development focused on cellulosic biomass, which can exist in more diverse geographic ranges (Somma et al. 2010). Bio-ethanol facilities could then be located in other areas of the U.S. without added transportation costs. A more intensive refining process involved in cellulosic ethanol production is worth the investment – cellulosic alcohol fuels have the capacity to be “cost-effective, environmentally beneficial, and have a greater energy output to input ratio than grain ethanol” (Solomon et al. 2007).

**Potential Cellulosic Biofuel Crops**

Among the wide variety of herbaceous energy crops currently being researched today, *Miscanthus x giganteus* (miscanthus) and *Panicum virgatum* (switchgrass) have been recognized as the most viable for bioenergy feedstocks. Both perennial grasses have great potential as biofuel crops because of exceptionally high yields, low energy inputs, nutrient use efficiency,
and resistance to cold temperatures. While living and as biofuels, *M. x giganteus* and *P. virgatum* also have the capacity to decrease levels of greenhouse gases being emitted into the atmosphere. They serve as renewable energy sources and can function as carbon sinks through soil carbon sequestration (Khanna et al. 2008; BERC 2010; USDOE 2006). In addition, *M. x giganteus* and *P. virgatum* utilize similar equipment and growth conditions as corn; this would make the transition to conventional cultivation practices an easier process (Khanna 2008).

*P. virgatum* has been extensively researched since the 1990s by the U.S. Department of Energy for biofuel crop development (Landis & Werling 2010). Ideal characteristics of *P. virgatum* include high productivity on an array of soil types and conditions, ability to benefit soil and water quality, as well as conservation efforts (BERC 2010). The warm-season grass is naturally occurring in tallgrass prairie that originally covered the Great Plains (Figure 3a-b); prairie soils are also known to exhibit *P. virgatum*. It historically grew with other key native tallgrass prairie species, for instance indiangrass, little bluestem, and big bluestem, as well as sunflowers and other forb plants (Rinehart 2010). After the widespread destruction of tallgrass prairie in the U.S., *P. virgatum* is routinely seen on marginal lands not suitable for annual cropping, along fencing, and riparian buffers (Rinehart 2010). Its capacity to adapt to a wide variety of environments has allowed the plant to evolve many ecotypes; lowland and upland morphological ecotypes are most widely accepted (Oklahoma State University 2010).
M. x giganteus is a rhizomatous perennial tallgrass hybrid that is produced naturally from the crossing of M. sacchariflorus and M. sinensis (Figure 4a-b) (BERC 2010). However, the hybrid does not generate viable seed and is established by inserting a piece of the root, called a rhizome, into the soil (BERC 2010). It only requires one initial planting in the fall and will rapidly grow in the following spring, having the ability to outcompete any present weed species (EBI 2010). M. x giganteus is non-native to the U.S. (Khanna et al. 2008). The characteristics that propose this species as an ideal biofuel crop also suggest, for some, the beginnings of an invasive weed (Pyter et al. 2010). Concerns of M. x giganteus potentially becoming an invasive species in the country have been mediated by the fact that this particular species cannot generate fertile pollen and can be used as a bioenergy feedstock in large-scale production systems (Pyter et al. 2010). The European Union has also been growing M. x giganteus for the past 20 years in extensive agricultural systems with no signs of invasiveness (Khanna et al. 2008).

Conversely, establishing M. x giganteus is expensive and requires planting from propagules rather than seed; the specialized machinery and equipment required for this task is currently nonexistent and is performed by hand (Pyter et al. 2010). Continued research in designing feasible rhizome propagation techniques is called for in order to commercialize M. x
*giganteus*. The highest annual biomass yields ever recorded in the U.S. and European agricultural research trials have been of *M. x giganteus*, without the presence of nitrogen fertilizers (EBI 2010). As of now, no confirmed pests or pathogens have been identified and additional research has been geared toward investigating what other species of Miscanthus can be used in agriculture (EBI 2010).

Since the 1960s, *M. x giganteus* has been extensively studied in Europe and used in the production of bioenergy, though introduction in the U.S. has been relatively recent (Lewandowski et al. 2003). European studies have suggested a variety of ways in which insect pests may impact biofuel production. Data provided by the U.S. Department of Energy and other sources have also contributed insight as to what species of insect pests may be expected in *P. virgatum* agriculture (Landis & Werling 2010). In addition, observations in a range of agricultural cropping systems have also been helpful in determining the types of arthropod pests that may infest *M. x giganteus* and *P. virgatum* crops. All of these sources provide useful information of potential insect pests that can then be applied to other biofuel crops. For instance,
studies have demonstrated that insects influence *P. virgatum* productivity after initial planting (Wolfe & Fiske 1995). After establishment, *P. virgatum* also has been observed to be susceptible to herbivorous arthropods such as crickets and grasshoppers (Parrish et al. 1999). A variety of plant diseases can be transmitted via insect feeding (Landis & Werling 2010). A study conducted by Huggett et al. (1999) found that the corn leaf aphid, *Rhopalosiphum maidis* Fitch, served as a vector for the successful transmission of the Barley Yellow Dwarf Virus (BYDV). A recent study by Prasifka et al. (2009) indicated fall armyworm, *Spodoptera frugiperda* (J.E. Smith), as a potential pest of *M. x giganteus* and *P. virgatum*. Collectively, this data poses evidence for potential obstacles that may arise due to insect pests in both grass species. Additional research is required that spans different geographic areas so that pest issues can be compared and better understood prior to the transition to large-scale agriculture.

**Prospective Insect Pests**

Thrips and aphids are major insect pests in the agricultural world (Huang 2010; van Emden & Harrington 2007). They damage plants by puncturing plant material with their mouthparts to feed on the sap contents, which causes wilting and discoloration (Frank 2010; van Emden & Harrington 2007). These arthropods attack a wide variety of plant hosts and can also act as vectors for plant diseases (Huang et al. 2010; Mueller et al. 2010). In particular, the western flower thrips (*Frankliniella occidentalis*) is considered a significant insect pest on a global scale (Reitz 2009). This species is part of the family Thripidae, order Thysanoptera and is known to attack hundreds of horticultural crops and weeds (Reitz 2009). The insect is small, elongated and winged in appearance (Figure 4a-b). Thrips, in general, develop in six stages that include the egg, two feeding larva stages, two non-feeding pupal intermediates, and adult (Figure 5b) (Reitz 2009). Females can lay up to 300 eggs in a lifetime (28 days) that are inserted into the stem, leaf, or flowering bodies of a plant and are hard to identify (Frank 2010). Eggs may require up to two to four days to hatch depending on the temperature of the environment (Reitz 2009). Thrips undergo pupation in the soil or flowering bodies of a plant and emerge with visible wings and antennae (Frank 2010). The rate of development is influenced by temperature and the host plant but can occur very rapidly; several generations can be produced in one growing season (Reitz 2009). The western flower thrips is also observed to spread plant viruses such as the tomato spotted wilt tospovirus (TSWV) and impatiens necrotic spot tospovirus (INSV) (Frank 2010). Goldbach and Peters reported in 1994 that TSWV had been estimated to cause
approximately one billion dollars in yield losses. More than 1,000 plant species in 84 families are vulnerable to TSWV (Parrella et al. 2003). The most significant damage contributed by the western flower thrips is considered to be the transmission of tospoviruses that reduce the productivity of the plant and can lead to death depending on the age of the inflicted individual (Reitz 2009; Frank 2010). Only the first and second nymph stages can contract plant diseases which then are dispersed to other plants by the adult thrips (Figure 5a) (Frank 2010).

**Figure 5a.** Adult Western Flower Thrips (Sparks & Riley 2010) **b.** Thrips Life Cycle (UCIPM 2010)

Over 4,700 species of aphids inhabit the planet and belong to the superfamily Apidoidea, family Aphididae (Remaudière & Remaudière 1997, as cited in van Emden & Harrington 2007). Of those species, 450 have been observed in the presence of crop plants (Blackman & Eastop 2000). Approximately 100 of these aphid species have perpetuated to major crop pests such as the melon/cotton aphid (*Aphis gossypii*), rose aphid (*Macrosiphum rosae*), and green peach aphid (*Myzus persicae*) (Figure 5a-b) (Greer 2000; van Emden & Harrington 2007). The largest subfamily, Aphidinae, contains a high level of herbaceous feeders which impacts agricultural
systems most significantly (Blackman & Eastop 2006). Some aphids feed on only one specific plant species, although others (the genera *Aphis* for instance) feed on multiple hosts (Illinois Natural History Survey 2010). While they consume a variety of annual and perennial vegetation, some can alternate between the different types (INHS 2010). Aphids all share similar morphological characteristics and are modified depending on the environment and host type of the species (van Emden & Harrington 2007). A small and soft-bodied structure and absence of wings are exhibited by most aphids (van Emden & Harrington 2007). These insects excrete honeydew (a sugar source) that is utilized by many other insects such as ants. They damage plants by puncturing the plant body and consuming the exuding sap. This feeding alters the plant tissue by drying up the leaves, forming galls (growth deformities), and stunting plant growth (INHS 2010). Aphid reproduction may occur through parthenogenesis for most or all of their life which allows unmated females to produce clonal (live) female offspring (van Emden & Harrington 2007). However, aphids can be viviparous (give birth to live young) and oviparous (lay eggs) during various periods of the year (van Emden & Harrington 2007). Male aphids are still unidentified in some species (Illinois Natural History Survey 2010). The ability of parthenogenesis can allow aphid populations to grow exponentially and exhibit high turn-over rates. Throughout the life cycle of aphids, little movement is involved and consists of feeding and producing offspring (van Emden & Harrington 2007). Migration and dispersal only occur in response to host plant death of the individual or the entire area, in addition to overpopulation of the species itself (van Emden & Harrington 2007). These stimuli trigger the emergence of winged aphids (alatae) that leave the area in search of healthy host plants (van Emden & Harrington 2007). Aphids can act as vectors for many plant diseases such as the alfalfa mosaic virus, cucumber mosaic virus, and tobacco streak virus (INHS 2010; Mueller et al. 2010).
As observed in a range of other agricultural crops, thrips and aphids have the capacity to damage many different plant hosts in the process of direct feeding and transmission of plant viruses. Decreases in crop productivity contribute to yield losses and economic deficit all over the world. Potential biofuel crops are at high risk of insect attack by these arthropods in particular because of their polyphagous (multiple plant hosts) feeding behavior. Large-scale bioenergy cropping systems may be devastated by thrips and aphid pests if proper management strategies are not established. Current pest strategies to combat the western flower thrips and varieties of aphid pest species in agriculture include: biological control agents (parasitoids and predators) and insecticides (Reitz 2009; Bethke et al. 1994). Greenhouse studies have also demonstrated the feasibility of utilizing integrated pest management (IPM) strategies such as insect growth regulators (IGRs) that kill thrips and aphids by disrupting their development and biorational pesticides that use various strains of fungus to kill these insects (Greer 2000). Biorational pesticides utilize moderately non-toxic materials that impact ecosystems on a lesser scale than commercial pesticides; major categories include: “botanicals, microbials, minerals, and synthetic materials” (Grubinger 2010). However, these pests have demonstrated insecticide resistances in many different crops due to constant exposure to an array of chemicals (Reitz 2009; Bethke et al. 1994). Examples of the types of damage thrips and aphids cause and the management strategies in place to combat them serve as a comparison of what may be developed for biofuel cropping systems.

Figure 6. Examples of Common Aphid Pest Species a. Green Peach Aphid (University of Florida 2010) b. Cotton Aphid (University of Tennessee 2010)
Purpose of Study

In an effort to better understand how herbivorous arthropod communities impact potential biofuel crops, research objectives are 1) to determine when *P. virgatum* and *M. x giganteus* crops would be most susceptible to thrips damage and 2) to examine aphid feeding preference among seven cellulosic biofuel grasses and five nitrogen fertilization rates in *P. virgatum*. This information will provide further insight into the times at which thrips populations fluctuate and also indicate the plant host suitability for each insect pest. The influence of cultivation practices such as nitrogen fertilization applications in correlation with aphid feeding preference will also be assessed. It is hypothesized that aphid and thrips communities have the ability to infest *M. x giganteus* and *P. virgatum* biofuel crops based on pest issues previously observed in other agricultural cropping systems. In addition, populations of the western flower thrips in *M. x giganteus* and *P. virgatum* plots are expected to peak during the highest point in the growing season when more biomass is available to support large numbers of insects. Finally, it is hypothesized that larger numbers of aphids would be observed on *P. virgatum* leaves of higher nitrogen content; in contrast to thrips, aphids are easily captured in the field and raised in laboratory settings.

Study Sites and Methods

Western flower thrips (*Frankliniella occidentalis*) populations inhabiting *M. x giganteus* and *P. virgatum* plots were monitored during the growing season (May through July 2010) at the Great Lakes Bioenergy Research Center (GLBRC) Intensive Experimental Site at Kellogg Biological Station. The 22 ha site studies ten different treatments that are replicated and subdivided into five blocks (Figure 7) (KBS LTER 2010). The GLBRC Intensive Experimental Site is a long-term experiment that contains two grain based crops (continuous corn and corn-soybean-canola), in addition to five cellulosic crops (*switchgrass*, *miscanthus*, poplar trees, native grass mix including four species, old field plant community, and restored prairie). Project goals for this site include observing the productivity of novel biofuel cropping systems, measuring yield and quality of biomass, assessing relationships between crops and microbial communities, nutrient requirements, as well as biogeochemical factors. Four plots (40 x 28 m) of *M. x giganteus* and *P. virgatum* were selected for insect sampling. In each plot, two yellow sticky traps (Seabright Laboratories Sticky Aphid Whitefly Trap) attached to step-in fence posts were
positioned randomly, a few meters apart from each other. All traps faced southward to avoid any directional effects on thrips population. Traps were collected on a weekly basis. Sticky traps were examined in the laboratory and counted to determine thrips population per plot.
Study Site 1

Figure 7. KBS GLBRC 2010 Intensive Field Site (LTER KBS 2010)

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The experiments were performed May through July 2010 under laboratory conditions to i) study the feeding preferences of the spotted cereal aphid (*Sipha elegans*; *Hemiptera: Aphididae*) on different biofuel grasses and ii) determine the effects of nitrogen fertilizations on aphid feeding and reproduction on *P. virgatum*. The setup for both experiments was similar in equipment and methodology. Leaves of different potential biofuel grasses (miscanthus, switchgrass, indiangrass, big bluestem, little bluestem, reed canarygrass, and prairie cordgrass) were collected from the GLBRC Biofuel Productivity Trial (Figure 8), and of five nitrogen fertilizer levels (0 lbs., 25 lbs., 75 lbs., 125 lbs., and 175 lbs.) from the GLBRC Switchgrass Nitrogen/Harvest Experimental Site (Figure 9). The GLBRC Biofuel Productivity experiment was established in May 2009 with an aim to determine the productivity of new biofuel crops. It contains 15 different biofuel crops replicated in four blocks. The GLBRC Switchgrass Nitrogen/Harvest Experimental Site established in June 2008 is a continuous *P. virgatum* system that receives nine different fertilization rates from 0 to 175 lbs./acre in order to demonstrate how nitrogen impacts plant productivity. Each plot is 4.6 m x 15.2 m and is replicated in four blocks.
### Study Site 2

**Figure 8.** KBS GLBRC 2010 Biofuel Productivity Experiment (KBS LTER 2010)

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Study Site 3

Figure 9. KBS GLBRC 2010 Switchgrass Nitrogen/Harvest Experiment (KBS LTER 2010)

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Leaves obtained from each site were then cut into approximately 3 cm strips. To check the feeding preference for different hosts or fertilization levels, one strip of each type was placed together in five Petri dishes (15 cm dia) lined with moistened white filter paper. Each host was arranged along the outer rim of the Petri dish equally spaced from one another. Ten female, non-apterous aphids were introduced to the center of each dish, giving the aphid choice of seven different grass species or five nitrogen levels at approximately the same distance from the aphids. Parafilm was then used to seal the Petri dishes. Measurements of aphid number and general location were recorded 24 hours after aphid introduction; the initial reaction of the aphids to different host plants was desired resulting in a 24 hour time period. This experiment had 5 replicates and was repeated 3 times (replication in time). Aphid fecundity (reproduction) in relation to different host types (grass sp. and fertilization levels) were assessed by introducing one adult female on two strips of each host type in three Petri dishes (6 cm dia) lined with moistened white filter paper. Parafilm was again used to seal the Petri dishes. Aphid number was counted after 4 days of aphid introduction; aphid proliferation requires more than a 24 hour time period. All Petri dishes were kept in drawers to deter light exposure and promote aphid activity. This experiment had 3 replicates and was repeated 4 times (replication in time).

**Statistical Analyses**

The results were analyzed by using a one-way analysis of variance (ANOVA) and separation of means was subjected to Student’s t-test using statistical software JMP, Version 7. SAS Institute Inc., Cary, NC, 1989-2007. Statistical significance was accepted if p-value was < 0.05.

**Results**

(a) Western Flower Thrips (*Frankliniella occidentalis*)

Weekly trap counts indicated the population of thrips peaked between late May and early June on *M. x giganteus* and *P. virgatum*, respectively (Figure 10). Significantly higher numbers of thrips (P<0.001) were caught on *P. virgatum* (ranged from 35.13 to 212.25 thrips/trap) compared to *M. x giganteus* (ranged from 5.63 to 117.25 thrips/trap). In addition, a gradual decline in thrips number is observed over the sampling dates (Figure 10).
Feeding preference of the cereal aphid was significantly higher on big bluestem (2.93 aphids/leaf strip) followed by \textit{M. x giganteus} (2.26 aphids/leaf strip) and \textit{P. virgatum} (2.13 aphids/leaf strip) (Figure 11); little bluestem was the least preferred host of the spotted cereal aphid with 0.33 mean number of aphids per leaf strip. Feeding preference correlates to the number of aphids found on the plant of study.

Reproduction (fecundity) was highest on \textit{M. x giganteus} (8.11 average offspring) followed by big bluestem (4.89 average offspring). The fecundity was lowest on Indian grass with average offspring of 2.33. Increasing but non-significant trends were observed in aphid feeding preference and fecundity with increasing plant nitrogen levels (Figure 12). The p-values reported for aphid fecundity and preference were .680 and .392, respectively.
Host Plant

Figure 11. Feeding preference and fecundity of cereal aphid on seven potential biofuel grasses

Figure 12. Feeding preference and fecundity to five nitrogen fertilization levels in *P. virgatum*
Discussion

Field and laboratory studies indicate that insect pests demonstrate preferences for certain biofuel crops. Weekly trap counts exhibited higher numbers of thrips in *P. virgatum* than *M. x giganteus* (Figure 10). In contrast to the hypothesis, populations of thrips in both biofuel grasses did not peak during the highest point of the growing season. The reason could be presence of other preferred hosts such as blooming summer weeds in the vicinity of plots.

**Insect Host Feeding Preference**

Aphids were observed to display similar feeding preferences in regards to *M. x giganteus* and *P. virgatum* (Figure 11). However, the reproductive success of aphids was greatest on *M. x giganteus*. A variety of factors may have influenced how thrips and aphids responded. The plant physiology specific to studied host plants may account for observed differences. For instance, *M. x giganteus* was observed to grow much higher and thicker and possessed leaves with greater width and length than *P. virgatum*. Perhaps thrips were able to colonize and survive much more easily in *P. virgatum* due to its low height and small leaves. In contrast, aphids may have found *M. x giganteus* more suitable for survival. *M. x giganteus* leaves provide more surface area and protection than *P. virgatum*. Larger numbers of aphids can escape predators by taking refuge on the underside of *M. x giganteus* leaves. In addition, thicker leaves can support the additional weight of offspring. Females may have sought *M. x giganteus* as a more suitable environment for the development of offspring than thin and open fields of *P. virgatum*.

Chemical differences between *P. virgatum* and *M. x giganteus* biomass could also reveal correlations pertaining to insect palpability of a host. For example, different ratios of carbon to nitrogen have been shown to influence the feeding behavior of insects (Coviella & Trumble 1999; Trumble & Butler 2009). *M. x giganteus* plots also demonstrated consistently lower thrips number than *P. virgatum* plots from the start of sampling. However, populations decrease even more so after June 7, 2010 and continue to remain under 25 thrips until the end of sampling (Figure 10). This decline may be attributed to the high productivity of *M. x giganteus* during this period.

Aphids are known to migrate when presented with stress (van Emden & Harrington 2007). Thrips may have responded similarly and encountered difficulties that inhibited survival in *M. x giganteus* as it grew. Symptoms of insect damage were also first observed in *M. x giganteus* plots. The sampling of *P. virgatum* did not occur until similar damage was noted. In
the presence of stress, thrips may have migrated to *P. virgatum* plots; close proximity existed (Figure 7). As the thrips community increased exponentially, biomass availability decreased and reduced thrips survival. However, thrips populations significantly increased July 5, 2010 in *P. virgatum* but returned to numbers encountered in the previous week. Larger populations could be supported by the reduction of thrips and increase of biomass. As soon as resources were depleted, numbers returned to a level that could be supported. As observed, insect pests exert certain pressures on *M. x giganteus* and *P. virgatum*. Thrips and aphids require certain needs that drive their preference for food and habitat. Potential biofuel crops will ultimately face varying levels of stress that will require each species to be managed differently.

*Influence of Nitrogen Fertilization on Insect Activity*

Though not statistically significant, the data suggest a trend that aphid feeding preference and fecundity increase in response to elevated nitrogen applications (Figure 12). Many studies have shown that fertilization rates in agriculture impact the predation of insect pests. For example, according to Chen et al. (2008), herbivorous arthropods were more abundant in cotton crops when treated with greater nitrogen fertilization applications. Data suggest that as the fertilizer level is increased in *P. virgatum* cultivation, the aphid count also increased. Research conducted by Chau and Heinz (2006) also demonstrated that reducing fertilization levels decreased thrips populations in chrysanthemum. Conducting additional trials would be useful in determining if higher nitrogen applications in *P. virgatum* do indeed increase aphid attack and damage.

Biofuel agriculture requires a middle ground that must be established in order to control populations of aphids and other damaging pests. For instance, high yields will be expected from fast growing crops like *P. virgatum* and *M. x giganteus* but may be more palatable to insects in response to high fertilization. To overcome these issues, a variety of conventional techniques could be utilized. For instance, insect growth regulators (IGRs) could be applied to fields in combination with fertilization applications to inhibit insect development and thus reduce crop loss. Biocontrol agents and biorational pesticides could also be used and managed with fertilizers to discourage insecticide/pesticide resistances in biofuel agriculture. Furthermore, thrips data also demonstrates that insect pests decline over time (Figure 10). Management practices and techniques may consequently be intensive at the beginning of the season, but require gradually fewer inputs and treatment throughout. Novel approaches to integrated pest management and
biofuel crop cultivation must be explored to determine how farmers can compromise high crop yield and the methods to achieving that biomass with the loss and damage presented by insect pests.

To improve the accuracy of this data, various methodological considerations can be implemented. Additional trials can be conducted to improve the sample size and obtain a better representation of the sample groups. Performing these experiments over multiple seasons - at the very start of the growing season to harvest - would also be advantageous. Furthermore, weather factors such as rainfall and temperature could be assessed in relation to thrips populations to help explain observed fluctuations; higher temperature and moisture content would create more favorable conditions for insect reproduction (Trumble & Butler 2009). The field of biofuel agriculture provides a range of studies that can further analyze the impact of insect pest communities. For instance, other insect pests familiar in the agricultural world can be evaluated as risks for biofuel crops. Insecticides, pesticides, and IPM strategies could also be examined in the field to assess their success in treating biofuel cropping systems. Although M. x giganteus and P. virgatum are the focus of many recent studies, a variety of potential biofuel crops are awaiting research. Insect pest communities may impact these other promising species and necessitate unique management strategies. Further research will be required to sufficiently analyze what biofuel crops will best suit our needs for the future.

**Implications and Future Research**

Biofuel crops are vulnerable to attack and disease by insect pests. Novel cultivation practices and management will need to be set in order to advance biofuel agriculture. Although susceptible to insect attack, M. x giganteus and P. virgatum continue to demonstrate ideal characteristics as bioenergy feedstocks. Properties of high yields, low energy inputs, nutrient use efficiency, and resistance to cold temperatures outweigh associated costs required to that advance these crops to successful alternative energies. The impact of the western flower thrips and spotted cereal aphid as biofuel pests also provides future implications for bioenergy cropping systems. Transferring biofuels to large-scale agriculture has not been adequately studied and could possibly hinder ecosystem services provided by arthropod communities.

Vital insect associations could be significantly altered in response to the conversion of marginal lands to biofuel production croplands. Arthropods like thrips and aphids are consumed by organisms higher in the food chain. Changes in the distribution and habitat could cause
detrimental population shifts of their consumers (Landis & Werling 2010). In addition, the exotic lineage of some of the proposed biofuel feedstocks may promote high productivity due to the absence of natural pests, though the interactions with native and invasive insects are unknown (Spencer & Raghu 2009). Studies associating biofuel production systems with arthropods have indicated that insects “cause shifts in biofuel crop community composition or productivity,” biofuels have the ability to “increase insecticide resistance” and “act as reservoirs for insect-transmitted diseases,” in addition to impacting the “pollination/pest control services to the surrounding landscape” (Landis & Werling 2010). The development of unique agricultural practices and integrated pest management systems will be required to commercialize biofuel agriculture. It is highly probable that the expansion of bioenergy cropping systems in North America will result in impacts to arthropod communities, both negative and beneficial. Known insect pests in current biofuel production will be significant as new pests are discovered. In regards to thrips and aphids, utilizing other pest information from established agricultural cropping systems will provide significant data for future studies. Fully understanding how arthropods influence biofuel crops through systematic research will be necessary to successfully achieve government initiatives in the search for alternative energies.
Literature Cited


