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Characterization of the Arthropod Communities and Aphid Feeding Behavior Associated with Perennial Warm-Season Grasses (Poaceae) Composition in Nebraska and Wisconsin

Kathryn M. Keller
University of Nebraska-Lincoln

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by

Kathryn M. Keller

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Kathryn Marie Keller, M.S.

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Advisers: Tiffany Heng-Moss and Thomas J. Weissling

Switchgrass, *Panicum virgatum* L., along with two other perennial warm-season grasses, big bluestem (*Andropogon gerardii* Vitman) and indiangrass, (*Sorghastrum nutans* L), compose a majority of the grasses found in North American tall grass prairies and have recently received attention as potential bioenergy feedstock. Limited research has been carried out on the relationship of arthropods on these three warm-season grasses in North America. Due to this limited research, the first objective of this research was to document the arthropods associated with switchgrass, big bluestem and indiangrass in Nebraska and Wisconsin over three sampling seasons. More than 10 arthropod orders and over 67 families were collected between the two locations with some of the most abundant families collected including: Carabidae, Chloropidae, Cicadellidae, Figitidae, and Thripidae.

Previous research has documented greenbugs (*Schizaphis graminum* Rondani) and yellow sugarcane aphids (*Sipha flava* Forbes) as potential pests of switchgrass, but limited information is available on the host suitability of big bluestem and indiangrass to these two aphid species. Therefore, the second objective of this research was to document aphid feeding preference among these three grass species through a series of choice

studies and to characterize greenbug feeding behaviors using the electric penetration graph (EPG) technique. Choice studies identified differences in the preference of two aphid species in response to the three grasses with switchgrass being most preferred by *Schizaphis graminum* at 1, 2 and 4 h; whereas switchgrass was the least preferred by *S. flava* starting at 24 h after aphid introduction. Feeding behavior studies of *S. graminum* on switchgrass, big bluestem and indiangrass indicated that greenbugs took significantly more time before achieving the first sieve-element phase (salivation and ingestion of sieve element phloem sap) when feeding on indiangrass compared to both switchgrass and big bluestem, suggesting resistance factors in indiangrass are associated with phloem tissue. These studies are the first to examine the feeding preference of *S. graminum* and *S. flava* on big bluestem and indiangrass. This research provides important baseline information about the arthropod communities associated with the three warm-season grasses, and advances our understanding of the plant-insect interactions within this system.

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Jeremiah 29:11

"For I know well the plans I have in mind for you, says the Lord, plans for your welfare, not for woe! Plans to give you a future full of hope."

GRANT INFORMATION

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Introduction

Switchgrass, *Panicum virgatum* L., is a perennial warm-season grass that has recently showed much potential as a bioenergy crop in the United States. Much of current switchgrass research has focused on the agronomic side and disease with limited research concentrating on the potential arthropods associated with switchgrass. Along with switchgrass there are two other warm-season grasses that have also demonstrated the potential as a bioenergy crop in the USA, big bluestem, *Andropogon gerardii* Vitman, and indiangrass, *Sorghastrum nutans* (L.). Together these three grasses compose a majority of the grasses found in North American tall grass prairies (Bouton 2008). Therefore the overall goals of this research were to characterize the arthropod communities associated with switchgrass, big bluestem and indiangrass, characterize the host preference for potential aphid pests, and elucidate aphid feeding behavior on these three grasses.

Specific Objectives

1) Conduct a survey of arthropods at the order and family levels for switchgrass, big bluestem, indiangrass and a low diversity mix (LDM)
2) Examine the host preference of yellow sugarcane aphid, *Sipha flava*, and the greenbug aphid *Schizaphis graminum*, on big bluestem, indiangrass and switchgrass
3) Describe the feeding behavior of the greenbug aphid on big bluestem, indiangrass and switchgrass

Literature Review

Switchgrass

Switchgrass is a warm-season perennial C_4 grass species native to the grasslands of North America, with a range from Mexico to Canada (Vogel 2004). Because of its wide range, switchgrass has evolved into several diverse populations resulting in a wide variation within the species (Vogel et al. 2011, Zalapa et al. 2011, Lu et al. 2013). Switchgrass has various characteristics that make it very useful in conservation, livestock production, and bioenergy feedstock (Vogel 2004). Switchgrass is known to grow to a height of half a meter to three meters, depending on the population, with most genotypes growing in a caespitose appearance (i.e. growing in dense clumps) (Bouton 2008). The basic chromosome number of switchgrass is 9, however several ploidy levels of switchgrass do exist, with tetraploid (2n = 4x= 36) and octoploids (2n = 8x = 72) being predominant (Moser and Vogel 1995, Bouton 2008, Vogel et al. 2011, Zalapa et al. 2011, Lu et al. 2013).

There are two distinct ecotypes of switchgrass, lowland and upland, which can be distinguished by their chloroplast markers (Hultquist et al. 1997, Young et al. 2012). The ecotypes are specific populations within the switchgrass species that are more adapted to a particular environment. The lowland ecotype is better adapted to grow in flood plains and has the potential to grow quicker than the upland ecotype (Vogel 2004). The upland ecotype, which is often shorter than lowland, does not grow as quickly and is found in areas that are not subject to flooding (Vogel 2004). The lowland ecotype is also usually a tetraploid, whereas the upland ecotype is often octoploid.

Liberty

'Liberty' is a lowland switchgrass ecotype that has been developed specifically for bioenergy production. Liberty development began in 1996 by Ken Vogel and USDA-ARS grass breeding program (Vogel et al. 2014). Two switchgrass cultivars were used in the initial crossing for Liberty: Summer (female parent cultivar) and Kanlow (male parent cultivar). Summer is a tetraploid, upland ecotype that is based on the germplasm collected in southeast Nebraska, and Kanlow is a tetraploid lowland cultivar that originated from a collection in Oklahoma (Vogel et al. 2014). Their goal with selecting the two parental lines was to improve the winter hardiness of switchgrass.

After three generations of breeding, Vogel et al. (2014) was able to maintain the winter hardiness of Summer and the high yield potential of Kanlow within Liberty. Due to these kept qualities, Liberty is now seen as the most elite and first of its kind switchgrass cultivar that is high yielding, can withstand the harsh Midwest winters, and an ideal bioenergy grass (Vogel et al. 2014). 'Liberty' has a typical lowland switchgrass phenotype, but with having Summer as the female in the original cross it has an upland cytoplasm (Hultquist et al. 1996). Liberty, a tetraploid cultivar, can be distinguished from other Kanlow switchgrass cultivars by its earlier maturity and chloroplast markers; it can be distinguished from Summer switchgrass cultivars by its lowland phenotype (Vogel et al. 2014). The USDA-ARS and the Agricultural Research Division of the University of Nebraska officially released Liberty on November 27, 2013 (Vogel et al. 2014). Despite these attributes, little is known about the potential insect pests associated with Liberty. This information is critical for the future development of Liberty for bioenergy production.

Bioenergy Feedstock Potential of Switchgrass

Traditionally, switchgrass is a main component of North American grassland prairies. It has been used in pastures as a rangeland crop for cattle as well as the conservation reserve program (CRP) for grassland conservation. Switchgrass also has the potential to produce nutritious hay if cut when seedheads are beginning to emerge. Because of its competitive nature, switchgrass is best managed as a monoculture. When planted in a mixture, switchgrass has a tendency to shade out other plants and out compete them for nutrients due to its complex rhizome system. When planted in a mixture, no more than 20% of the seed should be switchgrass (Vogel 2004).

Within the past twenty years the use of switchgrass as a potential biofuel source has come into consideration through a series of evaluations by the U.S. Department of Energy (US-DOE) (Vogel 1996, Vogel et al. 2002, Sarath et al. 2008). The biomass feedstocks that are currently in use produce ethanol from sugar- and starch-rich crops, such as maize, by fermenting the starch in the grains (*Zea mays* L.). However, there are some negative impacts from this type of ethanol production due to the requirements of a labor-intensive agricultural system and requiring high inputs (e.g. nitrogen fertilizer). These requirements may negatively impact the carbon dioxide (CO_2) and overall energy balance within the agricultural system (Jakob et al. 2009). Other factors such as drought and biodiversity loss may lead to even more negative effects on the environment (AGMRC 2015, Conca 2015).

Ethanol can be produced from other plant products as well, such as the fermentation of sugar in the cell walls, primarily cellulose and hemicellulose. Forage

crops, such as switchgrass, have a high cell wall content (Vogel 1996). Due to their high cell wall content, bioenergy feedstocks such as switchgrass, miscanthus, sorghum, big bluestem and indiangrass are promising candidates of future renewable energy solutions. This is because of their reduced need for annual input costs and lower needs of fossil fuels used for production, creating a more positive energy balance (Hill et al. 2006, Rooney et al. 2007, Heaton et al. 2008).

Switchgrass, along with other warm-season grasses such as big bluestem and indiangrass, has been selected as a promising candidate for bioenergy cropping for a wide variety of reasons, including: high levels of production across diverse landscapes; suitability for marginal and erosive land; low water and nutrient requirements; positive environmental benefits; and compatibility with modern farming practices (Sanderson et al. 1996, McLaughlin et al. 1999, Sanderson et al. 2004). It was documented in yield data from 2010 that land quality and soil texture do not appear to have a significant impact on the overall yield of switchgrass (Wullschleger et al. 2010). In addition, due to the extensive root system of switchgrass, it may help reduce the rates of erosion and runoff on potential marginal land (McLaughlin and Walsh 1998). All of this will aid in reducing nutrient loss in the soil, increased usage of soil carbon, and overall reduction in chemical usage compared to annual row crops (McLaughlin et al. 1994, Sanderson et al. 1996). It is estimated that with the production of herbaceous energy crops, such as switchgrass, big bluestem, and indiangrass, there would be a 95% reduction in rates of soil erosion compared to annual row crops (Hohenstein and Wright 1994). Life cycle analysis models that estimated ethanol production from switchgrass averaged 94% lower greenhouse gas (GHG) emission than from gasoline (Schmer et al. 2008)

The overall yield of switchgrass can vary depending on the location and cultivar, averaging 10 to 14 Mg ha^{-1}, although yields of up to 40 Mg ha^{-1} have been reported in select locations with increased fertilizer inputs and precipitation (Wullschleger et al. 2010). It is anticipated that yield rates will continue to improve with breeding efforts to help incorporate traits such as insect resistance and cold hardiness (Perlack et al. 2005, Bouton 2008). Bioenergy crops, such as switchgrass, big bluestem, and indiangrass, will depend not only on their biomass energy produced, but also on the energy required to grow the crop and convert it to usage energy. It is estimated to have an average energy ratio of 1.34 (i.e., for every joule used to produce ethanol from maize there is a 34% energy gain), with a best case scenario energy ratio of 1.53 for maize (i.e. 53% net energy gain) (Shapouri et al. 2003). However, similar studies done with switchgrass have indicated energy ratios of 4.43 (443% net energy gain) (McLaughlin and Walsh 1998) to greater than 5.40 (540% net energy gain.) (Schmer et al. 2008).

Big Bluestem

Big bluestem, *Andropogon gerardii* Vitman, is also a warm-season perennial C_4 grass species native to the grasslands of North America, having a range similar to that of switchgrass being found east of the Rocky Mountains. Big bluestem has traditional uses similar to switchgrass, including conservation, erosion control and as a forage crop for livestock (Wennerbery 2004). Big bluestem has a base chromosome number of x =10; with most populations being predominately hexaploid plants (2n= 60) (Boe et al. 2004). Big bluestem, growing to a height of 2 meters, can be distinguished from other warm-season grasses by the blue coloration on the culm, or the stem of the plant, and the 3-parted flower clusters that resemble a turkey's foot (Wennerbery 2004). Big bluestem

also has the potential to be a bioenergy crop due to the high cellulose content in the cell walls.

Indiangrass

Indiangrass, *Sorghastrum nutans* (L.) Nash, is another major component of the tallgrass vegetation and has traditionally been employed as a range crop for livestock, conservation and erosion control (Owsley 2011). It is usually in seeding mixtures with big bluestem, switchgrass and other C_4 grasses (Mitchell and Vogel 2004). This warm-season perennial C_4 grass species native to the grasslands of North America has a native range similar to switchgrass, being found throughout most of the United States and Canada. Indiangrass, with a base chromosome number of x = 10, is known to grow to a height of 1 to 1.5 meters and can be distinguished by its "rifle-sight" ligule, the outgrowth at the junction of the leaf and the leafstalk (Mitchell and Vogel 2004, Owsley 2011). Indiangrass also has the potential to be a bioenergy crop with a high cellulose content in the cell walls: however, very limited research has previously examined the arthropod community associated with this warm-season grass species.

Arthropods Associated with Switchgrass, Big Bluestem and Indiangrass

Grasses (family Poaceae) host a diverse array of arthropods. While switchgrass is one of the most well-studied native warm-season grasses, most of the research to date has primarily focused on improving the agronomic qualities and understanding abiotic and biotic stressors including understanding the viruses and other diseases that affect these grasses. Only a few studies have looked at the arthropod communities associated with these grasses (Boerner and Harris 1991, Gottwald and Adam 1998, Kindler and

Dalrymple 1999, McIntyre and Thompson 2003, Raun and Mitchell 2008, Schaeffer et al. 2011, Ullah 2012, Kempski 2013, Koch et al. 2014a, Koch et al. 2014b, Koch et al. 2014c, Prochaska 2015).

Schaffer et al. (2011) conducted a survey in the summers of 2007 and 2008, investigating the arthropod community associated with managed switchgrass fields at varying stand ages and found 84 families spanning 12 arthropod orders. Thysanoptera, Hymenoptera, and Coleoptera were the most abundant orders collected, composing 80% of all arthropods collected (Schaeffer et al. 2011). Some insects have only anecdotally been documented with pest potential in switchgrass, such as grasshoppers (Acrididae), where the problem can vary within a single site, year and population (Vogel 2004, Parrish and Fike 2005). Overall, very few studies have been published on insects and their pest status in switchgrass and other warm-season grasses such as big bluestem and indiangrass. Due to these grasses being relatively pest free in their native habitat, this often results in the common belief that switchgrass, big bluestem and indiangrass will require few pest management practices.

It was documented by Kindler and Dalrymple (1999) that yellow sugarcane aphids, *Sipha flava* Forbes, can feed and reproduce on switchgrass, suggesting the potential for severe damage. However in this study it was found that switchgrass was used as a host only in the absence of a more preferred host, and that aphid longevity and fecundity on switchgrass was much lower compared to the development and reproduction on other C_4 grass species (Kindler and Dalrymple 1999). In a native setting, the yellow sugarcane aphid is not likely to pose a serious threat to switchgrass given the preference of a more favorable host. However, when grown in a monoculture setting, there is a

potential for the yellow sugarcane aphid to become a serious pest to switchgrass, especially in the southern regions.

The bluestem gall midge (*Stenodisplosis wattsii* Gangè) was documented on switchgrass, along with other warm-season grasses such as big bluestem, little bluestem and indiangrass throughout Nebraska (Raun and Mitchell 2008). This study also reported the midge to be a significant pest in seed production fields. In 2008 a new species of gall midge, *Chilophaga virgate* Gangè (Diptera: Cecidomyiidae) was collected in South Dakota from switchgrass fields. *C. virgati* infests plant tillers and the infested tillers showed a reduction in length and produced only 35% of the mean weight of uninfested tillers when averaged across all cultivars (Boe and Gangè 2010).

In a study of avian feeding habits, McIntyre and Thompson (2003) observed grasshoppers (Acrididae and Tettigoniidae) were more prevalent in native grass stands than in stands with introduced grass species. While this study did not focus primarily on grasshopper abundance, it did show grasshoppers being found in switchgrass and other warm-season grasses, with a potential to cause significant biomass yield loss (McIntyre and Thompson 2003). Researchers in Germany also discovered thrips (Thysanoptera) could colonize switchgrass under drought stress conditions (Gottwald and Adam 1998). Collembola, although not recognized as a significant plant pest, were observed feeding on the microrrhize associated with switchgrass. However, they appeared to have little impact on the plant nutrient availability (Boerner and Harris 1991).

The tallgrass prairie, of which switchgrass, big bluestem and indiangrass are all a component, are hosts to numerous arthropod groups. The following insect orders are

common in native prairie settings: Orthoptera, Coleoptera, Hemiptera, and Thysanoptera. Dipteran and lepidopteran larvae have also been reported with prairie plants (Bruner 1899, Shelford 1963, Blocker 1969, Risser et al. 1981, Whiles and Charlton 2006). Some of these potential lepidopteran pests include the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), armyworm, *Mythimna* (*Pseudaletia*) *unipuncta* (Haworth), and three species of stem-boring moths, *Blastobasis repartella* (Dietz), *Haimbachia albescens* Capps (Crambidae) and *Papaipema nebris* (Guenée) (Noctuidae) (Adamski and Hodges 1996, Capinera 2005, Prasifka et al. 2009a, Prasifka et al. 2009b, Prasifka et al. 2011a, Prasifka et al. 2011b, Capinera 2013).

One of the most potential warm-season grass pest of interest has been the fall armyworm, *S. frugiperda*. It is a noctuid moth that can only successfully overwinter in the southern parts of Florida and Texas. Prasika et al. (2009b) documented the feeding and development of two strains of *S. frugiperda* on *Miscanthus* x *giganteus* and switchgrass, finding that *S. frugiperda* development on switchgrass was similar to other alternate hosts and in some cases even more favorable than alternative hosts (Prasifka et al. 2009b). The armyworm, *M. unipuncta*, is another important pest of pastures, grain crops and weedy grasses. Unlike *S. frugiperda*, *M. unipuncta* may be able to overwinter in areas further north such as Tennessee (Capinera 2013). Due to this northern range of the armyworm it may be able to infest warm-season grasses grown for biofuels earlier in the season. Prasifka et al. (2011a) performed and evaluated *M. unipuncta* defoliation experiments on Kanlow switchgrass and found that exceptionally high *M. unipuncta* densities (120-150/m^2) would only produce a reduction of 20% in plant biomass,

indicating that situations requiring insecticide control for *M. unipuncta* may be scarce (Prasifka et al. 2011a).

More potential warm-season grass pests include three stem-boring moths: *B. repartella*, *H. albescens*, and *P. nebris*. *B. repartella*, initially reported in South Dakota in 2004, was more extensively surveyed by Prasika et al. (2009a). The survey suggests *B. repartella* may be present in established switchgrass across the Midwestern US (Prasifka et al. 2009a). Prasika et al. (2011b) further documented *H. albescens* and *P. nebris* in switchgrass stands in Illinois and Iowa during 2010 (Prasifka et al. 2011b). For these three stem-boring moths documented on switchgrass it is unlikely that *B. repartella* and *H. albescens* will have a significant impact on switchgrass production with only mild stunting (usually < 5%); however, *P. nebris* may have a greater potential to damage switchgrass, as stalk borer larvae move between the stems and could kill several tillers during the first three growing months (Prasifka et al. 2011b). Although the moths do not currently appear to be a serious pest currently in warm-season grasses, several complications could alter the pest status of these stem-boring moths. Chemical management could be very difficult to use in the future, due to stem-borers living on the inside of the plant.

In addition to herbivores, arthropod predators in the orders Coleoptera, Diptera, Hemiptera and Araneae, have been found in these prairie habitats. Table 1 summarizes the arthropods documented in prairie habitats. Ants (Formicidae) are another group of beneficial arthropods that have been previously documented in prairie settings, helping with soil aeration and seed movement beneath the surface of the soil. Pollinators, such as

halictid bees, have also been documented in prairie habitats (Shelford 1963, Risser et al. 1981).

Until recently, few studies have looked at phloem-feeding insects that could be potential pests of switchgrass. These studies include members of the hemipteran families Aleyrodidae (whiteflies), Cicadellidae (leafhoppers), Delphacidae (planthoppers) and Aphididae (aphids), which are sometimes referred to as the most damaging pests worldwide (Hilder et al. 1995). Recently, studies in switchgrass have focused on the family Aphididae. Aphids are known as a major pest of agricultural crops worldwide and may be of particular importance for their ability to damage these crops by removing the photo assimilates along with their efficient ability to transmit several damaging plant viruses (Smith and Boyko 2007).

For switchgrass, big bluestem, and indiangrass to reach their full potential as bioenergy crops, the arthropod complex needs to be more thoroughly examined for these three warm-season grass species. Because little is known about the current arthropods associated with these three warm-season grasses, previous work has suggested that insect pests will emerge as these grasses are cultivated into a monoculture setting. This was shown to be the case with buffalograss, *Buchloe dactyloidea* (Nattall) Engelmann, another warm-season grass species. Like switchgrass, buffalograss was thought to be relatively pest free in native settings (DeShazer et al. 1992). However, as buffalograss turf production increased, pests began to emerge. In the late 1980s, the mealybugs, *Tridiscus sporoboli* (Cockerell) and Trionymus sp. were documented as potential pests of buffalograss in field and greenhouse production systems (Baxendale et al. 1994), with further studies revealing the western chinch bug, *Blissus occiduus* Barber, also causing

significant damage to buffalograss (Baxendale et al. 1999, Eickhoff et al. 2004, Eickhoff et al. 2006). However, beneficial arthropods (including ground beetles, spiders, hymenoptera parasitoids and hemipteran predators) have also been found in buffalograss to help minimize pest outbreaks (Heng-Moss et al. 1998, Carstens et al. 2007). Germplasm screenings have identified buffalograsses with resistant to mealybugs (Johnson-Cicalese et al. 1998) and the western chinch bug (Heng-Moss et al. 2002, Gulsen et al. 2005). Overall, it is clear that the development of switchgrass, big bluestem, or indiangrass as a bioenergy crop will require effective pest management strategies.

Greenbug

Aphids have been associated with switchgrass, big bluestem and indiangrass to a limited extent. Previous research by Koch et al. (2014a) indicated switchgrass as a suitable host for two aphid species: *Schizaphis graminum* (Randani) and *Sipha flava* Forbes. The greenbug, *Schizaphis graminum* (Randani), is parthenogenetic in nature. There are three nympal instars before the aphid molts into the adult stage, occurring in 7-9 days at temperatures between 60-80° F. An adult greenbug can produce up to five nymphs a day (Nuessly and Nagata 2005). This aphid has been observed to feed on more than 70 graminaceous species, including barley, bluegrass, maize, sorghum, switchgrass, wheat and wheatgrass (Michels Jr. 1986, Nuessly and Nagata 2005, Koch et al. 2014a).

The greenbug has been recognized as a pest of small grains for the past 150 years (Nuessly and Nagata 2005). There are currently 40 recognized species of Schizaphis worldwide, with seven found in North America (Blackman and Eastop 1984, Nuessly and Nagata 2005). The first report of greenbug in North America was documented in Virginia

in the early 1880s, with greenbug being found on wheat and barley (Webster and Phillips 1912, Nuessly and Nagata 2005). In Nebraska, the first report of greenbug damage was on sorghum during the 1968 growing season (Harvey and Hackerott 1969, Nuessly and Nagata 2005). The report of greenbug in Nebraska occurred before infestations spread throughout much of the grain production areas in North America (Harvey and Hackerott 1969, Nuessly and Nagata 2005).

Greenbug feeding initially causes yellow or red leaf spots. Continued feeding leads to general yellowing and reddening of the leaf, and eventual death of the leaf and root. Plant attributes such as yield, size and overall survival can be greatly impacted by greenbug herbivory on the plant (Nuessly and Nagata 2005). The greenbug can also serve as a vector of numerous plant pathogens such as barley yellow dwarf virus (Murphy 1959), sugarcane mosaic virus (Ingram and Summers 1938) and maize and dwarf mosaic virus (Nault and Bradley 1969). Insecticides are the current front line of small grain defense against greenbugs (Hays et al. 1999). However, the more economical long-term solution may include integrating plant resistance into the management strategy (Nuessly and Nagata 2005).

Koch et al. (2014a) studied the host suitability of four species of aphids: *Sipha flava* (Forbes), yellow sugarcane aphid (YSA); *Schizaphis graminum* (Randani), greenbug (GB); *Rhopallosiphum padi* (L.), bird cherry-oat aphid (BCOA); and *Diuraphis noxia* (Mordvilko), Russian wheat aphid (RWA). Screen studies showed that switchgrass did not serve as a suitable host for *R. padi* and *D. noxia* on four populations of switchgrass: Kanlow, Summer, KxS (Kanlow male, Summer female), and SxK (Summer male, Kanlow female), the last two populations being derived from crossing Kanlow (K)

and Summer (S) plants. However, these four switchgrass populations did serve as a suitable feeding and reproductive host for *S. flava* and *S. graminum* (Koch et al. 2014a). Another study demonstrated that feeding by greenbug and yellow sugarcane aphids can elicit a number of stress-related responses in Kanlow, Summer and KxS switchgrass populations. It was shown that Kanlow may develop defensive responses in the form of transcriptional changes, that may be part of the defensive cascade to help trigger a variety of responses, to greenbug and yellow sugarcane aphid feeding (Prochaska 2015).

Yellow Sugarcane Aphid

The yellow sugarcane aphid (YSA), *Sipha flava*, is native to North America, with it first being described in Illinois in 1884 (Forbes 1884). Populations soon spread throughout much of North America (Nuessly 2005). As with the greenbug, *S. flava* are also parthenogenetic, with nymphs going through four instars before emerging as adults. This process takes 8 days on sorghum and 18-22 days on sugarcane (Hentz and Nuessly 2004). *Sipha flava* can be found on cultivated row crops such as rice, maize, sorghum, and sugarcane, and can also be pests of plants within various genera of Graminea, including *Hordeum*, *Oryza*, *Panicum*, *Sorghum*, and *Triticum* (Nuessly 2005, Koch et al. 2014a).

Sipha flava feeding can result in the yellowing and reddening of plant leaves, and prolonged exposure to yellow sugarcane herbivory can lead to premature senescence of the leaves and plant death. Yield reductions may result from yellow sugarcane feeding at early plant stages and may also reduce tillering (Hall 2001). Yellow sugarcane is also

known to vector several plant pathogens including barley yellow dwarf virus (Garrett et al. 2004) and sugarcane mosaic potyvirus (Blackman and Eastop 1984).

Insecticides along with natural enemies such as predacious ants, ladybird beetles, and young spiders, have been shown to help aid in yellow sugarcane management. Accurate timing of insecticides is critical to avoid stand loss or yield (Nuessly 2005). Several sorghum varieties have also been shown to be susceptible to yellow sugarcane feeding (Starkes and Mirkes 1979). As is the case with greenbug, insecticides are the current front line of management for yellow sugarcane; however, future, more economically and environmentally friendly, approaches to management may include plant resistance.

Plant Resistance

According to Smith (2005), plant resistance to arthropods is "The sum of the constitutive genetically inherited qualities that result in a plant of one cultivar or species being less damaged than a susceptible plant lacking these qualities". Therefore, plant resistance to arthropods is a relative property, based on the response of resistance and susceptible plants to a certain pest species given similar conditions (Smith 1998). Currently there are hundreds of insect-resistant cultivars grown in the United States that offer considerable environmental and economical benefits and overall greatly increased food production (Smith 1998, 2005). Due to this, plant resistance has become a major focus of breeding efforts, and many of the major cereal crop cultivars now contain some level of arthropod-resistance. Plant resistance also provides a more environmentally friendly approach to pest management due to the lower pesticide usage. It was estimated

that the production of insect resistant alfalfa, barely, maize, and sorghum cultivars in the United States would allow for a 37% decrease in insecticide use (Shalk and Ratcliffe 1976). Furthermore, plant resistance has also been shown to decrease the spread of insect transmitted pathogens. It was shown that there was a significant reduction in the transmission (31% - 74%) of the watermelon mosaic virus in resistant lines of muskmelon, *Cucmis melo* L., to the melon aphid, *Aphis gossypii* Glover (Kishaba et al. 1992). Overall, plant resistance has been shown to be a very reliable strategy for managing insect pests.

Plant resistance may be further classified into three mechanisms: antibiosis, antixenosis, and tolerance. These three mechanisms were originally described by Painter (1951) and more accurately described as categories by Horber (1980) (Painter 1951, Horber 1980). Antibiosis is a plant quality that negatively affects the biology or life history of the arthropod trying to use that plant as a host (Smith 2005). Antibiosis may result from numerous plant defenses such as toxic allelochemicals, including ketones and alkaloids, to more so morphological and physical defenses such as trichome density, type and size. Even if the antibiosis response does not directly kill the insect pest, it will still cause significant reductions in the overall fitness, seen in a reduced body mass and size, and possibly fecundity (Smith 2005).

Antixenosis, as defined by Painter (1951), is “the presence of morphological or chemical plant factors that adversely alter arthropod behavior.” As a result of antixenosis, the arthropod pest may select a replacement plant to serve as its host plant. Some plant characteristics that attribute to antixenosis include a thickened epidermal layer, deterrent compounds, waxy deposits on leaves, or a change in trichome numbers or density (Smith

2005). Both antibiosis and antixenosis can place selection pressure on the arthropod pest, resulting in a possible biotype development.

A biotype is defined as populations within an arthropod species that differ in their ability to utilize a particular trait in a specific plant genotype (Wilhoit 1992, Smith 2005). There are more than 20 greenbug biotypes to date recognized largely for their ability to overcome various plant resistance strategies and for their ability to utilize several host plants (Nuessly et al. 2008, Bouktila et al. 2012). To date there have been no documented biotypes for *S. flava* (Hoelscher et al. 1997).

The third category of resistance, tolerance, is defined as the plants ability to withstand or recover from damage caused by arthropod populations equal to those found on susceptible cultivars (Smith 2005). In general, tolerance involves only plant characteristics and most likely does not affect the pest arthropod, making tolerance significantly different from antibiosis and antixenosis (Reese et al. 1994). There are six primary factors connected with plants expressing tolerance. These six factors include: increased net photosynthetic rate; high relative growth rate; increased branching/tillering after apical dominance release; pre-existing high levels of carbon from the root system; the ability to transfer stored carbon from the roots to the shoots; and increased oxidative enzyme activity (Gawrońska and Kiełkiewicz 1999, Strauss and Agrawal 1999, Heng-Moss et al. 2004, Smith 2005, Franzen et al. 2007).

A series of choice and no choice studies were conducted to document the categories of switchgrass resistance to yellow sugarcane and greenbug (Koch et al. 2014b). The two no-choice experiments determined antibiosis and tolerance responses to

yellow sugarcane and greenbug with three switchgrass populations: Kanlow (lowland ecotype), Summer (upland ecotype), and KxS. This study also found that Kanlow expressed resistance to both aphid species by showing high levels of antibiosis. In another study by Koch et al. (2014a), KxS showed low levels of antibiosis to yellow sugarcane, and Summer expressed tolerance to the greenbug. Experimental results also showed KxS lacking tolerance and antibiotic characteristics to greenbug, whereas the Summer population lacked tolerance and antibiotic characteristics to yellow sugarcane. Choice studies were used to evaluate the preference of yellow sugarcane and greenbug on the three switchgrass populations. These studies documented a lack of antixenosis for all three switchgrass populations when feed on by yellow sugarcane. However, with reference to greenbug, 24 hours after greenbug introduction there was a preference for the KxS population (Koch et al. 2014c).

The study of aphid feeding behavior with electronic penetration graphs (EPG)

Studying the feeding behavior of insects can shed light on a number of insect preferences and can help broaden our current knowledge of plant-insect interactions. Until the last couple of decades, it has been difficult to study the feeding behavior of insects with piercing-sucking mouthparts due to the inability to directly observe the stylets within plant tissue (Walker 2000). Studying the feeding behavior in chewing insects is often easier due to the fact that feeding can be easily observed. Because of this difficultly of observing piercing-sucking insect feeding behavior, special techniques have been developed to help monitor piercing-sucking insect feeding activity. The first technique was developed by Mclean and Kinsey (1964) to monitor and record aphid feeding and salivation. These initial feeding monitors used an alternating current (AC)

recording system. Later feeding monitors were developed with a direct current (DC) as described by Tjallingii (McLean and Kinsey 1964, Tjallingii 1978). Since then, both AC and DC recording systems have been used in studies related to plant resistance. However, the DC system has shown to provide a higher quality of waveform detail of an electronic penetration graph (EPG) and has allowed for measurements of inside-waveform frequencies, allowing different waveforms to be identified more easily (Tjallingii 2000, Van Helden and Tjallingii 2000). Due to this, the DC system has become more widely used in studies emphasizing in plant resistance.

The EPG technique allows for the recording of a specific waveform corresponding to the insect activities and the placement of the stylet tips within the plant tissues (Tjallingii 2006). The basic principle of EPG monitors includes the insect and the plant as part of an electrical circuit connected to a voltage source and input resistor. The output wire makes contact with the plant by inserting a stiff copper wire into the potting soil surrounding the plant, while the input of the EPG system makes contact with the insects through the connection of a small gold wire glued to the insect's dorsum with conductive adhesive (Walker 2000). After these output and input wires are in place, the insect is then introduced to the plant. Once the insect inserts its mouthparts into the plant this will complete the circuit, with current flowing from the voltage source, through the plant, through the insect, through the input resistor and then back to the voltage source.

Specific stylet positions and feeding behaviors were described and correlated to waveforms for many species with histology experiments (stylectomy) and revealed several important DC-EPG waveforms (A, B, C, E1, E2, F and G) (Tjallingii 1978, Kimmins and Tjallingii 1985, Tjallingii 1988, Spiller et al. 1990, Tjallingii 1990,

Tjallingii and Hogen-Esch 1993). The A, B, and C waveforms are all part of a pathway phase, in which occur intercellular stylet penetration and withdrawal, periods of no stylet movement and short intracellular punctures by stylet tips, also known as potential drops (waveform pd) (Prado and Tjallingii 1994, Jiang and Walker 2001). Waveform G, xylem sap ingestion, is related to water intake by water-deprived aphids (Spiller et al. 1990). Waveforms E1 and E2, representing the sieve element (phloem) phase, begin with initial watery salvation into the sieve element, followed by passive ingestion from phloem sap with simultaneous watery salvation (Reese et al. 2000).

Useful knowledge, such as plant resistance mechanisms, can be gained by monitoring EPG waveforms to determine aphid activity within plant tissues (Van Helden and Tjallingii 2000, Jiang and Walker 2001, Crompton and Ode 2010). Phloem based-resistance factors have been previously reported in many systems to aphids. EPG studies for resistant tomato lines (*Lycopersicon esculentum* Miller) with the resistant gene, *Mi*, suggested *Macrosiphum euphorbiae* (Thomas) phloem feeding was disrupted on resistant lines compared to the susceptible lines (Kaloshian et al. 2000). However, due to no significant differences being found in the time required for aphids to attain their first sieve element contact on resistant and susceptible plants, the reduction in duration of the sieve element phase activities was not a result of a physical barrier or possible plant chemistry preventing the aphid from discovering the sieve element (Kaloshian et al. 2000). Phloem-based resistance has been reported in many systems, including: *M. persicae* and *M. euphorbiae* on resistant *Solanum stoloniferum* Schltdl. & Bouché; *Aphis gossypii* on resistant *Cucumis melo* genotypes (Kennedy et al. 1978); *Myzus persicae* (Sulzer) on resistant *Prunus* genotypes (Sauge et al. 1998, Sauge et al. 2002); and *Aphis*

glycines Matsumura on resistant soybeans, *Glycine max* (L.) Merr (Diaz-Montano et al. 2007, Crompton and Ode 2010).

The cereal aphid *S. graminum* has been well studied with regards to its feeding behavior, particularly on wheat, sorghum and switchgrass (Campbell et al. 1982, Montllor et al. 1983, Dreyer et al. 1984, McCauley Jr. et al. 1990, Formusoh et al. 1992, Morgham et al. 1992, Goussian et al. 2005, Pereira et al. 2010, Koch et al. 2014c). Many of these studies have looked at the probing behavior of the greenbug on various plant treatments, however Montllor et al. (1983) examined the feeding behavior of two *S. graminum* biotypes (biotypes C and E) on resistant and susceptible sorghum lines and examined differences the feeding behavior of each biotype on the susceptible and resistant sorghum lines, especially in relation to total sieve element contact. In Koch et al. (2014c), *S. graminum* (biotype I) feeding behavior was monitored on three switchgrass populations: Kanlow, Summer and the experimental strain, KxS. *Schizaphis graminum* showed significantly less phloem ingestion on Kanlow than both Summer and KxS, suggesting resistance factors in Kanlow being in the phloem tissue (Koch et al. 2014c). To date, no EPG studies have looked at insect feeding behavior on other warm-season grasses, such as big bluestem and indiangrass. Thus, any future studies could provide valuable insights into possible aphid-resistance mechanisms. Studies of aphid feeding behavior could shed some light onto our current knowledge of plant-insect interactions and the relationship between the vectors (aphids) and viruses within this system.

Table 1. Selected arthropod families present in North American tallgrass prairie

Order	Family
Coleoptera	Carabidae
	Chrysomelidae
	Cocinellidae
	Curculionidae
	Scarabaeidae
Diptera	Asilidae
	Cecidomyiidae
	Chironomidae
	Cloropidae
	Tephritidae
	Syrphidae
Hemiptera	Anthocoridae
	Aphididae
	Blissidae
	Cicadellidae
	Lygaeidae
	Miridae
	Nabidae
	Pentatomidae
	Reduviidae
Hymenoptera	Formicidae
	Halicidae
Orthoptera	Acrididae
	Tetrigidae
	Tettigoniidae
	Gryllidae

(Bruner 1899, Shelford 1963, Blocker 1969, Risser et al. 1981, Whiles and Charlton 2006)

CHAPTER 2. IDENTIFICATION, SEASONAL ABUNDANCE, AND THE INFLUENCE OF STAND COMPOSITION ON ARTHROPODS INHABITING WARM-SEASON GRASSES IN NEBRASKA AND WISCONSIN

Introduction

Switchgrass, *Panicum virgatum* L., is a warm-season perennial C_4 grass species native to the grasslands of North America, with a range from Mexico to Canada (Vogel 2004). Switchgrass, along with two other warm-season grasses, big bluestem (*Andropogon gerardii* Vitman) and indiangrass (*Sorghastrum nutans* (L.)) compose a majority of the grasses found in the North American tall grass prairies (Bouton 2008). Historically switchgrass, along with big bluestem and indiangrass has been utilized as a component of a diversity of programs, including: (1) biomass production in prairies, (2) conservation reserve program (CRP) for grassland conservation, (3) grassed waterways and (4) prairie restoration efforts. However, over the past 20 years bioenergy production has emerged as a new use for warm-season grasses such as switchgrass. Switchgrass, along with other warm-season grasses such as big bluestem and indiangrass, has been selected as a promising candidate for bioenergy cropping for a wide variety of reasons, including: high levels of production across diverse landscapes; suitability for marginal and erosive land; low water and nutrient requirements; positive environmental benefits; and compatibility with modern farming practices (Sanderson et al. 1996, McLaughlin et al. 1999, Sanderson et al. 2004). Switchgrass also has cell walls composed primarily of cellulose and hemicellulose, making it a potential energy crop for cellulostic ethanol production (Vogel 2004).

Despite the numerous arthropod surveys completed, it is still estimated that up to half of arthropods inhibiting prairies await description (Arenz and Joern 1996). Arthropod surveys completed by both Bruner (1899) and Blocker (1969) in Nebraska and Kansas prairies were foundational; however, these studies are decades old and did not

focus on a specific grass species. Another survey was conducted in 2007 and 2008 provided a comprehensive assessment of the arthropods of switchgrass in Nebraska as well as the influence of stand age on the composition and abundance of selected arthropods (Schaeffer et al. 2011). Yet, for these warm-season grasses to reach their full potential as a bioenergy crop additional arthropod surveys need to be completed to determine the arthropod communities associated with these grasses in both a monoculture and a mixed setting. Therefore, the objectives of this research were to identify the arthropods associated with switchgrass, big bluestem and indiangrass in both Nebraska and Wisconsin and assess the impact of stand age and stand type on the arthropod communities associated with these three warm-season grasses. Given the perennial nature of these grasses, they have the potential to be in production for up to 10 years; therefore, establishing the seasonal abundance of selected arthropod groups will be fundamental in developing arthropod monitoring programs as well as gaining insight on potential arthropod pests. Overall, this information will be vital in the warm-season grass cropping system.

Materials and Methods

Arthropod Survey. *Study 1* - During the summers of 2013, 2014, and 2015 samples were collected from replicated stands (0.84 ha/stand) of the following warm-season perennial grasses: switchgrass (Liberty population) big bluestem (mixture of Bonanza and Goldmine populations) and a low diversity mix (LDM; mixture of Bonanza big bluestem, Scout indiangrass and Trailway sideoats grama). There were three replicates of each of the three warm-season grasses, composing nine total stands for sampling. Stands were established from seed at the Agricultural Research and Development Center (ARDC) at the University of Nebraska-Lincoln extension site located near Mead, NE in spring 2012. The fields were in soybeans in the year prior to the grass establishment, with no-till seeding of the grass seeds into the soybean stubble. Soil in the area consisted of a silty clay loam. A total of 44.0, 64.7, and 73.8 cm of rain fell during the sampling periods in 2013, 2014, and 2015, respectively. The vegetation surrounding the sampling sites was primarily bromegrass (*Bromus inermis* L.). Biomass was determined during the late summer/fall. Harvest dates for each year were 19 November 2013, 29 November 2014, and 7 November 2015. Nitrogen fertilizer was applied to all plots in the spring of 2013, 2014, and 2015 at the rate of 113.5kg/ha. The fertilizer dates for each year were 14 May 2013, 2 May 2014, and 13 April 2015.

Plots were established in a completely randomized design with 3 replications of each stand type with 6 subsamples within each replication, creating eighteen subsamples for each of the three grass stands. Samples were collected on a grid pattern at least 27 m apart (Figure 2.1). Arthropod samples were collected every two weeks throughout the growing season during all three years. Because these are warm-season grasses, sampling

was initiated at the end of May as the grasses began active growth for the season. Sampling was discontinued at the end of September or early October due to declining temperatures and reduced arthropod activity. Specific sampling dates for 2013, 2014 and 2015 are summarized in Table 2.1. Two sampling techniques, pitfall traps and sticky traps, were used for each sampling site. These methods served to collect arthropods from multiple levels within the warm-season grass canopy.

Pitfall traps – Pitfall traps were constructed from a 474 mL plastic Solo® cup (Solo Cup Company, Highland Park, IL), a 207 mL Hy-Vee (Hy-Vee, Inc., West Des Moines, IA) plastic punch cup as a funnel, and a 204 mL plastic Solo® cup filled with propylene glycol antifreeze as a killing solution (Morrill 1975). Arthropods were retrieved from traps every two weeks and stored in 75% ethyl alcohol until counted. These traps provided a biweekly count of the surface-dwelling arthropods present in the warm-season grass during the collecting season.

Sticky traps – One double-sided 7.6 x 12.7 cm yellow sticky card (Whitmore Monitoring Cards, Hummert International, Earth City, MO) was placed at a height of 1 m at the top of a wooden garden stake driven into the ground. Sticky cards were oriented north-south and placed at the center of each sampling site. Cards were collected every two weeks in a clear plastic sandwich bag and were returned to the laboratory for processing.

Study 2 - During the summer of 2013, 2014, and 2015 samples were also collected from CenUSA Bioenergy plots located at the UNL ARDC near Mead, NE and at the University of Wisconsin Arlington Research Station located 22 miles north of Madison,

WI. Samples were collected from stands of the following warm-season perennial grasses: switchgrass, big bluestem, and indiangrass. Within these plots were several genotypes of each grass type. There were 22 switchgrass genotypes, 12 big bluestem genotypes, and 12 genotypes of indiangrass. None of these genotypes were combined as each composed an individual plot within the overall plot of all three grasses sampled. The total switchgrass plot size was 0.051 ha for switchgrass, total big bluestem plot size was 0.031 ha and total indiangrass plot size was 0.031 ha.

Stands in both states were established in the spring of 2012 from seed. The fields were in soybeans in the year prior to the grass establishment, with no-till seeding of the grass seeds into the soybean stubble. In the Nebraska location the soil in the area consisted of a silty clay loam and a total of 44.0, 64.7, and 73.8 cm of rain fell during the sampling periods in 2013, 2014, and 2015, respectively. The vegetation surrounding the sampling sites was primarily bromegrass (*Bromus inermis* L.). In the Wisconsin location the soil in the area consisted of a Plano silty loam and a total of 45.9, 32.9, and 43.6 cm of rain fell during the sampling periods in 2013, 2014, and 2015, respectively. The vegetation surrounding the sampling sites near Arlington, WI was turf type fescue. Plots in both states were managed for overall biomass production with the biomass being harvested in the late summer/fall. Harvest dates for each year in Nebraska were 19 November 2013, 29 November 2014, and 7 November 2015. For Wisconsin, the harvest dates are as follows: 23 October 2013, 20 October 2014, and 5 November 2015. Nitrogen fertilizer was applied to all plots in both states every spring at the rate of 113.5kg/ha. For Nebraska plots the fertilizer was applied at the following dates: 14 May 2013, 2 May

2014, and 13 April 2015. In Wisconsin, the fertilizer was applied mid May-early June (no dates applicable).

Sampling sites were on a grid pattern at least 4.5 m apart. Three samples were collected from each stand type (Figure 2.2). Arthropod samples were collected every two weeks throughout the growing season during all three years. Because these are warm-season grasses, sampling was initiated at the end of May as the grasses began active growth for the season. Sampling was discontinued at the end of September or early October due to declining temperatures and reduced arthropod activity. Specific sampling dates for 2013, 2014 and 2015 are shown in Table 2.1 and Table 2.2. Two sampling techniques, pitfall traps and sticky traps, were used at each sampling site. These methods served to collect arthropods from multiple levels within the warm-season grass canopy.

Reference Collection. A family-level reference collection of the arthropods associated with switchgrass was initially established by Schaeffer et al. (2011); throughout 2013, 2014, and 2015 big bluestem, indiangrass and LDM were added to this reference collection with the addition of any new families collected in these grasses. As thousands of arthropods can be collected in a field survey of a warm-season grass habitat, identifying and monitoring every collected species was not feasible due to time and labor constraints. Thus, the following criteria were used for including collected arthropods in the reference collection: 1) overall abundance/prevalence throughout the season – was the arthropod collected on multiple occasions or in significant numbers at some point during the growing season; 2) had the arthropod previously been documented in prairie or switchgrass habitats; and 3) was the arthropod a known or suspected pest or beneficial in

warm-season grass habitats. Based on this reference collection, arthropod families were selected for further seasonal abundance and influence on stand age studies.

Results

Arthropods associated with warm-season grasses. Over the course of three sampling seasons, families of arthropods were collected from over 10 orders of insects as well as non-insect groups including arachnids (Table 2.3 – Table 2.11). Coleoptera, Diptera and Thysanoptera were the most abundant orders for three of the years, usually composing over 75-80% of all arthropods collected. Another 5-10% was consistently composed of Hemiptera and Hymenoptera. With the remaining 10% consisting of the orders Araneae, Lepidoptera, Neuroptera, Orthoptera and Opiliones. Tables 2.12 - 2.38 provide a comprehensive list of all of the collected families for all three seasons and locations.

Sticky traps collected the greatest number of total arthropods, primarily thrips (Thripidae) and grass flies (Chloropidae) (Table 2.39 – Table 2.41). Pitfall traps were most effective at collecting mobile, surface-dwelling arthropods, such as ground beetles (Carabidae) and sap beetles (Nitidulidae) (Table 2.39 – Table 2.41).

Seasonal Abundance and Influence of Stand Age and Type of Selected Arthropods.

The seasonal abundance and influence of stand age and type were assessed for the following arthropod groups: Acrididae, Tettigonidae, Carabidae, Chloropidae, Chrysomelidae, Cicadellidae, Coccinellidae, Formicidae, Gryllidae, Nitidulidae, Scarabaeidae, Staphylinidae, Tephritidae, Thripidae, Parasitic Hymenoptera, Araneae and Opiliones.

Acrididae and Tettigonidae. Short- and long-horned grasshoppers collections were relatively low over the three sampling seasons. Grasshoppers were collected in both sticky traps and pitfall traps with a higher abundance of grasshoppers being collected in sticky traps for Study 1 for all three sampling seasons, and a peak abundance usually occurring in the middle of the sampling season (data not shown). Very few, if any, grasshoppers were collected in either sticky traps or pitfall traps for Study 2.

Sweep net samples may be more effective in collecting herbivorous arthropods, like grasshoppers; however, sweep net samples are particularly sensitive to variances in plant density and can be destructive to plant structure (McIntyre and Thompson 2003). Sweep net samples were collected in this study; however, relatively low numbers of grasshoppers were collected with this sampling tactic (data not shown). Overall, grasshopper numbers were not sufficient to determine the influence of stand composition on grasshoppers.

Carabidae. Pitfall traps were designed to collect surface-dwelling arthropods; therefore, this was the preferred method for collecting ground beetles. In 2013 there were over 6,600 collected in pitfall traps in all three of the sampling studies, along with over 3,800 collected in 2014 and over 3,400 collected in 2015. The higher numbers of ground beetles collected in the 2013 season may be due to their high degree of mobility allowing them to colonize new food resources, or it may reflect previous populations from prior land use of soybeans. In Study 1 there was a general increase in ground beetle numbers as the collecting season progressed for 2013 and 2014. For the 2015 collecting season there were peaks in ground beetle numbers at the beginning and end of the collecting season (Figure 2.3). Similar trends were observed for the Nebraska CenUSA plots with a general

increase for the 2013 and 2014 collecting season, whereas there was a spike in numbers at the beginning, middle and end of the collecting season for 2015 (Figure 2.4). For Study 2 at the Wisconsin site, there was a peak in the middle of the collecting season for 2013, 2014 and 2015 (Figure 2.5).

Multiple peaks throughout a collecting season may suggest most likely several populations or generations of ground beetles were active in the warm-season grasses during the collecting season. This study measured the overall abundance of ground beetles and did not record species present throughout the season. Therefore, these data represent not only predatory species, but also seed-feeding beetles such as *Harpalus* sp. Further examination at the species level is needed to determine the composition of predatory and seed-feeding beetles.

Chloropidae. Grass fly larvae are known to feed within the stems of grasses and have the potential to weaken the stems and reduce overall grass production. Grass flies were collected on sticky traps throughout the 2013, 2014 and 2015 collecting seasons. In Study 1, grass fly numbers generally increased as the collecting season progressed for the 2013 and 2014 collecting season. A peak in the middle of the 2015 was observed with over 1,000 grass flies being collecting on sticky traps. Overall grass flies collected in Study 1 seemed to prefer switchgrass in the beginning of the season, switching their preference to LDM by the second half of the collecting season (Figure 2.6). However, in Study 2 at the Nebraska site, no apparent trends were observed during the 2013, 2014 and 2015 collecting season (Figure 2.7). Wisconsin peaked in grass fly numbers at the end of the collecting season in 2013; however, an opposite trend was observed for the 2014 and

2015 colleting seasons with over 400 grass flies collected during the first and second collection dates (Figure 2.8).

Chrysomelidae. Leaf beetles were collected from both pitfall traps and sticky traps, although the majority of leaf beetles were collected in sticky traps. In Study 1, leaf beetles numbers peaked in the middle of the 2013 collecting season with over 1,400 leaf beetles being collected. Fewer beetles were collected during the 2014 and 2015 collecting season, though there was a rise in numbers at the end of each collecting season (data not shown). Analysis of Study 2 plots in Nebraska indicated a general increase in leaf beetle populations as the collecting season progressed for 2013, 2014, and 2015 (data not shown). Wisconsin plots displayed no observable trends during the 2013, 2014, and 2015 collecting seasons (data not shown).

Cicadellidae. Pitfall traps and sticky traps were both effective for collecting leafhoppers. Leafhoppers were collected throughout the season and are known to be a pest for other crops. In Study 1, the data suggests no general trends during the 2013 and 2015 collecting season; however, there was a decrease in populations as the collecting season progressed for 2014 (Figure 2.9). The Nebraska plots in 2013, 2014, and 2015 displayed no overall trend in Study 2 (Figure 2.10). In Wisconsin, there were more leafhoppers collected at the end of the 2013 collecting season with 45 leafhoppers being collected in indiangrass at collection date 7. Lower numbers of leafhoppers were collected with no apparent trends for 2014 and 2015 (Figure 2.11).

Coccinellidae. Both pitfall and sticky traps collected lady beetles with the majority of the lady beetles being collected on sticky traps; however, relatively low

numbers of lady beetles were collected in both sampling studies. While lady beetles were collected throughout the season, insufficient numbers prevented trends in seasonal abundance or influence of stand type from being determined (data not shown).

Formicidae. Ants were collected in both pitfall traps and sticky traps with more than 50% being collected in pitfall traps. With the sticky cards being located at a height of 1 m, the traps were not situated to accurately sample ants. Ants were collected throughout the season in pitfall traps. Data from Study 1 revealed that ant populations declined rapidly after the end of August for both the 2013 and 2014 collecting season. For the 2015 season ant populations remained consistent throughout the sampling season (data not shown). In the Nebraska plots in Study 2, very few ants were collected during 2013 and 2014; however, during 2015 there was a spike of over 50 ants collected in the middle of the season (data not shown). For the CenUSA Wisconsin plots, very low numbers of ants were collected for all three sampling seasons, preventing trends from being determined (data not shown).

Gryllidae. Crickets were collected in both pitfall traps and sticky traps, but pitfall traps appeared to be the most effective method for cricket collection. In Study 1, cricket numbers were very low for the first three collecting dates and then numbers began to increase for the duration of the collecting season during 2013, 2014, and 2015 (Figure 2.12). Study 2 Nebraska plots had very low numbers collected for the first three collecting dates with an increase after the third date for the rest of the collecting season for 2013, 2014, and 2015 (Figure 2.13). Very low numbers of crickets were collected in all three sampling seasons and these insufficient numbers prevented trends from being observed in Wisconsin (Figure 2.14). Overall, there was an increase in cricket numbers

collected as the stand age developed, suggesting that crickets may be more abundant once the grass stand is further established.

Nitidulidae. Sap beetles were collected in both pitfall traps and sticky traps throughout the collecting seasons with over 90% collected using pitfall traps. Sap beetles are often considered minor pests; however, large numbers may cause significant damage. In Study 1, peaks were seen at collection dates 7 and 8, with a maximum peak of 221 sap beetles collected in LDM at collection date 7, during the 2013 season. For the 2014 and 2015 collecting season, sap beetle numbers increased until the middle of the collecting season and then decrease for the remainder of the season. In 2015, sap beetles appeared to initially prefer switchgrass and switching this preference to LDM later in the collecting season (Figure 2.15). In Study 2 at Nebraska, no apparent trends were observed, but collected sap beetle numbers did increase for each collecting season (Figure 2.16). In Wisconsin very low numbers of sap beetles were collected during all three collecting seasons (Figure 2.17).

Scarabaeidae. Adult scarab beetles were collected throughout the season in pitfall traps. The Study 1 sampling study numbers fluctuated throughout all three seasons, ranging from zero to over 80. In 2013 and 2015 scarab beetles seemed to prefer LDM by the end of the collecting seasons (Figure 2.18). In Study 2, the Nebraska scarab numbers also fluctuated for all three collecting seasons, ranging from zero to five (Figure 2.19). Scarab numbers were very low and fluctuated for all three collecting seasons, ranging from zero to 14 in Wisconsin (Figure 2.20).

Staphylinidae. Rove beetles were collected in both pitfalls and sticky traps. However, since rove beetles are surface dwelling insects, sticky traps are not likely to accurately reflect rove beetle populations, making pitfalls the more preferred method for collection. In Study 1, there were fluctuations in rove beetle numbers, ranging from zero to 78 for all three collecting seasons (Figure 2.21). In Study 2 Nebraska plots, rove beetle collected numbers also fluctuated for all three collecting seasons ranging from zero to 56 (Figure 2.22). The numbers of rove beetles observed in Wisconsin plots were much lower than in Nebraska with overall numbers also varying throughout the three seasons (Figure 2.23). In both Study 1 and 2, the number of rove beetles collected increased for each collecting season, indicating that rove beetles may be more prevalent on more established grass stands.

Tephritidae. Tephritid flies were collected on sticky traps during the 2013, 2014, and 2015 collecting seasons. Tephritid numbers fluctuated throughout all three sampling seasons for both Study 1 and Study 2. In Study 1, collected tephritid flies ranged from zero to 60. In Study 2 Nebraska plots, the collected tephritid flies ranged from zero to 10. With Wisconsin the collected tephritid flies ranged for all three seasons from zero to a peak of 83 being collected in switchgrass on collection date 3 in 2015 (data not shown). For both studies collected tephritid fly numbers increased for each collecting season, indicating that tephritid flies may be more abundant on older grass stands.

Thripidae. Very large numbers of thrips were collected on sticky cards in 2013, 2014, and 2015, throughout each collecting seasons. In Study 1, thrips numbers were much higher in the beginning of the collecting season, with over 5,000 thrips being collected at collection date 3 in LDM in 2013, and slowly decreased during the collecting

season for 2013, 2014 and 2015 (Figure 2.24). The Study 2 Nebraska plots showed a similar trend in the 2013 and 2015 collecting season with thrips numbers being much higher at the start of the collecting season and then declining throughout the season. There was no apparent trend with thrips numbers peaking in the middle of the collecting season and low at the start and the end of the collecting season for 2014 (Figure 2.25) Thrips numbers were much higher in the beginning of the collecting season and declined over time in all three sampling seasons in Wisconsin (Figure 2.26).

Overall, thrips numbers collected in all three studies decreased with each collecting season, meaning that thrips may potentially prefer newly established grass stands; however, more research could help determine if the observed differences represent consistent trends or seasonal variations between 2013, 2014 and 2015.

Parasitic Hymenoptera. The parasitoid wasps collected were in the following families: Braconidae, Figitidae, Ichneumonidae, Mutillidae, Mymaridae, Platygastridae, Scelionidae and Scollidae, with Figitidae and Mymaridae composing a majority of the parasistic hymenoptera collected for both studies. Both Figuitidae and Mymaridae are known to parasitize a wide variety of insect orders. Parasitic hymenoptera were collected on sticky cards for all three collecting seasons. In Study 1 parasitoid populations indicated trends showing an increase in population numbers at the end of the collecting season for all three sampling seasons (data not shown). Nebraska plots in Study 2 showed no trends with numbers varying each collecting season (data not shown). In Wisconsin no trends were observed with collected parasitoid numbers fluctuating each collecting season (data not shown). Overall for both Study 1 and 2 there were higher numbers of

parasitoids collected in the 2014 sampling season compared to 2013 and 2015. This could be partially due to the increased rainfall from 2013 to 2014.

Araneae. Spiders were collected in both pitfall and sticky traps during all three collecting seasons. Pitfall traps tended to select for the highly mobile, surface-dwelling spiders such as Lycosidae, whereas the sticky traps collected more of the canopy-dwelling spiders such as Clubionidae, so it is most effective to use both sampling methods to determine overall spider populations.

In Study 1, the numbers fluctuated for the 2013 season; however, in the 2014 and 2015 collecting seasons, spider numbers peaked in the beginning and declined as the season progressed (data not shown). For Study 2 in Nebraska, spider numbers collected fluctuated for all three collecting seasons, ranging from zero to 29 spiders collected (data not shown). Wisconsin plots showed no observable trends for all three collecting season with numbers fluctuating between zero and 23 (data not shown).

Opiliones. Harvestmen were collected in both pitfall traps and sticky traps, with the majority of harvestmen collected in the pitfall traps, making this the preferred sampling method. In Study 1, very low numbers of harvestmen were collected in 2013, with numbers increasing for the 2014 and 2015 collecting seasons. In the 2014 and 2015 seasons, harvestmen numbers increased as the collecting season progressed, reaching numbers as high as 45 at date 8 in LDM in 2015 (data not shown). Very low numbers of harvestmen were observed in Study 2 Nebraska plots during all three collecting seasons, with a peak of five harvestmen collected at date 5 in big bluestem in 2015 (data not shown). Wisconsin plots revealed much higher numbers of harvestmen during all three

seasons. Harvestmen numbers for all three seasons were highest at the beginning of the collecting seasons and declined with time, with a peak of 105 harvestmen collected from indiangrass at collection date 1 in 2013 (data not shown).

Discussion

Over 67 arthropod families were collected in association with warm-season grasses during the 2013, 2014 and 2015 growing seasons for all three sampling locations. Seventeen of these families were monitored throughout the three collecting seasons. These groups were further classified into: incidental arthropods, potential arthropods pests or beneficial arthropods. The arthropods that did not directly feed on the grass plants or were not known to be predators or parasitioids were classified as incidental arthropods. Presumably, these arthropods have no direct impact on the biomass production of these grasses or on potential arthropod pests. However, they may play a vital role in the ecosystem, such as aiding with pollination or decomposition. Crickets, as scavengers, were classified as incidental arthropods.

Potential arthropod pests are classified as arthropods that cause direct damage to the grass plant. Arthropods in this category include: grasshoppers, chloropid flies, leaf beetles, leafhoppers, sap beetles, scarab beetles and thrips. Four groups of potential pests were abundant throughout this study: chloropid fly larvae which are known to feed within stems, impacting the overall biomass production; scarab beetles which have the potential to remove large amounts of biomass with high populations; thrips and leafhoppers which remove photosynthates therefore decreasing biomass production. Thrips and leafhoppers also have the potential to transmit a variety of plant diseases (Triplehorn and Johnson 2005).

There were several beneficial arthropods collected as well in this study. Predators such as ground beetles, harvestmen, rove beetles, and spiders prey on several arthropods.

These predatory groups were often found in large numbers and may be feeding on a wide variety of arthropod hosts. Hymenoptera parasitoids were also collected and can have both a broad and narrow range of hosts. Parasitioids found in the families Ichneumonidae, Braconidae, and Figitidae are generalists that parasitize eggs from a wide variety of orders. Mymarid and scelionid wasps also parasitize eggs in numerous arthropod orders. However, platygastrids only parasitize Sternorrhyncha and larval cecidomyiid midges (Triplehorn and Johnson 2005).

Parasitoid wasps numbers peaked during the second collecting season for all three sampling locations, therefore it is uncertain if parasitic hymenoptera will play a specific role in maintaining pest populations in warm-season grasses. However, other generalist predators such as ground beetles, harvestmen, rove beetles and spiders were present in high numbers throughout all three collecting seasons. It is difficult to know which predatory group will play the most significant role with warm-season grass production by aiding in pest reduction until the specific pest species have been identified. This is also true when it comes to pest-specific interactions, such as those including parasitoids.

Not all beneficial arthropods serve as parasitoids or predators. Ants for example perform a variety of benefits to the grass ecosystem. Ants help aerate the soil, which in turn helps promote healthy plant growth. Ants can also help reseed stands. In prairie settings, ants move seed around in tunnels underground and therefore help with reseeding (Risser et al. 1981). Certain ant species can also serve as predators and help reduce pest populations.

This study provides comparative, fundamental information on arthropods associated with switchgrass, big bluestem, indiangrass and a mixed grass stand in both the Nebraska and Wisconsin landscape. The reference collection includes many of the arthropod orders and families previously documented in association with prairie grasses by Bruner (1899), Weaver and Fitzpatrick (1934), Shelford (1963), Blocker (1969), Risser et al. (1981), Whiles and Charlton (2006) and Schaeffer et al. (2011). In a previous study, it was concluded that the big bluestem gall midge, *Stenodisplosis wattsii* Gangè, is a pest of switchgrass in Nebraska (Raun and Mitchell 2008). In our study, only one cecidomyiid was collected, in 2013 from a switchgrass field in Nebraska; however, this could be due to a lack of accurate sampling techniques for cecidomyiids.

Stand age appeared to influence some but not all of the arthropod groups. For example, thrips numbers decreased with each year of sampling, indicating thrips may prefer younger grass stands. Thrips numbers were usually much higher at the beginning of each sampling season and then declined with time over the season. The role of stand age on the arthropod community needs to be further evaluated to better understand its impact on select arthropod groups.

Stand type also appears to have influenced some arthropod groups. Although for most arthropod groups there were similar numbers collected in each of the stand types, some groups did show a preference to certain stand types. Chlorpoid flies were collected in higher numbers for the LDM and indiangrass fields in all three sampling locations during the 2014 collecting year. Tephritid flies were more prominent in the switchgrass stands for all three sampling locations among all three collecting seasons. The role played

by stand type remains unclear and needs to be further investigated to determine the impact of stand type on potential arthropod groups.

Seasonal abundance records and information on the influence of stand age and stand type on arthropod abundance can form the basis for developing an effective arthropod monitoring program. When another native warm-season grass, buffalograss, was transferred into a monoculture production setting new pests unexpectedly emerged (Baxendale et al. 1999). Surveys of potential buffalograss pests and their natural enemies in the hopes of developing successful management strategies were only initiated in response to these new emerging pest outbreaks (Heng-Moss et al. 1998). The goal of this research is to be proactive when it comes to developing baseline information on arthropod groups found in warm-season grasses that can be further developed into sustainable management strategies for potential pest outbreaks.

This study also provides important baseline information that can be further expanded upon to broaden our knowledge of switchgrass and other warm-season grass arthropods. The seasonal abundance and stand type arthropod information can be expanded upon to include additional arthropod pests and their natural enemies. Additional studies could be carried out for a longer duration to determine if similar trends are observed on population abundance. Overall, this study combined with future studies will shed light on the arthropod complex associated with warm-season grasses and will be useful in the development of effective pest management strategies.

Long-term implications of this research include the possibility of a changing landscape, potentially more grasses being a part of the future ecosystem. With this

possibility being taken into consideration, it becomes evident that there will need to be research to address these long-term effects of potentially using these grasses as a biofuel source. This research will potentially allow us to be ahead of the curve when it comes to these grass systems with regards to potential pests. Plant resistance is an important form of control, and further research will be needed to determine further potential pests and beneficial arthropods associated in these grass stands.

It is suggested that lignin, a component of the cell wall, content may be reduced in switchgrass, without detrimental affects on the yield and still allow for resistance (Whetten and Sederoff 1995, Vanholme et al. 2010, Dowd et al. 2012). There was also a recent study published concerning the change in climate patterns over the next several years (Bathke et al. 2014). The landscape in Nebraska could be very different in Nebraska due to increased temperatures and less rainfall amounts due to these increased temperatures. There is also predicted to be a longer growing season with the projected frost season projected to begin two weeks later by the end of the century (Bathke et al. 2014).This study is important not only from the bioenergy aspect of switchgrass, big bluestem, and indiangrass, but also for the lignin content and the potential climate changes in Nebraska.

Figure 2.1 – Design layout for Study 1

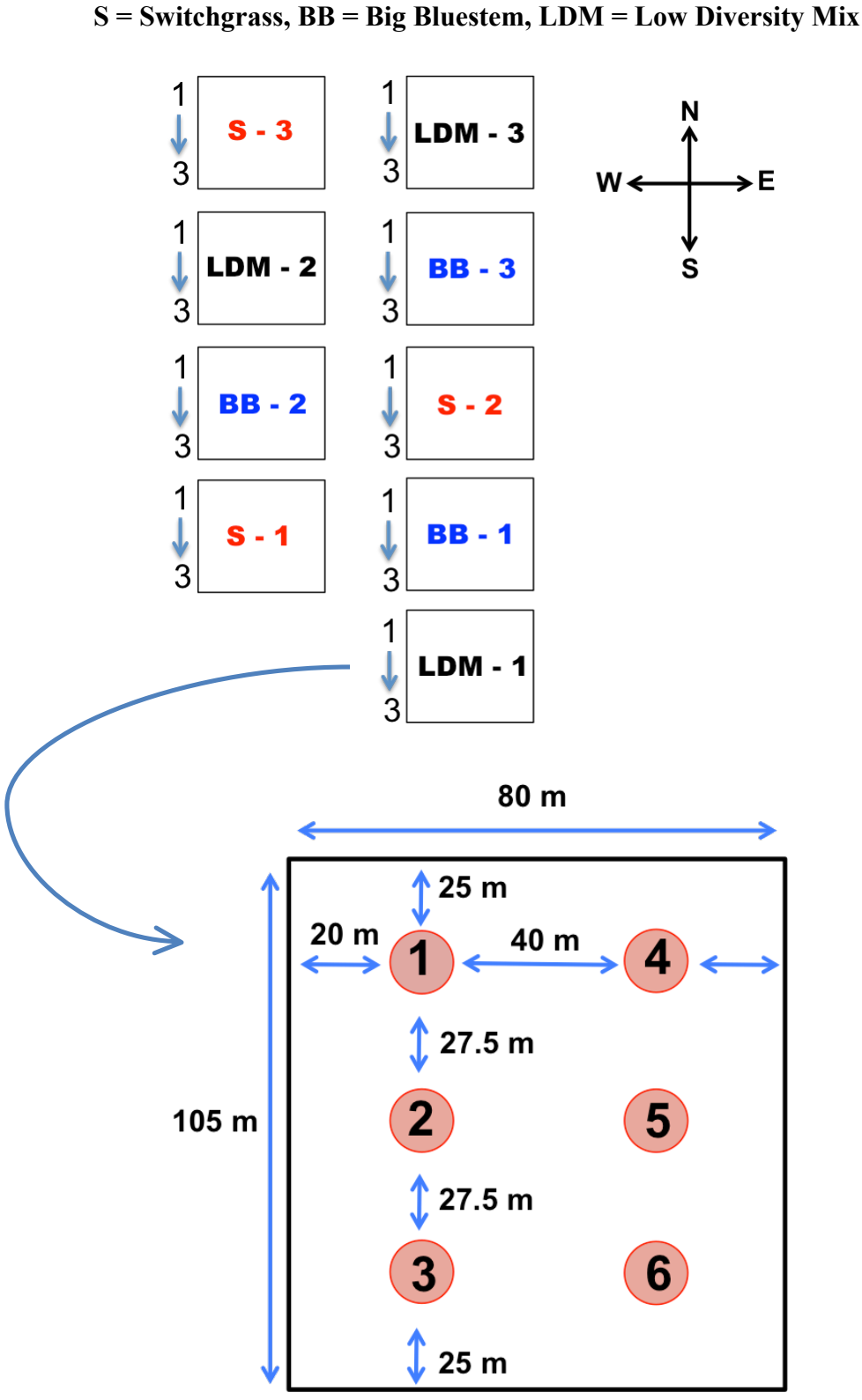


Figure 2.2 – Design layout for Study 2

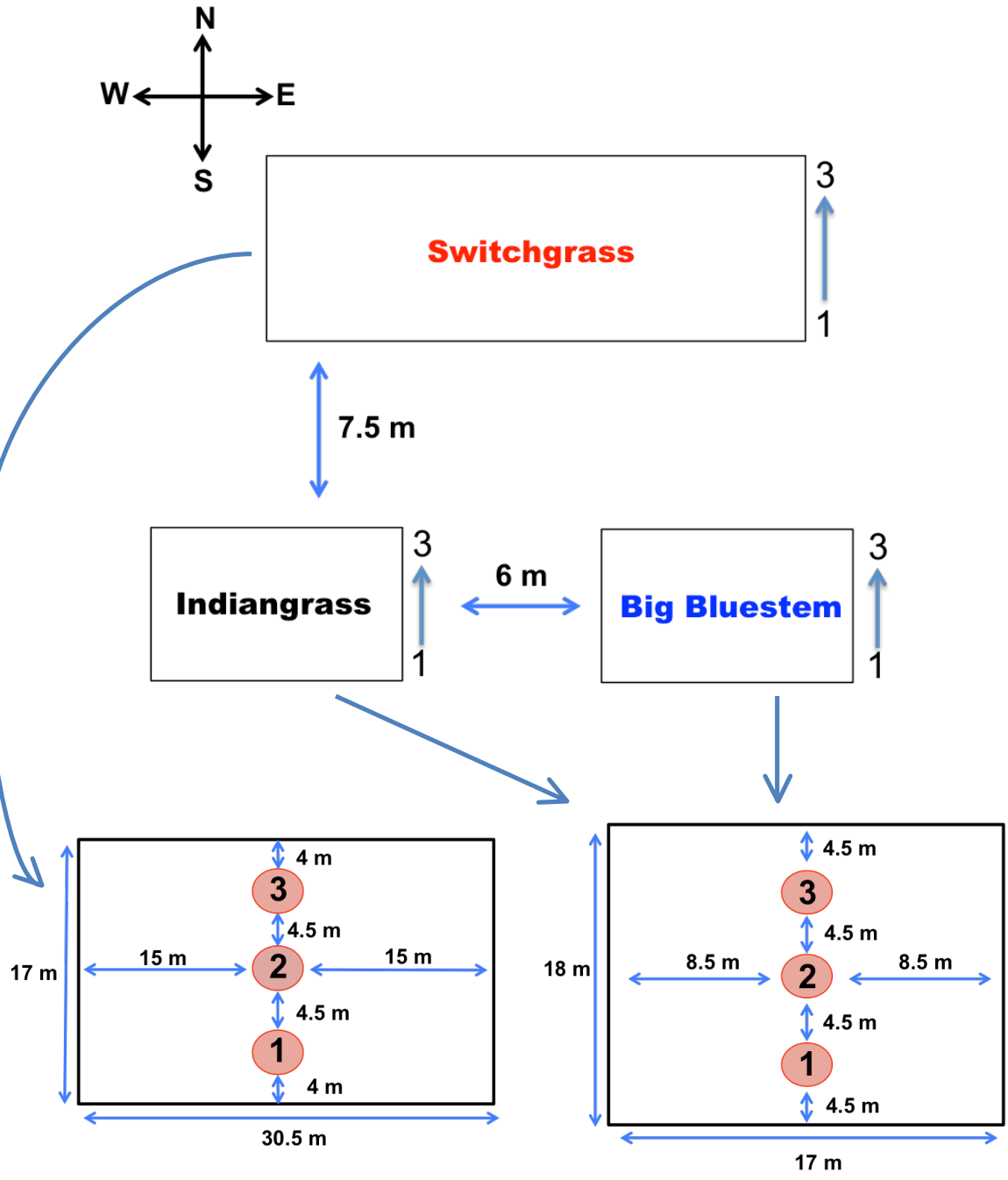


Table 2.1 – Collection dates for pitfall and stick traps in 2013, 2014 and 2015 near Mead, NE.

	Collection Date								
Year	1	2	3	4	5	6	7	8	9
2013	6/3	6/17	7/1	7/15	7/31	8/12	8/30	9/13	9/27
2014	6/11	6/25	7/9	7/23	8/6	8/20	9/3	9/17	--
2015	6/10	6/22	7/8	7/22	8/5	8/19	9/3	9/17	--

*Nebraska CenUSA plots not sampled until second sampling date for 2013

Table 2.2 – Collection dates for pitfall and stick traps in 2013, 2014 and 2015 near Madison, WI.

	Collection Date							
Year	1	2	3	4	5	6	7	8
2013	6/3	6/17	7/1	7/15	7/29	8/12	8/26	9/9
2014	6/25	7/9	7/23	8/6	8/20	9/3	9/17	10/1
2015	6/18	7/2	7/16	7/30	8/13	--	9/10	--

*No samples collected for dates 6 and 8 in 2015

Table 2.3 – Total number of selected arthropod orders collected from select warm-season grass stands in 2013 near Mead, NE.

Arthropod Order	Switchgrass	Big Bluestem	LDM	Total	% total
Thysanoptera	7448	12551	13257	33256	40.7
Diptera	8325	9219	9185	26729	32.7
Coleoptera	4773	3444	4112	12329	15.1
Hemiptera	1341	1356	1460	4157	5.09
Hymenoptera	609	1803	962	3374	4.13
Orthoptera	147	171	249	567	0.69
Lepidoptera	107	145	208	460	0.56
Araneae	153	67	161	381	0.47
Neuroptera	94	69	103	266	0.33
Collembola	74	8	63	145	0.18
Opiliones	6	0	10	16	0.02

Table 2.4 – Total number of selected arthropod orders collected from select warm-season grass stands in 2014 near Mead, NE.

Arthropod Order	Switchgrass	Big Bluestem	LDM	Total	% total
Diptera	11874	12978	1624	41098	41.12
Thysanoptera	10865	10768	7984	29617	29.65
Coleoptera	2757	2584	2905	8246	8.25
Hymenoptera	2384	3829	966	7179	7.18
Hemiptera	1245	1938	2658	5841	5.84
Orthoptera	994	1506	1267	3767	3.77
Collembola	1116	734	864	2714	2.72
Araneae	232	300	208	740	0.74
Lepidoptera	48	121	116	285	0.28
Opiliones	61	85	116	262	0.25
Neuroptera	88	66	44	198	0.19

Table 2.5– Total number of selected arthropod orders collected from select warm-season grass stands in 2015 near Mead, NE.

Arthropod Order	Switchgrass	Big Bluestem	LDM	Total	% total
Diptera	5535	6534	6446	18515	36.61
Thysanoptera	2649	2966	2604	8219	16.25
Coleoptera	2607	1924	2304	6835	13.5
Orthoptera	1660	2222	2244	6126	12.11
Hemiptera	1597	2196	2220	6013	11.89
Hymenoptera	658	1766	947	3371	6.67
Araneae	215	149	145	509	1.0
Collembola	64	44	221	329	0.65
Opiliones	68	128	124	320	0.63
Lepidoptera	50	106	92	248	0.49
Neuroptera	12	45	33	90	0.18

Table 2.6 – Total number of selected arthropod orders collected in switchgrass, big bluestem and indiangrass in CenUSA plots in 2013 near Mead, NE.

Arthropod Order	Switchgrass	Big Bluestem	Indiangrass	Total	% total
Thysanoptera	2966	1226	1665	5857	64.58
Diptera	502	497	653	1652	18.21
Coleoptera	266	200	108	574	6.33
Hemiptera	137	138	233	508	5.6
Lepidoptera	67	47	55	169	1.86
Hymenoptera	61	43	29	133	1.47
Orthoptera	63	7	29	99	1.09
Araneae	10	28	19	57	0.63
Neuroptera	9	5	6	20	0.22
Collembola	0	0	0	0	0
Opiliones	0	0	0	0	0

Table 2.7 – Total number of selected arthropod orders collected in switchgrass, big bluestem and indiangrass in CenUSA plots in 2014 near Mead, NE.

Arthropod Order	Switchgrass	Big Bluestem	Indiangrass	Total	% total
Diptera	1250	1127	2054	4431	49.1
Thysanoptera	629	652	682	1963	21.75
Coleoptera	381	359	157	897	9.94
Hymenoptera	525	286	70	881	9.76
Orthoptera	195	117	53	365	4.04
Hemiptera	148	63	98	309	3.42
Araneae	15	16	23	54	0.59
Lepidoptera	11	23	17	51	0.57
Collembola	19	13	18	50	0.55
Neuroptera	5	5	12	22	0.24
Opiliones	1	0	0	1	0.01

Table 2.8 – Total number of selected arthropod orders collected in switchgrass, big bluestem and indiangrass in CenUSA plots in 2015 near Mead, NE.

Arthropod Order	Switchgrass	Big Bluestem	Indiangrass	Total	% total
Diptera	692	1606	1484	3782	48.39
Thysanoptera	406	534	444	1384	17.7
Coleoptera	432	301	209	942	12.05
Hemiptera	327	235	139	701	8.97
Orthoptera	162	211	82	455	5.82
Hymenoptera	124	136	66	326	4.17
Araneae	49	28	18	95	1.22
Lepidoptera	14	23	34	71	0.91
Neuroptera	4	18	12	34	0.44
Opiliones	9	11	4	24	0.3
Collembola	1	0	0	1	0.01

Table 2.9 – Total number of selected arthropod orders collected in switchgrass, big bluestem and indiangrass in CenUSA plots in 2013 near Arlington, WI.

Arthropod Order	Switchgrass	Big Bluestem	Indiangrass	Total	% total
Thysanoptera	2835	2569	3608	9012	48.14
Diptera	2375	1930	2497	6802	36.34
Coleoptera	535	456	388	1379	7.37
Opiliones	141	176	198	515	2.75
Hemiptera	58	142	151	351	1.88
Lepidoptera	274	2	3	279	1.49
Araneae	82	49	64	195	1.04
Hymenoptera	41	29	36	106	0.57
Orthoptera	19	10	7	36	0.19
Neuroptera	15	8	3	26	0.14
Collembola	0	13	5	18	0.09

Table 2.10 – Total number of selected arthropod orders collected in switchgrass, big bluestem and indiangrass in CenUSA plots in 2014 near Arlington, WI.

Arthropod Order	Switchgrass	Big Bluestem	Indiangrass	Total	% total
Diptera	1957	1724	1783	5464	49.42
Thysanoptera	1412	1466	1307	4185	37.85
Hymenoptera	115	167	129	411	3.72
Coleoptera	93	139	168	400	3.61
Opiliones	55	99	99	253	2.28
Orthoptera	40	28	44	112	1.01
Araneae	24	44	36	104	0.94
Hemiptera	34	33	35	102	0.92
Collembola	8	2	1	11	0.10
Neuroptera	2	3	3	8	0.07
Lepidoptera	1	2	3	6	0.05

Table 2.11– Total number of selected arthropod orders collected in switchgrass, big bluestem and indiangrass in CenUSA plots in 2015 near Arlington, WI.

Arthropod Order	Switchgrass	Big Bluestem	Indiangrass	Total	% total
Diptera	1467	1432	1710	4609	49.36
Thysanoptera	619	1135	1181	2935	31.43
Coleoptera	183	447	178	808	8.65
Opiliones	81	174	192	447	4.79
Hymenoptera	51	90	38	179	1.92
Orthoptera	28	57	49	134	1.44
Araneae	35	35	29	99	1.06
Hemiptera	23	48	24	95	1.02
Collembola	24	0	1	25	0.27
Lepidoptera	3	0	1	4	0.04
Neuroptera	1	0	1	2	0.02

Table 2.12 – Arthropod Families collected in bioenergy Switchgrass (Liberty) near Mead, NE in 2013. 60 Families were collected during the 2013 sampling season.

Arthropod Order	Family
Blattodea	Blattidae
Coleoptera	Cantharidae
	Carabidae
	Chrysomelidae
	Cleridae
	Coccinellidae
	Colydiidae
	Curculionidae
	Elateridae
	Histeridae
	Mordellidae
	Nitidulidae
	Phengodidae
	Scarabaeidae
	Silphidae
	Silvanidae
	Staphylinidae
Collembola	Isotomidae
Diptera	Bibionidae
	Calliphoridae
	Cecidomyiidae
	Chloropidae
	Dolichopodidae
	Muscidae
	Syrphidae
	Tachinidae
	Tephritidae
Hemiptera	Anthocoridae
	Aphididae
	Cicadellidae
	Cydnidae
	Lygaeidae
	Membracidae
	Miridae
	Nabidae
	Pentatomidae
	Psyllidae
Hymenoptera	Apidae
	Braconidae
Hymenoptera (cont.)	Figitidae
	Formicidae
	Ichneumonidae
	Mymaridae
	Scelionidae
Lepidoptera	Arctiidae
	Noctuidae
	Pieridae
Neuroptera	Chrysopidae
Orthoptera	Acrididae
	Gryllidae
	Rhaphidophoridae
	Tettigoniidae
Thysanoptera	Thripidae
Araneae	Clubionidae
	Gnaphosidae
	Lycosidae
	Salticidae
	Tetragnathidae
	Thomasidae
Opilliones	Phalangiidae

Table 2.13 – Arthropod Families collected in big bluestem near Mead, NE in 2013. 41 Families were collected during the 2013 sampling season.

Arthropod Order	Family
Coleoptera	Cantharidae
	Carabidae
	Chrysomelidae
	Coccinellidae
	Colydiidae
	Elateridae
	Histeridae
	Lampyridae
	Nitidulidae
	Phengodidae
	Scarabaeidae
	Silphidae
	Staphylinidae
Collembola	Isotomidae
Diptera	Calliphoridae
	Chloropidae
	Dolichopodidae
	Muscidae
	Syrphidae
	Tephritidae
Hemiptera	Anthocoridae
	Aphididae
	Cercopidae
	Cicadellidae
	Membracidae
	Miridae
	Pentatomidae
	Psyllidae
Hymenoptera	Apidae
	Braconidae
	Figitidae
	Formicidae
	Ichneumonidae
	Vespidae
Lepidoptera	Noctuidae
	Pieridae
Neuroptera	Chrysopidae
Orthoptera	Gryllidae
Thysanoptera	Thripidae

Arthropod Order	Family
Araneae (cont.)	Lycosidae
	Tetragnathidae

Table 2.14 – Arthropod Families collected in low diversity mix (LDM) near Mead, NE in 2013. 61 Families were collected during the 2013 sampling season.

Arthropod Order	Family
Coleoptera	Cantharidae
	Carabidae
	Chrysomelidae
	Cleridae
	Coccinellidae
	Colydiidae
	Elateridae
	Histeridae
	Lampyridae
	Mordellidae
	Nitidulidae
	Phengodidae
	Scarabaeidae
	Silphidae
	Staphylinidae
Collembola	Isotomidae
Diptera	Calliphoridae
	Chloropidae
	Dolichopodidae
	Muscidae
	Tephritidae
	Syrphidae
Hemiptera	Anthocoridae
	Aphididae
	Cicadellidae
	Lygaeidae
	Membracidae
	Miridae
	Nabidae
	Pentatomidae
	Psyllidae
	Reduviidae
Hymenoptera	Apidae
	Braconidae
	Figitidae
	Formicidae
	Ichneumonidae
	Mymaridae
	Platgastridae

Arthropod Order	Family
Hymenoptera (cont.)	Scollidae
	Vespidae
Lepidoptera	Noctuidae
	Nymphalidae
	Pieridae
	Pyralidae
Neuroptera	Chrysopidae
	Hemerobiidae
Orthoptera	Acrididae
	Gryllidae
	Tettigoniidae
Thysanoptera	Thripidae
Araneae	Clubionidae
	Dysderidae
	Gnaphosidae
	Lycosidae
	Oxyopidae
	Philodromidae
	Salticidae
	Tetragnathidae
	Thomasidae
Opilliones	Phalangiidae

Table 2.15 – Arthropod Families collected switchgrass from CenUSA plots near Mead, NE in 2013. 40 Families were collected during the 2013 sampling season.

Arthropod Order	Family
Coleoptera	Cantharidae
	Carabidae
	Chrysomelidae
	Coccinellidae
	Colydiidae
	Elateridae
	Lampyridae
	Nitidulidae
	Phengodidae
	Scarabaeidae
	Silphidae
	Staphylinidae
Diptera	Calliphoridae
	Chloropidae
	Dolichopodidae
	Muscidae
	Syrphidae
Hemiptera	Anthocoridae
	Aphididae
	Cicadellidae
	Cydnidae
	Geocoridae
	Membracidae
	Pentatomidae
	Psyllidae
Hymenoptera	Apidae
	Braconidae
	Figitidae
	Formicidae
	Ichneumonidae
	Scollidae
Lepidoptera	Noctuidae
	Pieridae
Neuroptera	Chrysopidae
Orthoptera	Acrididae
	Gryllidae
Thysanoptera	Thripidae
Araneae	Lycosidae
	Oxyopidae
Araneae (cont.)	Thomasidae

Table 2.16 – Arthropod Families collected big bluestem from CenUSA plots near Mead, NE in 2013. 44 Families were collected during the 2013 sampling season.

Arthropod Order	Family
Coleoptera	Carabidae
	Cerambycidae
	Chrysomelidae
	Coccinellidae
	Colydiidae
	Curculionidae
	Elateridae
	Histeridae
	Lampyridae
	Mordellidae
	Nitidulidae
	Scarabaeidae
	Staphylinidae
Diptera	Calliphoridae
	Chloropidae
	Dolichopodidae
	Muscidae
	Tephritidae
	Syrphidae
Hemiptera	Anthocoridae
	Aphididae
	Cicadellidae
	Cydnidae
	Geocoridae
	Lygaeidae
	Miridae
	Nabidae
	Pentatomidae
	Psyllidae
Hymenoptera	Apidae
	Braconidae
	Figitidae
	Formicidae
	Ichneumonidae
	Mutillidae
Lepidoptera	Noctuidae
	Pieridae
Neuroptera	Chrysopidae
Orthoptera	Acrididae
Orthoptera (cont.)	Gryllidae
Thysanoptera	Thripidae
Araneae	Clubionidae
	Lycosidae
	Thomasidae

Table 2.17 – Arthropod Families collected in indiangrass from CenUSA plots near Mead, NE in 2013. 48 Families were collected during the 2013 sampling season.

Arthropod Order	Family
Coleoptera	Cantharidae
	Carabidae
	Cerambycidae
	Chrysomelidae
	Cleridae
	Coccinellidae
	Colydiidae
	Curculionidae
	Elateridae
	Lampyridae
	Mordellidae
	Scarabaeidae
	Staphylinidae
Diptera	Calliphoridae
	Chloropidae
	Dolichopodidae
	Muscidae
	Tephritidae
	Sarcophagidae
	Syrphidae
Hemiptera	Anthocoridae
	Aphididae
	Cicadellidae
	Coreidae
	Lygaeidae
	Miridae
	Nabidae
	Pentatomidae
	Psyllidae
Hymenoptera	Apidae
	Braconidae
	Figitidae
	Formicidae
	Ichneumonidae
	Mutillidae
Lepidoptera	Noctuidae
	Pieridae
	Pyralidae
Neuroptera	Chrysopidae
Neuroptera (cont.)	Hemerobiidae
Orthoptera	Acrididae
	Gryllidae
	Tettigoniidae
Thysanoptera	Thripidae
Araneae	Gnaphosidae
	Lycosidae
	Salticidae
	Thomasidae

Table 2.18 – Arthropod Families collected in swichgrass from CenUSA plots near Arlington, WI in 2013. 38 Families were collected during the 2013 sampling season.

Arthropod Order	**Family**
Coleoptera	Carabidae
	Chrysomelidae
	Coccinellidae
	Colydiidae
	Curculionidae
	Elateridae
	Lampyridae
	Nitidulidae
	Scarabaeidae
	Staphylinidae
Diptera	Calliphoridae
	Chloropidae
	Muscidae
	Tephritidae
	Sarcophagidae
	Syrphidae
Hemiptera	Anthocoridae
	Aphididae
	Cicadellidae
	Lygaeidae
	Membracidae
	Miridae
	Nabidae
Hymenoptera	Apidae
	Braconidae
	Figitidae
	Formicidae
	Ichneumonidae
Lepidoptera	Noctuidae
	Pyralidae
Neuroptera	Chrysopidae
Orthoptera	Gryllidae
Thysanoptera	Thripidae
Araneae	Lycosidae
	Salticidae
	Tetragnathidae
	Thomasidae
Opilliones	Phalangiidae

Table 2.19 – Arthropod Families collected in big bluestem from CenUSA plots near Arlington, WI in 2013. 40 Families were collected during the 2013 sampling season.

Arthropod Order	Family
Coleoptera	Cantharidae
	Carabidae
	Chrysomelidae
	Cleridae
	Coccinellidae
	Colydiidae
	Curculionidae
	Elateridae
	Lampyridae
	Nitidulidae
	Scarabaeidae
	Staphylinidae
Collembola	Isotomidae
Diptera	Calliphoridae
	Chloropidae
	Muscidae
	Tephritidae
	Syrphidae
Hemiptera	Anthocoridae
	Aphididae
	Cicadellidae
	Membracidae
	Miridae
	Psyllidae
Hymenoptera	Apidae
	Braconidae
	Figitidae
	Formicidae
	Ichneumonidae
Lepidoptera	Noctuidae
	Pieridae
	Pyralidae
Neuroptera	Chrysopidae
Orthoptera	Gryllidae
Thysanoptera	Thripidae
Araneae	Agelenidae
	Lycosidae
	Tetragnathidae
	Thomasidae
Opilliones	Phalangiidae

Table 2.20 – Arthropod Families collected in indiangrass from CenUSA plots near Arlington, WI in 2013. 40 Families were collected during the 2013 sampling season.

Arthropod Order	Family
Coleoptera	Carabidae
	Chrysomelidae
	Cleridae
	Coccinellidae
	Curculionidae
	Elateridae
	Lampyridae
	Mordellidae
	Nitidulidae
	Scarabaeidae
	Staphylinidae
Collembola	Isotomidae
Diptera	Calliphoridae
	Chloropidae
	Muscidae
	Tephritidae
	Syrphidae
Hemiptera	Anthocoridae
	Aphididae
	Cercopidae
	Cicadellidae
	Lygaeidae
	Miridae
	Nabidae
	Psyllidae
Hymenoptera	Apidae
	Braconidae
	Figitidae
	Formicidae
Lepidoptera	Pieridae
	Pyralidae
Neuroptera	Chrysopidae
	Hemerobiidae
Orthoptera	Acrididae
	Gryllidae
Thysanoptera	Thripidae
Araneae	Clubionidae
	Lycosidae
	Thomasidae
Opilliones	Phalangiidae

Table 2.21 – Arthropod Families collected in bioenergy switchgrass (Liberty) near Mead, NE in 2014. 64 Families were collected during the 2014 sampling season.

Arthropod Order	Family
Coleoptera	Cantharidae
	Carabidae
	Cerambycidae
	Chrysomelidae
	Coccinellidae
	Colydiidae
	Curculionidae
	Elateridae
	Histeridae
	Lampyridae
	Meloidae
	Mordellidae
	Nitidulidae
	Scarabaeidae
	Silphidae
	Staphylinidae
Collembola	Isotomidae
Diptera	Agromyzidae
	Calliphoridae
	Chloropidae
	Dolichopodidae
	Muscidae
	Tephritidae
	Scatopsidae
	Syrphidae
Hemiptera	Anthocoridae
	Aphididae
	Cercopidae
	Cicadellidae
	Cydnidae
	Derbidae
	Geocoridae
	Lygaeidae
	Membracidae
	Miridae
	Nabidae
	Pentatomidae
	Reduviidae
Hymenoptera	Apidae
Hymenoptera (cont.)	Braconidae
	Figitidae
	Formicidae
	Ichneumonidae
	Mymaridae
	Platgastridae
	Scelionidae
Lepidoptera	Noctuidae
	Pieridae
	Pyralidae
Neuroptera	Chrysopidae
	Hemerobiidae
Orthoptera	Acrididae
	Gryllidae
	Tettigoniidae
Thysanoptera	Thripidae
Araneae	Clubionidae
	Gnaphosidae
	Lycosidae
	Oxyopidae
	Philodromidae
	Salticidae
	Theridiiae
	Thomasidae
Opilliones	Phalangiidae

Table 2.22 – Arthropod Families collected in big bluestem near Mead, NE in 2014. 67 Families were collected during the 2014 sampling season.

Arthropod Order	Family
Coleoptera	Buprestidae
	Cantharidae
	Carabidae
	Cerambycidae
	Chrysomelidae
	Coccinellidae
	Colydiidae
	Curculionidae
	Elateridae
	Histeridae
	Lampyridae
	Meloidae
	Mordellidae
	Nitidulidae
	Scarabaeidae
	Silphidae
	Staphylinidae
	Trogidae
Collembola	Isotomidae
	Sminthuriidae
Diptera	Calliphoridae
	Chloropidae
	Dolichopodidae
	Muscidae
	Tachinidae
	Tephritidae
	Syrphidae
Hemiptera	Anthocoridae
	Aphididae
	Blissidae
	Cercopidae
	Cicadellidae
	Cydnidae
	Derbidae
	Geocoridae
	Lygaeidae
	Membracidae
	Miridae
	Nabidae

Arthropod Order	Family
Hemiptera (cont.)	Pentatomidae
Hymenoptera	Apidae
	Braconidae
	Figitidae
	Formicidae
	Ichneumonidae
	Mymaridae
	Platgastridae
	Vespidae
Lepidoptera	Hesperiidae
	Noctuidae
	Nymphalidae
	Pieridae
	Pyralidae
Neuroptera	Chrysopidae
Orthoptera	Acrididae
	Gryllidae
	Tettigoniidae
Thysanoptera	Thripidae
Araneae	Clubionidae
	Gnaphosidae
	Lycosidae
	Oxyopidae
	Philodromidae
	Salticidae
	Tetragnathidae
	Thomasidae
Opilliones	Phalangiidae

Table 2.23 – Arthropod Families collected in low diversity mix (LDM) near Mead, NE in 2014. 66 Families were collected during the 2014 sampling season.

Arthropod Order	Family
Coleoptera	Cantharidae
	Carabidae
	Cerambycidae
	Chrysomelidae
	Cleridae
	Coccinellidae
	Colydiidae
	Curculionidae
	Elateridae
	Histeridae
	Lampyridae
	Mordellidae
	Nitidulidae
	Scarabaeidae
	Silphidae
	Staphylinidae
Collembola	Isotomidae
	Sminthuriidae
Diptera	Agromyzidae
	Calliphoridae
	Chloropidae
	Dolichopodidae
	Muscidae
	Tephritidae
	Scatopsidae
	Syrphidae
Hemiptera	Anthocoridae
	Aphididae
	Blissidae
	Cercopidae
	Cicadellidae
	Cydnidae
	Derbidae
	Geocoridae
	Lygaeidae
	Membracidae
	Miridae
	Nabidae
	Pentatomidae
Hymenoptera	Apidae
	Braconidae
	Figitidae
	Formicidae
	Ichneumonidae
	Mymaridae
	Platgastridae
	Mutillidae
Lepidoptera	Hesperiidae
	Noctuidae
	Pieridae
	Pyralidae
Neuroptera	Chrysopidae
	Hemerobiidae
Orthoptera	Acrididae
	Gryllidae
	Tettigoniidae
Thysanoptera	Thripidae
Araneae	Clubionidae
	Gnaphosidae
	Lycosidae
	Oxyopidae
	Philodromidae
	Salticidae
	Tetragnathidae
	Thomasidae
Opilliones	Phalangiidae

Table 2.24 – Arthropod Families collected in switchgrass from CenUSA plots near Mead, NE in 2014. 43 Families were collected during the 2014 sampling season.

Arthropod Order	Family
Coleoptera	Carabidae
	Chrysomelidae
	Coccinellidae
	Colydiidae
	Elateridae
	Lampyridae
	Nitidulidae
	Scarabaeidae
	Silphidae
	Staphylinidae
Collembola	Isotomidae
Diptera	Chloropidae
	Muscidae
	Tephritidae
	Syrphidae
Hemiptera	Anthocoridae
	Aphididae
	Cicadellidae
	Cicadidae
	Derbidae
	Lygaeidae
	Membracidae
	Miridae
	Nabidae
	Pentatomidae
Hymenoptera	Braconidae
	Figitidae
	Formicidae
	Ichneumonidae
	Mymaridae
	Platgastridae
Lepidoptera	Noctuidae
	Pieridae
Neuroptera	Chrysopidae
Orthoptera	Acrididae
	Gryllidae
	Tettigoniidae
Thysanoptera	Thripidae
Araneae	Clubionidae
Araneae (cont.)	Gnaphosidae
	Lycosidae
	Philodromidae
Opilliones	Phalangiidae

Table 2.25– Arthropod Families collected in big bluestem from CenUSA plots near Mead, NE in 2014. 37 Families were collected during the 2014 sampling season.

Arthropod Order	**Family**
Coleoptera	Cantharidae
	Carabidae
	Chrysomelidae
	Coccinellidae
	Colydiidae
	Elateridae
	Lampyridae
	Nitidulidae
	Scarabaeidae
	Silphidae
	Staphylinidae
Collembola	Isotomidae
Diptera	Calliphoridae
	Chloropidae
	Muscidae
	Tephritidae
	Syrphidae
Hemiptera	Anthocoridae
	Cicadellidae
	Cicadidae
	Derbidae
	Lygaeidae
	Membracidae
	Pentatomidae
Hymenoptera	Braconidae
	Figitidae
	Formicidae
	Ichneumonidae
	Mymaridae
	Platgastridae
Lepidoptera	Noctuidae
	Pieridae
Neuroptera	Chrysopidae
Orthoptera	Gryllidae
Thysanoptera	Thripidae
Araneae	Lycosidae
	Thomasidae

Table 2.26 – Arthropod Families collected in indiangrass from CenUSA plots near Mead, NE in 2014. 39 Families were collected during the 2014 sampling season.

Arthropod Order	**Family**
Coleoptera	Cantharidae
	Carabidae
	Chrysomelidae
	Coccinellidae
	Colydiidae
	Elateridae
	Histeridae
	Lampyridae
	Nitidulidae
	Scarabaeidae
	Staphylinidae
Collembola	Isotomidae
Diptera	Chloropidae
	Dolichopodidae
	Muscidae
	Tephritidae
	Syrphidae
Hemiptera	Anthocoridae
	Aphididae
	Cicadellidae
	Derbidae
	Lygaeidae
	Membracidae
	Pentatomidae
Hymenoptera	Braconidae
	Figitidae
	Formicidae
	Mymaridae
Lepidoptera	Noctuidae
	Pieridae
Neuroptera	Chrysopidae
	Hemerobiidae
Orthoptera	Acrididae
	Gryllidae
Thysanoptera	Thripidae
Araneae	Clubionidae
	Lycosidae
	Salticidae
	Thomasidae

Table 2.27 – Arthropod Families collected in switchgrass from CenUSA plots near Arlington, WI in 2014. 34 Families were collected during the 2014 sampling season.

Arthropod Order	**Family**
Coleoptera	Carabidae
	Chrysomelidae
	Curculionidae
	Elateridae
	Nitidulidae
	Scarabaeidae
	Staphylinidae
Collembola	Isotomidae
Diptera	Calliphoridae
	Chloropidae
	Dolichopodidae
	Muscidae
	Tephritidae
	Scatopsidae
	Syrphidae
Hemiptera	Aphididae
	Cicadellidae
	Lygaeidae
	Miridae
	Nabidae
	Pentatomidae
Hymenoptera	Braconidae
	Figitidae
	Formicidae
	Ichneumonidae
	Mymaridae
Lepidoptera	Pieridae
Neuroptera	Chrysopidae
Orthoptera	Gryllidae
Thysanoptera	Thripidae
Araneae	Gnaphosidae
	Lycosidae
	Theridiidae
Opilliones	Phalangiidae

Table 2.28 – Arthropod Families collected in big bluestem from CenUSA plots near Arlington, WI in 2014. 42 Families were collected during the 2014 sampling season.

Arthropod Order	Family
Coleoptera	Cantharidae
	Carabidae
	Chrysomelidae
	Coccinellidae
	Curculionidae
	Elateridae
	Lampyridae
	Nitidulidae
	Scarabaeidae
	Staphylinidae
Collembola	Isotomidae
Diptera	Calliphoridae
	Chloropidae
	Dolichopodidae
	Muscidae
	Tephritidae
	Scatopsidae
	Syrphidae
Hemiptera	Aphididae
	Cicadellidae
	Miridae
	Reduviidae
Hymenoptera	Apidae
	Braconidae
	Figitidae
	Formicidae
	Ichneumonidae
	Mymaridae
	Platgastridae
Lepidoptera	Pieridae
	Pyralidae
Neuroptera	Chrysopidae
Orthoptera	Acrididae
	Gryllidae
	Tettigoniidae
Thysanoptera	Thripidae
Araneae	Agelenidae
	Clubionidae
	Lycosidae
Araneae (cont.)	Theridiiae
	Thomasidae
Opilliones	Phalangiidae

Table 2.29 – Arthropod Families collected in indiangrass from CenUSA plots near Arlington, WI in 2014. 44 Families were collected during the 2014 sampling season.

Arthropod Order	Family
Coleoptera	Cantharidae
	Carabidae
	Chrysomelidae
	Cleridae
	Coccinellidae
	Curculionidae
	Elateridae
	Nitidulidae
	Scarabaeidae
	Staphylinidae
Collembola	Isotomidae
Diptera	Calliphoridae
	Chloropidae
	Dolichopodidae
	Muscidae
	Tephritidae
	Scatopsidae
	Syrphidae
Hemiptera	Anthocoridae
	Aphididae
	Cicadellidae
	Membracidae
	Miridae
	Reduviidae
Hymenoptera	Braconidae
	Figitidae
	Formicidae
	Ichneumonidae
	Mymaridae
	Platgastridae
Lepidoptera	Noctuidae
	Pieridae
	Pyralidae
Neuroptera	Chrysopidae
	Hemerobiidae
Orthoptera	Acrididae
	Gryllidae
	Tettigoniidae
Thysanoptera	Thripidae
Araneae	Agelenidae
	Lycosidae
	Theridiiae
	Thomasidae
Opilliones	Phalangiidae

Table 2.30 – Arthropod Families collected in bioenergy switchgrass (Liberty) near Mead, NE in 2015. 59 Families were collected during the 2015 sampling season.

Arthropod Order	Family
Coleoptera	Cantharidae
	Carabidae
	Cerambycidae
	Chrysomelidae
	Coccinellidae
	Colydiidae
	Curculionidae
	Elateridae
	Histeridae
	Lampyridae
	Mordellidae
	Nitidulidae
	Phengodidae
	Scarabaeidae
	Silphidae
	Staphylinidae
Collembola	Isotomidae
Diptera	Calliphoridae
	Chloropidae
	Dolichopodidae
	Muscidae
	Tephritidae
	Syrphidae
Hemiptera	Aphididae
	Blissidae
	Cercopidae
	Cicadellidae
	Cydnidae
	Derbidae
	Lygaeidae
	Membracidae
	Miridae
	Nabidae
	Pentatomidae
	Reduviidae
Hymenoptera	Apidae
	Braconidae
	Figitidae
	Formicidae
Hymenoptera (cont.)	Ichneumonidae
	Mymaridae
	Platgastridae
Lepidoptera	Noctuidae
	Pieridae
	Pyralidae
Neuroptera	Chrysopidae
	Hemerobiidae
Orthoptera	Acrididae
	Gryllidae
	Tettigoniidae
Thysanoptera	Thripidae
Araneae	Clubionidae
	Gnaphosidae
	Lycosidae
	Oxyopidae
	Philodromidae
	Tetragnathidae
	Thomasidae
Opilliones	Phalangiidae

Table 2.31 – Arthropod Families collected in big bluestem near Mead, NE in 2015. 62 Families were collected during the 2015 sampling season.

Arthropod Order	Family
Coleoptera	Cantharidae
	Carabidae
	Cerambycidae
	Chrysomelidae
	Coccinellidae
	Colydiidae
	Curculionidae
	Elateridae
	Histeridae
	Lampyridae
	Mordellidae
	Nitidulidae
	Phengodidae
	Scarabaeidae
	Silphidae
	Staphylinidae
Collembola	Isotomidae
Diptera	Agromyzidae
	Calliphoridae
	Chironomidae
	Chloropidae
	Dolichopodidae
	Muscidae
	Tephritidae
	Syrphidae
Hemiptera	Anthocoridae
	Aphididae
	Cercopidae
	Cicadellidae
	Cydnidae
	Derbidae
	Lygaeidae
	Membracidae
	Miridae
	Nabidae
	Pentatomidae
	Reduviidae
Hymenoptera	Apidae
	Braconidae

Arthropod Order	Family
Hymenoptera (cont.)	Figitidae
	Formicidae
	Ichneumonidae
	Mymaridae
	Platgastridae
Lepidoptera	Noctuidae
	Pieridae
	Pyralidae
Neuroptera	Chrysopidae
	Hemerobiidae
Orthoptera	Acrididae
	Gryllidae
	Tettigoniidae
Thysanoptera	Thripidae
Araneae	Clubionidae
	Gnaphosidae
	Lycosidae
	Oxyopidae
	Philodromidae
	Salticidae
	Tetragnathidae
	Thomasidae
Opilliones	Phalangiidae

Table 2.32 – Arthropod Families collected in low diversity mix (LDM) near Mead, NE in 2015. 59 Families were collected during the 2015 sampling season.

Arthropod Order	Family
Coleoptera	Cantharidae
	Carabidae
	Chrysomelidae
	Cleridae
	Coccinellidae
	Colydiidae
	Curculionidae
	Elateridae
	Histeridae
	Lampyridae
	Mordellidae
	Nitidulidae
	Phengodidae
	Scarabaeidae
	Silphidae
	Staphylinidae
Collembola	Isotomidae
Diptera	Agromyzidae
	Calliphoridae
	Chloropidae
	Dolichopodidae
	Muscidae
	Tephritidae
	Syrphidae
Hemiptera	Anthocoridae
	Aphididae
	Cercopidae
	Cicadellidae
	Derbidae
	Geocoridae
	Membracidae
	Miridae
	Nabidae
	Pentatomidae
Hymenoptera	Apidae
	Braconidae
	Figitidae
	Formicidae
	Ichneumonidae

Arthropod Order	Family
Hymenoptera (cont.)	Mymaridae
	Platgastridae
Lepidoptera	Noctuidae
	Pieridae
	Pyralidae
Neuroptera	Chrysopidae
	Hemerobiidae
Orthoptera	Acrididae
	Gryllidae
	Tettigoniidae
Thysanoptera	Thripidae
Araneae	Clubionidae
	Gnaphosidae
	Lycosidae
	Oxyopidae
	Philodromidae
	Salticidae
	Tetragnathidae
	Thomasidae
Opilliones	Phalangiidae

Table 2.33 – Arthropod Families collected in switchgrass from CenUSA plots near Mead, NE in 2015. 41 Families were collected during the 2015 sampling season.

Arthropod Order	Family
Coleoptera	Carabidae
	Chrysomelidae
	Coccinellidae
	Colydiidae
	Curculionidae
	Elateridae
	Histeridae
	Nitidulidae
	Phengodidae
	Scarabaeidae
	Staphylinidae
Collembola	Isotomidae
Diptera	Calliphoridae
	Chloropidae
	Dolichopodidae
	Muscidae
	Tephritidae
	Syrphidae
Hemiptera	Aphididae
	Cicadellidae
	Derbidae
	Lygaeidae
	Membracidae
	Pentatomidae
Hymenoptera	Braconidae
	Figitidae
	Formicidae
	Ichneumonidae
Lepidoptera	Noctuidae
	Pieridae
Neuroptera	Chrysopidae
Orthoptera	Acrididae
	Gryllidae
Thysanoptera	Thripidae
Araneae	Gnaphosidae
	Lycosidae
	Oxyopidae
	Philodromidae
	Tetragnathidae
Araneae (cont.)	Thomasidae
Opilliones	Phalangiidae

Table 2.34 – Arthropod Families collected in big bluestem from CenUSA plots near Mead, NE in 2015. 42 Families were collected during the 2015 sampling season.

Arthropod Order	Family
Coleoptera	Carabidae
	Cerambycidae
	Chrysomelidae
	Coccinellidae
	Colydiidae
	Curculionidae
	Elateridae
	Histeridae
	Lampyridae
	Nitidulidae
	Phengodidae
	Scarabaeidae
	Silphidae
	Staphylinidae
Diptera	Calliphoridae
	Chloropidae
	Dolichopodidae
	Muscidae
	Tephritidae
	Syrphidae
Hemiptera	Aphididae
	Cicadellidae
	Derbidae
	Lygaeidae
	Membracidae
	Miridae
	Pentatomidae
Hymenoptera	Braconidae
	Figitidae
	Formicidae
	Ichneumonidae
Lepidoptera	Noctuidae
	Pieridae
Neuroptera	Chrysopidae
	Hemerobiidae
Orthoptera	Gryllidae
	Tettigoniidae
Thysanoptera	Thripidae
Araneae	Lycosidae
Araneae (cont.)	Oxyopidae
	Thomasidae
Opilliones	Phalangiidae

Table 2.35 – Arthropod Families collected in indiangrass from CenUSA plots near Mead, NE in 2015. 41 Families were collected during the 2015 sampling season.

Arthropod Order	Family
Coleoptera	Carabidae
	Chrysomelidae
	Coccinellidae
	Colydiidae
	Curculionidae
	Elateridae
	Histeridae
	Lampyridae
	Nitidulidae
	Phengodidae
	Scarabaeidae
	Silphidae
	Staphylinidae
Diptera	Calliphoridae
	Chloropidae
	Dolichopodidae
	Muscidae
	Tephritidae
	Syrphidae
Hemiptera	Anthocoridae
	Aphididae
	Cicadellidae
	Derbidae
	Membracidae
	Miridae
	Pentatomidae
Hymenoptera	Braconidae
	Figitidae
	Formicidae
	Ichneumonidae
Lepidoptera	Noctuidae
	Pieridae
Neuroptera	Chrysopidae
	Hemerobiidae
Orthoptera	Acrididae
	Gryllidae
	Tettigoniidae
Thysanoptera	Thripidae
Araneae	Lycosidae
Araneae (cont.)	Thomasidae
Opilliones	Phalangiidae

Table 2.36 – Arthropod Families collected in switchgrass from CenUSA plots near Arlington, WI in 2015. 35 Families were collected during the 2015 sampling season.

Arthropod Order	**Family**
Coleoptera	Cantharidae
	Carabidae
	Cerambycidae
	Chrysomelidae
	Coccinellidae
	Curculionidae
	Elateridae
	Nitidulidae
	Scarabaeidae
	Staphylinidae
Collembola	Isotomidae
Diptera	Calliphoridae
	Chloropidae
	Dolichopodidae
	Muscidae
	Tephritidae
	Sphaeroceridae
	Syrphidae
Hemiptera	Cicadellidae
	Lygaeidae
	Nabidae
Hymenoptera	Braconidae
	Figitidae
	Formicidae
	Mymaridae
	Platygastridae
Lepidoptera	Noctuidae
	Pieridae
Neuroptera	Chrysopidae
Orthoptera	Gryllidae
Thysanoptera	Thripidae
Araneae	Lycosidae
	Tetragnathidae
	Thomasidae
Opilliones	Phalangiidae

Table 2.37 – Arthropod Families collected in big bluestem from CenUSA plots near Arlington, WI in 2015. 35 Families were collected during the 2015 sampling season.

Arthropod Order	**Family**
Coleoptera	Cantharidae
	Carabidae
	Chrysomelidae
	Coccinellidae
	Curculionidae
	Elateridae
	Lampyridae
	Nitidulidae
	Scarabaeidae
	Staphylinidae
Collembola	Isotomidae
Diptera	Calliphoridae
	Chloropidae
	Dolichopodidae
	Muscidae
	Tephritidae
	Sphaeroceridae
	Syrphidae
Hemiptera	Cicadellidae
	Membracidae
	Miridae
Hymenoptera	Braconidae
	Figitidae
	Formicidae
	Ichneumonidae
	Mymaridae
Neuroptera	Chrysopidae
Orthoptera	Acrididae
	Gryllidae
Thysanoptera	Thripidae
Araneae	Lycosidae
	Theridiiae
	Thomasidae
Opilliones	Phalangiidae

Table 2.38 – Arthropod Families collected in indiangrass from CenUSA plots near Arlington, WI in 2015. 37 Families were collected during the 2015 sampling season.

Arthropod Order	**Family**
Coleoptera	Cantharidae
	Carabidae
	Cerambycidae
	Chrysomelidae
	Coccinellidae
	Curculionidae
	Elateridae
	Lampyridae
	Nitidulidae
Collembola	Isotomidae
Diptera	Calliphoridae
	Chloropidae
	Dolichopodidae
	Muscidae
	Tephritidae
	Sphaeroceridae
	Syrphidae
Hemiptera	Anthocoridae
	Aphididae
	Cercopidae
	Cicadellidae
	Lygaeidae
	Membracidae
Hymenoptera	Braconidae
	Figitidae
	Formicidae
	Ichneumonidae
	Mymaridae
Lepidoptera	Noctuidae
Neuroptera	Hemerobiidae
Orthoptera	Acrididae
	Gryllidae
Thysanoptera	Thripidae
Araneae	Lycosidae
	Salticidae
	Thomasidae
Opilliones	Phalangiidae

Table 2.39 – Total number of Arthropod Families collected in switchgrass, big bluestem, and low diversity mix from near Mead, NE by two techniques in 2013, 2014 and 2015.

	2013		2014		2015	
Arthropod	**Pitfall**	**Sticky trap**	**Pitfall**	**Sticky Trap**	**Pitfall**	**Sticky Trap**
Acrididae	5	4	26	7	36	11
Carabidae	5,805	76	3,265	86	2,905	23
Chloropidae	--	17,552	--	25,224	--	11,833
Chrysomelidae	163	3,223	95	800	98	189
Cicadellidae	111	1,657	3,710	517	1,767	875
Coccinellidae	41	205	26	159	27	42
Formicidae	47	26	173	11	172	238
Gryllidae	572	--	3,715	--	6,042	1
Nitidulidae	953	7	584	1	1,928	--
Scarabaeidae	380	--	583	1	454	1
Staphylinidae	98	6	405	6	630	15
Tettigonidae	--	8	2	4	1	24
Tephrititdae	--	116	--	89	--	185
Thripidae	--	33,256	--	29,617	--	8,219
Parasitic Hymenoptera	--	3,174	--	6,468	--	2,934
Araneae	213	168	527	213	471	38
Opiliones	5	11	129	133	259	61

Table 2.40 – Total number of Arthropod Families collected in switchgrass, big bluestem, and indiangrass from CenUSA plots near Mead, NE by two techniques in 2013, 2014 and 2015.

	2013		2014		2015	
Arthropod	**Pitfall**	**Sticky trap**	**Pitfall**	**Sticky Trap**	**Pitfall**	**Sticky Trap**
Acrididae	8	--	3	--	5	1
Carabidae	264	16	360	2	299	5
Chloropidae	--	1,275	--	3,884	--	3,244
Chrysomelidae	2	44	4	29	10	40
Cicadellidae	2	227	8	99	54	142
Coccinellidae	--	10	1	7	1	4
Formicidae	11	4	37	2	79	2
Gryllidae	59	--	368	1	400	--
Nitidulidae	6	2	339	1	335	--
Scarabaeidae	9	--	5	--	23	--
Staphylinidae	3	23	66	4	177	3
Tettigonidae	--	1	--	1	2	--
Tephrititdae	--	7	--	16	--	27
Thripidae	--	5,857	--	1,963	--	1,384
Parasitic Hymenoptera	--	66	--	837	--	229
Araneae	31	26	43	11	92	3
Opiliones	--	--	--	1	24	--

Table 2.41 – Total number of Arthropod Families collected in switchgrass, big bluestem, and indiangrass from CenUSA plots near Arlington, WI by two techniques in 2013, 2014 and 2015.

	2013		2014		2015	
Arthropod	**Pitfall**	**Sticky trap**	**Pitfall**	**Sticky Trap**	**Pitfall**	**Sticky Trap**
Acrididae	1	--	11	1	7	--
Carabidae	561	31	267	1	257	3
Chloropidae	--	4,805	--	2,507	--	3,104
Chrysomelidae	8	572	4	5	18	398
Cicadellidae	3	162	20	46	35	36
Coccinellidae	2	18	--	2	--	3
Formicidae	39	--	30	--	48	1
Gryllidae	34	--	102	--	127	--
Nitidulidae	11	2	13	--	8	--
Scarabaeidae	13	2	19	--	7	1
Staphylinidae	48	8	16	1	16	1
Tettigonidae	--	--	1	1	--	--
Tephrititdae	6	133	1	166	21	239
Thripidae	--	9,012	--	4,185	--	2,935
Parasitic Hymenoptera	--	57	--	314	--	128
Araneae	187	8	96	8	96	3
Opiliones	513	2	251	2	446	1

Figure 2.3 - Total number of ground beetles (Carabidae) collected in pitfall traps in three warm-season grass stands near Mead, NE in 2013, 2014 and 2015.

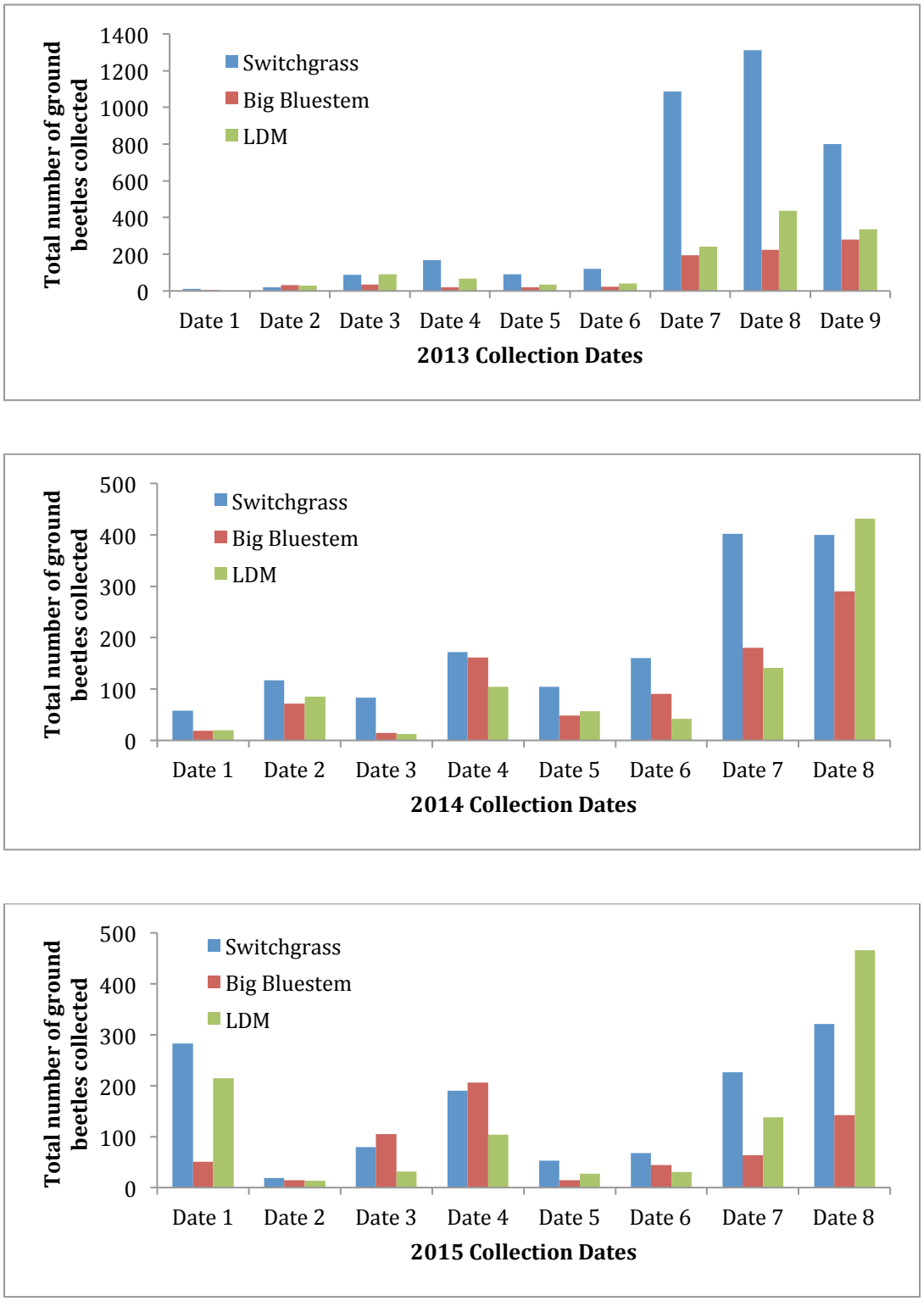
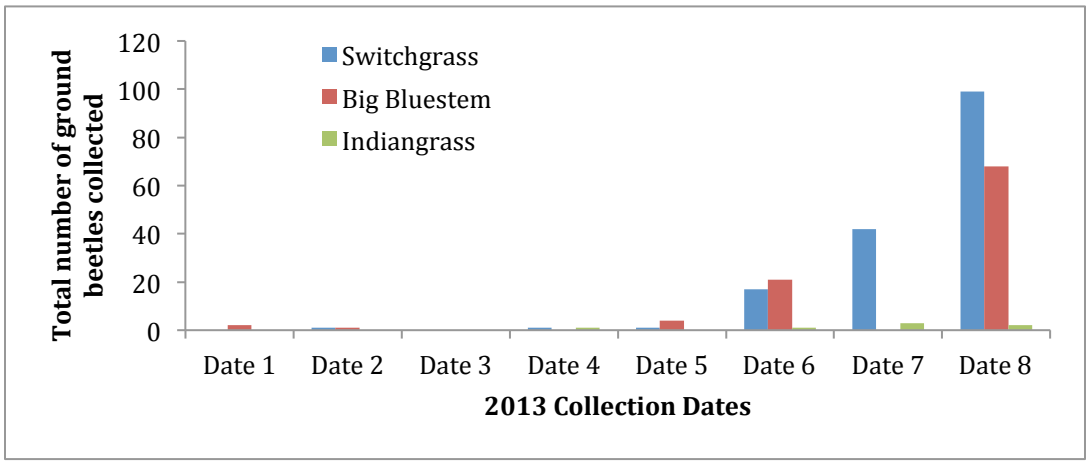
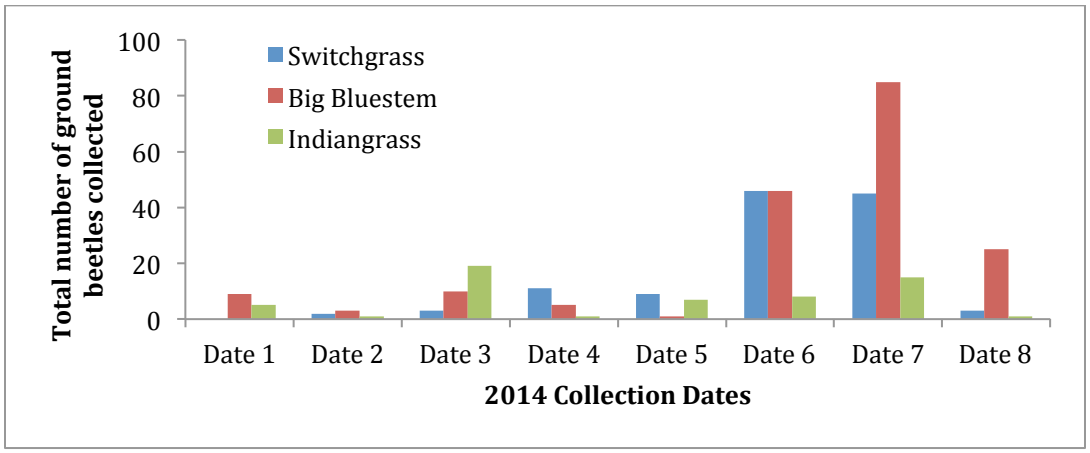


Figure 2.4 - Total number of ground beetles (Carabidae) collected in pitfall traps in switchgrass, big bluestem and indiangrass in CenUSA plots near Mead, NE in 2013, 2014 and 2015.





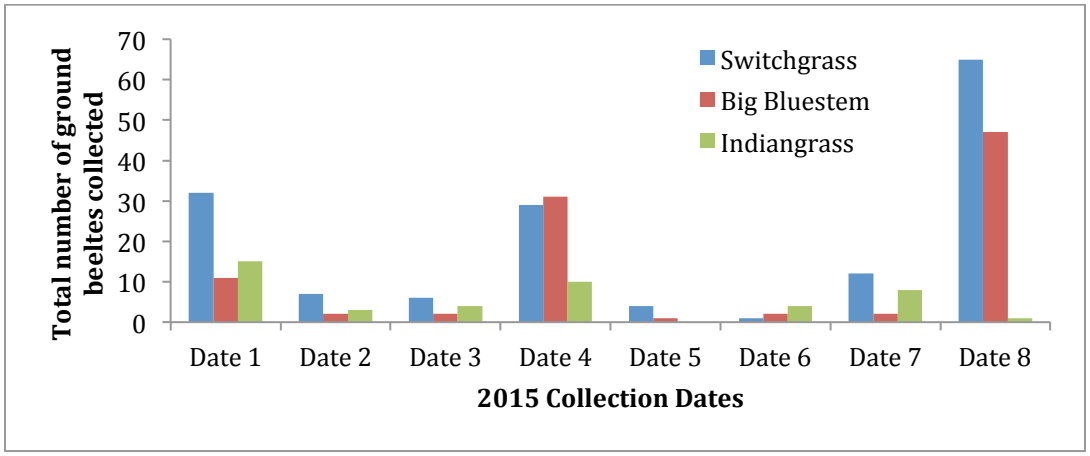
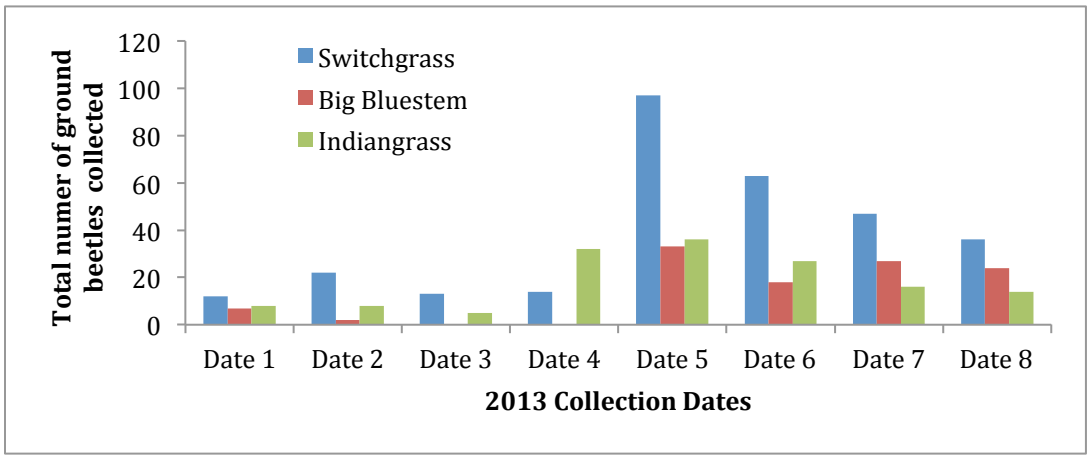
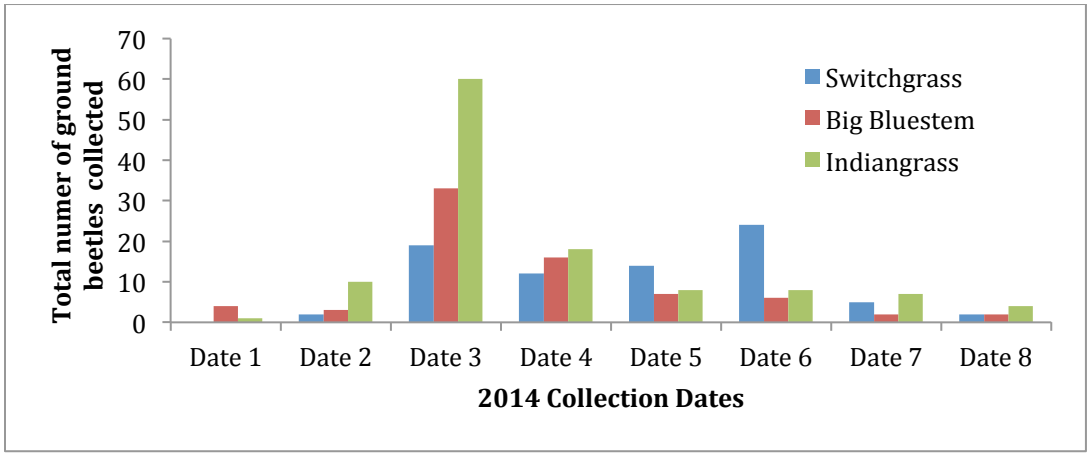


Figure 2.5 - Total number of ground beetles (Carabidae) collected in pitfall traps in switchgrass, big bluestem and indiangrass in CenUSA plots near Arlington, WI in 2013, 2014 and 2015.





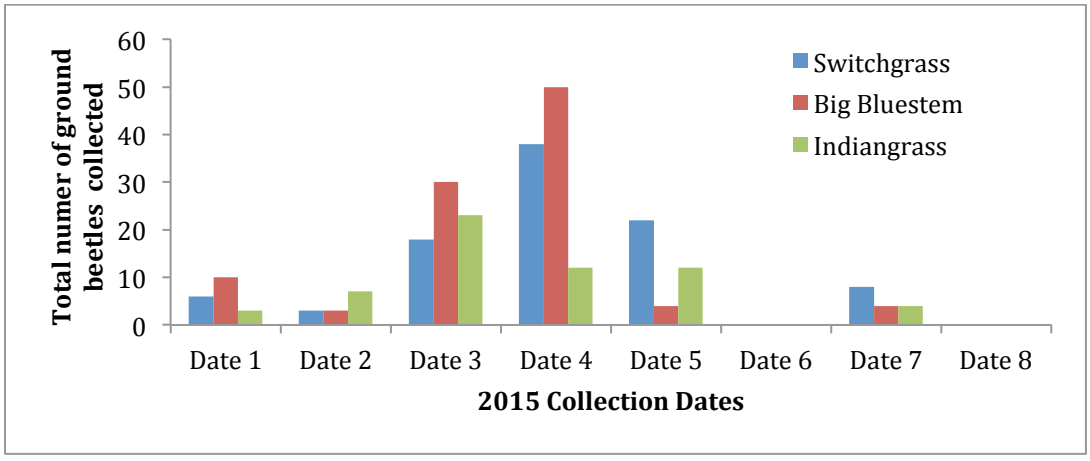


Figure 2.6 - Total number of grass flies (Chloropidae) collected on sticky cards in three warm-season grass stands near Mead, NE in 2013, 2014 and 2015.

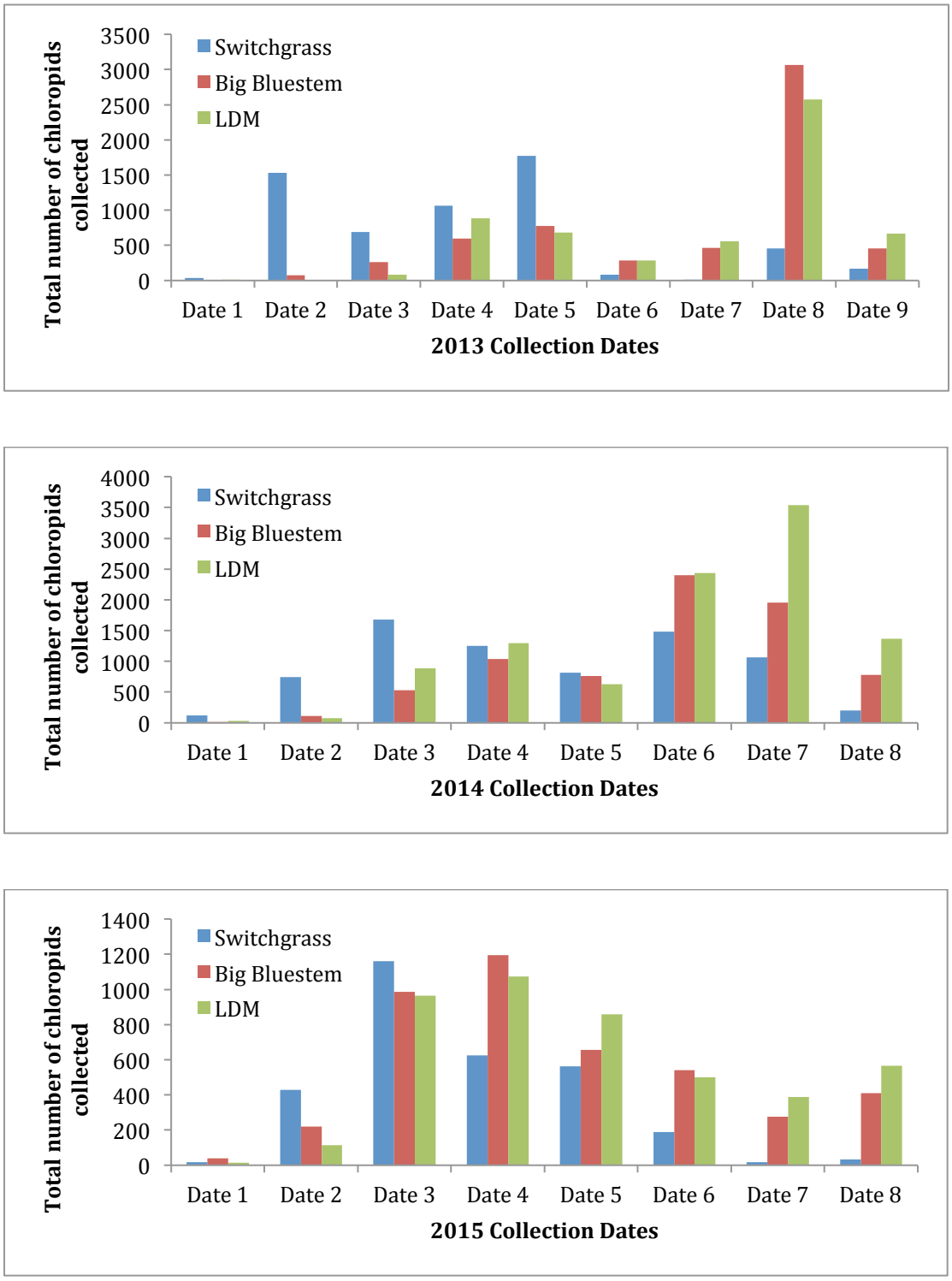
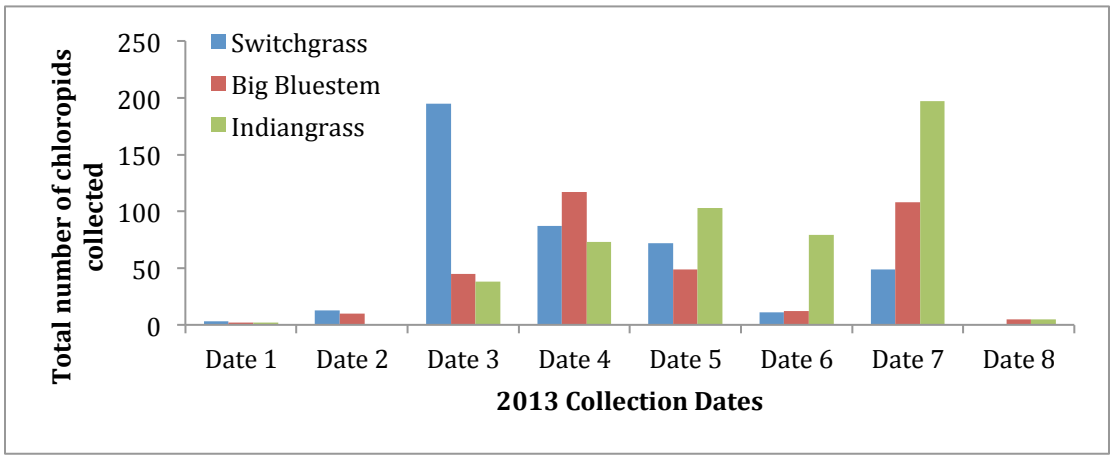
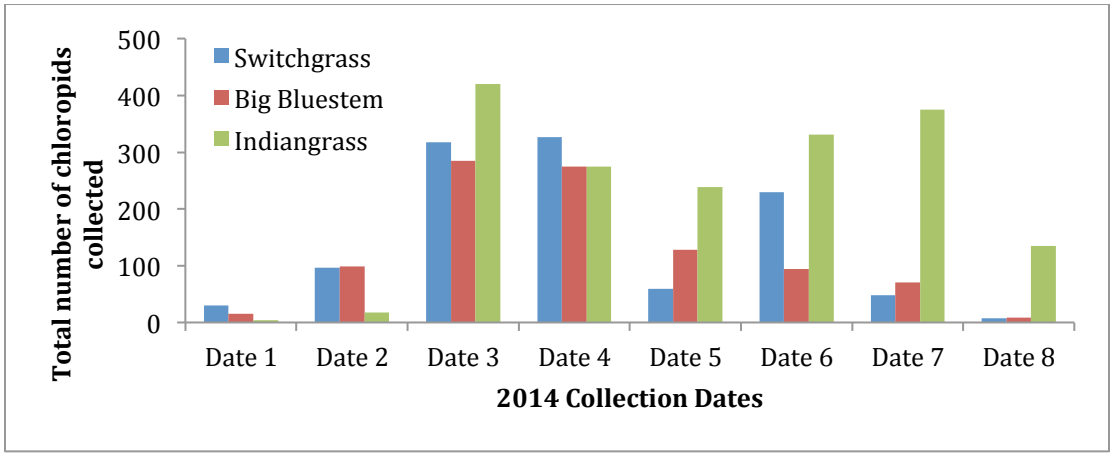


Figure 2.7 - Total number of grass flies (Chloropidae) collected in sticky cards in switchgrass, big bluestem and indiangrass in CenUSA plots near Mead, NE in 2013, 2014 and 2015.





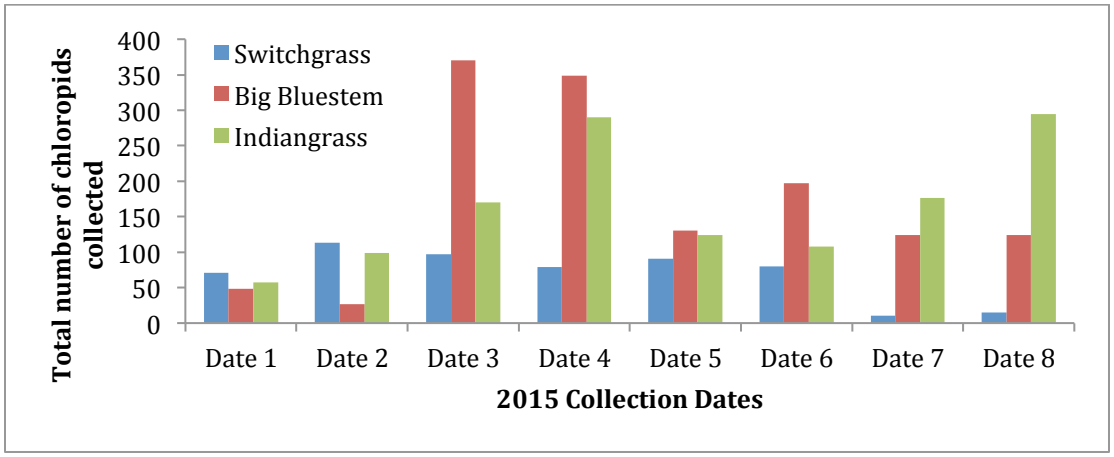
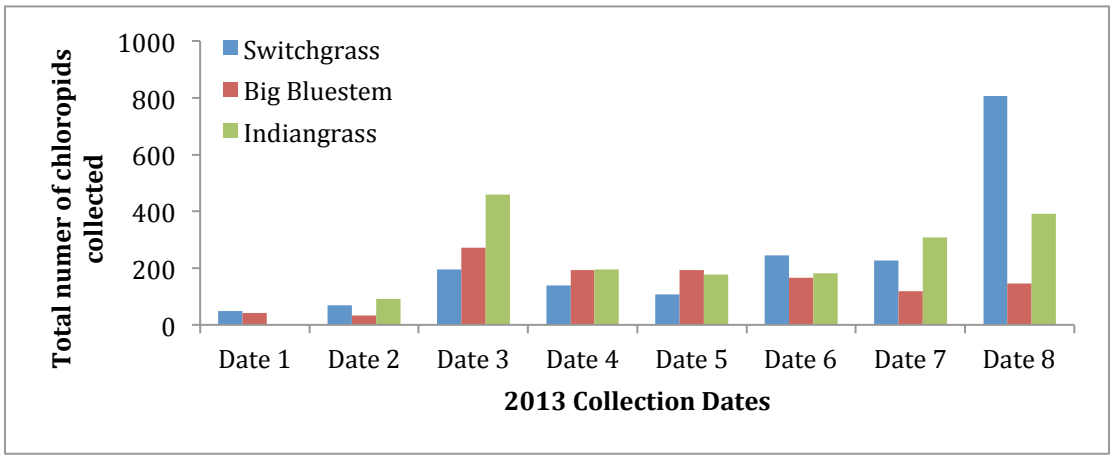
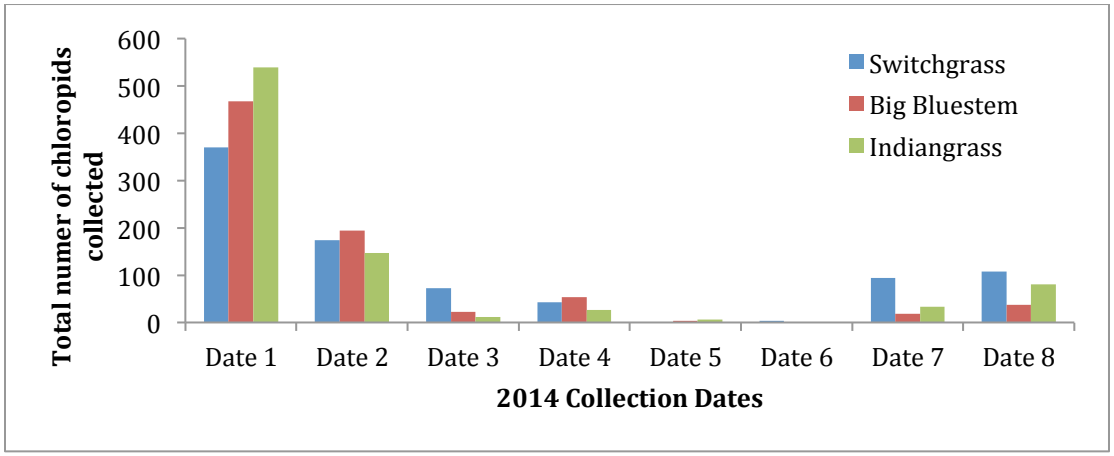


Figure 2.8 - Total number of grass flies (Chloropidae) collected on sticky traps in switchgrass, big bluestem and indiangrass in CenUSA plots near Arlington, WI in 2013, 2014 and 2015.





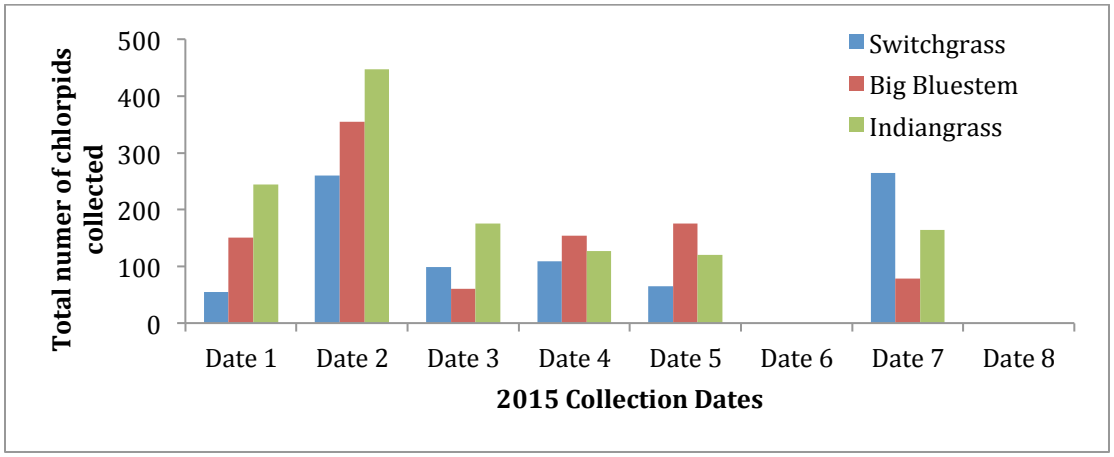


Figure 2.9 - Total number of leafhoppers (Cicadellidae) collected on sticky traps in three warm-season grass stands near Mead, NE in 2013, 2014 and 2015.

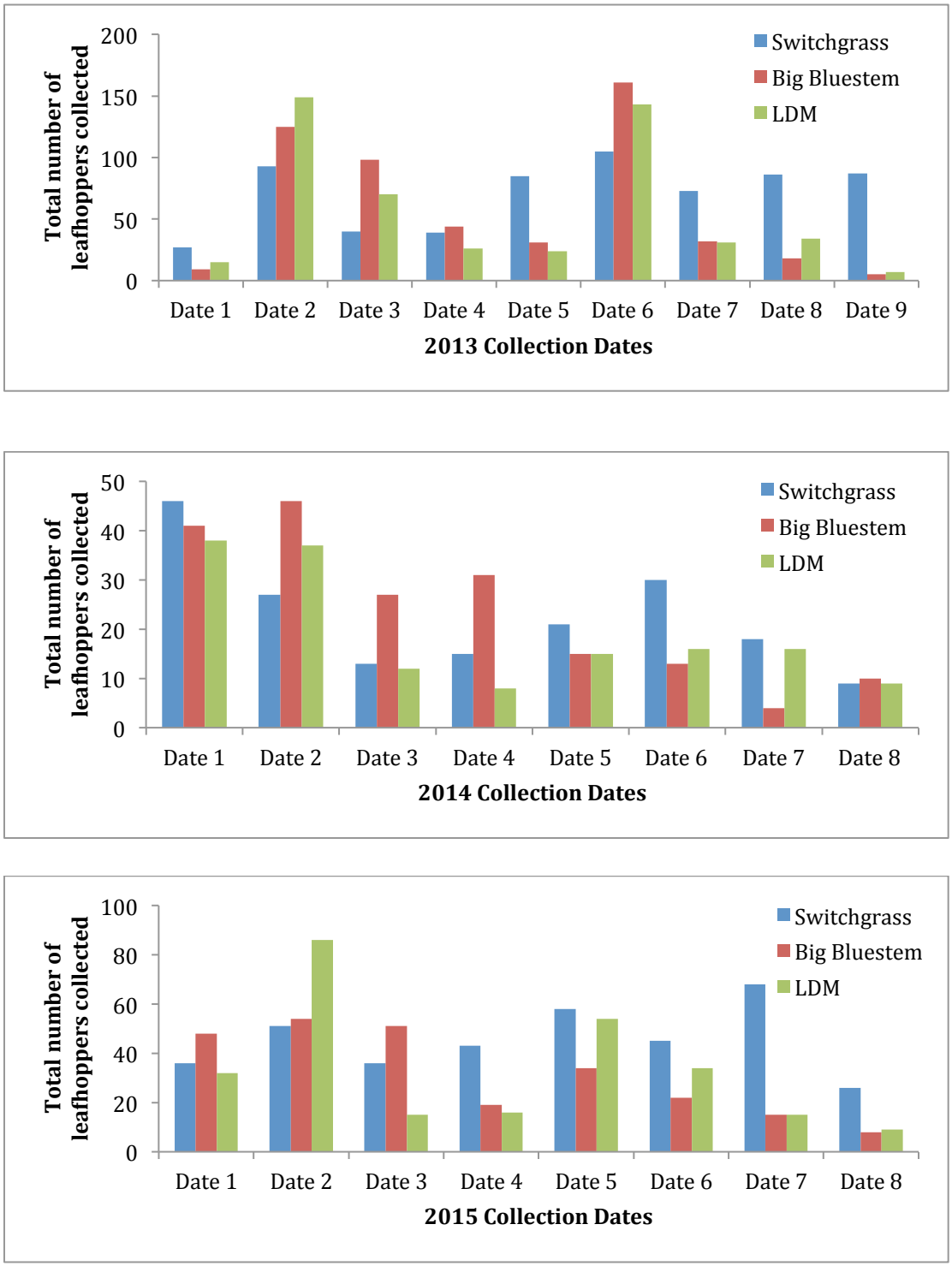
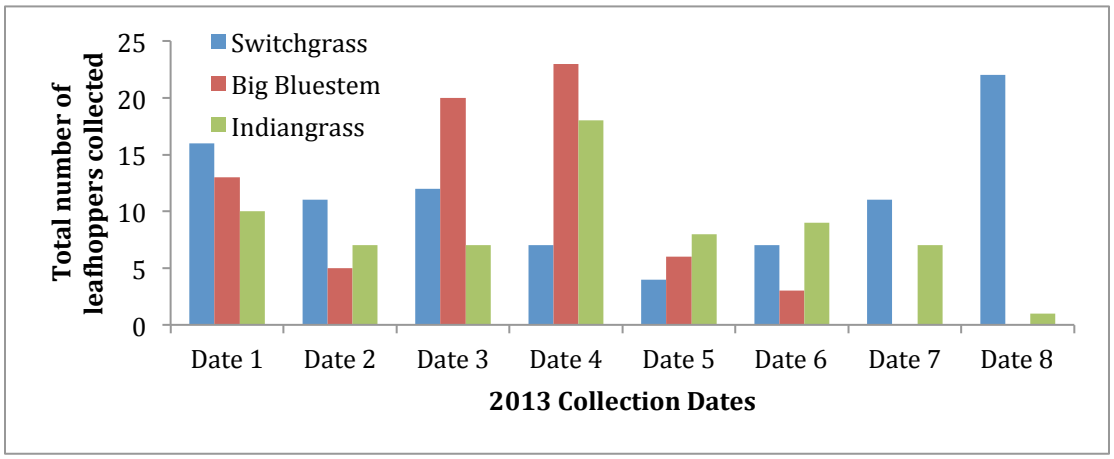
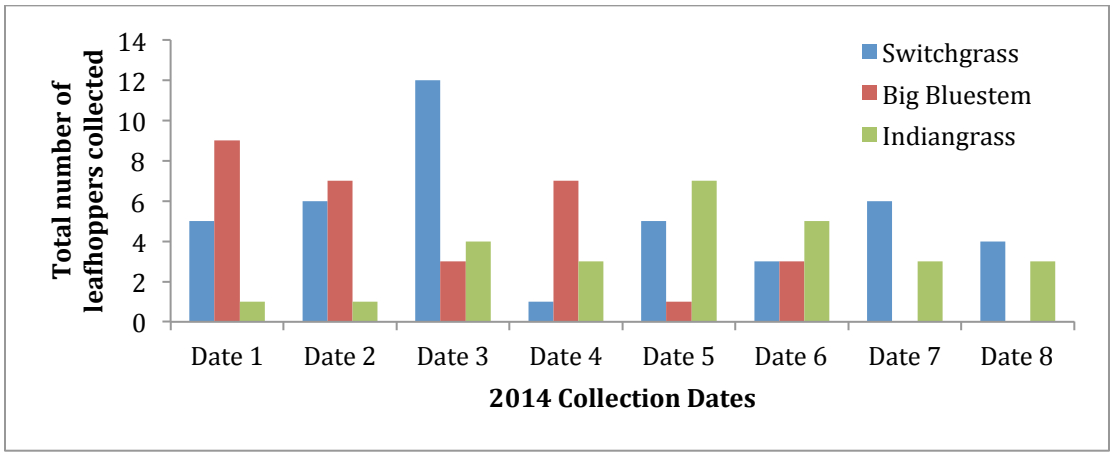


Figure 2.10 - Total number of leafhoppers (Cicadellidae) collected in sticky traps in switchgrass, big bluestem and indiangrass in CenUSA plots near Mead, NE in 2013, 2014 and 2015.





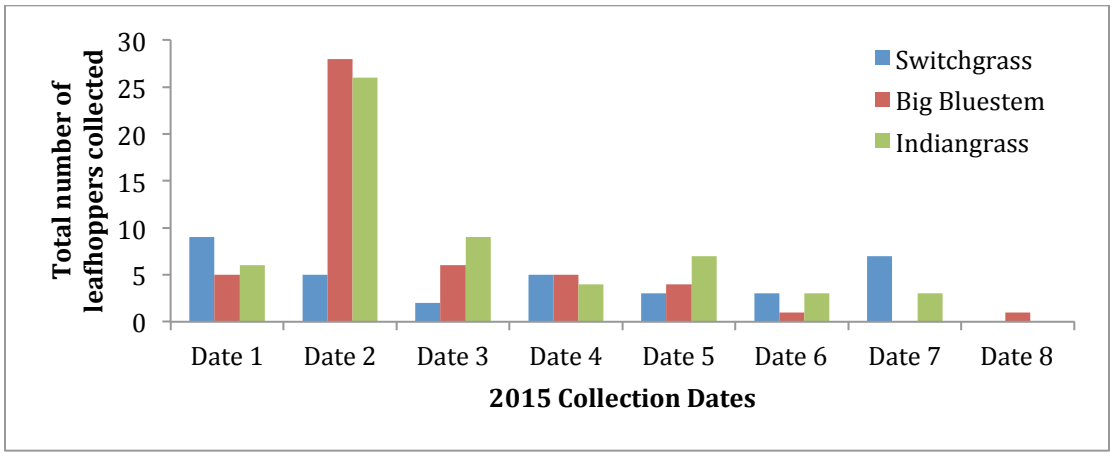
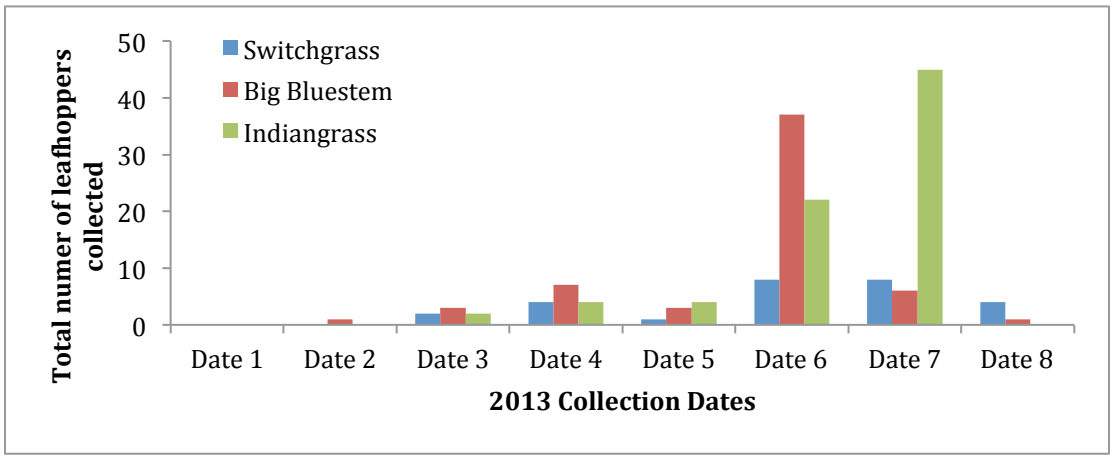
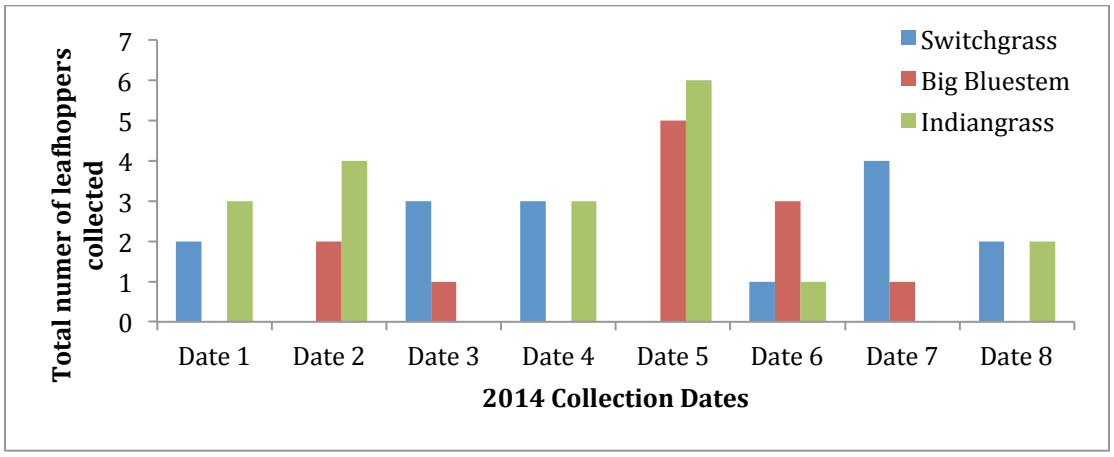


Figure 2.11 - Total number of leafhoppers (Cicadellidae) collected on sticky traps in switchgrass, big bluestem and indiangrass in CenUSA plots near Arlington, WI in 2013, 2014 and 2015.





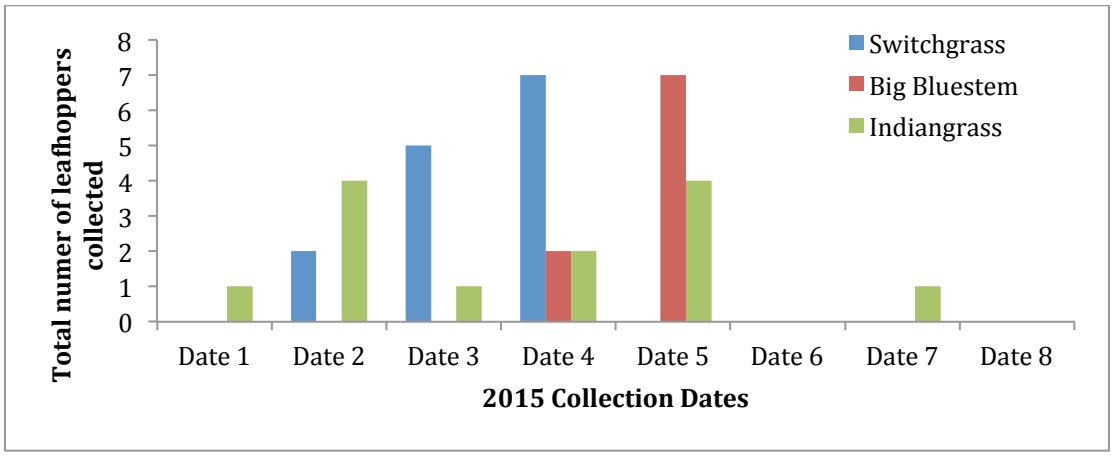
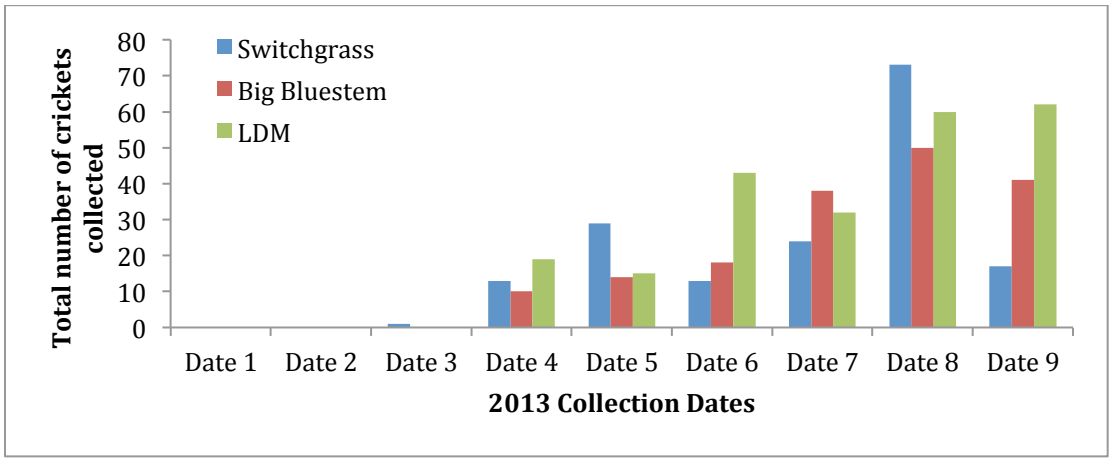
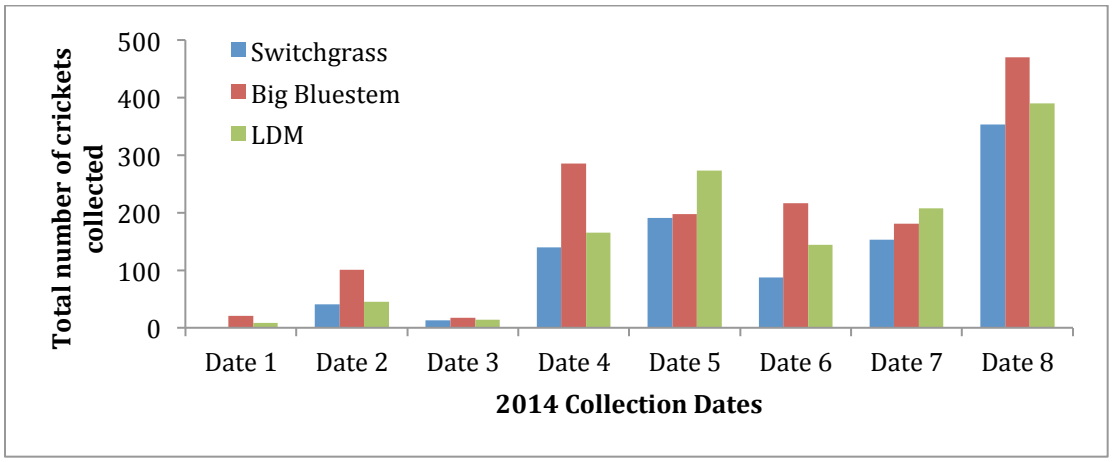


Figure 2.12 - Total number of crickets (Gryllidae) collected in pitfall traps in three warm-season grass stands near Mead, NE in 2013, 2014 and 2015.





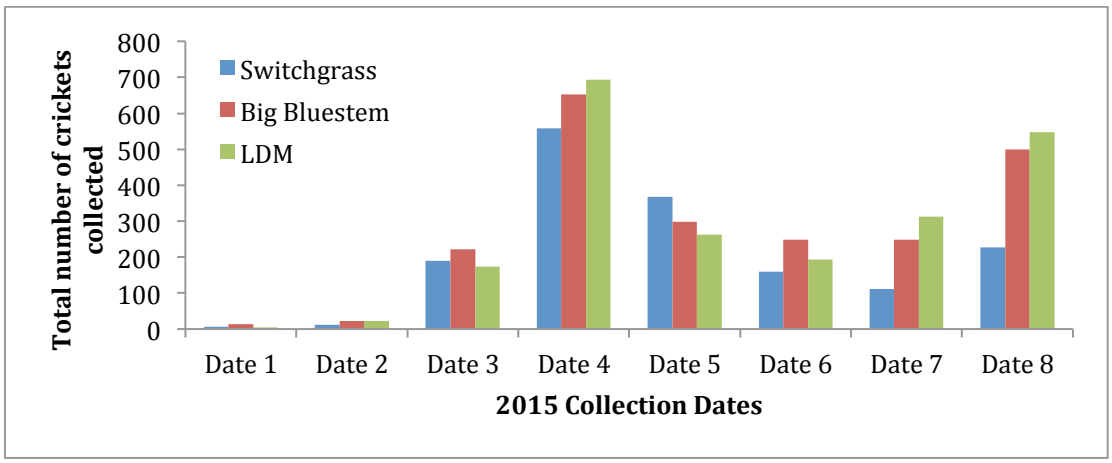


Figure 2.13 - Total number of crickets (Gryllidae) collected in pitfall traps in switchgrass, big bluestem and indiangrass in CenUSA plots near Mead, NE in 2013, 2014 and 2015.

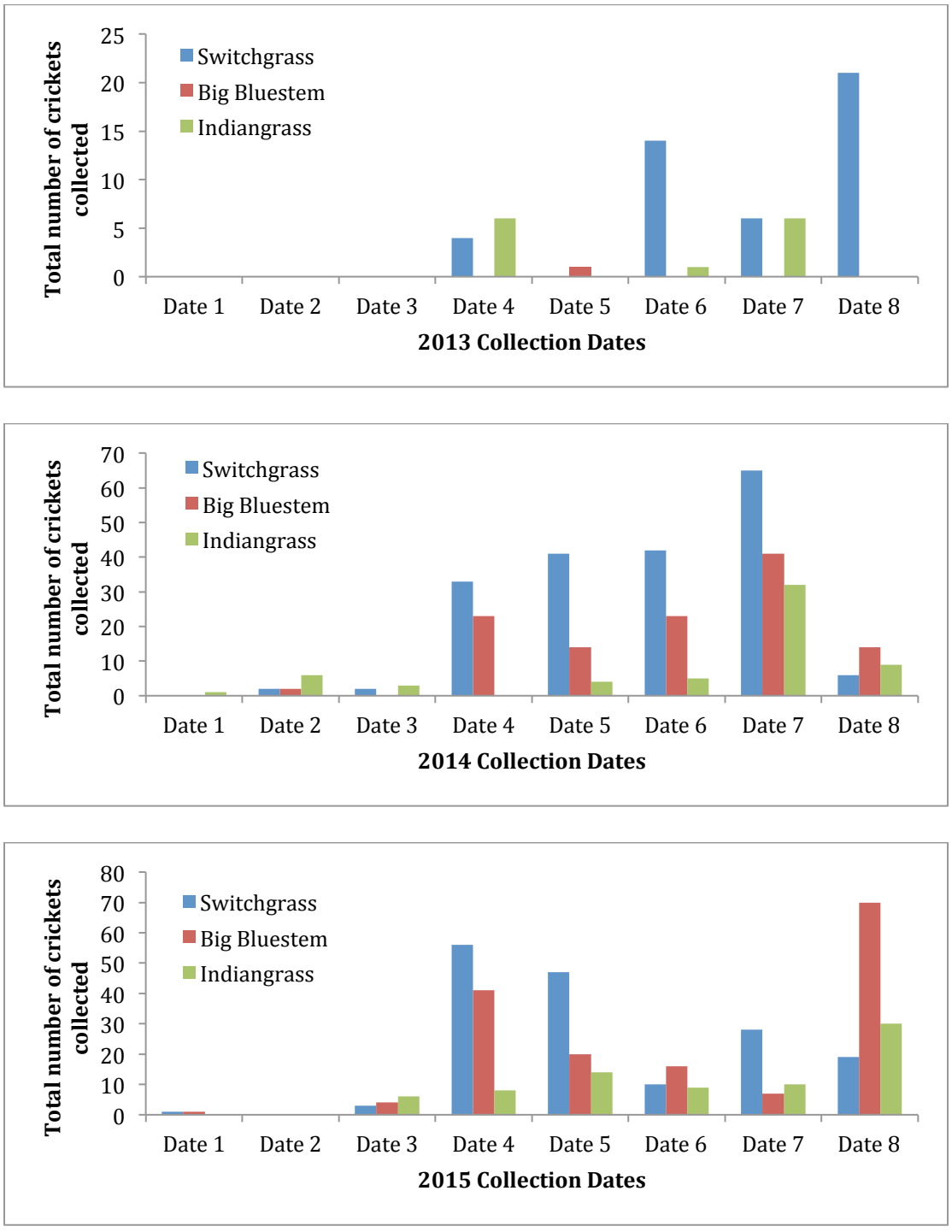


Figure 2.14 - Total number of crickets (Gryllidae) collected in pitfall traps in switchgrass, big bluestem and indiangrass in CenUSA plots near Arlington, WI in 2013, 2014 and 2015.

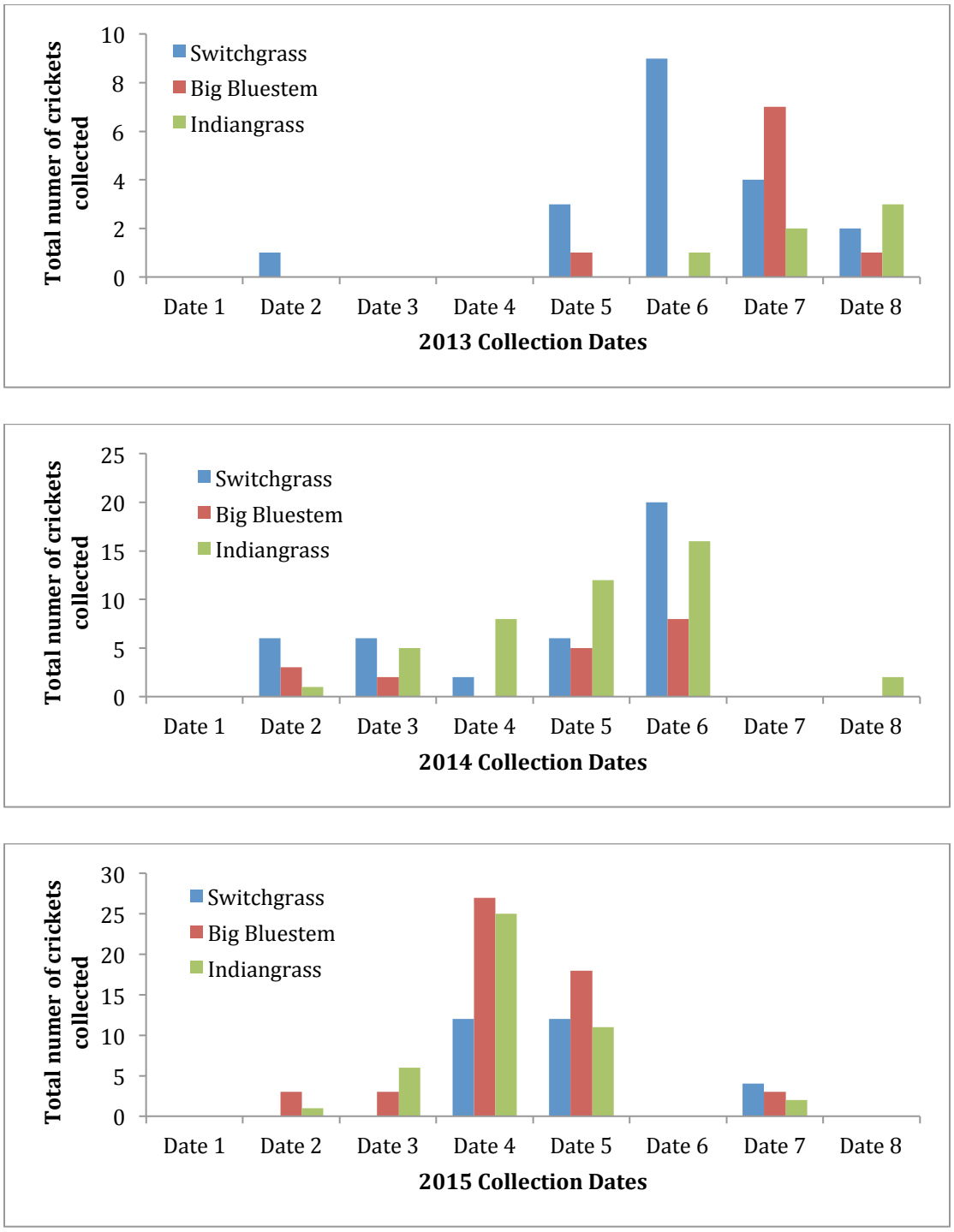
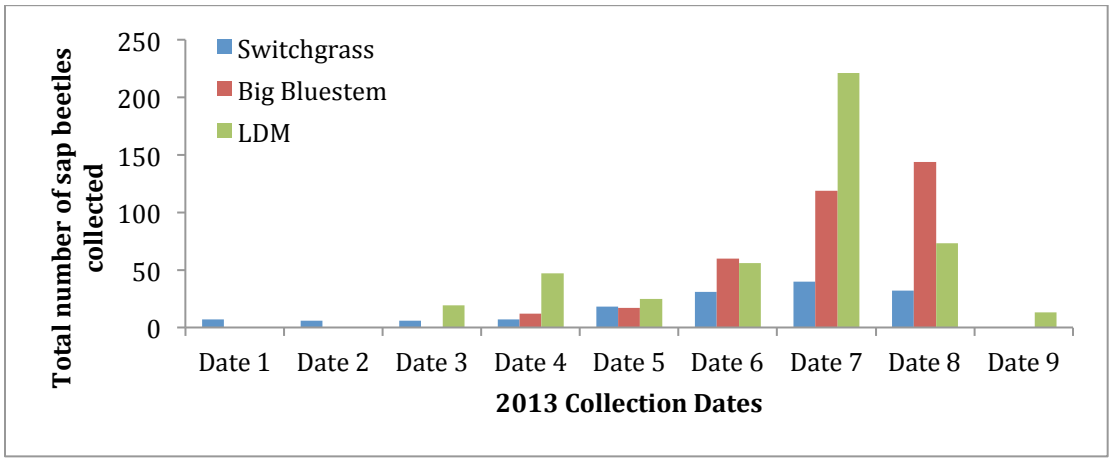


Figure 2.15 - Total number of sap beetles (Nitidulidae) collected in pitfall traps in three warm-season grass stands near Mead, NE in 2013, 2014 and 2015.



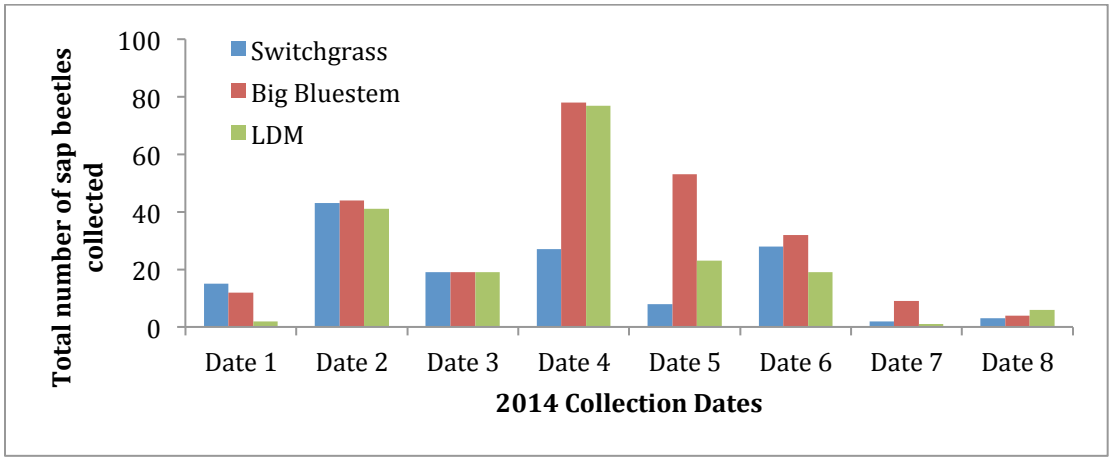


Figure 2.16 - Total number of sap beetles (Nitidulidae) collected in pitfall traps in switchgrass, big bluestem and indiangrass in CenUSA plots near Mead, NE in 2013, 2014 and 2015.

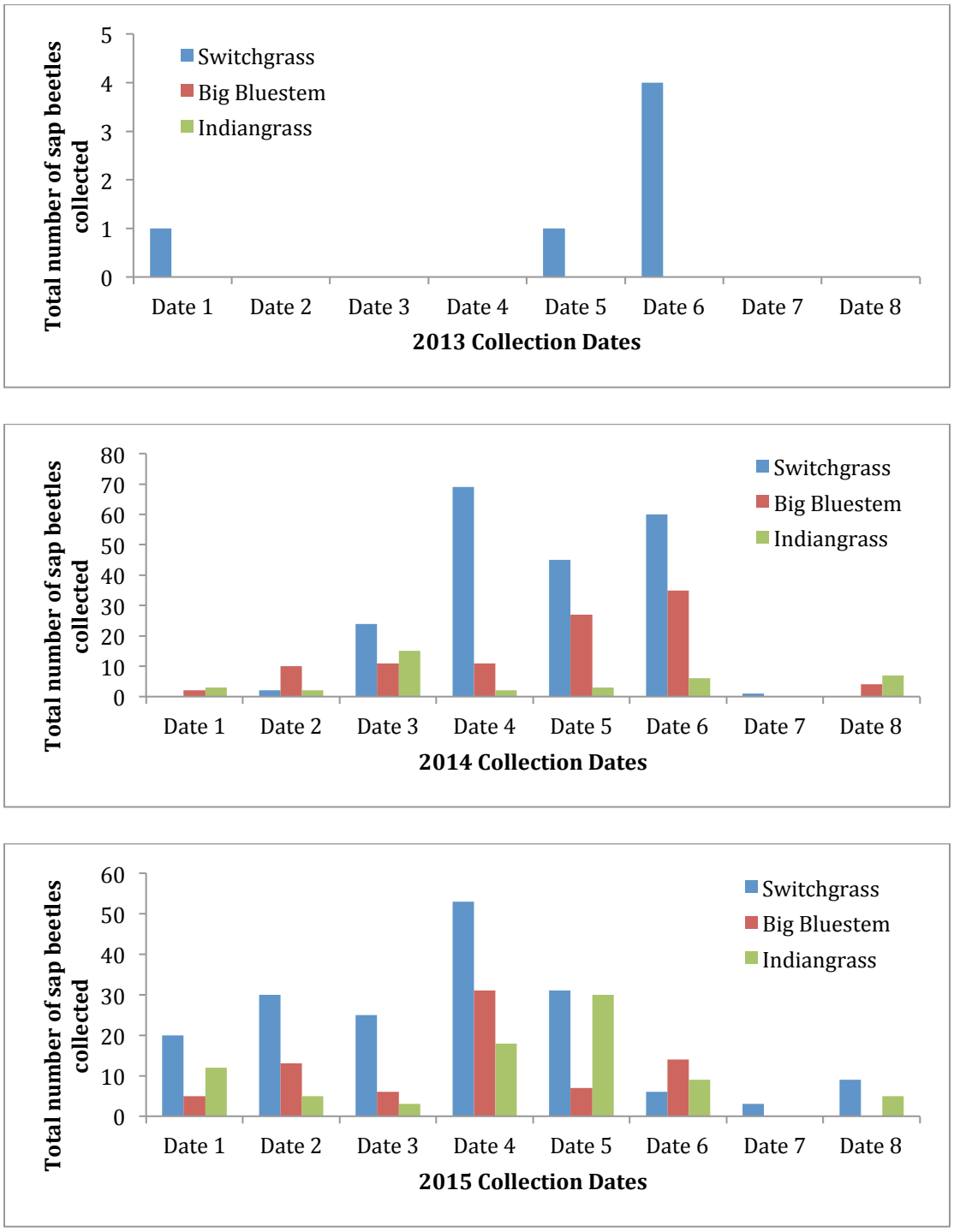


Figure 2.17 - Total number of sap beetles (Nitidulidae) collected in pitfall traps in switchgrass, big bluestem and indiangrass in CenUSA plots near Arlington, WI in 2013, 2014 and 2015.

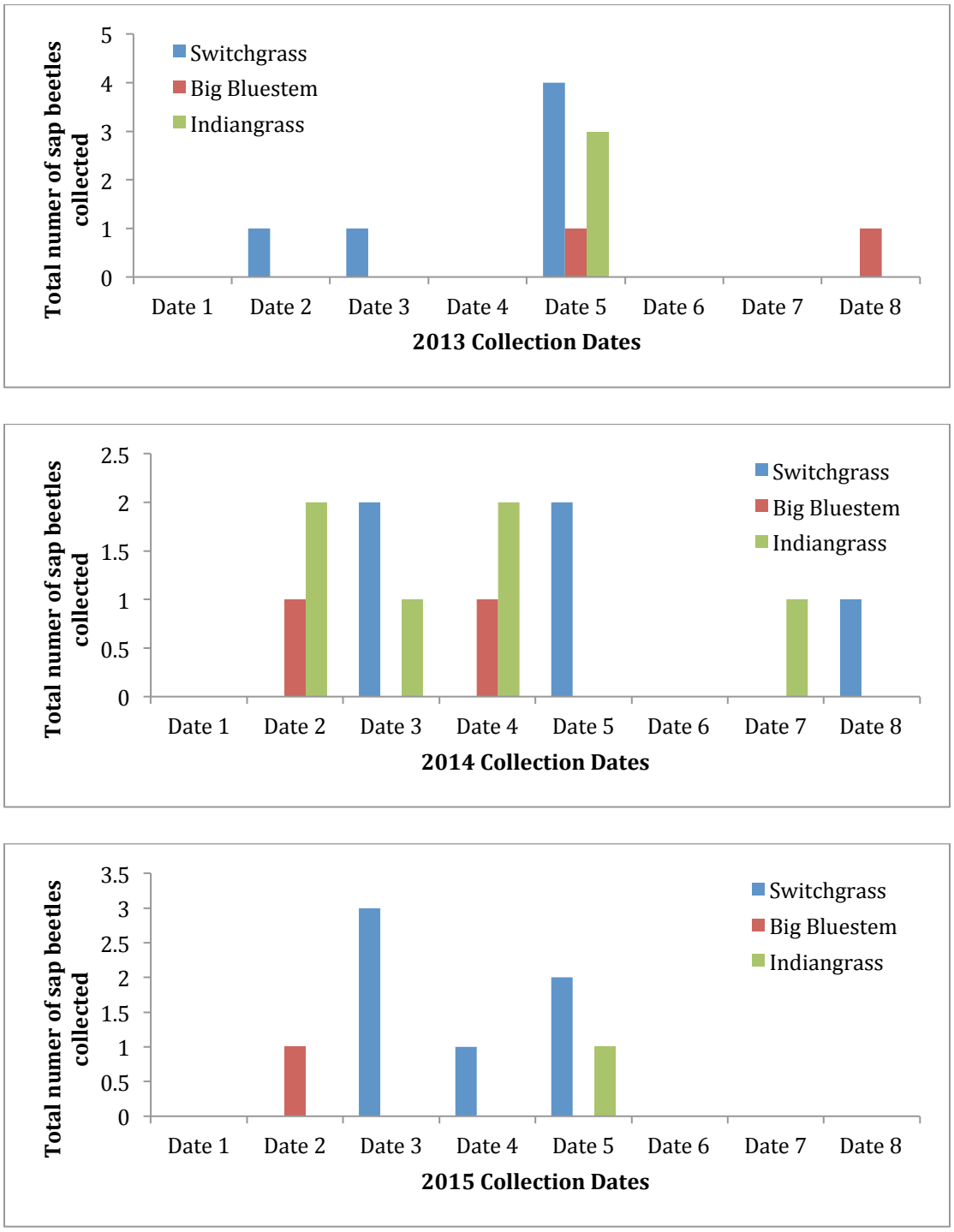
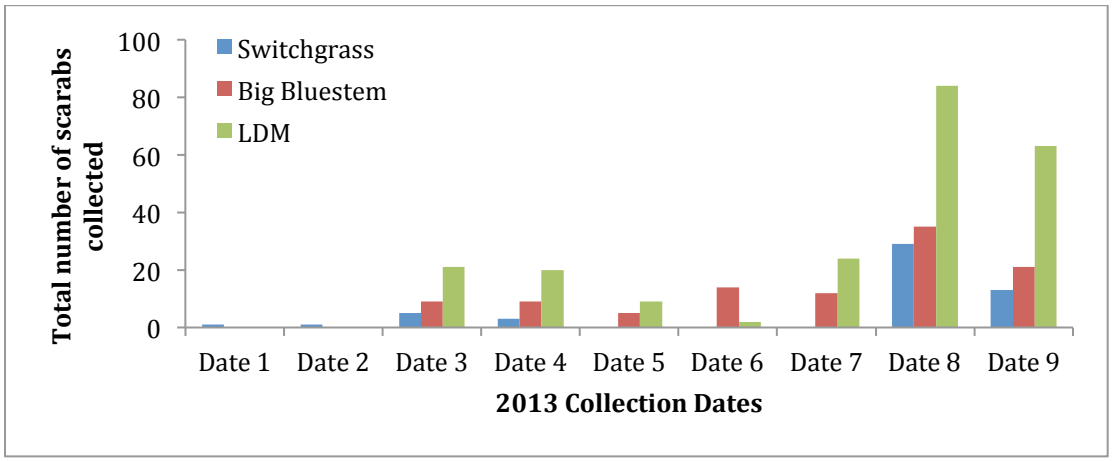
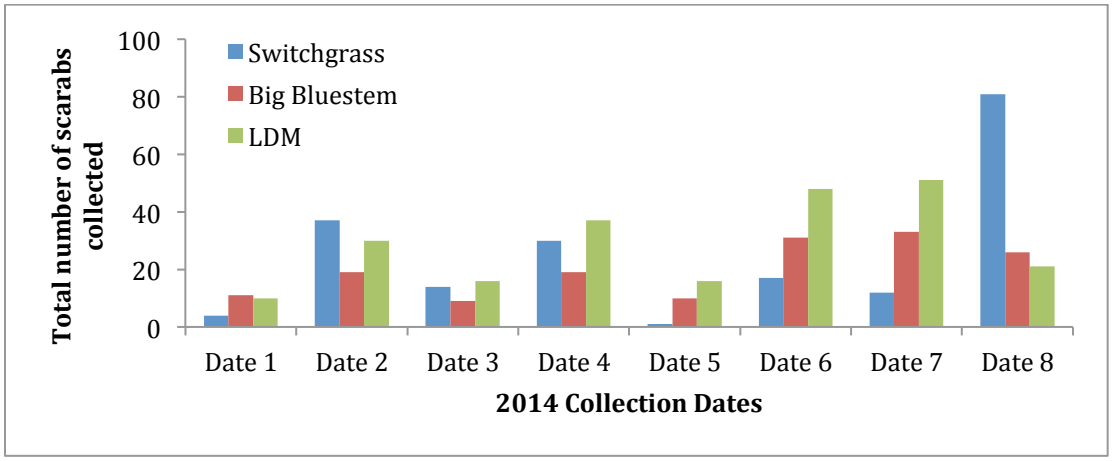


Figure 2.18 - Total number of scarabs (Scarabaeidae) collected in pitfall traps in three warm-season grass stands near Mead, NE in 2013, 2014 and 2015.





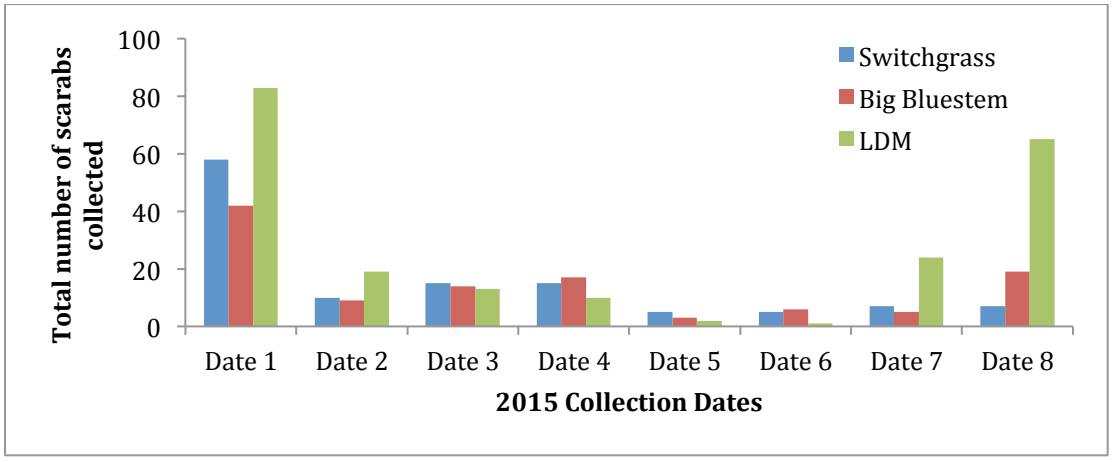
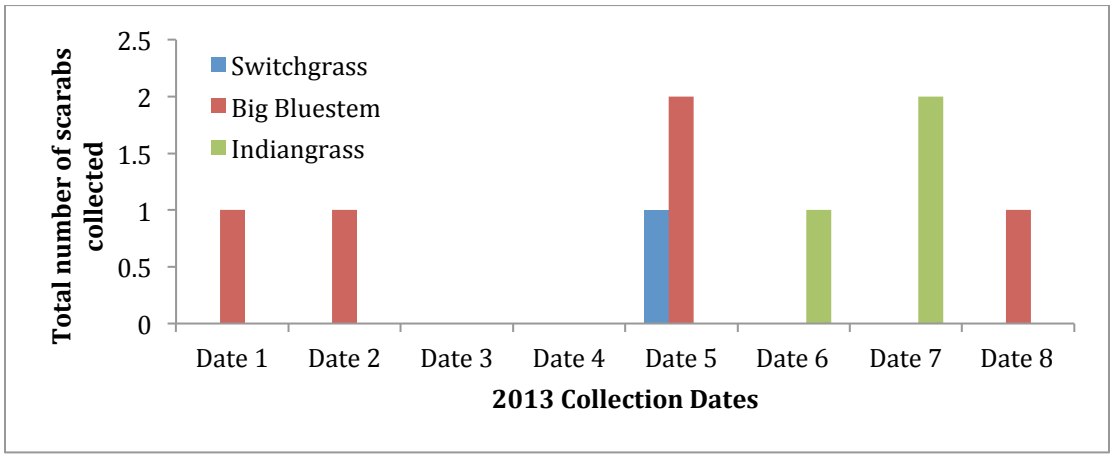
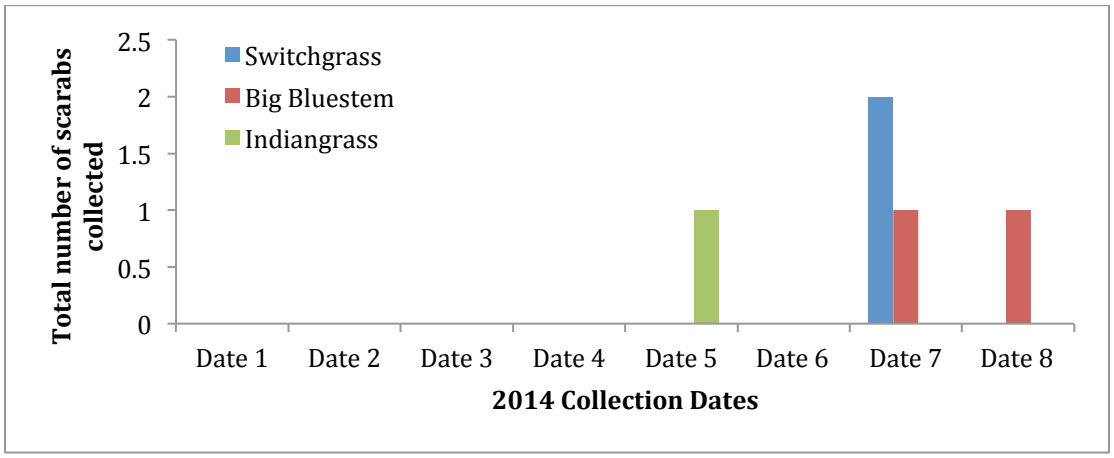


Figure 2.19 - Total number of scarabs (Scarabaeidae) collected in pitfall traps in switchgrass, big bluestem and indiangrass in CenUSA plots near Mead, NE in 2013, 2014 and 2015.





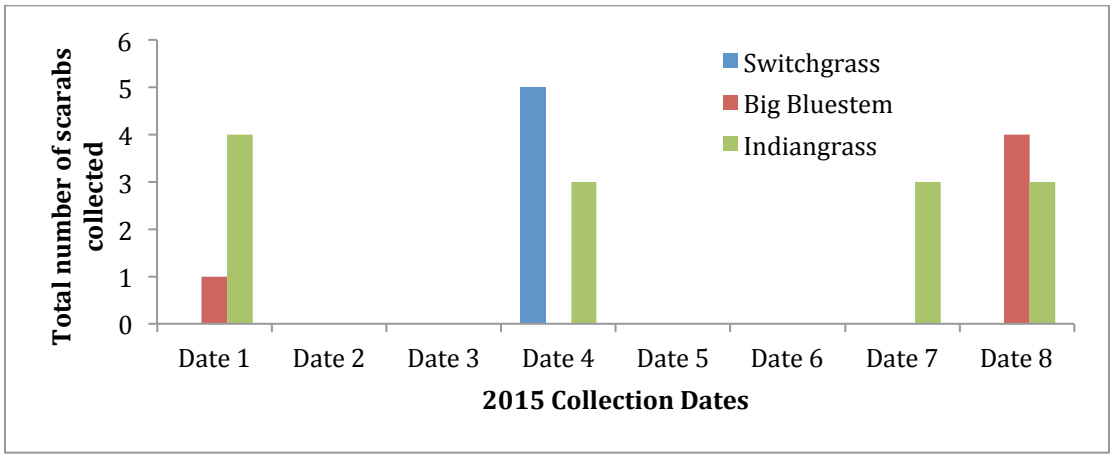


Figure 2.20 - Total number of scarabs (Scarabaeidae) collected in pitfall traps in switchgrass, big bluestem and indiangrass in CenUSA plots near Arlington, WI in 2013, 2014 and 2015.

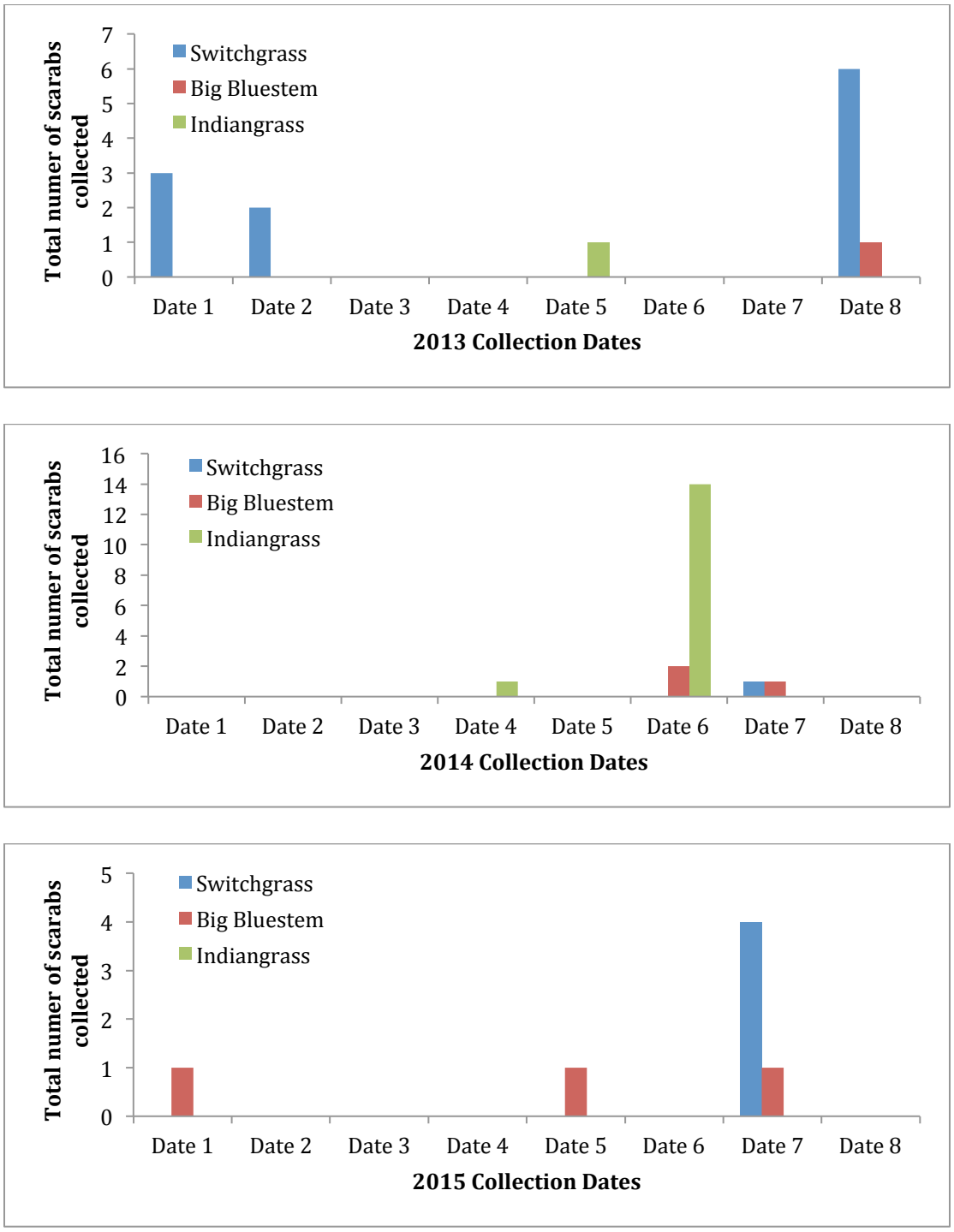
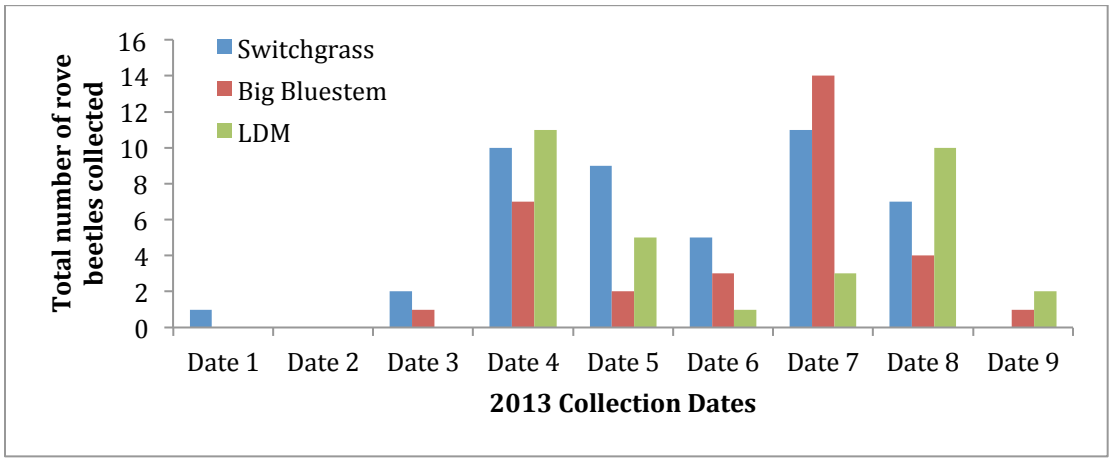
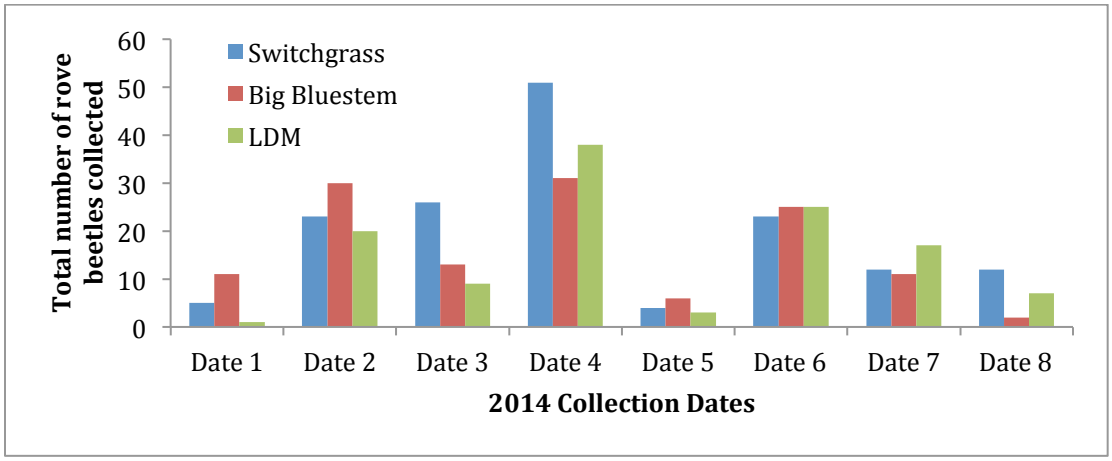


Figure 2.21 - Total number of rove beetles (Staphylinidae) collected in pitfall traps in three warm-season grass stands near Mead, NE in 2013, 2014 and 2015.





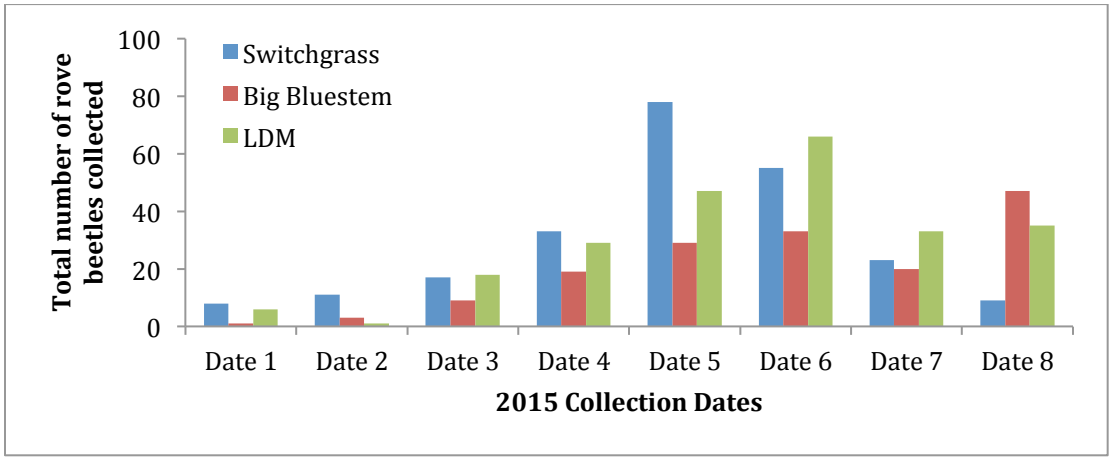


Figure 2.22 - Total number of rove beetles (Staphylinidae) collected in pitfall traps in switchgrass, big bluestem and indiangrass in CenUSA plots near Mead, NE in 2013, 2014 and 2015.

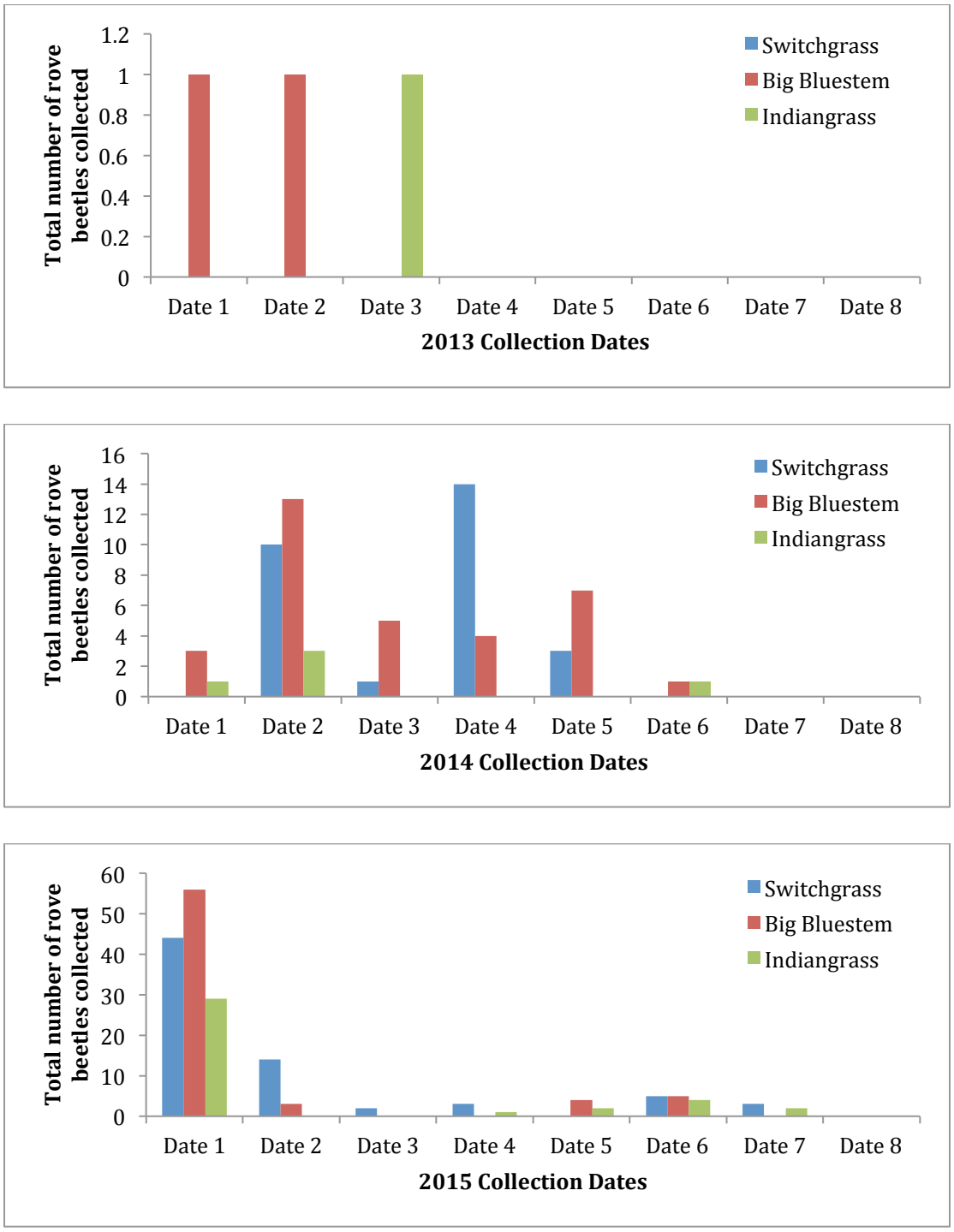


Figure 2.23- Total number of rove beetles (Staphylinidae) collected in pitfall traps in switchgrass, big bluestem and indiangrass in CenUSA plots near Arlington, WI in 2013, 2014 and 2015.

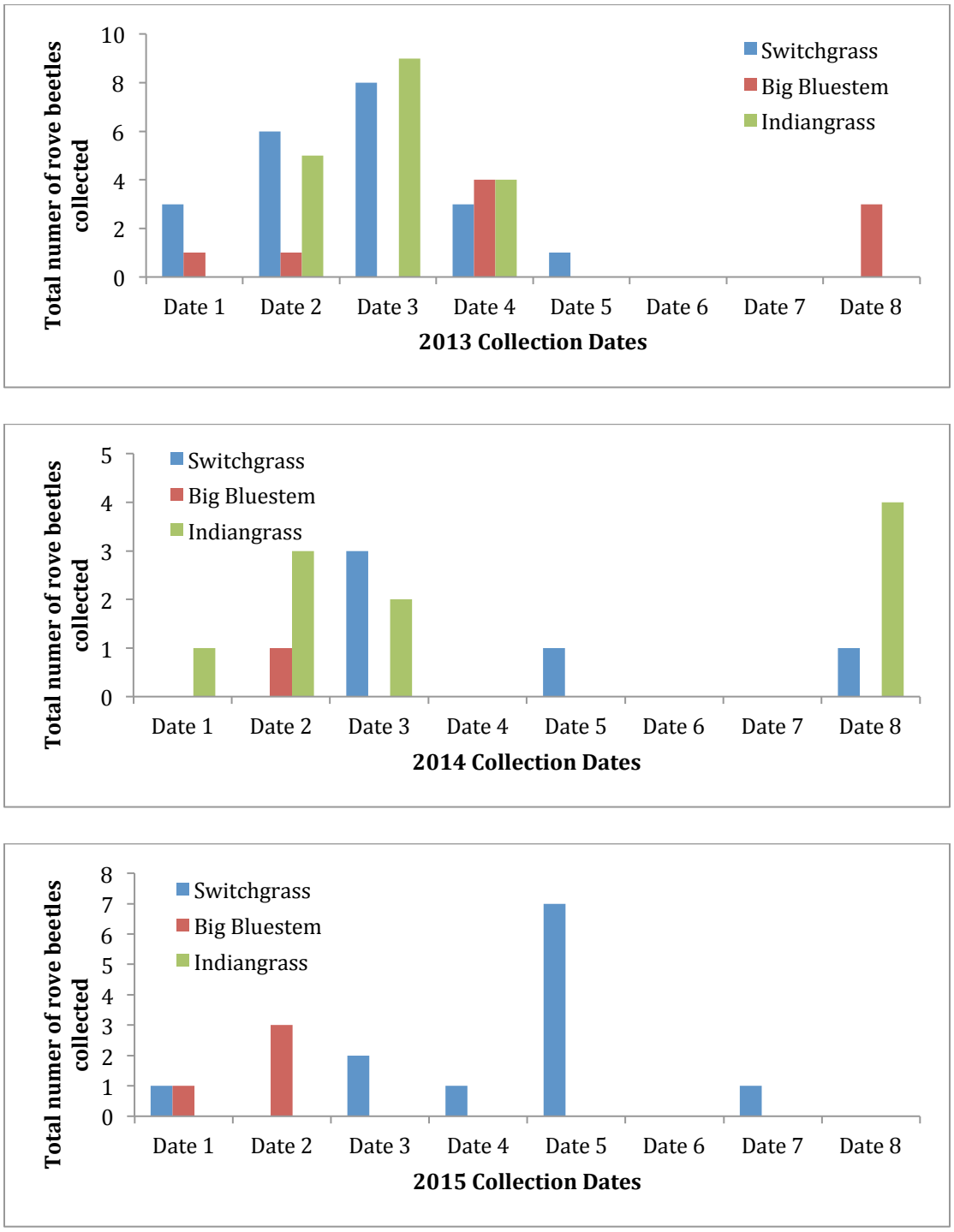


Figure 2.24 - Total number of thrips (Thripidae) collected in sticky traps in three warm-season grass stands near Mead, NE in 2013, 2014 and 2015.

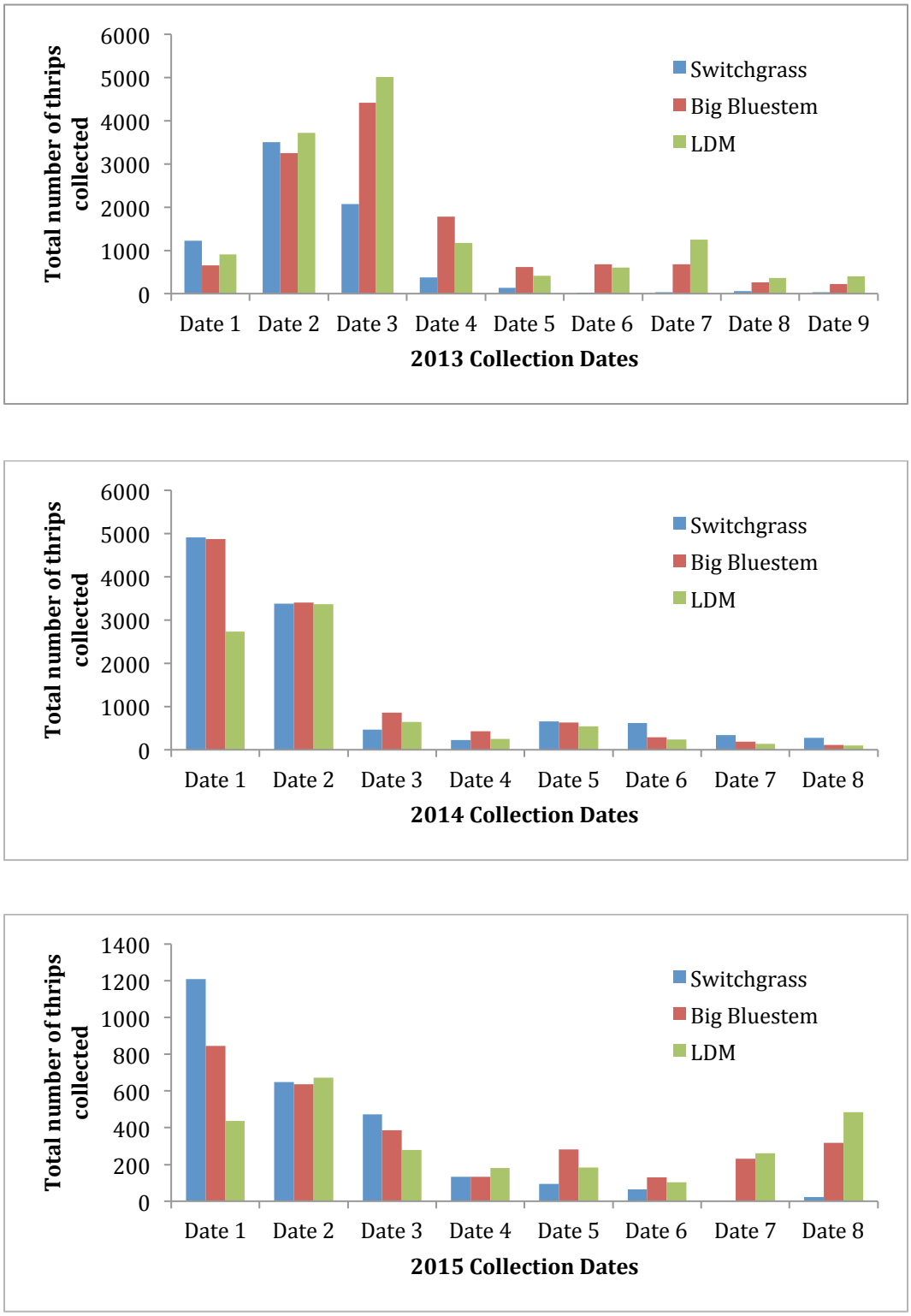


Figure 2.25 - Total number of thrips (Thripidae) collected in sticky traps in switchgrass, big bluestem and indiangrass in CenUSA plots near Mead, NE in 2013, 2014 and 2015.

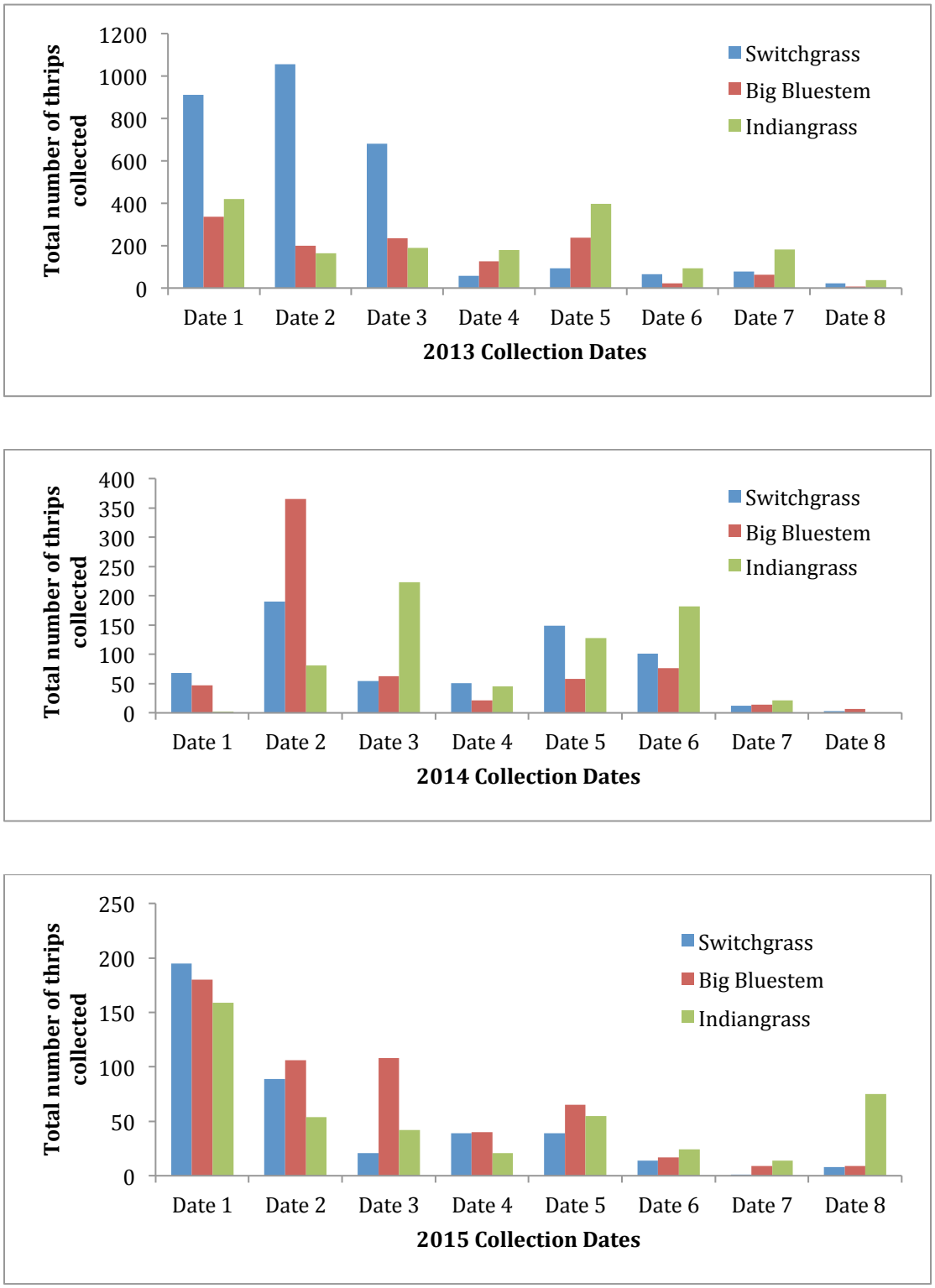
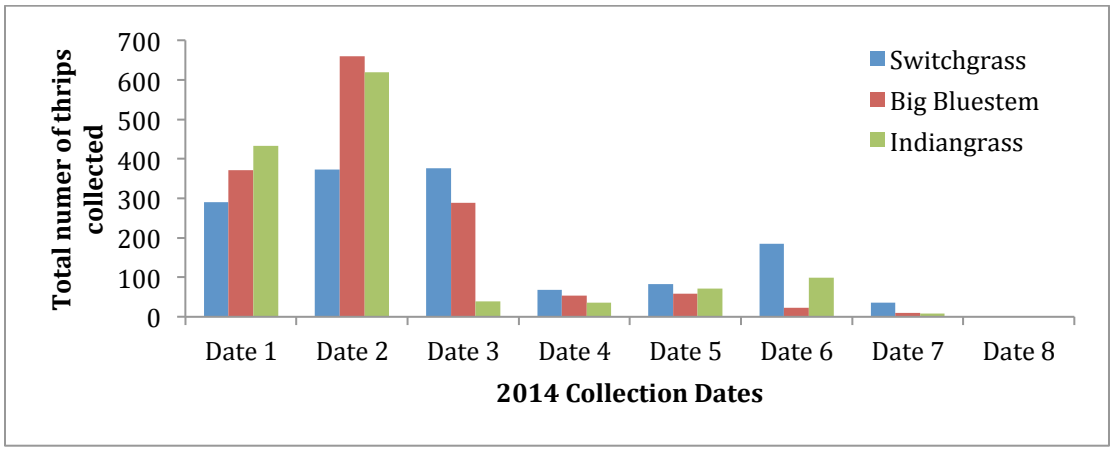
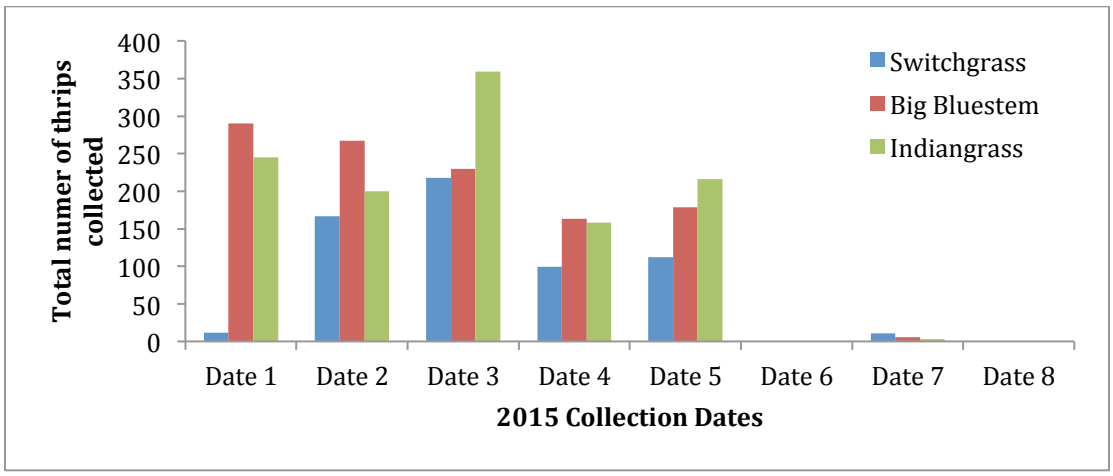


Figure 2.26 - Total number of thrips (Thripidae) collected in sticky traps in switchgrass, big bluestem and indiangrass in CenUSA plots near Arlington, WI in 2013, 2014 and 2015.





CHAPTER 3. CHARACTERIZATION OF GREENBUG FEEDING BEHAVIOR AND APHID (HEMIPTERA: APHIDIDAE) HOST PERFERENCE IN RELATIONSHIP TO WARM-SEASON GRASSES

Introduction

Switchgrass, *Panicium virgatium* L., along with big bluestem, *Andropogon gerardii* Vitman, and indiangrass, *Sorghastrum nutans* (L.) Nash, compose a majority of the grasses found in North American tallgrass prairies (Bouton 2008). Currently all three grasses are used for livestock forage, erosion control, and in conservation reserve programs (CRP) for grassland conservation (Sanderson et al. 2004, Vogel 2004, Wennerbery 2004, Owsley 2011). Switchgrass, along with big bluestem and indiangrass, have recently been selected as potential candidates for herbaceous bioenergy crops, for a wide variety of reasons including: high levels of production across diverse landscapes; suitability for marginal and erosive land; low water and nutrient requirements; positive environmental benefits; and compatibility with modern farming practices (Sanderson et al. 1996, McLaughlin et al. 1999, Sanderson et al. 2004, Bouton 2008). Nevertheless, further examination of the potential arthropod pests needs to be completed for these grasses to develop into their full potential as bioenergy crops.

Through both indirect and direct factors, insects contribute considerably to crop losses worldwide. Non-insecticidal pest management strategies, such as plant resistance, have gained interest as a possible mechanism for controlling potential insect pests (Smith 2005). Koch et al. (2014b) revealed resistance in four tetraploid switchgrass populations to two potential aphid pest species, *Schizaphis graminum* (Rondani) and *Sipha flava* (Forbes). Aphids are significant pests of crops and cause damage to the plants by removing photo assimilates and vectoring numerous plant pathogens (Smith and Boyko 2007). Aphids will feed on the plant by inserting their piercing-sucking mouthpart stylets into the plant tissue in search of the phloem sieve element (Prado and Tjallingii 1994,

Tjallingii 2006, Smith and Boyko 2007). This stylet penetration by aphids can play a significant role in host plant acceptance or rejection (Tjallingii 1994, Prado and Tjallingii 1997, Diaz-Montano et al. 2007). Aphid stylet penetration can be monitored by an electrical method called electrical penetration graph (EPG) technique (Tjallingii 1978). This technique first used an alternating current (AC) developed by McLean and Kinsey (1964). Several years later Tjallingii (1978) established a direct current (DC) based monitor. This technique allows for the monitoring of an aphid's probing activities by producing signal waveforms that correspond to specific probing behaviors and location of the insect's stylet tips within the plant tissue (Tjallingii 2006). Monitoring aphid stylet placement within the plant tissue is a useful tool in helping to determine possible resistance mechanisms at the plant tissue level (Van Helden and Tjallingii 2000, Jiang and Walker 2001, Crompton and Ode 2010).

Schizaphis graminum and *S. flava* have been documented colonizing over 50 graminaceous hosts for each species (Michels Jr. 1986, Kindler and Dalrymple 1999). Previous work has also documented switchgrass as a potential host for *S. graminum* and *S. flava* (Kindler and Dalrymple 1999, Burd et al. 2012, Koch et al. 2014a, Prochaska 2015), and demonstrated varying levels of tolerance and antibiosis in three select switchgrass populations to *S. graminum* and *S. flava* (Koch et al. 2014b). The feeding behavior of *S. graminum* feeding on wheat, *Triticum aestivum* L., sorghum, *Sorghum bicolor* (L.) Moench, and switchgrass was previously documented using the EPG technique (Campbell et al. 1982, Montllor et al. 1983, Pereira et al. 2010, Koch et al. 2014c).

The EPG technique has been used to study the feeding behavior of aphids on a wide variety of host plants; however, no research to date has documented the feeding behavior of aphids on big bluestem or indiangrass. Similarly, there have been no reports on the potential presence of antixenosis in big bluestem or indiangrass to aphids. Due to this absence of knowledge, the objective of this research was to determine host preference of *S. graminum* and *S. flava*, and to analyze the feeding behavior of *S. graminum* on select switchgrass, big bluestem and indiangrass populations.

Materials and Methods

Plant Material. Screening studies consisted of 17 switchgrass populations, 4 big bluestem populations, and 10 indiangrass populations. All warm-season grass populations selected for the screening study were part of a multi-state comprehensive biomass yield test study (Table 3.1). Choice studies and EPG recordings consisted of the following three susceptible plant populations selected from screening studies: switchgrass (Summer), big bluestem experimental strain (MW5A C1), and indiangrass experimental strain (SN HZ 4 C1).

Insects. *Schizaphis graminum* (biotype I) and *S. flava* were used for screening, host preference, and EPG studies. Colonies for both aphids were initially obtained from Dr. John D. Burd, USDA-ARS in Stillwater, Oklahoma. The *S. graminum* colony was maintained on a susceptible sorghum cultivar 'BCK60', in a plant growth chamber at 25 ± 2°C with a photoperiod of 16:8 (L:D) h. *Sipha flava* could not successfully be kept in a growth chamber; therefore, this colony was maintained in the greenhouse at 25 ± 7°C and 16:8 (L:D) h, on the same sorghum cultivar 'BCK60', within a wooden-frame cage ventilated with organdy fabric (63.5 cm length x 40.6 cm width x 83.8 cm height).

Screening Studies. A series of screening studies were performed to determine host suitability of *S. flava* to 17 switchgrass populations, 4 big bluestem populations, and 10 indiangrass populations at the development stage of V2 (2^{nd} leaf stage) as described by Moore et al. (1991) (Moore et al. 1991) (Table 3.1). The switchgrasses Kanlow (resistant) and Summer (susceptible) were included as controls (Koch et al. 2014b, Prochaska 2015). The experimental design for each study was completely randomized with 10

replicates per population. Plants were grown in SC-10 Super Cell Single Cell Cone-tainers (3.8 cm diameter by 21 cm deep) (Stuewe & Sons, Inc., Corvallis, OR) containing a Fafard Growing Media (Mix No. 3B) (Conrad Fafard, Awawam, MA). Cone-tainers were placed in 7 by 14 con-tainer trays and maintained in a greenhouse at 25 ± 7°C with supplemented LED lights (Pro 325, Lumigrow, Novato, CA) to produce a photoperiod of 16:8 (L:D) h. After emergence plants were thinned to one plant per cone-tainer. Plants were fertilized every two weeks with a soluble (20:10:20 N-P-K) fertilizer. Ten apterous, adult aphids were transferred to each plant with a fine paintbrush, and then caged by tubular plastic cages (4 cm diameter by 46 cm height) covered with organdy fabric to confine the aphids. After aphid introduction, plants were maintained in a greenhouse at 25 ± 7°C and 16:8 (L:D) h. Plants were evaluated every seven days post infestation by counting the total number of aphids and performing a visual damage rating on a 1-5 scale. Plant damage ratings served as a visual assessment of the injury sustained by aphid feeding (Smith et al. 1994). The damage rating scale was adopted from Heng-Moss et al. (2002), Koch et al. (2014a), and Koch et al. (2014b) where 1 = 10% or less of the leaf area damaged; 2 = 11-30 % of the leaf area damaged; 3 = 31-50% of the leaf area damaged; 4 = 51-70% of the leaf tissue damaged; 5 = 71% or more of the leaf area damaged and the plant near death. Plant damage was characterized by chlorosis, a reddening discoloration, or desiccation of the leaf. Experiments were terminated when the average damage rating reached 3 for a given population or when aphid numbers plateaued across all populations of switchgrass, big bluestem and indiangrass. Susceptible and tolerant plants were determined based on total aphid number on the plant and damage ratings of the plants. To further characterize damage ratings, populations were grouped

into one of four levels of resistance: highly susceptible (HS, mean damage ratings ≥ 4); moderately susceptible (MS, mean damage ratings ≥ 3 but < 4); moderately resistant (MR, mean damage ratings ≥ 2 but <3); and highly resistant (HR, mean damage ratings <2) (Heng-Moss et al. 2002, Pierson et al. 2010, Koch et al. 2014a).

Non-Preference Studies. Choice studies were performed for both *S. graminum* and *S. flava* to assess aphid preference among the three susceptible populations of switchgrass, big bluestem and indiangrass selected from the screening study. Plants were grown in plastic nursery pots (9 cm in diameter by 9 cm in depth) containing a Fafard Growing Media (Mix No. 3B) (Conrad Fafard, Awawam, MA). One seed of each susceptible population of switchgrass (Summer), big bluestem experimental strain (MW5A C1), and indiangrass experimental strain (SN HZ 4 C1) was planted near the perimeter of the pot. Within a pot, seeds for each population were equally spaced from each other and from the center of the pot (5.2 cm between grasses and 3 cm from center), and randomly oriented with relation to each other. Plants were maintained in a greenhouse as previously described until the plants reached the V2 development stage. Plants were fertilized every two weeks with a soluble (20:10:20 N-P-K) fertilizer.

Each individual pot served as an experimental unit, where one plant of switchgrass, big bluestem and indiangrass was represented in each pot. Prior to introduction, aphids were placed in a petri dish and starved for approximately one h. Following the pre-treatment, 50 adult apterous aphids were introduced onto filter paper (1.5 cm in diameter) in the center of the arena. Pots were then arranged within a heavy-duty plastic flat (50.8 cm length x 35.6 cm width x 7.6 cm depth) filled with water to prevent aphids from moving among pots. The number of aphids was visually documented

on each treatment (e.g., switchgrass, big bluestem, and indiangrass) at 1, 2, 4, 8, 24, 48, and 72 h after aphid introduction. Experiments were conducted in a controlled laboratory setting at 25 ± 5°C with continuous light. The experimental design was a randomized complete block with 10 replications per choice study.

Statistical analysis. Choice studies were analyzed as a repeated measures design using generalized linear mixed model analyses (PROC GLIMMIX) and a Poisson distribution to identify differences in aphid preference among the three susceptible grass populations (SAS/STAT 2013), with the pot arena set-up being the repeated measure. Where appropriate, means were separated using Fisher's least significant difference (LSD) procedure (α=0.05).

EPG Recordings. For the feeding behavior study, plants were grown and maintained in cone-tainers as previously described for the choice studies. After emergence, plants were thinned to one plant per cone-tainer. Switchgrass, big bluestem, and indiangrass plants were grown to the V2 developmental stage for all recordings and were selected based on uniformity. Before recordings, plants were transferred from the greenhouse to the laboratory (25 ± 5°C), and allowed to acclimate for approximately 24 h.

Feeding behavior of *S. graminum* (biotype I) was evaluated using the EPG-DC system described by Tjallingii (1978). Recordings were performed using a Giga-8 EPG model (EPG Systems, Wageningen, The Netherlands) with a 10^9 Ω resistance amplifier and an adjustable voltage. Output from the EPG was digitized at a sample rate of 100 Hz (100 samples per s) per channel using a built-in data logger (DI-710, Dataq Instruments

Inc., Akron, OH) and recorded on a computer with EPG acquisition software (Stylet+EPG Systems, Wageningen, The Netherlands). Voltage was monitored for fluctuations on the computer and adjusted at ± 5 V as needed, while the gain was adjusted from 50x-100x in order to improve the quality of the recordings.

Adult, apterous *S. graminum* were placed in a petri dish and denied food 1 h prior to initiating recordings to increase the likelihood of feeding, and to allow resheathing of their stylets (Annan et al. 2000). An individual plant and insect were integrated to complete an electrical circuit using a copper electrode, stuck in the soil of the potted plant, and a gold wire (99.99%, 10 μm diameter and 2-3 cm in length) (Sigmund Cohn Corp., Mount Vernon, NY) attached to the dorsum of the aphid with silver conductive glue (4 mL water with one drop of Triton X-100, 4 g water soluble glue (Scotch clear paper glue, non-toxic; 3M, St. Paul, MN), 4 g silver flake [: 99.95%, size: 8-10 μm, Inframat Advanced Materials, Manchester, CT]). The opposite end of the gold wire was attached to a 24-gauge copper wire (≈ 2 cm length), which was soldered to a copper nail (1.6 mm x 19.0 mm). After the aphids were fixed to the gold wire, the electrode was inserted into the EPG probe. The EPG probe was an amplifier with a one-giga-ohm input resistance and 50x gain (Tjallingii 1985, 1988). At the completion of the starvation period, wired aphids were placed on the adaxial side of the newest, fully developed leaf. Aphid placement was considered successful if the aphid was able to move freely on the surface of the leaf. All plants, EPG probes, and plant electrodes were placed inside one of two Faraday cages, constructed from aluminum mesh wire with an aluminum frame and base (61 cm x 61 cm x 76 cm), in order to protect the EPG's internal conductors from electrical and environmental noise (Crompton and Ode 2010). Recordings were made on

eight plants simultaneously, with at least one plant of each of the susceptible switchgrass, big bluestem, and indiangrass populations represented in each recording. The feeding behavior of *S. graminum* was recorded for 15 h with 20 replications per grass population. Recordings began mid-afternoon and were maintained under continuous fluorescent light.

Feeding behavior parameters and experimental design. EPG procedures were followed according to Van Helden and Tjallingii (2000), while EPG waveforms were differentiated and categorized according to Reese et al. (2000). The waveforms were grouped into three main behavioral phases: pathway phase, xylem, and phloem (sieve element phase) (Prado and Tjallingii 1994, Reese et al. 2000, Tjallingii 2006). The pathway phase (waveforms A, B, and C) is characterized by intercellular stylet penetration and withdrawal, periods of no stylet movement, and brief intracellular punctures by stylet tips, also known as potential drops (waveform pd) (Prado and Tjallingii 1994, Jiang and Walker 2001). For simplification, differences between waveforms A, B, and C were not defined in the study and the three waveforms were generically labeled as waveform C (Garzo et al. 2002, Alverez et al. 2006). Waveforms F (stylet penetration difficulties) were not always common in recordings and were included in pathway phase whenever they were observed (Diaz-Montano et al. 2007). The xylem phase (waveform G) occurs when the stylet tips are in the xylem tissue and is characterized by the aphid drinking from the xylem elements (Spiller et al. 1990, Tjallingii 1990). The xylem often provides fewer nutrients than the phloem sap and more commonly occurs in a water-deprived aphid (Spiller et al. 1990, Powell and Hardie 2002). The sieve element involves salivation secretions (waveforms E1) and ingestion of phloem sap (waveforms E2). Waveforms E1 and E2 can be difficult to distinguish from

one another, thus the waveforms were combined and labeled generally as waveform E to depict general penetration activities of *S. graminum* in phloem tissues (Annan et al. 1997, Annan et al. 2000).

EPG feeding behavior parameters were selected from the Sarria Excel Notebook (Sarria et al. 2009). The calculated parameters included the mean time from start of recording to first probe (elapsed time of placement of aphid on the plant to insertion of mouthparts) and first sieve element phases; time from first aphid probe to first sieve element phase; total number of potential drops, pathway phases (n-PP), sieve element phases, xylem phases, non-probing events, and probes after first sieve element phases; sum of duration of pathway phases, sieve element phases, xylem phases, non-probing events, first probe, and first sieve element phase; potential phloem ingestion index (PPII) and percent of aphis with sustained phloem ingestion (E>10 min).

Statistical analysis. EPG files were annotated by waveform and the duration of each was calculated in a Microsoft Excel Workbook. Data were combined, separated by switchgrass, big bluestem, indiangrass populations, aphid number (replication), and converted to comma-separated values (CSV). The combined data were checked for errors using a beta-program designed for SAS software (SAS/STAT 2013). Once errors in waveform labeling were corrected, the data were tested for significance using an analysis of variance (ANOVA) generalized linear mixed models (PROC GLIMMIX). When appropriate, means were separated using Fischer's least significant difference (LSD) test (α=0.05). Normality was assessed for all parameters using graphical analysis of the residuals and a Shapiro-Wilk test (Shapiro and Francia 1972). Data that did not fit a normal distribution was fit to a lognormal or gamma distribution.

Results

Screening Studies. The populations were grouped into one of four levels of resistance based on damage ratings (Table 3.2). Based on these damage ratings the most susceptible plants (HS) were used for non-preference and EPG studies. The switchgrass findings were similar to those found by Koch et al. (2014a) with Summer receiving a resistance level of HS and Kanlow receiving a resistance rating of HR. The big bluestem population MW5A C1 was found to be highly susceptible (HS). All of the indiangrass populations displayed some level of susceptibility to *S. flava*, with the experimental strain SN HZ 4 C1 being the most susceptible (HS). Based on these damage ratings the most susceptible plants (HS) were selected for non-preference and EPG studies.

Non-Preference Studies. For Study 1, a significant interaction between grass type and evaluation time was detected (Figure 3.1) ($F=1.97$; $df=12,180$; $p=0.03$). Significant differences in the number of aphids were detected at 1, 2, and 4 h after *S. graminum* introduction with switchgrass having significantly more aphids than both big bluestem and indiangrass at all three time points (Estimates – 1 h: 1 big bluestem vs. switchgrass, -1.23 and indiangrass vs. switchgrass, -1.07; 2 h: big bluestem vs. switchgrass, -0.65 and indiangrass vs. switchgrass, and -0.58; 4 h: big bluestem vs. switchgrass, -0.52 and indiangrass vs. switchgrass, -0.49). The greatest *S. graminum* preference was observed at 1 h after introduction with switchgrass having two times as many aphids than indiangrass, and three times as many aphids than big bluestem (7.9 ± 1.4, 2.7 ± 0.7, and 2.3 ± 0.7, respectively). No significant differences were detected between big bluestem and indiangrass at any time point.

A significant interaction between grass type and evaluation time was again detected for Study 2 (Figure 3.2) (F=2.01; df=12,180; p=0.03). Significant differences were detected at 8, 24, 48 and 72 h after *S. flava* introduction (Estimates – 8 h: big bluestem vs. switchgrass, 0.87; 24 h: big bluestem vs. switchgrass, 1.11; 48 h: big bluestem vs. switchgrass, 2.19 and indiangrass vs. switchgrass, 0.85; 72 h: big bluestem vs. switchgrass, 2.08 and indiangrass vs. switchgrass, 0.94). At 8 and 24 h switchgrass had significantly fewer aphids than big bluestem; however, switchgrass was not significantly different from indiangrass at 8 and 24 h post aphid introduction. At 48 and 72 h switchgrass contained significantly fewer aphids than big bluestem and indiangrass. The greatest difference for *S. flava* was detected at 48 h after introduction with big bluestem having over a 9-fold higher mean aphid number (± SEM) than switchgrass (5.4 ± 1.5, 0.6 ± 0.5, respectively). Indiangrass had a mean aphid number of more than 8-fold greater than switchgrass at 48 h after *S. flava* introduction (4.9 ± 1.4, 0.6 ± 0.5, respectively).

EPG Study.

Parameters for time and duration of pattern segments. Grass populations did not have a significant influence on time to first probe (F=0.93; df=2, 35; p=0.4); however, significant differences were detected for time to first sieve element phase (F=3.44; df=2, 53; p=0.04) (Table 3.1). Indiangrass was significantly different from big bluestem for the first sieve element phase. Specifically, aphids spent more time reaching the sieve element phase for the first time compared to big bluestem. After feeding was initiated there were no significant differences for the following parameters: total duration of SE phases (F=0.41; df=2, 53; p=0.7), total duration of first probe (F=0.11; df=2, 57; p=0.9), and

duration of first sieve element phase (F=0.65; df=2, 53; p=0.53). Significant differences were also detected for time from the first probe to first sieve element phase (F=3.34; df=53,2; p=0.04) with the aphids feeding on indiangrass taking significantly more time to reach their first sieve element phase compared to aphids feeding on big bluestem. No significant differences in time from first probe to first sieve element phase were observed between switchgrass and indiangrass or big bluestem.

The total duration of pathway phases was significantly different (F=9.06; df=1, 57; p=0.004) with aphids feeding on indiangrass spending significantly more time in pathway phases than aphids feeding on switchgrass and big bluestem. Total duration of xylem phases was also significantly different (F= 3.32; df=2,52; p=0.04), specifically aphids feeding on indiangrass spent significantly less time in xylem compared to aphids feeding on big bluestem. The parameter of total duration of non-probing events was also significantly different (F=5.37; df=2, 57; p=0.007). Aphids feeding on switchgrass spent significantly more time non-probing compared to aphids feeding on big bluestem and indiangrass.

Parameters linked to stylet pathway and xylem ingestion activities. Significant differences were detected among grass populations in mean number of potential drops (F=3.50; df=2, 57; 0.04) (Table 3.2). For the mean number of pathway phases, switchgrass (172.8 ± 14.2) had significantly fewer than indiangrass (267.7 ± 19.7); however, for all other parameters there were no significant differences among the three grasses (Table 3.2).

Parameters linked to sieve element phases (Table 3.2). No significant differences in sieve element phase numbers among the three grass populations were detected ($F=1.49$; $df=2, 53$; $p=0.23$) (Table 3.2). There also was not a significant difference detected in potential phloem index (PPII) ($F=0.61$; $df=2, 53$; $p=0.55$), and in the percentage of *S. graminum* showing sustained phloem ingestion ($E > 10$ min.).

Discussion

Screening studies documented varying levels of susceptibility within the grass populations evaluated. All populations had some level of resistance, either a highly or moderately resistant, except for the indiangrass populations screened, which were only moderately to highly susceptible. Results were comparable to Koch et al. (2014b) and Prochaska et al. (2015) with switchgrass populations of Kanlow and Summer being highly resistant and highly susceptible to *S. flava*, respectively.

Choice studies for *S. graminum* showed a preference for the susceptible switchgrass (Summer) at 1, 2, and 4 h after aphid introduction, relative to big bluestem and indiangrass. However, at 4 h post aphid introduction there was a clear movement of *S. graminum* from switchgrass to big bluestem and indiangrass, with all three grass populations having similar *S. graminum* densities for the duration of the study. This may indicate that external plant cues, such as plant volatiles or the presence of pubescence (Smith 2005) do not play an obvious role in the preference of grasses for *S. graminum*, since aphids settled on all grass types equally 8 h after aphid introduction. This was also supported by the lack of significant differences in the EPG parameter for time to first probe, with the mean time (± SEM) for *S. graminum* to initiate probing on switchgrass (9.57 ± 3.1 minutes), being similar to big bluestem (16.9 ± 7.9 minutes), and indiangrass (17.2 ± 8.2 minutes).

Resistance factors in the mesophyll and epidermis may be indicated by a large number of test probes and an increased time to reach the initial sieve element phase (Alverez et al. 2006). There was a significant difference found for *S. graminum* to reach

the first sieve element phase, with aphids probing on big bluestem reaching the sieve elements significantly faster (249.6 ± 40.8 minutes) than aphids feeding on indiangrass (388.4 ± 45.3 minutes). Another parameter that is also useful for determining plant resistance is the time from first probe to first sieve element phase. This parameter corrects for any differences in the time for the aphid to reach the initial sieve element phase due to postpone probing likely caused by an epidermal factor. Significant differences were noted for mean time of *S. graminum* to reach the first sieve element phase following the first probe. Aphids feeding on big bluestem took significantly less time (232.8 ± 40.3 minutes) compared to indiangrass (370.3 ± 42.6 minutes) to reach the first sieve element phase following the first probe. Finally, there were no significant differences among grass populations for the mean number of pathway phases. However, there was a significant difference with the total duration of pathway phases, with *S. graminum* feeding on indiangrass spending significantly more time (619.0 ± 31.5 minutes) in pathway compared to switchgrass (492.4 ± 30.6 minutes). Spending significantly more time in pathway suggests potential phloem resistance in indiangrass. Similar results were found by Chen et al. (1997) when examining the feeding behavior of the melon aphid, *Aphis gossypii* Glover, on isogenic lines of melon (*Cucumis melo* L.). *Aphis gossypii* spent significantly more time in pathway on the isogenic line containing the *Vat* resistance gene compared to the lines not containing the *Vat* gene (Chen et al. 1997).

Aphids began probing at similar times; however, *S. graminum* feeding on indiangrass took significantly more time to reach sieve element tissues, suggesting partial resistance with the mesophyll. However, once the aphid's stylets reached the phloem

tissue, greenbug behavior did not differ among the three grasses. These findings compare favorably with Jiang et al. (2001) where significant differences in the feeding behavior of the sweetpotato whitefly, *Bemisia tabaci* (Gennadius) on resistant (*Mi* gene) and susceptible tomatoes were observed for total duration of non-probing events and time to first sieve-element phase. *Bemisia tabaci* spent significantly more time in the non-probing phase on the resistant plants and reached the first sieve-element phase significantly faster on the susceptible plant (Jiang et al. 2001).

No differences were detected in aphid access to phloem sieve elements, total duration of sieve element phases, potential phloem ingestion index (PPII), or aphids showing sustained phloem feeding (> 10 minutes). The PPII is a corrected index used to determine the acceptability of phloem, measuring the percentage of time the insect spends in sieve elements, with the registration time to the first sieve element subtracted (Girma et al. 1992, Van Helden and Tjallingii 2000). Collectively, these data display no overall phloem resistance factors being associated with the grass populations used for EPG recordings.

Choice studies for *S. flava* found no significant preference among the three grasses until 8 h after aphid introduction. Initial lack of preference may simply be due to aphids searching for a host plant for the influence of plant properties acting as a repellant or an attractant for *S. flava*. Several plant properties can act as attractants or repellants. For example, plant volatiles released in a close proximity to the plants surface may act as a possible attractant or repellant for aphids (Smith 2005, Powell et al. 2006, Le Roux et al. 2008). Trichome numbers may also influence aphid behavior and increased trichome

densities have been shown to negatively impact herbivore populations (Agrawal 1999, Kessler and Baldwin 2002, Horgan et al. 2009).

Overall this research provides the first detailed account of *S. graminum* and *S. flava* feeding behaviors on big bluestem and indiangrass. It also supplements the findings of Koch et al. (2014c) with relation to *S. graminum* and *S. flava* feeding behaviors on switchgrass. However, preconditioning *S. graminum* on sorghum for all EPG recordings rather than preconditioning them on a susceptible KxS switchgrass population, as done by Koch et al. (2014c), likely resulted in the distinct differences observed in aphid feeding behavior for the switchgrass (Summer) population. Resistance in aphids can be species-specific (Tjallingii 2006); therefore, future studies should focus on the feeding of *S. flava* on various warm-season grasses to determine if there are possible resistance mechanisms. Future studies should also examine feeding behavior on different developmental stages of the three grasses. As indicated by Alvarez et al. (2006), host acceptance by aphids was strongly dependent on the developmental stages of the plant or leaves, in regards to *Myzus persicae* (Sulzer) feeding on 20 *Solanum* spp. genotypes (Alverez et al. 2006). Identifying possible resistance mechanisms in plants will play a vital role in overall plant resistance and should be studied in more detail to expand our current knowledge of plant resistance mechanisms.

Long-term implications of this research include the possibility of a changing landscape, potentially more grasses being a part of the future ecosystem. With this possibility being taken into consideration it becomes evident that there will need to be research to address these long-term impacts and this research will potentially allow us to

be ahead of the curve when it comes to these grass systems with regards to potential pests such as *S. graminum* and *S. flava*.

There was also a recent study published concerning the change in climate patterns over the next several years. The landscape in Nebraska could be very different in Nebraska due to increased temperatures and less rainfall amounts due to these increased temperatures. There is also predicted to be a longer growing season with the projected frost season projected to begin two weeks later by the end of the century (Bathke et al. 2014). With plant resistance becoming a more ideal option for pest management, this research has laid the foundation for future studies as they begin to further investigate this plant-insect interaction.

Table 3.1 Switchgrass, Big Bluestem and Indiangrass Populations used in screening studies, originating from CenUSA biomass yield test plots.

Switchgrass Populations	Big Bluestem Populations	Indiangrass Populations
Check strains:	**Check strains:**	**Check strains:**
Cave-in-Rock	Rountree*	Warrior*
Shawnee	Goldmine	Scout
Summer	Bonanza*	Chief*
Sunburst	Kaw	Rumsey
Kanlow	Niagara*	Tomahawk
Experimental strains:	Sunnyview*	Holt
Kanlow N2	Champ	**Experimental strains:**
Summer Late Mat. C2	**Experimental strains:**	Oto C3 HYLD-HDMD C4
KxS HP1 NETO2 C2	Kaw HYLD-HDMD C5*	NE 54 HYLD-HDMD C3
NE 2010 x HYLD-HDMD C1	Pawnee HYLD-HDMD C5*	HoltxOto Early HYLD-HDMD C3
CIR C4	Bambo C1*	SN HZ 4 C1
KxS HP1 NETO2 C1	MW5A C1	SN HZ 5 C1
KxS HP1 High Yield C1	MW58 C1*	Oto 2648
Kanlow N1 Late Mat-High Yield	WBB 12L*	
Kanlow N1 Early Mat-High Yield	BB-17-101*	
Blade EG1102*		
Blade EG2101*		
NFSG10-02*		
NFSG10-11*		
NL 93-2		
NL 94 C2-1		
NO94 C2-4*		
NSL 2009-1		

*Population not screened due to low germination issues

Table 3.2 Characterization of resistance levels based on damage ratings for *Sipha flava* in screening study performed on switchgrass, big bluestem and indiangrass populations (2nd leaf stage).

Grass Population	Resistance level	Grass Population	Resistance level
Switchgrass:		**Big Bluestem:**	
CIR C4	HR	Goldmine	MR
Kanlow	HR	Kaw	MR
Kanlow N2	HR	Champ	MS
NL 93-2	HR	MW5A C1	HS
Cave-in-Rock	MR	**Indiangrass:**	
Kanlow N1 Early Mat-High Yield	MR	Holt	MS
Kanlow N1 Late Mat-High Yield	MR	HoltxOto Early HYLD-HDMD C3	MS
NE 2010 x HYLD-HDMD C1	MR	NE 54 HYLD-HDMD C3	MS
NL 94 C2-1	MR	Oto C3 HYLD-HDMD C4	MS
NSL 2009-1	MR	Oto 2648	MS
Shawnee	MR	Rumsey	MS
Summer Late Mat. C2	MR	Tomahawk	MS
Sunburst	MR	Scout	MS
KxS HP1 NETO2 C1	MS	SN HZ 5 C1	MS
KxS HP1 High Yield C1	MS	SN HZ 4 C1	HS
KxS HP1 NETO2 C2	MS		
Summer	HS		

HR, highly resistant; MR, moderately resistant; MS, moderately susceptible; HS, highly susceptible

Table 3.3 Comparison of EPG parameters (mean ± SEM) for time and duration of pattern segments for 15 h of *Schizaphis graminum* feeding on switchgrass, big bluestem and indiangrass populations (2nd leaf stage).

Feeding Variable	Mean ± SEM[a]		
	Switchgrass	Big Bluestem	Indiangrass
Time to 1st probe[b]	9.57 ± 3.1a	16.9 ± 7.9a	17.2 ± 8.2a
Time to 1st SE[1] phase	252.7 ± 34.9ab	249.6 ± 40.8b	388.4 ± 45.3a
Time from 1st probe to 1st SE phase	243.7 ± 35.8ab	232.8 ± 40.3b	370.3 ± 42.6a
Duration of pathway phases[b]	492.4 ± 30.6b	557.9 ± 40.1b	619.0 ± 31.5a
Duration of xylem phases	66.1 ± 15.0ab	81.8 ± 13.2a	39.31 ± 6.0b
Duration of SE phases	83.6 ± 26.7a	128.05 ± 33.2a	122.1 ± 37.4a
Duration of NP[2] events	264.9 ± 40.3a	153.1 ± 32.5b	129.6 ± 15.5b
Duration of 1st probe	70.0 ± 21.1a	71.9 ± 19.53a	59.6 ± 14.6a
Duration of 1st SE phase	11.6 ± 6.7a	11.3 ± 7.2a	19.0 ± 15.1a

[a]Treatment means within the same row followed by the same letter indicate no significant differences ($P \leq 0.05$), LSD test.
[b]Time and duration calculated in minutes
[1] Sieve element
[2] Non-probing

Table 3.4 Comparison of EPG parameters (mean ± SEM) for stylet activities for 15 h of *Schizaphis graminum* feeding on switchgrass, big bluestem and indiangrass populations (2nd leaf stage).

Feeding Variable	Mean ± SEM[a]		
	Switchgrass	Big Bluestem	Indiangrass
Potential drops	172.8 ± 14.2b	222.3 ± 25.0ab	246.7 ± 19.7a
Pathway phases	24.3 ± 2.3a	24.2 ± 2.8a	26.6 ± 2.8a
Xylem phases	2.4 ± 0.4a	2.6 ± 0.4a	2.7 ± 0.4a
SE[1] phases	7.0 ± 1.0a	5.4 ± 0.9a	5.0 ± 0.9a
NP[2] events	15.2 ± 2.0a	16.9 ± 2.7a	19.3 ± 2.4a
Probes after 1st SE phase	8.8 ± 1.6a	9.5 ± 1.7a	9.5 ± 1.7a
Potential phloem ingestion index (PPII)	13.1 ± 4.2a	21.8 ± 5.4a	27.0 ± 7.8a
% of aphids showing sustained ingestion (E > 10min.)	50 (10/20)a	60 (12/20)a	55 (11/20)a

[a]Treatment means within the same row followed by the same letter indicate no significant differences ($P \leq 0.05$), LSD test.
[1] Sieve element
[2] Non-probing

Figure 3.1 – Comparison of *Schizaphis graminum* preference among switchgrass, big bluestem and indiangrass. * Denotes significant differences ($P \leq 0.05$), LSD test.

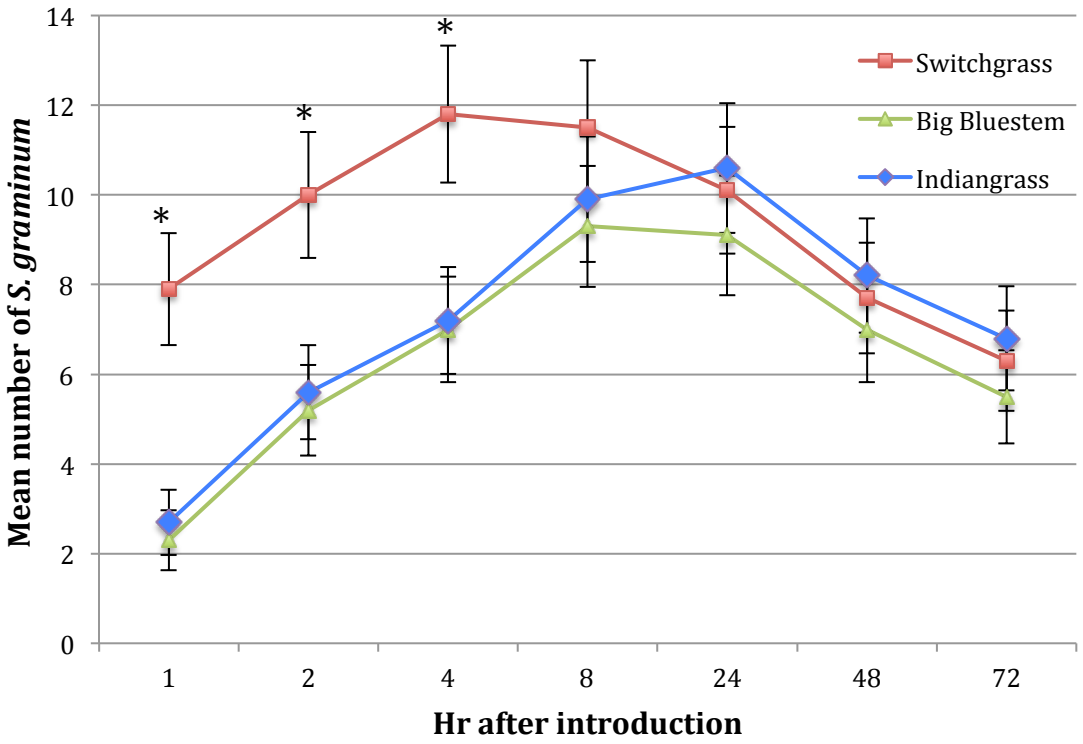
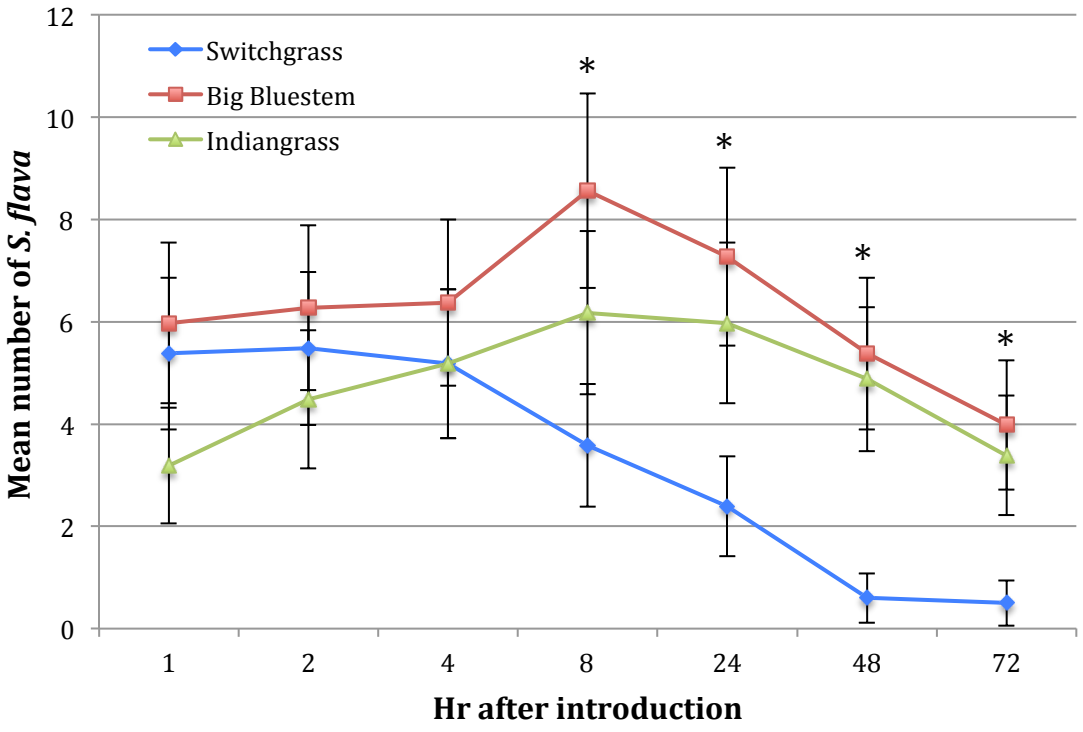


Figure 3.2 – Comparison of *Sipha flava* preference among switchgrass, big bluestem and indiangrass. * Denotes significant differences ($P \leq 0.05$), LSD test.



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