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Characterization of Cereal Aphid Resistance in Tetraploid Switchgrass Populations (*Panicum virgatum* L.)

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CHARACTERIZATION OF CEREAL APHID RESISTANCE IN TETRAPLOID SWITCHGRASS POPULATIONS (PANICUM VIRGATUM L.)

By:
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A THESIS

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Characterization of cereal aphid resistance in tetraploid switchgrass populations

(Panicum virgatum L.)

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University of Nebraska, 2013

Advisers: Tiffany Heng-Moss and Jeff Bradshaw

Switchgrass, Panicum virgatum L., is a perennial warm-season grass that has been identified as a model species for the development of bioenergy crops in the United States. The objectives of this research were to evaluate selected switchgrass populations for host suitability and differential resistance to potential aphid pests, determine the categories (antibiosis, antixenosis, and/or tolerance) of resistance among selected switchgrass populations, and elucidate Schizaphis graminum (Rondani) feeding behavior on resistant and susceptible switchgrasses. Screens for host suitability of two switchgrass populations, Summer and Kanlow, and two experimental strains, KxS and SxK, revealed all switchgrasses were unsuitable feeding and reproductive hosts to Rhopalosiphum padi (L.), and Diuraphis noxia (Mordvilko). Both Sipha flava (Forbes) and S. graminum were able to establish on all switchgrasses tested with differential levels of resistance among the switchgrasses. Two no-choice studies, performed to characterize the categories of resistance (antibiosis and tolerance) to S. flava and S. graminum, demonstrated that Kanlow possesses high levels of antibiosis to both aphids, while KxS possesses low-to-moderate levels of antibiosis to S. flava. Functional plant loss indices indicated that
tolerance is an important category of resistance for Summer to *S. graminum*. Two choice studies evaluated *S. graminum* and *S. flava* preference for switchgrass populations, with a third study to assess *S. graminum* feeding behavior using the electrical penetration graph (EPG) technique. Choice studies for *S. flava* indicated no preference by aphids for any of the switchgrass populations. However, *S. graminum* displayed a preference for KxS at 24 h after aphid introduction. Feeding behavior studies for *S. graminum* on switchgrass indicated that aphids had significantly less phloem ingestion on Kanlow than both KxS and Summer, suggesting that resistance factors in Kanlow are associated with the phloem tissue. These studies are the first attempt to analyze the categories of resistance in switchgrass and provide critical information for characterizing the mechanisms of resistance and improving our knowledge of the plant-insect interactions within this system.
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Chapter 1

Introduction and Thesis Objectives

Switchgrass, *Panicum virgatum* L., a perennial warm-season grass, has demonstrated great potential as a bioenergy crop in the USA. Much research has focused on the agronomic development of switchgrass, while potential insects that may limit production have received little attention. Nonetheless, it is likely that large-scale plantings of this species will result in insect infestations that could negatively impact establishment and yields. One strategy that may be imperative in mitigating potential pest problems is the development of plant resistance. Little is known about plant resistance categories and mechanisms in switchgrass to insects, although preliminary research indicates resistance factors are present to potential lepidopteran and aphid pests. Therefore, the focus of this research was to identify potential aphid pests, characterize the categories (antibiosis, antixenosis, and/or tolerance) of resistance among switchgrass populations, and elucidate aphid feeding behavior on resistant and susceptible switchgrasses.
Objectives:

1) Evaluate selected switchgrass populations for resistance to determine host suitability and plant damage differences to four potential aphid pests: *Schizaphis graminum* (Rondani), *Rhopalosiphum padi* (L.), *Rhopalosiphum maidis* (Fitch), and *Sipha flava* (Forbes).

2) Characterize the categories of resistance (antibiosis, antixenosis, and tolerance) among selected switchgrass populations.

3) Elucidate *S. graminum* feeding behavior on resistant and susceptible switchgrass populations using the electronic penetration graph (EPG) technique.
Switchgrass

Switchgrass, *Panicum virgatum* L., is a perennial, polyploid, warm-season grass whose native habitat includes the tallgrass prairies throughout much of North America, east of the Rocky Mountains (Vogel 2004, Mitchell et al. 2008, 2012). Switchgrass is one of the dominant components of North American prairies, and is considered one of the “big three” grasses, along with indiangrass, *Sorghastrum nutans* (L.) Nash, and big bluestem, *Andropogon gerardii* Vitman, which compose the greatest percentage of the species found in tallgrass prairies (Bouton 2008). Switchgrass may reach up to three meters in height, with most genotypes caespitose in appearance (i.e., they grow in dense clumps) with short rhizomes which may form a loose sod over time (Vogel 2004, Bouton 2008). Switchgrass is widely adapted to North America, and is found growing natively in the continent from 20° north latitude to over 55° north latitude and east of 100° west longitude (Moser and Vogel 1995, Vogel 2004, Bouton 2008). Due in large part to its relatively broad geographic distribution, switchgrass has evolved into multiple, diverse populations resulting in significant natural variation, morphological diversity, and ploidy levels (Vogel et al. 2011, Zalapa et al. 2011, Lu et al. 2013). The basic chromosome number of switchgrass is 9, and although multiple ploidy levels exist, tetraploids (2n = 4x = 36) and octoploids (2n = 8x = 72) predominate (Moser and Vogel 1995, Sanderson et al. 1996, Bouton 2008). Taxonomically, switchgrass is characterized by two distinct ecotypes, lowland and upland, which are distinguishable based on chloroplastic markers (Hultquist et al. 1997, Young et al. 2012). Lowland ecotypes are generally taller, coarser, better adapted for growth in flood plains, and may grow faster as opposed to upland
ecotypes (Vogel 2004). Further, lowland ecotypes are generally tetraploids, while upland
cotypes are often octoploids.

**Switchgrass As a Bioenergy Feedstock**

Traditionally, much of the research on switchgrass has focused on its use as a
rangeland forage crop. Switchgrass has been seeded in pastures and rangeland, in both
pure stands and mixtures, in the U.S. for more than 70 years (Vogel 2004), and was
undoubtedly used long before that in its native state as an unmanaged forage crop
(Parrish and Fike 2005). Accordingly, the early agronomic work on switchgrass focused
mainly on forage value and forage yield. However, more recently switchgrass has been
identified as model species for the development of herbaceous bioenergy production,
based on a series of evaluations by the U.S Department of Energy (US-DOE) (Vogel
1996, Vogel et al. 2002, Sarath et al. 2008). Currently, biomass feedstocks are used to
produce ethanol from sugar- and starch-rich crops, such as maize (*Zea mays* L.), by
fermenting the starch in grains; however, these crops are generally produced in labor-
intensive agricultural systems and require high inputs (e.g., nitrogen fertilization) and
may negatively impact the overall energy and CO₂ balance within the production system
(Jakob et al. 2009). However, ethanol can also be produce from other plant products, such
as fermentation of sugars in plant cell walls, which are the most abundant plant materials,
while forage crops, including switchgrass, excel in plant cell wall production (Vogel
1996). Consequently, dedicated cellulosic biofuels, such as switchgrass, are a promising
component of future renewable energy solutions and may provide a more efficient and
sustainable energy resource, because their reduced need for annual inputs minimizes cost
and fossil fuels used in production, leading to a more positive energy balance (Hill et al. 2006, Heaton et al. 2008).

Among potential herbaceous energy crops, switchgrass was selected as one of the most promising candidates for bioenergy cropping due to its large number of desirable attributes including: high productivity across diverse environments, suitability for marginal and erosive land, relatively low water and nutrient requirements, positive environmental benefits, and compatibility with conventional farming practices (Sanderson et al. 1996, McLaughlin et al. 1998, Sanderson et al. 2004). Wullschleger et al. (2010) noted from yield data collected across the U.S. for switchgrasses that soil texture and land quality do not appear to have a significant impact on yield for switchgrass. This is a particularly relevant point since much of the land that has been suggested for switchgrass production is marginal or erosive land. Furthermore, switchgrass may reduce soil erosion rates and runoff on that marginal land, due to its extensive and well-developed root system (McLaughlin and Walsh 1998). That is, switchgrass may reduce the loss of soil nutrients, increase incorporation of soil carbon, and reduce use of agricultural chemicals compared to annual row crops (McLaughlin et al. 1994, Sanderson et al. 1996). Hohenstein and Wright (1994) estimated an approximate 95% reduction in soil erosion rates in the production of herbaceous energy crops, including switchgrass, relative to traditional annual row crops. Furthermore, life-cycle analysis models estimated that ethanol produced from switchgrass averaged 94% lower greenhouse gas (GHG) emissions than from gasoline (Schmer et al. 2008).

Long-term sustainability of bioenergy crops will depend not only on the energy produced by the biomass, but also on the energy required to grow the crop and convert it
to usable energy. Shapouri et al. (2003) estimated an average energy ratio of 1.34 and a best-case scenario energy ratio of 1.53 for maize (i.e., for every joule used to produce ethanol from maize, there is a 34% or 53% energy gain, respectively) (Shapouri et al. 2003). However, similar studies with switchgrass have indicated an energy ratio from 4.43 (443% net energy gain) (McLaughlin and Walsh 1998) to greater than 5.40 (540% net energy gain) (Schmer et al. 2008). Currently, switchgrass yields vary greatly between locations and cultivars, with yields frequently in the range of 10 to 14 Mg ha\(^{-1}\); however, yields of nearly 40 Mg ha\(^{-1}\) in select locations with relatively high fertilizer input and high precipitation have been reported (Wullschleger et al. 2010). Further, because switchgrass as a species is barely removed from the wild, from a crop-improvement standpoint, it is expected that yields will continue to increase with further breeding efforts (Perlack et al. 2005, Bouton 2008).

**Potential Insect Pests of Switchgrass**

Generally, grasses (Poaceae) host a variety of different pests, belonging to multiple insect orders. Grass foliage-feeding insects belong primarily to the orders Orthoptera, Lepidoptera, Coleoptera (especially Chrysomelidae and Curculionidae), Hymenoptera, and Phasmida (Tscharntke and Greiler 1995). Other important insect pests include sap-feeders in Hemiptera and Thysanoptera, and stem-boring insects primarily within Diptera, Hymenoptera, Lepidoptera, and Coleoptera (Tscharntke and Greiler 1995). Schaeffer et al. (2011) conducted a survey of the arthropod community associated with managed switchgrass fields in Nebraska and recorded 84 families across 12 arthropod orders. By far the most abundant orders were Thysanoptera, Hymenoptera, and Coleoptera, representing more than 80% of all arthropods collected (Schaeffer et al.
Some insects have only anecdotally been documented with pest potential in switchgrass, such as grasshoppers (Acrididae) (Vogel 2004, Parrish and Fike 2005). However, very few studies have been published on insects and their pest status in switchgrass, largely because many warm-season grasses generally appear to be relatively pest free in their native habitat, resulting in the common belief that switchgrass will require few insect pest management practices (Moser et al. 2004, Parrish and Fike 2005, Prasifka et al. 2009a).

One important potential switchgrass pest of interest has been the fall armyworm, *Spodoptera frugiperda* (J.E. Smith). *Spodoptera frugiperda* is a noctuid moth native to the tropical regions of the western hemisphere. In the U.S., *S. frugiperda* can only successfully overwinter in the southernmost parts of Florida and Texas. *Spodoptera frugiperda* has a wide range of host plants, with over 80 different host species recorded, including many grasses (Capinera 2005). Prasifka et al. (2009b) assessed the feeding and development of two strains of *S. frugiperda* on both switchgrass and *Miscanthus x giganteus*, finding that *S. frugiperda* development on switchgrass was consistent with other alternate hosts, and in some cases even compared favorably to other alternate hosts.

Armyworm, *Mythimna (Pseudaletia) unipuncta* (Haworth) is a cosmopolitan insect and may be an important pest of pasture and weedy grasses, as well as several grain crops, including maize (Capinera 2013). Furthermore, *M. unipuncta* may be able to overwinter in areas on the U.S. as far north as Tennessee, unlike *S. frugiperda*. This entails that *M. unipuncta* may be able to infest switchgrass grown for biofuels much earlier in the season, when tillers may be more susceptible and are still small enough to be consumed by relatively few larvae (Prasifka et al. 2009b). Evaluations to determine
relative feeding and development of *M. unipuncta* on field grown ‘Cave-In-Rock’ switchgrass and maize demonstrated that *M. unipuncta* was able to successfully complete development on the tested switchgrasses, however the relative value appeared to be lower for the switchgrass cultivar, with longer developmental time and lower 10-day mass relative to maize (Prasifka et al. 2011a). However, defoliation experiments for *M. unipuncta* on ‘Kanlow’ switchgrass suggested that exceptionally high *M. unipuncta* densities (120-150/m²) would only produce around a 20% reduction in plant biomass, implying that scenarios requiring insecticide or other control of *M. unipuncta* may be uncommon (Prasifka et al. 2011a).

Other lepidopterans may also emerge as important switchgrass pests, with recent reports of three stem-boring moths. *Blastobasis repartella* (Dietz) was first observed as a potential pest of switchgrasses in South Dakota in 2004 and more extensively surveyed in 2009 by Prasifka et al (2009a). *Blastobasis repartella* was originally documented feeding in ‘Dacotah’ and Cave-In-Rock switchgrass; however, subsequent surveys revealed the moth in a wide range of cultivars. *Blastobasis repartella* was originally described from two male specimens collected near Denver, Colorado in 1910 (Adamski and Hodges 1996); however, no information of the biology of the moth existed until studies in 2009. Reports suggest that *B. repartella* may be ubiquitous in established switchgrass across the midwestern U.S. (Prasifka et al. 2009a) and that the moth is apparently restricted to switchgrass (Adamski et al. 2010). Prasifka et al. (2011b) further characterized two additional lepidopteran stem borers of switchgrass, *Haimbachia albescens* Capps (Crambidae) and *Papaipema nebris* (Guenée) (Noctuidae), in Illinois and Iowa during 2010. In addition to *B. repartella*, results indicated that *P. nebris* infestations in
switchgrass might also be relatively common across the midwestern U.S., with *B. repartella* more abundant in more established switchgrass and *P. nebris* most abundant in newly established stands (Prasifka et al. 2011b). Although *H. albescens* appeared to be uncommon, switchgrass was established as a feeding host for the species, with information on the host plants of *H. albescens* previously unknown (Prasifka et al. 2011b). Currently, reports indicate that *B. repartella* and *H. albescens* likely have minimal impact on switchgrass production, with only mild stunting (typically <5%); however, *P. nebris* may present a greater potential to damage switchgrass, as stalk borer larvae often move between stems, and may kill several tillers during the first 3 months of growth (Prasifka et al. 2011b). Although the three stem-boring moths do not appear to present a serious threat to switchgrass currently, several complications could impact the potential pest status of these insects. Because stem-borers live almost exclusively inside the plant, chemical control with insecticides can be very difficult.

In 2008, a new species of gall midge, *Chilophaga virgati* Gagné (Diptera: Cecidomyiidae) was collected from switchgrass fields in South Dakota. Significant differences were documented between switchgrass cultivars for *C. virgati* infestation, although the mean percentage of tillers infested across all cultivars was 13 and 14% in 2008 and 2009, respectively (Boe and Gagné 2010). In addition, *C. virgati* infested tillers were markedly reduced in length and produced only 35% of the mean weight of uninfested tillers, averaged across all cultivars (Boe and Gagné 2010).

Aphids have also been documented in association with switchgrass, albeit to a very limited extent. Kindler and Dalrymple (1999) evaluated over 50 species of warm- and cool-season grasses for the relative development and reproduction of yellow
sugarcane aphid, *Sipha flava* (Forbes). Switchgrass supported moderate *S. flava* populations compared to all host grasses tested; however, when compared to more economically important hosts, such as sorghum, *Sorghum bicolor* (L.) Moench, barley, *Hordeum vulgare* L., and wheat, *Triticum aestivum* L., *S. flava* fecundity and longevity was among the lowest for switchgrass (Kindler and Dalrymple 1999). Another study with *S. flava*, noted that a switchgrass cultivar, ‘Alamo’, was one of the most resistant of all grass species tested in Hawaii (Miyasaka et al. 2007).

Further screens of various grasses showed that switchgrass was a very inefficient or non-host for two aphid species, the English grain aphid, *Sitobion avenae* (F.), and the apple grain aphid, *Rhopalosiphum oxyacanthae* (Schrank) (Coon 1959). Accordingly, only 20% of *S. avenae* nymphs were able to survive on switchgrass for 6 days, while no adult *S. avenae* or *R. oxyacanthae* in any developmental stage survived the evaluation (Coon 1959). Kieckhefer (1984) evaluated the preference and reproduction of *Schizaphis graminum* (Rondani), *Rhopalosiphum padi* (L.), *Rhopalosiphum maidis* (Fitch), and *S. avenae* on warm-season grasses, finding none of the aphids reproduced successfully on neither seedling nor mature switchgrass (Kieckhefer 1984).

The most detailed description of aphid performance on switchgrass to date tested several switchgrass cultivars to a variety of important cereal aphids, demonstrating that *S. graminum* (biotypes I and Florida), *R. padi*, *R. maidis*, and *S. flava* all established on the switchgrasses tested (Burd et al. 2012). Burd et al. (2012) noted that both biotypes of *S. graminum* and *R. maidis* were particularly virulent to the two-week-old switchgrasses tested, resulting in significant injury or death of the plants; however, evaluations for switchgrass plants at 4 weeks of age, showed that *R. padi* was either unable or less
successful at colonizing the switchgrasses, while all aphids were less virulent. Furthermore, the switchgrasses produced fewer leaves following infestations when compared to uninfested controls, with *S. graminum* biotype I and the Florida biotype producing the greatest effect with 50 to 65% fewer leaves produced and 70 to 80% less leaf biomass, respectively (Burd et al. 2012).

Aphids are major insect pests of agricultural crops around the world and may be of particular importance for their ability to damage crops by removing photo assimilates and their efficient ability to transmit numerous devastating plant viruses (Smith and Boyko 2007). Aphids are phloem sap feeders with piercing-sucking mouthparts that efficiently facilitate the delivery of virions into plant cells (Ng and Perry 2004). Collectively, insects are the most common of the vectors of plant viruses, while aphids alone account for the transmission of 50% of the insect-transmitted viruses (Nault 1997, Ng and Perry 2004). Schrotenboer et al. (2011) noted that switchgrass could accumulate barley yellow dwarf virus (BYDV) infections, transmitted by many important cereal aphids, quickly under both natural and greenhouse conditions. Furthermore, more developed and productive cultivars were preferentially selected by *R. padi* and were also most susceptible to the PAV strain of BYDV (Schrotenboer et al. 2011). Although the impact that important viruses, such as BYDV, may have on switchgrass grown for biofuels is poorly understood, BYDV has been shown to significantly reduce biomass production in other native perennial grasses (Malmstrom et al. 2005). Further complicating the potential interactions between switchgrass and pests and/or pathogens is the prospective of inadvertently producing more susceptible genotypes to pests and pathogens with breeding efforts for increases biomass and biofuel conversion properties.
Although many of the insects documented in switchgrass currently do not appear to pose an immediate threat, the recent discovery of new species and description of previously poorly understood species suggests an incomplete understanding of the ecology within this system. Further, while current knowledge of potential insect pest of switchgrass populations being developed for biomass production may be limited, previous work suggests that insect pests will emerge as production is increased in monoculture settings that are not as obvious in small and more diverse settings (Mitchell et al. 2008, Prasifka et al. 2009a, Prasifka and Gray 2012). Thus, it is clear that the development of switchgrass as sustainable bioenergy feedstock will require effective pest management strategies. Furthermore, Prasifka et al. (2011a) states that it is important to note that yields of dedicated biomass crops depend primarily on the size of vegetative components, rather than reproductive components as in grain crops, thus economic thresholds could be significantly different for the same pests on fuel crops compared to food crops.

**Plant Resistance**

According to Smith (2005), “Plant resistance 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.” Accordingly, plant resistance to insects is a relative property, based on the comparative response of resistant and susceptible plants to the pest insect, given similar conditions (Smith 1998). Currently, hundreds of insect-resistant cultivars are grown in the U.S., where they offer substantial economic and environmental benefits and have greatly advanced food production (Smith 1998, 2005). Consequently, plant resistance has become a major focus of breeding efforts.
and many of the major cereal crop cultivars now possess levels of insect-resistance. Insect-resistant plants also provide an attractive means for managing insect pests because they may reduce insecticide application, resulting in the reduction of input costs and harsh chemicals in the environment. Schalk and Ratcliffe (1976) estimated that the production of insect resistant alfalfa, barley, maize, and sorghum cultivars in the U.S. allowed for a 37% decrease in insecticide application. Furthermore, plant resistance has been demonstrated to reduce the spread of insect transmitted pathogens. Kishaba et al. (1992) demonstrated a significant reduction (31% - 74%) in the transmission of watermelon mosaic virus in resistant lines of muskmelon, *Cucumis melo* L., to the melon aphid, *Aphis gossypii* Glover. Plant resistance may even improve the efficiency of insect biological control agents as well, effectively synergizing the interactions between the insect-resistant plants and natural enemies by decreasing the vigor of the insect pest (Quisenberry and Schotzko 1994, Smith 1998, 2005). Collectively, this has made plant resistance one of the most effective and sustainable strategies for controlling insect pests.

Generally, plant resistance may be further distinguished into three categories, as originally described by Painter (1951): antibiosis, antixenosis, and tolerance. Antibiosis describes some plant quality that adversely affects the biology or life history of an arthropod attempting to utilize the plant as a host (Smith 2005). In general, antibiosis may result from a number of plant mechanisms ranging from the production of toxic allelochemicals, such as alkaloids and ketones, to morphological and physical defenses, including trichome size, type or density. Further, even if the effect of an antibiotic response does not immediately kill the insect pest, significant reductions in overall fitness may be conferred by reduced body size and mass, and/or fecundity (Smith 2005).
Antixenosis is a term that describes any plant characteristic that affects the behavior of an arthropod pest, and is typically expressed as non-preference. According to Smith (2005), Antixenosis may be conferred by physical barriers, including thickened plant epidermal layers, waxy deposits on leaves, stems, or fruits, or a change in trichome structure or density, not present on susceptible plants. Plant chemicals may also be important among antixenotic plants by acting as repellants to deter pests from feeding or ovipositing. As a result of antixenotic factors, arthropod pests may abandon their efforts to consume, ingest or oviposit on an otherwise palatable plant (Smith 2005). According to Smith (1998), “tolerance is characterized by properties that allow a resistant plant to yield more biomass than a susceptible plant, due to the ability to withstand or recover from insect damage caused by insect populations equal to those on plants of a susceptible cultivar.” In general, tolerance involves only plant characteristics and does not likely affect the pest arthropod, and is therefore significantly different from antixenosis and antibiosis (Reese et al. 1994). Mechanisms for tolerance may include factors such as increased net photosynthetic rate, high relative growth rate, and pre-existing high levels of carbon stored in roots (Strauss and Agrawal 1999). Experiments in sorghum hybrids, a related warm-season grass, showed that photosynthetic rates of resistant sorghum plants were unaffected by S. graminum feeding for short durations, while susceptible plants had a significant reduction in photosynthetic rates; however, the tolerance of the resistant plants was overcome with longer durations of S. graminum feeding (Nagaraj et al. 2002). Further, Nagaraj et al (2002) suggested that the tolerance might be the result of the inability of salivary toxins from S. graminum to interact with specific targets in the host plant or longer times needed to cause injury in resistant lines. Moreover, tolerance
generally offers several advantages over antibiosis and antixenosis; specifically, arthropod populations are not reduced from exposure to tolerant plants as they typically are on antibiotic and antixenotic plants. As a result, pest populations are more likely to remain avirulent to plant resistance genes of tolerant plants, since the selection pressure placed on the pest populations is assumed to be significantly less than the characteristically high pressure from antibiosis (Smith 2005).

To date, limited work has been conducted to investigate plant resistance among switchgrass populations to potential insect pests. Dowd and Johnson (2009) noted that the apparent lack of insect pest problems in switchgrass suggests that insect resistance genes are present. In recent studies to evaluate switchgrass for resistance, differential levels of resistance documented among switchgrass populations to *S. frugiperda* (Dowd and Johnson 2009, Dowd et al. 2012). In a screen of both tetraploid and octoploid upland switchgrass cultivars in multiple developmental stages, the cultivar ‘Dacotah’ was consistently among the most heavily damaged cultivars by *S. frugiperda* feeding, while ‘Trailblazer’ showed the highest levels of resistance in the seedling stage and ‘Blackwell’ was among the most resistant cultivars among older plants (Dowd and Johnson 2009). Furthermore, Dowd and Johnson (2009) examined representatives of several classes of resistance genes reported to confer resistance to caterpillars and diseases in other systems, and noted difference among switchgrass cultivars in expression of two main peroxidase isozymes, as well as differences in the sequence for cationic peroxidase, which is homologous to cationic peroxidase in maize-associated insect resistance. Previous research has indicated that peroxidases play an important role in the plant’s response to biotic and abiotic stresses in a number of systems (Ni et al. 2001, Heng-Moss...
et al. 2004). Studies by Heng-Moss et al. (2004) indicated that resistant buffalograsses, *Buchloë dactyloides* (Nuttall) Engelmann, may be able to tolerate chinch bug, *Blissus occiduus* Barber, feeding by increasing peroxidase activity. Three resistant buffalograsses (NE91-118, Cody, and Tatanka) all had increased levels of peroxidase activity at various time points, after *B. occiduus* introduction, relative to uninfested controls; however peroxidase activity for the susceptible buffalograss (378) remained similar between infested and uninfested plants (Heng-Moss et al. 2004). Similar findings have also been reported for susceptible and resistant wheat and barley varieties to *Diuraphis noxia* (Mordvilko), demonstrating a general up-regulation of peroxidase genes in tolerant plants challenged by aphids, but not in the susceptible varieties (Ni et al. 2001, Franzen et al. 2007, Gutsche et al. 2009). The exact role of oxidative enzymes has not been completely elucidated and peroxidases could potentially be playing multiple roles in the tolerant plant’s defense response, such as triggering the production of hydrogen peroxide, which can act as a downstream signaling molecule for plant defense reactions to insect injury, or the efficient removal of reactive oxygen species (ROS), potentially allowing tolerant plants to sustain less tissue damage than susceptible plants as a result (Hildebrand et al. 1986, Heng-Moss et al. 2004, Passardi et al. 2005, Gutsche et al. 2009). Nonetheless, the exact implication of differences in switchgrass peroxidase isozymes in plant resistance is uncertain, and in need of further studies to determine any potential role in resistance to insects.

DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) is a major hydroxamic acid found in many grass crops including wheat and maize, and confers toxicity to many important insect pests including *S. graminum* and *R. padi* (Corcuera
Some indirect evidence suggests that DIMBOA may be present in switchgrass (Lin et al. 2008) and, accordingly, could be an important resistance factor in switchgrass as well; however, further studies are needed to confirm this evidence. Lee et al. (2009) characterized three steroidal saponins produced by switchgrass, including protodioscin. Subsequent studies demonstrated that protodiodcin inhibited growth of Helicoverpa zea Boddie (corn earworms) and S. frugiperda by 28.8 and 29.4%, respectively (Dowd et al. 2011). Correspondingly, Prasifka et al. (2011a) noted that older leaves of Kanlow had high levels of protodioscin and were more resistant to M. unipuncta; however, exact mechanisms of saponin toxicity are poorly understood.

One of the major impediments the biochemical conversion of switchgrass biomass into liquid fuels is lignin content (Dien et al. 2008); however, the development of switchgrasses with reduced lignin content may consequently have detrimental effects on plant resistance as well. Lignin is the generic term for a large group of aromatic polymers that may serve as a matrix around the polysaccharide components of some plant cell walls, providing additional rigidity and strength (Whetten and Sederoff 1995, Vanholme et al. 2010), and has also been implicated as a resistance factor against several insect pests (Dowd et al. 2012). Dowd and Johnson (2009) also noted in screens that no correlation seemed evident between plant resistance to S. frugiperda and lignin content, with ‘Trailblazer’, which was developed for better forage quality and lower lignin, having among the highest levels of resistance in the screen. Similarly, screens for resistance to S. frugiperda among hybrid crosses between ‘Summer’ and ‘Kanlow’ switchgrasses showed little correlation between plant resistance and lignin content, with modest correlation occurring only in early season (spring green up) plants (Dowd et al. 2012). Accordingly,
current information suggests that reduced lignin content may not adversely affect yield or other production factors, with other important resistance mechanisms present in switchgrasses.

Currently, no studies have evaluated resistance categories and mechanisms in switchgrasses to aphids in any detail; however, aphids are among the most important insect pests of world agriculture. Plant resistance to aphids may be particularly valuable since many pest aphid species are resistant to many insecticides, including important cereal pests such as *S. graminum* (Devonshire and Field 1991, Zhu et al. 2000). Additionally, plant resistance could play an intimate role in virus transmission by aphids in switchgrass. Previous work has demonstrated that some persistently transmitted viruses such as barley yellow dwarf virus are phloem restricted and typically requires several hours of feeding before a healthy aphid may acquire the virus, or transmit it to a healthy plant (Power 1991, Prado and Tjallingii 1994). Accordingly, resistant plants that limit phloem feeding by the aphid, either through antibiosis or antixenosis, may reduce the vector efficiency of aphids for the transmission of phloem-based, persistent viruses. However, increased probing has also been associated with resistant plants and the tendency to produce many short and separate probes on resistant plants could be responsible for an increase in non-persistent virus transmission, which may be acquired or transmitted by the aphid in as little as seconds (Kaloshian et al. 2000). Because aphids lack chemosensory organs on their stylets, sampling of sap from individual cells (likely sampling each cell encountered during stylet penetrations) plays an important role in host acceptance, and as a result, non-persistent virus transmission (Tjallingii 1994, Nault 1997). Accordingly, Powell et al. (1992) showed a positive correlation with acquisition
and inoculation of the potato virus Y potyvirus, and acquisition of beet mosaic potyvirus with cell membrane punctures by *Brachycaudus helichrysi* (Kaltenbach) and *Drepanosiphum platanoides* (Shrank). Therefore, it may be important to understand feeding behavior of sucking insects in switchgrass.

**Electronic penetration graphs (EPG) for the study of aphid feeding behavior**

Traditionally, the feeding behavior of insects with piercing-sucking mouthparts has been difficult to study due to most of the relevant behaviors occurring within the food substrate and not being directly observable (Walker 2000). As a result, specialized techniques have been developed to help elucidate the relevant feeding behavior and activities of piercing-sucking insects. The first electronic feeding monitor was developed by McLean and Kinsey (1964) to record aphid feeding and salivation. The first feeding monitors used an alternating current (AC) recorder system, with a direct current (DC) based monitor later described by Tjallingii (1978). Both AC and DC based systems have been used for studies of insect feeding behavior in relation to plant resistance; however, the DC-based system is able to provide better waveform details of an electronic penetration graph (EPG) and allow measurements of inside-waveform frequencies, allowing more and different waveforms to be identified easily (Tjallingii 2000, Van Helden and Tjallingii 2000). Consequently, the DC system has become a popular tool for insect feeding behavior studies with emphasis on plant resistance, allowing for more detailed conclusions.

The EPG technique allows the recording of signal waveforms corresponding to different insect activities and the position of the stylet tips within the plant tissues (Tjallingii 2006). The basic principle of EPG monitors is simple and involves the
inclusion of an insect and plant in an electrical circuit, connected to a voltage source and an input resistor. The output wire makes contact with plant by connecting to a stiff copper wire inserted into the potting soil in which the plant is rooted, while the input of the EPG system makes contact with the insect by connecting to a fine gold wire that is glued to the insect’s dorsum with conductive adhesive (Walker 2000). The insect is then introduced to the plant, and once the insect inserts its stylets into the plant, the circuit is completed, where current flows from the voltage source, through plant, through the insect, through the input resistor, and back to the voltage source. Further, the biological (plant-insect) component presents an electrical resistance to the flow of current through the system, where changes in the resistance of the system corresponds to specific biological activity and stylet position (Walker 2000). Specific feeding behaviors and stylet positions 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). Waveforms A, B, and C are all characteristic of the pathway phase, in which intercellular stylet penetration and withdrawal, periods of no stylet movement, and brief intracellular punctures by stylet tips, also known as potential drops (waveform pd), occur (Prado and Tjallingii 1994, Jiang and Walker 2001). Xylem sap ingestion was also defined by the characteristic waveform G, and is related to water intake by water-deprived aphids (Spiller et al. 1990). Collectively, waveforms E1 and E2 represent the sieve element (phloem) phase, specifically watery salivation into sieve elements and passive ingestion of phloem sap with concurrent salivation, respectively (Reese et al. 2000).
Accordingly, by using waveform comparisons to distinguish aphid stylet activities and position, insight may be gained into potential resistance mechanisms as well as plant tissue location of resistance factors (Van Helden and Tjallingii 2000, Jiang et al. 2001, Crompton and Ode 2010). Garzo et al. (2002) studied the feeding behavior of *A. gossypii* on susceptible and resistant melon genotypes (*Cucumis melo* L.) and found resistance factors in both pre-phloem and phloem tissue. Aphid feeding on resistant lines demonstrated longer non-probing time, increased number of probes, shorter duration of the probes, an increased number of short probes, and an increased number of probes before reaching the phloem, collectively indicating either chemical or physical deterrents present in the epidermis and mesophyll (Garzo et al. 2002). However, phloem-based resistance factors were also indicated by significantly shorter duration of the phloem ingestion phase (E2 pattern) on the resistant genotypes, relative to the susceptible entries (Garzo et al. 2002). Garzo et al. (2002) also suggested that the resistance mechanism found on melon genotype (TGR-1551) at the phloem level appeared to be physical because aphids that reached the phloem were typically unable to start ingestion, and therefore cannot detect the presence of any chemical deterrent compound. Similarly, other work has demonstrated evidence for potential physical phloem barriers, whereby large deposition of callose were detected around stylet sheaths produced by *A. gossypii* when feeding on the AR-5 resistant melon genotype (Shinoda 1993).

Furthermore, phloem-based resistance factors have been commonly reported in many systems to aphids. Electronic penetration graph studies for resistant tomato lines (*Lycopersicon esculentum* Miller) with the resistance gene, *Mi*, indicated that *Macrosiphum euphorbiae* (Thomas) phloem feeding was disrupted on resistant lines.
relative to the susceptible lines (Kaloshian et al. 2000). However, the reduction in
duration of sieve element phase activities was not a result of physical barriers or plant
chemistry preventing the aphid from locating the sieve element, since there was no
significant difference in the time required for aphids to achieve their first sieve element
contact on resistant and susceptible plants (Kaloshian et al. 2000). Phloem-based
resistance has been reported in many systems including: *Myzus persicae* (Sulzer) on
resistant *Prunus* genotypes (Sauge et al. 1998, 2002); *M. persicae* and *M. euphorbiae* on
resistant *Solanum stoloniferum* Schltdl. & Bouché; *A. gossypii* on resistant *C. melo*
genotypes (Kennedy et al. 1978); and *Aphis glycines* Matsumura on resistant soybeans,
*Glycine max* (L.) Merr. (Diaz-Montano et al. 2007, Crompton and Ode 2010).

Furthermore, the feeding behavior of the cereal aphid *S. graminum* has been well
characterized, especially on sorghum and wheat (Campbell et al. 1982, Montllor et al.
1992, Goussain et al. 2005, Pereira et al. 2010). Much of the literature for *S. graminum*
feeding behavior has addressed the effects of various plant treatments on *S. graminum*
probing; however, Montllor et al. (1983) evaluated the feeding of two *S. graminum*
biotypes (biotypes C and E) on resistant and susceptible sorghum lines and determined
differences between the biotypes in feeding behavior among the sorghum genotypes,
especially in relation to sieve element access and acceptance. However, currently no EPG
studies have been performed for insect feeding behavior on switchgrass; thus any future
studies using this technique could provide valuable insights into aphid-resistance
mechanisms. Studies of aphid feeding behavior may help identify resistance mechanisms
and are imperative to improving our knowledge of the plant-insect interaction and the relationship between vectors (aphids) and viruses within this system.
CHAPTER 2

Evaluation of tetraploid switchgrass populations (*Panicum virgatum* L.) for host suitability and differential resistance to four cereal aphids.

**Introduction**

One major challenge modern society faces is overcoming its dependence on fossil fuels, and their impending exhaustion, by implementing sustainable and renewable energy resources. An approach to improving the overall energy economy of the US is through the production of energy from plant-based products, or biofuels (Parrish and Fike 2005). The Biomass Research and Development Technical Advisory Committee, under the Biomass Research and Development Act of 2000, established the goal of replacing 30 percent of the U.S. petroleum consumption with biomass-derived energy by the year 2030 (Perlack et al. 2005). However, biomass currently supplies about four percent of the total energy consumption in the United States, producing over four quadrillion Btu of energy in 2011 (US-EIA, 2012). As the push for the development of efficient and sustainable biofuel crops continues, one important species, which has been identified as a model herbaceous biomass crop, is switchgrass (*Panicum virgatum* L.) (Vogel 1996, Vogel et al. 2002).

Switchgrass is a perennial, polyploid, warm-season grass native to tallgrass prairies of North America, east of the Rocky Mountains (Vogel 2004, Mitchell et al. 2008, 2012). Because of its relatively broad geographic distribution, switchgrass has evolved into multiple, diverse populations resulting in significant natural variation, morphological diversity, and ploidy levels (Vogel et al. 2011, Zalapa et al. 2011, Lu et al. 2013). Further, switchgrass is characterized by two distinct ecotypes, lowland and upland, distinguishable based on chloroplastic markers (Hultquist et al. 1997, Young et
Lowland ecotypes are generally taller, coarser, adapted for growth in flood plains, and exhibit considerably greater yield potential as opposed to upland ecotypes. Further, hybrids between certain upland and lowland tetraploid populations display heterosis for biomass yields (Martinez-Reyna and Vogel 2008). In their native habitat, many warm-season grasses generally appear to be relatively pest free, resulting in the common belief that switchgrass will require few insect pest management practices (Moser et al. 2004, Parrish and Fike 2005, Prasifka et al. 2009a). As a result, despite the attention switchgrass has received in recent years for its development as biomass feedstock, potential insect pests have been largely ignored.

While current knowledge of potential insect pest of switchgrass populations being developed for biomass production is lacking, previous work suggests that insect pests will emerge, particularly as production is increased in monoculture settings (Mitchell et al. 2008, Prasifka et al. 2009a). Fall armyworm, Spodoptera frugiperda (J.E. Smith), and armyworm, Mythimna unipuncta (Haworth), have both been demonstrated to complete development on switchgrass, however, in both cases development was delayed relative to corn (Prasifka et al. 2009b, Prasifka et al. 2011a). The stem-boring moths, Papaipema nebris (Guenée) and Haimbachia albescens Capps, have also been documented in switchgrass recently (Prasifka et al. 2011b), while a fifth moth, Blastobasis repartella (Dietz), appears to be a monophagous stem-borer restricted to switchgrass (Prasifka et al. 2009a, Adamski et al. 2010). The recent rediscovery of B. repartella, which appears to be relatively common in switchgrass, is particularly interesting and suggests a lack of knowledge of the insect fauna associated with switchgrass rather than the absence of insect pests, which has generally been assumed (Adamski et al. 2010). Grasshoppers
(Acrididae) have also been anecdotally documented as defoliators of switchgrass (Vogel 2004, Parrish and Fike 2005).

Likewise, few studies to date have examined insects with piercing-sucking mouth parts on switchgrass. Kindler and Dalrymple (1999) noted the development and reproduction of yellow sugarcane aphid, *Sipha flava* (Forbes), on various warm- and cool-season grasses including switchgrass. However, another study with *S. flava*, noted that a switchgrass cultivar, ‘Alamo’, was one of the most resistant of all grass species tested in Hawaii (Miyasaka et al. 2007). Additionally, a screen of various grasses showed that switchgrass was a very inefficient or non-host for the English grain aphid, *Sitobion avenae* (F.) (= *Macrosiphum granarium* [Kirby.]), and the apple grain aphid, *Rhopalosiphum oxyacanthae* (Schrank) (= *R. fitchii* [Sanderson]) (Coon 1959).

Kieckhefer (1984) evaluated the preference and reproduction of *Schizaphis graminum* (Rondani), *Rhopalosiphum padi* (L.), *Rhopalosiphum maidis* (Fitch), and *S. avenae* on warm-season grasses, finding none of the aphids reproduced successfully on mature switchgrass, while only *R. padi* had moderate reproductive success on seedling stage switchgrass. Finally, Burd et al. (2012) tested several switchgrass cultivars to a variety of aphids, demonstrating that *S. graminum* (biotypes I and Florida), *R. padi*, *R. maidis*, and *S. flava* all established on the switchgrasses tested (Burd et al. 2012). However, conflicting information seems to be present in these few characterizations, while thorough studies addressing aphids as potential pests of switchgrass are lacking. Therefore, with such limited information on potential insect pests of switchgrass, additional studies are needed to help bridge the gap in our knowledge of the plant-insect interactions within switchgrass.
Additionally, in order to develop sustainable biomass yields from switchgrass, effective insect pest management strategies will be essential. One of the most effective and sustainable strategies for controlling insect pests affecting switchgrass is plant resistance (Dowd and Johnson 2009). Insect resistant cultivars may be able to produce high yields by either negatively affecting the biology and/or behavior of the insect, or by tolerating the injury of the insect pest. In this study, we evaluated selected switchgrass populations for susceptibility to four potential aphid pests (*S. flava*, *S. graminum*, *R. padi*, and *Diuraphis noxia* [Mordvilko]), to determine host suitability and plant damage differences.

**Materials and Methods**

**Plant material.** All screening studies consisted of two cultivars and two experimental strains. Kanlow is a lowland-tetraploid population that originated from switchgrass collected near Wetumka, OK (Alderson and Sharp 1994, Vogel and Mitchell 2008). Summer is an upland-tetraploid population, derived from plants collected near Nebraska City, NE (Alderson and Sharp 1994, Vogel and Mitchell 2008). The two experimental strains, KxS HP1 C1 High Yield and SxK HP1 C1 High Yield strains, were produced by reciprocal matings between Kanlow and Summer plants, followed by selection among the F1 progeny for winter survival and vigor, and followed by two generations of random mating to stabilize the populations and then one generation of selection for seedling vigor at six weeks after planting and high biomass yields. These experimental strains, which will be referred to hereafter as KxS and SxK, were developed by Dr. Kenneth Vogel, USDA-ARS, Lincoln, NE who also provided seed of the cultivars.
Summer plants were the seed parents for the KxS population while Kanlow plants were the seed parents of the SxK populations.

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 cone-tainer trays and maintained in a greenhouse at 25 ± 7°C with the lighting augmented by 400-W Metal Halide lamps to produce a photoperiod of 16:8 (L:D) h. After emergence, plants were thinned down to one plant per cone-tainer. Plants were fertilized every two weeks with a soluble (20:10:20 N-P-K) fertilizer.

**Insect colonies.** The switchgrasses were evaluated for their host suitability to four aphid species: *S. flava*, *S. graminum* (biotype I), *D. noxia*, and *R. padi*. All four aphid colonies were obtained from Dr. John D. Burd, USDA-ARS in Stillwater, Oklahoma. The *S. flava*, *R. padi*, and *D. noxia* colonies were maintained on a continuous supply of ‘Haxby’ barley plants, while *S. graminum* was maintained on a susceptible sorghum cultivar ‘BCK60’. The *S. graminum* colony was maintained in a plant growth chamber at 25 ± 2°C with a photoperiod of 16:8 (L:D) h, while *D. noxia* and *R. padi* were similarly maintained in a growth chamber, but at 20 ± 2°C and 16:8 (L:D) h. However, *S. flava* could not successfully be kept in a growth chamber, thus the colony was maintained in the greenhouse at 25 ± 7°C and 16:8 (L:D) h within clear plastic cages, approximately 12.5 cm diameter and ventilated with organdy fabric.

**Screening Studies.** *Screening studies 1, 2, 3 & 4.* Four screening studies were performed to assess host suitability of four switchgrass populations (Kanlow, Summer, KxS, and SxK) to *S. graminum* (screens 1 & 2) and *S. flava* (screens 3 & 4) at two host
developmental stages (2\textsuperscript{nd} and 5\textsuperscript{th} leaf stage). For screens involving \textit{S. graminum}, the susceptible sorghum BCK60, was included in a similar developmental stage to provide a control and a relative comparison of the success of the aphids with a well-known standard. Similarly, a barley cultivar, ‘Haxby’, was included for all screens with \textit{S. flava} for the same purpose. The experimental design was a completely randomized design with 10 replicates per population per screen. Five apterous, adult aphids were transferred to each plant with a fine paintbrush, and then caged with tubular plastic cages (4 cm diameter by 46 cm height) with vents 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 twice weekly by counting the total number of aphids and performing a visual damage rating on a 1-5 scale. Damage ratings served as a visual assessment of the injury sustained to the plant by aphid feeding (Smith et al. 1994). The damage rating scale was adopted from Heng-Moss et al. (2002), 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 area damaged; and 5 = 71\% or more of the leaf area damaged and the plant near death. Plant damage was characterized by chlorosis, a reddish discoloration, or desiccation of the leaf. Experiments were terminated once the mean damage rating reached 4 for any given population, or when aphid numbers and damage ratings plateaued across all populations of switchgrass.

To better describe aphid injury over time, cumulative aphid days were derived from the aphid counts. Cumulative aphid days (CAD) were calculated using the following formula: $CAD = \sum_{i=1}^{n} \left( \frac{N_i + N_{i+1}}{2} \right) \times T$, where $N_i$ is the total number of aphids on a plant at a given evaluation date $i$, $N_{i+1}$ is the total number of aphids on same plant on
the subsequent evaluation date, and $T$ is the number of days between the two evaluation
dates (Hanafi et al. 1989). To further characterize damage ratings, populations were
grouped into one of four levels of resistance: highly susceptible (HS, mean damage
ratings $\geq 4$); moderately susceptible (MS, mean damage ratings $\geq 3$ but $< 4$); moderately
resistant (MR, mean damage ratings $\geq 2$ but $< 3$); and highly resistant (HR, mean damage
ratings $< 2$) (Heng-Moss et al. 2002, Pierson et al. 2010).

Screening studies 5, 6, 7 & 8. Four additional screens measured the host
suitability of the same switchgrass populations to $D. noxia$ (screens 5 & 6) and $R. padi$
(screens 7 & 8). Methods as described above for $S. graminum$ and $S. flava$ were
attempted, however efforts to establish $D. noxia$ and $R. padi$ on the switchgrass
populations were unsuccessful. Thus, in order to confirm switchgrass as a non-feeding
and reproductive host for $D. noxia$ and $R. padi$, methods were adjusted to assess aphid
fecundity over a shorter period of 5 days. Plants were evaluated in the same
developmental stages as screening studies 1-4, with the barley cultivar, Haxby, serving as
the control for all screens involving $D. noxia$ and $R. padi$. The experimental design was a
completely randomized design with 10 replicates per population per screen. Small clip
cages were made with two, heavy duty, double-stick foam tape squares (25.4 mm by 25.4
mm by 1.5 mm) (3M Co., St. Paul, MN). Two foam tape squares were placed together to
provide additional depth, and holes, 1.6 cm in diameter, were cut in the center of the
squares. Square cages were then stuck to a leaf and a small organdy fabric square placed
opposite the square cage on the leaf to seal one side. The square cage was infested with
10 apterous, adult aphids, and organdy fabric placed over the top half to completely seal
the cage. After 5 days, the cages were opened and both adult aphids and nymphs were
counted to assess survival and reproduction. Notes were made of evidence of localized damage within the cage.

**Statistical analysis.** Generalized linear mixed model analyses (PROC GLIMMIX, SAS Institute 2011) were conducted for all aphid counts and damage ratings to measure population differences. The effect each population of switchgrass had on the cumulative aphid days was determined using square root transformed data to meet the assumptions of the generalized linear mixed model analysis. Where appropriate, means were separated using Fisher’s least significant difference (LSD) procedure ($\alpha = 0.05$).

**Results**

**Screening Studies.** *Screening Studies 1 & 2.* Significant differences were found for CAD among the populations of switchgrass in both the 2nd and 5th leaf stages for *S. graminum* (Table 2.1) (Screen 1 CAD: $F = 32.28; \text{df} = 3, 36; \text{P} < 0.0001$; Screen 2 CAD: $F = 5.89; \text{df} = 3, 33; \text{P} = 0.003$). Further, the relative ranking for all four populations of switchgrass was similar for both the 2nd (screen 1) and 5th (screen 2) leaf stages with respect to CAD. In the 2nd leaf stage, KxS had the highest CAD ($\pm$ SEM) response, with a value of $1582.6 \pm 301.9$, which was at least 10-fold greater than all other switchgrass populations. However, Summer, SxK, and Kanlow were not significantly different in the 2nd leaf stage. In the 5th leaf stage, KxS, SxK, and Summer were all statistically similar with relatively high CAD’s; however, Kanlow was significantly lower than all other populations with a CAD ($\pm$ SEM) of $212.6 \pm 210.4$. At the 5th leaf stage, KxS, SxK, and Summer all had a CAD value more than 10-fold greater than Kanlow. Both Summer and SxK were characterized by a relatively low CAD in the 2nd leaf stage and high CAD in the 5th leaf stage. Sorghum supported large densities of aphids in both the early and late
developmental stage with mean CAD’s (± SEM) of 1534.0 ± 160.5 and 45596.7 ± 4298.6, respectively. However, the purpose of sorghum was to provide a well-documented check in the study, thus data is presented here, but not considered in the analysis among switchgrass populations.

No significant differences were measured among switchgrass populations for damage ratings in the 2nd leaf stage for *S. graminum* (Screen 1 damage: $F = 0.81; \text{df} = 3, 35; P = 0.5$). However, in the 5th leaf stage, Kanlow, with a mean damage rating of 1.15, was found to have a significantly lower damage rating than all other switchgrass populations (Screen 2 damage: $F = 4.36; \text{df} = 3, 33; P = 0.01$). The low damage rating for Kanlow in the 5th leaf stage is consistent with the very low CAD, relative to all other switchgrass populations. Although not statistically significant, Kanlow also had the lowest mean damage rating for *S. graminum* in the 2nd leaf stage screen. Further, Kanlow was the only population to have consistently low values for both damage ratings and CAD in both leaf stages, suggesting that Kanlow may have resistance, specifically antibiosis, to *S. graminum* relative to Summer, KxS, and SxK.

Based on the damage ratings, both SxK and KxS were determined to be moderately resistant (MR) to *S. graminum* in 2nd and 5th leaf stages (Table 2.3). Kanlow was moderately resistant in the 2nd leaf stage, however in the 5th leaf stage, it was the only population to show strong resistance to damage and was characterized as highly resistant (HR). Summer was the only switchgrass population to be characterized as moderately susceptible (MS), with a damage rating (± SEM) of 3.10 ± 0.41 in the 2nd leaf stage. However, in the 5th leaf stage, Summer was classified as moderately resistant (MR). In general, none of the switchgrass populations sustained high degrees of injury from *S.*
graminum infestations, with damage being fairly moderate. Conversely, the sorghum check received very high damage ratings, with a mean rating of 5.0 in both developmental stages.

**Screening Studies 3 & 4.** Significant differences were found among switchgrass populations for CAD and damage ratings in both 2\textsuperscript{nd} and 5\textsuperscript{th} leaf screens for *S. flava* (Table 2.2) (Screen 3 CAD: F = 5.44; df = 3, 34; P = .0036; Screen 3 damage: F = 24.45; df = 3, 34; P < .0001; Screen 4 CAD: F = 6.69; df = 3, 34; P = .0011; Screen 4 damage: F = 10.05; df = 3, 34; P < .0001). However, the relative ranking of the populations was not consistent for either parameter between screens. Although not significantly different from KxS, Kanlow had the lowest CAD in Screen 3 (2\textsuperscript{nd} leaf stage), with a mean (± SEM) of 145.0 ± 43.0. SxK and Summer were not statistically different from each other and had the highest CAD (± SEM) in the 2\textsuperscript{nd} leaf stage screen, with 453.7 ± 56.4 and 366.5 ± 55.7 respectively. KxS fell relatively in the middle, and was not significantly different from either group.

The screen for *S. flava* in the 5\textsuperscript{th} leaf stage showed Kanlow to have the lowest CAD again, although it was not significantly different from SxK. Likewise, Summer had the greatest CAD (± SEM) of 1369.2 ± 148.9. However, a significant difference could not be determined between Summer and KxS. Comparing between the 2\textsuperscript{nd} leaf stage and 5\textsuperscript{th} leaf stage screens, SxK and KxS did not demonstrate a constant pattern in CAD, while Summer and Kanlow were consistently high and low, respectively.

Damage ratings for *S. flava* on both leaf stages of switchgrass were significant, with trends generally following the same pattern as CAD. At the 2\textsuperscript{nd} leaf stage, SxK and Summer showed the most damage, with ratings (± SEM) of 4.80 ± 0.20 and 4.56 ± 0.18
respectively, though Summer was not significantly different from KxS either. Similarly, in the 5th leaf stage, damage ratings were greatest for Summer, being the only population to exhibit a mean damage rating (± SEM) over three, at 3.94 ± 0.24. Conversely, SxK had more moderate damage in the 5th leaf stage, along with KxS. While not significantly different from SxK, once again, Kanlow showed the least damage with a mean rating (± SEM) of 2.00 ± 0.25.

Generally, the damage observed for S. flava on all switchgrass populations was much greater than damage resulting from S. graminum infestations, with a few exceptions, as evidenced by the ratings. Damage ratings for S. flava resulted in Summer, SxK, and KxS all being characterized as susceptible (HS, HS, and MS, respectively) when infested in the 2nd leaf stage (Table 2.3). However, in the 5th leaf stage, only Summer was characterized as susceptible (MS), while Kanlow, KxS, and SxK were all moderately resistant (MR).

Barley had higher CAD values in both developmental stages than was observed in switchgrass with S. flava. In the 2nd and 5th leaf stage screen, barley had a CAD (± SEM) of 1281.2 ± 161.2 and 2530.8 ± 727.2, respectively. Further, mean damage ratings (± SEM) for S. flava on the 2nd and 5th leaf stages of barley was 3.22 ± 0.28 and 3.39 ± 0.44, respectively. As with sorghum in screens 1 and 2, barley served as the check for S. flava; however, this data is not considered in the analysis among switchgrasses.

Screening studies 5, 6, 7, & 8. Although attempts were made to establish R. padi and D. noxia on the selected switchgrass populations, efforts were not successful using either whole plants or clip cages. After infestation, subsequent evaluations showed all aphids dead, with few exceptions, and little to no reproduction having occurred. The only
exception was for *R. padi* on 2\textsuperscript{nd}-leaf stage switchgrass, with moderate survival noted in a few cases. However, both aphids did survive and reproduce on barley in both developmental stages. *R. padi* produced aphid densities (± SEM) of 101.3 ± 6.6 and 44.6 ± 5.5 aphids per cage in the early and late developmental stage, respectively. Similarly, *D. noxia* produced aphid densities (± SEM) of 75.1 ± 8.1 and 56.5 ± 12.6 aphids per cage in the respective stages. Thus, when compared to aphid numbers documented on barley using the same methods, it was determined that none of the switchgrass populations in this study were suitable reproductive or feeding hosts for *R. padi* and *D. noxia* (data not presented).

**Discussion and Conclusions**

A total of eight screening studies were completed to evaluate switchgrass for resistance to four species of aphids. Although switchgrass was determined not to be a suitable feeding and reproductive host for *R. padi* and *D. noxia*, interesting results were discovered for *S. graminum* and *S. flava*. Kanlow demonstrated the greatest resistance across all screens for *S. graminum* and *S. flava*, consistently yielding low CAD and damage ratings. These data demonstrate that Kanlow possesses relatively strong resistance relative to the other three populations of switchgrass. Further, the overall low aphid fecundity and survival on Kanlow, measured by CAD, suggests that the category of resistance for Kanlow is antibiosis. Antibiosis is a category of resistance whereby some quality(s) possessed by a plant negatively affects the biology or life history of the insect (Smith 2005, Dogramaci et al. 2007).

Kanlow’s resistance is corroborated by the consistently low damage ratings. Based on damage ratings alone, Kanlow was classified as moderately resistant to highly
resistant across all screens. Additionally, Kanlow was the only switchgrass population to be characterized as resistant in all evaluations. Although more explicit parameters were not quantified for plant biomass yield in this study, previous works have shown that damage rating scales may provide insights into plant resistance, and thus may have a correlation to plant yield. Smith et al. (1994) noted several variations on visual rating scales and their application for determining host plant tolerance. Further, it is especially important to note the potential presence of multiple-species resistance in Kanlow. According to Smith (2005), multi-species resistance has traditionally been difficult to develop and/or identify; however it provides many advantages and is much more economically and ecologically valuable. Consequently, this may make Kanlow an attractive candidate as development continues to pursue switchgrass genotypes with increased plant yield, particularly under biotic stress factors.

Summer was consistently one of the most susceptible populations of switchgrass tested, with the exception of the screen for *S. graminum* in the 2*nd* leaf stage where its CAD was the lowest among all populations. However, in all other screens for *S. graminum* and *S. flava*, Summer routinely had large CAD values. Additionally, summer generally had the highest damage ratings recorded and was statistically significant among the highest ratings in each screen. The consistently high damage ratings for Summer may further help explain some of the inconsistent CAD values observed in Summer. Specifically, Summer often showed damage much faster and at much lower aphid densities than the other populations. Summer generally showed chlorosis, and in some cases tissue necrosis, and aphid populations suffered due to a lack of healthy tissue for feeding. Because CAD integrates aphid population density over time, it may mask aphid
accumulation rates (e.g., aphid density may have built up rapidly and then collapsed as a result). This was especially apparent with *S. flava*, which tended to cause more damage, quickly to all switchgrass populations screened. Another factor that undoubtedly contributed to some of the variation observed is the significant genetic diversity among the switchgrasses tested. Each population consists of multiple genotypes and, as a result, large variations may be anticipated.

Results from screens for both *S. graminum* and *S. flava* revealed KxS generally had high CAD, yet moderate damage ratings. KxS had greater than 10-fold the CAD of all other populations for *S. graminum* in the 2\textsuperscript{nd} leaf stage; however, damage ratings in that screen were consistent with all switchgrass despite the significantly greater aphid pressure. Similarly, with *S. flava*, KxS displayed CAD values statistically similar to the highest in each leaf stage. However, in both cases KxS also had significantly lower damage ratings than the other populations with the largest CAD values, except for Summer in the 2\textsuperscript{nd} leaf stage which was not statistically different. These data suggest that tolerance may be present in KxS, an important category of resistance to both *S. graminum* and *S. flava*.

There was no clear trend for SxK. When infested with *S. graminum*, CAD values were relatively low in the 2\textsuperscript{nd} leaf stage, but high in the 5\textsuperscript{th}. Otherwise, for *S. flava*, SxK had a relatively high CAD in the 2\textsuperscript{nd} leaf stage, however, a moderate to low CAD in the 5\textsuperscript{th}. Damage ratings for SxK were relatively modest and were classified as moderately resistant (MR) in all screens except with *S. flava*, infested at the 2\textsuperscript{nd} leaf stage. In that case, SxK was classified as highly susceptible (HS) with a mean damage rating (± SEM) of 4.80 ± 0.20 and most plants nearing death.
The resistance of the KxS and SxK populations in relationship to their Summer and Kanlow parents indicate that they inherited some resistance to *S. graminum* and *S. flava* from their Kanlow parent. The resistance appears to be quantitative. There did not appear to be a distinct difference among the two experimental populations for insect resistance based on the female or cytoplasm source. Crosses between Summer and Kanlow plants are being made to improve winter tolerance of the resulting high yielding populations for use in the northern states of the USA where Kanlow is unadapted. These results indicate that there will also be some insect resistance benefits.

These studies provide valuable baseline information concerning the host suitability of switchgrass to potential aphid pests and the plant-insect interactions within a system that has been largely overlooked. We were able to demonstrate with these studies that switchgrass is a suitable host for at least two species of aphids, *S. graminum* and *S. flava*, which could become important pests under the right conditions. Previous studies had described switchgrass as an unsuitable feeding and reproductive host for *S. graminum* (although it is unknown what biotype(s) the author used) (Kieckhefer 1984). In addition, we were able to demonstrate that differential resistance is present among these four tetraploid switchgrass populations. In another study, differential resistance was also found among several octaploid switchgrass cultivars to *S. frugiperda*, with the cultivars ‘Trailblazer’ and ‘Blackwell’ being the most resistant (Dowd and Johnson 2009). The information gained here and in other studies will prove useful to more detailed analysis aimed at dissecting the mechanisms underlying the plant-insect interactions within this system.
As with any cropping system, developing switchgrass into a sustainable bioenergy crop will require effective pest management strategies. Loss of biomass yield through insect damage has the potential to significantly impact the profitability and, ultimately, the long-term sustainability of switchgrass as a bioenergy crop. Further, getting ahead of the curve within this system is made even more critical, since biomass loss due to insect damage could provide a serious setback to this nascent sector. This work helps to provide a foundation for the development of sustainable pest management strategies, by identifying differential resistance in switchgrass populations to two important aphid species. Plant resistance is an important form of control, and further research will be needed to identify potential new pests in switchgrass and explore both the categories of resistance as well as the mechanisms involved. Collectively, this will better understanding of plant-insect interactions and provide advance guidance to deal with any emerging insect threats.
Table 2.1. Mean ± SEM cumulative aphid days (CAD) and damage ratings for switchgrass populations infested with *S. graminum* in the 2\(^{nd}\) (Screen 1) and 5\(^{th}\) (Screen 2) leaf stages.

<table>
<thead>
<tr>
<th>Switchgrass population</th>
<th>2(^{nd}) Leaf Stage</th>
<th>5(^{th}) Leaf Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAD</td>
<td>Mean damage rating(^1)</td>
</tr>
<tr>
<td>KxS</td>
<td>1582.6 ± 301.9a</td>
<td>2.67 ± 0.47a</td>
</tr>
<tr>
<td>SxK</td>
<td>133.1 ± 55.7b</td>
<td>2.70 ± 0.33a</td>
</tr>
<tr>
<td>Summer</td>
<td>65.7 ± 9.6b</td>
<td>3.10 ± 0.41a</td>
</tr>
<tr>
<td>Kanlow</td>
<td>109.3 ± 34.5b</td>
<td>2.20 ± 0.44a</td>
</tr>
</tbody>
</table>

Treatment means within the same column followed by the same letter indicate no significant differences (P > 0.05), LSD test.

\(^1\)Damage ratings based on 1-5 scale, 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 area damaged; and 5 = 71% or more of the leaf area damaged and the plant near death (Heng-Moss et al. 2002).
Table 2.2. Mean ± SEM cumulative aphid days (CAD) and damage ratings for switchgrass populations infested with *S. flava* in the 2\textsuperscript{nd} (Screen 3) and 5\textsuperscript{th} (Screen 4) leaf stages.

<table>
<thead>
<tr>
<th>Switchgrass population</th>
<th>2\textsuperscript{nd} Leaf Stage</th>
<th>5\textsuperscript{th} Leaf Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAD</td>
<td>Mean damage rating(^1)</td>
</tr>
<tr>
<td>SxK</td>
<td>453.7 ± 56.4(^a)</td>
<td>4.80 ± 0.20(^a)</td>
</tr>
<tr>
<td>Summer</td>
<td>366.5 ± 55.7(^a)</td>
<td>4.56 ± 0.18ab</td>
</tr>
<tr>
<td>KxS</td>
<td>296.5 ± 103.3ab</td>
<td>3.89 ± 0.51b</td>
</tr>
<tr>
<td>Kanlow</td>
<td>145.0 ± 43.0b</td>
<td>1.5 ± 0.27c</td>
</tr>
</tbody>
</table>

Treatment means within the same column followed by the same letter indicate no significant differences (P > 0.05), LSD test.

\(^1\)Damage ratings based on 1-5 scale, 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 area damaged; and 5 = 71% or more of the leaf area damaged and the plant near death (Heng-Moss et al. 2002).
Table 2.3. Characterization of resistance levels based on damage ratings for *S. graminum* and *S. flava* in Screens 1, 2, 3, & 4.

<table>
<thead>
<tr>
<th>Switchgrass population</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; Leaf Stage</th>
<th>5&lt;sup&gt;th&lt;/sup&gt; Leaf Stage</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; Leaf Stage</th>
<th>5&lt;sup&gt;th&lt;/sup&gt; Leaf Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer</td>
<td>MS</td>
<td>MR</td>
<td>HS</td>
<td>MS</td>
</tr>
<tr>
<td>SxK</td>
<td>MR</td>
<td>MR</td>
<td>HS</td>
<td>MR</td>
</tr>
<tr>
<td>KxS</td>
<td>MR</td>
<td>MR</td>
<td>MS</td>
<td>MR</td>
</tr>
<tr>
<td>Kanlow</td>
<td>MR</td>
<td>HR</td>
<td>HR</td>
<td>MR</td>
</tr>
</tbody>
</table>

HS, highly susceptible; MS, moderately susceptible; MR, moderately resistant; HR, highly resistant.
CHAPTER 3

Categories of resistance to greenbug and yellow sugarcane aphid (Homoptera: Aphididae) in three tetraploid switchgrass populations.

Introduction

Switchgrass, *Panicum virgatum* L., a perennial warm-season grass native to the tallgrass prairie regions of North America, east of the Rocky Mountains, is being developed as a bioenergy crop for marginal soils in the USA (Sarath et al. 2008, Vogel et al. 2011). It is a polyploid species, with a range of ploidys (Costich et al. 2010), however the tetraploid switchgrasses, which occur as upland or lowland ecotypes possess the best yield attributes (Hultquist et al. 1997, Vogel et al. 2011, Young et al. 2012). In addition, hybrids between certain upland and lowland tetraploid populations display heterosis for biomass yields (Martinez-Reyna and Vogel 2008).

While switchgrass has received increased agronomic attention, it is likely that large-scale plantings of this species will result in insect infestations that could negatively impact establishment and yields. As an example, in the related native warm-season perennial, buffalograss, *Buchloë dactyloides* (Nuttall) Engelmann, an emergence in multiple important pests was demonstrated with increased use of this species as a turfgrass (Baxendale et al. 1999, Heng-Moss et al. 2002). Likewise, recent work has demonstrated that insect problems may occur, particularly as production increases (Mitchell et al. 2008, Dowd and Johnson 2009, Prasifka et al. 2009a, Adamski et al. 2010). In 2004, a poorly understood species, *Blastobasis repartella* (Dietz), was rediscovered, and appears to be a monophagous stem-borer restricted to switchgrass (Prasifka et al. 2009a, Adamski et al. 2010). Four additional lepidopterans, *Spodoptera frugiperda* (J.E. Smith) (Dowd and Johnson 2009, Prasifka et al. 2009b, Nabity et al. 2010).
Dowd et al. 2012, *Mythimna unipuncta* (Haworth) (Prasifka et al. 2011a), *Papaipema nebris* (Guenée), and *Haimbachia albescens* Capps (Prasifka et al. 2011b) have also been recently documented on various populations of switchgrass. Burd et al. (2012) demonstrated that multiple switchgrass populations are suitable hosts to several important cereal aphids including: *Schizaphis graminum* (Rondani) (greenbug), *Rhopalosiphum padi* (L.) (bird-cherry oat aphid), *R. maidis* (Fitch) (corn leaf aphid), and *Sipha flava* (Forbes) (yellow sugarcane aphid). Additionally, other important insect pests have been more incidentally documented in association with switchgrass, including grasshoppers (Acrididae) (Vogel 2004, Parrish and Fike 2005). As a result, the prevalence of those and other potential pests in switchgrass may increase as the agricultural landscape changes to accommodate increased production of bioenergy feedstocks (Bouton 2008, Mitchell et al. 2008).

One of the most effective and sustainable strategies for controlling insect pests has been the development of insect-resistant plants. According to Smith (1998, 2005), hundreds of insect-resistant cultivars are currently grown in the U.S., offering substantial economic and environmental benefits. Insect-resistant plants provide an attractive pest management strategy by reducing insecticide application, resulting in the reduction of input costs and harsh chemicals in the environment. Further, plant resistance may improve the efficiency of insect biological control agents, synergizing the interactions between the insect-resistant plants and natural enemies by decreasing the vigor of the insect pest (Smith 1998, 2005). Dowd and Johnson (2009) noted that the apparent lack of insect pest problems in switchgrass suggested that insect resistance genes are present. Differential resistance has been documented among switchgrass populations to potential
insect pests including, *S. frugiperda* (Dowd and Johnson 2009, Dowd et al. 2012), as well as *S. flava* and *S. graminum* (Chapter 2). Further, Dowd et al. (2012) were able show that multiple resistance mechanisms may be at work; however, the categories and relative levels of resistance being expressed among these populations have remained undocumented.

Anitbiosis, antixenosis, and tolerance are important categories of resistance and have all been used as tactics for integrated pest management. Insect resistant switchgrasses may effectively contribute to pest management strategies by negatively affecting the pest insect’s biology (antibiosis), behavior (antixenosis), and/or by tolerating or repairing (tolerance) the injury resulting from the insect pest. Identifying these categories is critical for characterizing the biological mechanisms of resistance and improving our knowledge of the plant-insect interactions within this system. The objective of this research was to characterize the categories (antibiosis and tolerance) and relative levels of antibiosis and tolerance of selected tetraploid switchgrass populations to two potential aphid pests (*S. flava* and *S. graminum*).

**Materials and Methods**

**Plant material.** Two no-choice studies were performed to evaluate the categories and relative levels of antibiosis and tolerance of three switchgrass populations: Kanlow (lowland cultivar), Summer (cultivar) (Alderson and Sharp 1994, Vogel and Mitchell 2008), and a hybrid between Kanlow and Summer plants, hereafter referred to as KxS, developed by Dr. Kenneth Vogel, USDA-ARS, Lincoln, NE, by intermating Kanlow (male) and Summer (female) plants to produce hybrids (Martinez-Reyna and Vogel 2008,
Vogel and Mitchell 2008). Seeds for all populations were provided by Dr. Kenneth Vogel.

**Insect colonies.** The switchgrasses were evaluated for the categories of resistance to *S. flava* and *S. graminum* (biotype I). Aphid colonies were obtained from Dr. John D. Burd, USDA-ARS in Stillwater, Oklahoma. The *S. flava* colony was maintained on a continuous supply of ‘Haxby’ barley plants, while *S. graminum* was maintained on a susceptible sorghum cultivar ‘BCK60’. Both colonies were maintained in the greenhouse at 25 ± 7°C and 16:8 (L:D) h within clear plastic cages, approximately 12.5 cm diameter and ventilated with organdy fabric. Attempts were made to condition aphids on the same switchgrass population and developmental stage they were to be tested on for at least one week; however, efforts to condition enough of either aphid species on Kanlow were unsuccessful. As a result, all aphids were conditioned for at least one week prior to the beginning of each study on Summer, which had preliminarily been identified as the susceptible population (Chapter 2), in the 2nd leaf stage.

**Category Studies.** Two no-choice studies were performed to identify the presence of antibiosis and/or tolerance in three switchgrass populations (Summer, Kanlow, and KxS) to *S. graminum*, biotype I (Study 1) and *S. flava* (Study 2). In each study, the susceptible sorghum BCK60, was included as a control (in a similar developmental stage), to provide a well-known standard for both aphids. The experimental design for both studies was a completely randomized design with a 3 by 4 factorial (three levels of aphid infestation and three switchgrass populations, plus sorghum) and 10 replications. At the onset of both experiments, plants within a population were placed into groups of three according to similar height and quality.
Plants within the group were then randomly assigned an infestation level of 0, 5, or 10 aphids. This provided an uninfested control (0), a low infest level (5), and a high infest level (10). The corresponding number of apterous, adult aphids was transferred to each plant with a fine paintbrush and then caged with tubular plastic cages (4 cm diameter by 46 cm height) with vents 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.

*Tolerance Evaluation.* Plants were rated for aphid damage twice a week by using a visual damage rating on a 1-5 scale. Damage ratings served as a visual assessment of the injury sustained by the plant from aphid feeding (Smith et al. 1994). The damage rating scale was adopted from Heng-Moss et al. (2002), 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 area damaged; and 5 = 71% or more of the leaf area damaged and the plant near death. Plant damage was characterized by chlorosis, a reddish discoloration, or desiccation of the leaf. Experiments were terminated 21 days after initial aphid introduction, at which point, mean damage ratings had reached 4 for a given population, or aphid numbers and damage ratings plateaued across all populations of switchgrass. Plant heights and dry weights were then determined at the conclusion of each experiment. Plant biomasses were determined after placing the plant material in an oven 60°C for one week.

Aphid damage ratings, plant heights, and biomasses were used to calculate two functional plant loss indices (FPLIs) (Panda and Heinrichs 1983, Wu et al. 1986, Smith et al. 1994, Heng-Moss et al. 2003) to assess the relative levels of tolerance among the
selected switchgrass populations. The FPLIs were calculated using the following formulae:

\[
FPLI_{\text{(biomass)}} = 1 - \left( \frac{\text{biomass of infested plant}}{\text{biomass of control plant}} \right) \times \left( 1 - \frac{\text{damage rating}}{5} \right) \times 100
\]

\[
FPLI_{\text{(height)}} = 1 - \left( \frac{\text{height of infested plant}}{\text{height of control plant}} \right) \times \left( 1 - \frac{\text{damage rating}}{5} \right) \times 100
\]

In both FPLIs, lower values indicate the presence of tolerance, while higher values indicate a lack of tolerance.

**Antibiosis Evaluation.** The same plants used in the tolerance studies were also evaluated for the presence antibiosis. To assess antibiosis, aphids were introduced to the plants at two infestation levels (5 and 10) and confined (as described above). To evaluate the plants’ effect on aphid fecundity and survival, aphids were counted on each plant 7 and 14 days after aphid introduction. Because aphid counts at a given time only provide a snapshot in time, the plants’ effects on aphid multiplication over time were also evaluated by performing aphid counts twice a week (during evaluations for plant damage) for the duration of the experiment and calculating cumulative aphid days (CAD) using the following formula: 

\[
\text{CAD} = \sum_{i=1}^{n} \left( \frac{N_i + N_{i+1}}{2} \right) \times T, \text{ where } N_i \text{ is the total number of aphids on a plant at a given evaluation date } i, \text{ } N_{i+1} \text{ is the total number of aphids on the same plant on the subsequent evaluation date, and } T \text{ is the number of days between the two evaluation dates (Hanafi et al. 1989).}
\]

As described above, evaluations were performed for 21 days.

**Statistical analysis.** Generalized linear mixed model analyses (PROC GLIMMIX, SAS Institute 2011) were conducted for each functional plant loss index.
(FPLI) to detect population differences in switchgrass tolerance to aphid feeding. For the antibiosis evaluations, the mean number of aphids at 7 and 14 days after infest was analyzed as a repeated measures design using generalized linear mixed model analyses (PROC GLIMMIX). Cumulative aphid days (CAD), used to detect the effect that each population of switchgrass had on aphids over time, was analyzed using generalized linear mixed model analyses (PROC GLIMMIX) after a square root transformation of the data to meet the assumptions of the generalized linear mixed model analysis. Where appropriate, means were separated using Fisher’s least significant difference (LSD) procedure ($\alpha = 0.05$).

**Results**

**Tolerance Studies.** *Tolerance Study 1.* Statistically significant differences were detected among the grasses for the FPLI index based on plant biomass with *S. graminum*, for both infestation levels (Figure 3.1) (5-aphids: $F = 8.13$; df $= 3, 72$; $P < 0.0001$; 10-aphids: $F = 8.53$; df $= 3, 72$; $P < 0.0001$). The FPLI based on plant biomass was highest for the susceptible sorghum check at both infestation levels; however, KxS was not significantly different from sorghum at either the 5-aphid or 10-aphid infestation level. Summer had significantly lower FPLI values at both infestation levels than sorghum, as well as KxS at the higher infestation level. Kanlow had significantly lower FPLI values than any of the other grass treatments; however, as demonstrated by the results for antibiosis, that can likely be attributed to its strong antibiotic response. Thus, FPLI values for Kanlow were deemed to be skewed, and are not presented.

Significant differences in the FPLI index based on plant height were also detected among the grasses evaluated for the two *S. graminum* infestation levels (Figure 3.2) (5-
aphids: \( F = 6.65; \) df = 2, 54; P < 0.003; 10-aphids: \( F = 7.88; \) df = 2, 54; P = 0.001).

Similar to the FPLIs for biomass, FPLI values based on plant height were highest for the susceptible sorghum check and KxS, with no significant differences detected between sorghum and KxS at either infestation level. Again, Summer had significantly lower FPLI values at both infestation levels than sorghum and KxS. Collectively, the FPLI values based on plant biomass and plant height indicate the presence of tolerance in Summer to *S. graminum*.

**Tolerance Study 2.** Significant differences in the FPLI index based on plant biomass for *S. flava* were also detected among grasses for both levels of infestation (Figure 3.3) (5-aphids: \( F = 9.80; \) df = 3, 71; P < 0.0001; 10-aphids: \( F = 12.09; \) df = 3, 71; P < 0.0001). The susceptible sorghum had the highest FPLI values for *S. flava* at both infestation levels. The FPLI values for Summer were not significantly different from sorghum at either infestation level, indicating a lack of tolerance. The mean FPLI value for KxS at the 5-aphid infestation level was significantly lower than both Summer and sorghum. For the 10-aphid infestation level, the FPLI value for KxS was significantly lower than sorghum, however it was not significantly different from Summer. Again, Kanlow had the lowest FPLI values among grasses; however, it is not presented since the low FPLI values were considered to be a product of its strong antibiotic effect.

Significant differences in the FPLI based on plant height were also detected among treatments for both *S. flava* infestation levels (Figure 3.4) (5-aphids: \( F = 16.59; \) df = 3, 72; P < 0.0001; 10-aphids: \( F = 21.40; \) df = 3, 72; P < 0.0001). At the 5-aphid infestation level, sorghum had a mean FPLI value significantly higher than both Summer and KxS. However, the mean FPLI was significantly lower for KxS than Summer for the
low infestation level. Sorghum also produced the highest FPLI value at the 10-aphid infestation level; however, it was not significantly different from Summer for that infestation level. No significant difference was detected between KxS and Summer for FPLI at the high infestation level, however the FPLI value for KxS was significantly lower than sorghum.

**Antibiosis Studies. Antibiosis Study 1.** Significant differences were detected among the three switchgrass populations and sorghum for the mean number of *S. graminum* at both infestation levels (Table 3.1) (5 aphids: F = 12.23; df = 3, 72; P < 0.0001; 10 aphids: F = 7.05; df = 3, 72; P = 0.0003). The susceptible sorghum cultivar BCK60, included in this evaluation as a check, consistently had the highest mean number of *S. graminum* among the grasses tested at all time points and infestation combinations. However, at the 14-day and 10-aphid infestation level, no significant differences were detected among any of the switchgrasses and sorghum for mean aphid numbers, despite sorghum supporting at least twice as many aphids as any switchgrass population. This was likely the result of the large variation among replications for that treatment combination. Also, aphid counts were generally skewed among the susceptible grasses within the 14-day, 10-aphid infestation level due to aphid populations overwhelming the susceptible plants, resulting in the reduction of plant quality and subsequent decline in aphid numbers. Thus, that treatment combination was found to be less meaningful than others.

At the 7-day time point and 5-aphid infestation level, KxS was not significantly different from the susceptible sorghum, with mean aphid numbers (± SEM) of 34.2 ± 5.2 and 45.2 ± 4.6, respectively. Further, KxS consistently supported the most *S. graminum*
among the three switchgrass populations tested at all treatment combinations, and had significantly more aphids than both Kanlow and Summer at both 7 and 14 days for the 5-aphid infestation level. The mean *S. graminum* (± SEM) for KxS was 38.3 ± 7.6 at the 7-days, 10-aphid infestation level; however, it did not support aphid numbers that were significantly higher than Summer, which had 28.5 ± 6.8 aphids.

The mean number of *S. graminum* among switchgrass populations was consistently the lowest for Kanlow at all treatment combinations. Kanlow supported significantly fewer aphids than Summer and KxS within both the 5-aphid and 10-aphid infestation levels at the 7-day evaluation, with mean aphid numbers (± SEM) of 7.3 ± 3.6 and 8.2 ± 1.8, respectively. Kanlow also had significantly fewer aphids than KxS at the 14-day, 5-aphid infestation level, with mean aphid numbers (± SEM) of 4.4 ± 2.5 and 51.8 ± 24.3, respectively, for the two populations. Although no significant differences were detected among any of the grasses at the 14-day, 10-aphid infestation level, Kanlow supported a mean aphid number (± SEM) of 1.3 ± 0.7; less than one-tenth of the aphids supported by the next lowest population, Summer, with 14.7 ± 6.8 mean aphids.

Cumulative aphid days (CAD) were also significant at both 5-aphid and 10-aphid infestation levels with *S. graminum* (Table 3.3) (5 aphids: $F = 27.19; df = 3, 72; P < 0.0001$; 10 aphids: $F = 17.20; df = 3, 72; P < 0.0001$). Generally, CAD for *S. graminum* supported the results for mean aphid numbers at 7, and 14 days after aphid introduction. At both the low and high infestation level, the susceptible sorghum check was significantly higher than any of the switchgrasses with mean CADs (± SEM) of 998.9 ± 133.4 and 883.8 ± 116.5, respectively. Although not significantly different from Summer at the 10-aphid infestation level, KxS produced the largest response among the three
switchgrass populations, with mean CADs (± SEM) of 614.0 ± 170.7 and 412.7 ± 76.8 at the 5-aphid and 10-aphid infestation levels, respectively. The mean CAD response for Kanlow was significantly lower than both Summer and KxS at both 5-aphid and 10-aphid infestation levels. Kanlow had a mean CAD (± SEM) of 73.0 ± 31.6 at the 5-aphid infestation level and 70.1 ± 10.7 at the 10-aphid infestation level. Overall, the mean CADs for Kanlow were less than one-half of those for Summer and KxS at both infestation levels. No significant differences were detected between infestation levels within the switchgrass populations and sorghum (F = 0.98; df = 3, 72; P = 0.41).

*Antibiosis Study 2.* Significant differences were also detected among switchgrass populations and sorghum for the mean number of *S. flava* at both infestation treatment levels (Table 3.2) (5 aphids: F = 14.63; df = 3, 72; P < 0.0001; 10 aphids: F = 9.95; df = 3, 72; P < 0.0001). A significant interaction between treatment and infestation level (F = 3.03; df = 3, 72; P < 0.035), and treatment and time (evaluation date after infestation) (F = 6.13; df = 3, 72; P < 0.001) was also detected. Results for the mean aphid numbers at 7 and 14 days after infestation were similar between the *S. graminum* and *S. flava* evaluations; however, the relative rank of KxS and Summer was generally exchanged between the two studies. The susceptible sorghum check was consistently among the highest of all grasses for the mean number of *S. flava* at all time points and infestation combinations. At 7 days after aphid introduction, sorghum had significantly higher mean aphid numbers at both the 5-aphid and 10-aphid infestation levels than all switchgrass populations. However, at the 14-day mark, sorghum was not significantly different from Summer for the 5-aphid infestation level, or Summer and KxS for the 10-aphid infestation level.
When considering the 5-aphid infestation level, Summer had significantly more *S. flava* than all other switchgrass populations at both the 7-day and 14-day evaluations, with mean aphid numbers of 28.1 ± 6.1 and 110.9 ± 24.5, respectively. For the 10-aphid infestation level, Summer was not significantly different from KxS at either time point; however, both had significantly greater mean aphid numbers than Kanlow at both the 7-day and 14-day evaluations.

Similar to the results for *S. graminum*, Kanlow consistently had the lowest mean aphid numbers for *S. flava* as well. Although Kanlow was not significantly different from KxS for the 5-aphid infestation level at either time point, KxS had nearly a 2-fold higher mean aphid number (± SEM) than Kanlow at the 7-day evaluation (11.2 ± 2.7 and 6.3 ± 3.2, respectively), and over a 3-fold difference at the 14-day mark (33.4 ± 7.8 and 10.7 ± 5.3, respectively). For the 10-aphid infestation level, Kanlow produced significantly fewer aphids at both evaluation dates than Summer and KxS. Further, for the 10-aphid infestation level and both evaluation dates, the mean number of aphids for Kanlow was less than one-sixth of those for either of the other populations of switchgrass.

Significant differences were also detected among the grasses for CADs at both the 5-aphid and 10-aphid infestation levels with *S. flava* (Table 3.3) (5 aphids: $F = 14.26$; df = 3, 72; $P < 0.0001$; 10 aphids: $F = 20.99$; df = 3, 72; $P < 0.0001$). Over the duration of the experiment, Summer sustained the highest number of *S. flava* for both the 5-aphid and 10-aphid infestation levels among switchgrasses. For the 5-aphid infestation level, Summer had a CAD (± SEM) of 1694.2 ± 310.5, which was significantly higher than any other switchgrass. Summer also had the highest CAD for the 10-aphid infestation level, with a CAD (± SEM) of 2471.7 ± 268.8; however, that was not significantly different
from KxS within the same level, which had a CAD (± SEM) of 1763.4 ± 266.2. Kanlow produced the lowest CADs for both the 5-aphid and 10-aphid infestation levels, with mean CADs (± SEM) of 198.5 ± 86.5 and 283.9 ± 132.0, respectively. At both infestation levels, CADs for Kanlow were significantly less than both KxS and Summer. Further, Summer sustained CADs that were more than 8-fold higher than the CADs for Kanlow within both infestation levels.

Discussion and Conclusions

Two no-choice studies were completed to assess the relative levels of antibiosis and tolerance among three populations of switchgrass to two important aphid species. Antibiosis studies evaluated aphid survival and multiplication among the switchgrasses. Significant differences were found in mean aphid numbers at both the 7-day and 14-day evaluations, suggesting that antibiosis is an important category of resistance in switchgrass to both *S. graminum* and *S. flava*. In evaluations for both species of aphid, Kanlow consistently had the lowest mean aphid numbers within all time points and infestation levels. Further, mean aphid numbers remained stable for Kanlow across all treatment combinations, regardless of infestation level or date. Kanlow also supported the lowest mean CADs within both infestation levels of *S. graminum* and *S. flava*, among all grasses tested. Collectively, these data demonstrate that Kanlow did have adverse effects on the reproduction and/or survival of both aphid species tested. These results are important and demonstrate that Kanlow possesses multiple-species resistance. Multi-species resistance is an important quality; however, it has traditionally been difficult to identify/develop (Smith 2005). According to Smith (2005), “multiple-species resistance provides many advantages and is much more economically and ecologically valuable
because it may result in a greater reduction in the total amount of insecticides applied to
the system than reductions resulting from a cultivar with resistance to only one pest, and
may be helpful in avoiding the emergence of a secondary pest species as a primary pest”.
Accordingly, this makes Kanlow an attractive candidate for providing traits that can be
used to improve switchgrass germplasm for bioenergy.

Interestingly, Summer and KxS had different relative responses depending on the
aphid species evaluated. In general, results indicate the presence of tolerance, and
possibly low levels of antibiosis, in Summer to *S. graminum*. However, for *S. flava*, the
data suggest that KxS possesses low levels of antibiosis along with possible low levels of
tolerance. Both the infestation level (as indicated by CAD) and time (indicated by mean
aphid numbers) seem to be important in the evaluation of Summer for antibiosis. Mean
aphid numbers demonstrated that Summer resulted in a reduction of *S. graminum* over
time. At the early evaluation time, Summer had significantly more *S. graminum* than
Kanlow for both infestation levels. However, at 14-days after aphid introduction, aphid
numbers had declined and Summer was not significantly different from the resistant
population, Kanlow. However, results for CAD demonstrated that Summer did support a
significantly higher number of *S. graminum* than Kanlow throughout the experiment.
Further, CAD for *S. graminum* showed that Summer was not significantly different from
KxS for the high infestation level. Altogether, this suggests that Summer may posses
moderate levels of antibiosis to *S. graminum* at lower infestation levels and over time.

The more prominent category of resistance to *S. graminum* within Summer seems
to be tolerance. Both FPLI values (plant biomass and plant height) indicate the presence
of tolerance in Summer to *S. graminum*. The FPLI values for *S. graminum* demonstrated
that KxS was not significantly different from the susceptible sorghum, indicating the lack of tolerance. However, Summer had significantly lower FPLI values (based on plant height) than KxS at both infestation levels, and significantly lower FPLI (based on plant biomass) than KxS at the 10-aphid infestation level. It is particularly noteworthy that Summer did not have a significantly different CAD from KxS for the higher infestation level, yet still had significantly lower FPLI values within that infestation level for both indices. Based on these data, tolerance is an important category of resistance to *S. graminum* for Summer.

Evaluations for *S. flava* demonstrated that Summer was the susceptible population relative to the other switchgrasses, with consistently high aphid numbers, CAD values, and FPLI values. Generally, these data indicate that Summer lacks both antibiosis and tolerance to *S. flava*. However, the results for mean aphid numbers and CAD indicate that KxS possesses a moderate level of antibiosis to *S. flava*, relative to Summer. In all parameters for antibiosis, KxS had significantly lower values than Summer at the low infestation level. However, at the high infestation level, the antibiotic effect of KxS appears to be overcome by *S. flava* and no significant differences for aphid populations exist between KxS and Summer. While KxS did have significantly lower FPLI values for both plant biomass and plant height than Summer, significant differences only occurred at the low infestation levels. However, KxS also supported significantly fewer *S. flava* at the low infestation level than Summer, making it difficult to determine if low FPLI values were a result of plant tolerance, or simply less aphid pressure. At the high infestation level of *S. flava*, no significant differences were detected among Summer and KxS for FPLI values.
Interestingly, while Kanlow had a consistent antibiotic response to both aphid species, Summer and KxS had an inverse response with *S. graminum* and *S. flava*. This indicates multiple resistance mechanisms are present in switchgrass and raises interesting questions about the inheritance of the insect-resistance genes. It is also noteworthy that data for both FPLIs were consistent for both aphid species. While biomass yield is a particularly important parameter for a bioenergy feedstock, indices based on both plant biomass and plant height proved to be effective measures for assessing switchgrass tolerance with consistent responses.

This research provides valuable information and represents the first attempt at categorizing resistance in switchgrass. These studies demonstrated that both antibiosis and tolerance are important categories of resistance in tetraploid switchgrass populations being developed as bioenergy feedstocks. Most notably, we demonstrated that Kanlow possesses multiple-species resistance, with strong antibiotic effects against both *S. graminum* and *S. flava*, while tolerance is an important category of resistance for Summer to *S. graminum*. Collectively, this work provides a foundation for future investigation into insect-resistant switchgrasses to improve our understanding of the antibiotic and tolerant mechanisms involved.

The development of switchgrass with resistance to insects offers potential for proactively managing insect pests of biomass crops with an environmentally and economically sustainable solution. Identifying the categories of resistance is critical for understanding the underlying mechanisms of resistance and improving our knowledge of the plant-insect interactions within this system. Furthermore, identification of the
categories and mechanisms of resistance is critical for preserving resistance traits and developing integrated pest management strategies.
Table 3.1. Mean ± SEM\(^1\) number of *S. graminum* among switchgrass populations and sorghum at 7 and 14 days after initial introduction of 5 or 10 aphids.

<table>
<thead>
<tr>
<th>Switchgrass population</th>
<th>7 days after aphid introduction</th>
<th>14 days after aphid introduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 aphid infestation level</td>
<td>10 aphid infestation level</td>
</tr>
<tr>
<td>Kanlow</td>
<td>7.3 ± 3.6a</td>
<td>8.2 ± 1.8a</td>
</tr>
<tr>
<td>Summer</td>
<td>18.5 ± 3.8b</td>
<td>28.5 ± 6.8b</td>
</tr>
<tr>
<td>KxS</td>
<td>34.2 ± 5.2c</td>
<td>38.3 ± 7.6b</td>
</tr>
<tr>
<td>Sorghum</td>
<td>45.2 ± 4.6c</td>
<td>86.7 ± 8.4c</td>
</tr>
</tbody>
</table>

\(^1\)Treatment means within the same column followed by the same letter indicate no significant differences (P > 0.05), LSD test.
Table 3.2. Mean ± SEM$^1$ number of *S. flava* among switchgrass populations and sorghum at 7 and 14 days after initial introduction of 5 or 10 aphids.

<table>
<thead>
<tr>
<th>Switchgrass population</th>
<th>7 days after aphid introduction</th>
<th>14 days after aphid introduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 aphid infestation level</td>
<td>10 aphid infestation level</td>
</tr>
<tr>
<td>Kanlow</td>
<td>6.3 ± 3.2a</td>
<td>7.8 ± 2.9a</td>
</tr>
<tr>
<td>KxS</td>
<td>11.2 ± 2.7a</td>
<td>48.8 ± 10.4b</td>
</tr>
<tr>
<td>Summer</td>
<td>28.1 ± 6.1b</td>
<td>59.7 ± 8.9b</td>
</tr>
<tr>
<td>Sorghum</td>
<td>78.2 ± 6.3c</td>
<td>117.6 ± 13.5c</td>
</tr>
</tbody>
</table>

$^1$Treatment means within the same column followed by the same letter indicate no significant differences (P > 0.05), LSD test.
Table 3.3. Mean ± SEM\(^1\) cumulative aphid days (CAD) over duration of the study for switchgrass populations and sorghum infested with *S. graminum* and *S. flava* (5 and 10 aphid infest levels).

<table>
<thead>
<tr>
<th>Switchgrass population</th>
<th>5 aphid infestation level</th>
<th>10 aphid infestation level</th>
<th>5 aphid infestation level</th>
<th>10 aphid infestation level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kanlow</td>
<td>73.0 ± 31.6a</td>
<td>70.1 ± 10.7a</td>
<td>198.5 ± 86.5a</td>
<td>283.9 ± 132.0a</td>
</tr>
<tr>
<td>Summer</td>
<td>206.5 ± 53.5b</td>
<td>358.8 ± 123.3b</td>
<td>1694.2 ± 310.5c</td>
<td>2471.7 ± 268.8c</td>
</tr>
<tr>
<td>KxS</td>
<td>614.0 ± 170.7c</td>
<td>412.7 ± 76.8b</td>
<td>604.6 ± 122.2b</td>
<td>1763.4 ± 266.2bc</td>
</tr>
<tr>
<td>Sorghum</td>
<td>998.9 ± 133.4d</td>
<td>883.8 ± 116.5c</td>
<td>1033.1 ± 33.3bc</td>
<td>1229.1 ± 181.2b</td>
</tr>
</tbody>
</table>

\(^1\)Treatment means within the same column followed by the same letter indicate no significant differences (P > 0.05), LSD test.
Figure 3.1. FPLI values for *S. graminum* evaluations based on plant biomass (5 and 10 aphid infestation levels). Bars with the same letter in the same case are not significantly different (P > 0.05), LSD test. Lower FPLI value indicates tolerance.
Figure 3.2. FPLI values for *S. graminum* evaluations based on plant height (5 and 10 aphid infestation levels). Bars with the same letter in the same case are not significantly different (P > 0.05), LSD test. Lower FPLI value indicates tolerance.
Figure 3.3. FPLI values for *S. flava* evaluations based on plant biomass (5 and 10 aphid infestation levels). Bars with the same letter in the same case are not significantly different (P > 0.05), LSD test. Lower FPLI value indicates tolerance.
Figure 3.4. FPLI values for *S. flava* evaluations based on plant height (5 and 10 aphid infestation levels). Bars with the same letter in the same case are not significantly different (*P > 0.05*), LSD test. Lower FPLI value indicates tolerance.
CHAPTER 4

Characterization of greenbug feeding behavior and aphid (Hemiptera: Aphididae) host preference in relation to resistant and susceptible tetraploid switchgrass populations.

Introduction

Switchgrass, *Panicum virgatum* L., is a perennial, polyploid warm-season grass native to tallgrass prairies of North America, east of the Rocky Mountains (Vogel 2004, Mitchell et al. 2008, 2012). Traditionally, switchgrass has been important and widely used for conservation plantings, as well as livestock forage (Sanderson et al. 2004, Vogel 2004). Recently, switchgrass has also been identified as a model species for its development as an herbaceous energy crop, due largely to its perennial growth habit, relatively low production inputs, and broad adaptability to a wide range of growing conditions (Bouton 2008, Sanderson and Adler 2008, Sarath et al. 2008). However, long-term sustainability of switchgrass as a bioenergy feedstock will require efforts directed at improved biomass yield under a variety of biotic and abiotic stress factors.

Insect pests contribute significantly to crop losses worldwide through both direct and indirect injury. However, one of the most effective methods for controlling these insect pests is plant resistance (Smith 2005, Smith and Boyko 2007). Differential resistance to potential insect pests has been demonstrated in various tetraploid and octoploid switchgrass populations. Dowd and Johnson (2009) found differential resistance among several octoploid switchgrass populations to *Spodoptera frugiperda* with ‘Trailblazer’ and ‘Blackwell’ being the most resistant. Differential resistance was also demonstrated among four tetraploid switchgrass populations to two key aphid species, *Schizaphis graminum* (Rondani) and *Sipha flava* (Forbes) (Chapters 2 and 3).
Further studies also revealed that multiple insect resistance mechanisms appeared to be present in switchgrass plants derived from a cross between an upland and a lowland tetraploid population (Dowd et al. 2012).

Aphids are particularly important crop pests, and may cause plant damage by removing photo assimilates and transmitting an array of plant viruses (Smith and Boyko 2007). During feeding, the salivary styles of the aphid’s piercing-sucking mouthparts penetrate plant tissue to feed on phloem sieve elements (Tjallingii 2006, Smith and Boyko 2007). Furthermore, stylet penetrations by aphids also play an essential role in host plant acceptance or rejection (Tjallingii 1994, Prado and Tjallingii 1997, Diaz-Montano et al. 2007), and can be monitored electronically by the electrical penetration graph (EPG) technique (Tjallingii 2006). The EPG technique was first described by McLean and Kinsey (1964), using an alternating current (AC) recorder system, and later by Tjallingii (1978), using a direct current (DC) based monitor. The EPG technique allows the recording of signal waveforms corresponding to different insect activities and the position of the stylet tips within the plant tissues (Tjallingii 2006). Further, when considered in combination, stylet activities and position may be useful in determining the kind of resistance mechanisms that may be involved at the plant tissue level (Van Helden and Tjallingii 2000, Jiang et al. 2001, Crompton and Ode 2010).

Previous work has identified switchgrass as a suitable feeding and reproductive host for several aphid species, including *S. graminum* and *S. flava* (Kindler and Dalrymple 1999, Burd et al. 2012, Chapter 2). Both *S. graminum* and *S. flava* are important pests of grasses and cereals in North America with over 50 documented graminaceous hosts for each species (Michels Jr. 1986, Kindler and Dalrymple 1999).
Prior studies on the categories of resistance have shown differential levels of antibiosis and tolerance among select tetraploid switchgrass populations to *S. graminum* and *S. flava* (Chapter 3). Additionally, the EPG technique has been used previously to study *S. graminum* feeding behavior on other resistant and susceptible grasses, especially wheat, *Triticum aestivum* L., (Pereira et al. 2010) and sorghum, *Sorghum bicolor* (L.) Moench, (Campbell et al. 1982, Montllor et al. 1983, Dreyer et al. 1984).

Although the EPG technique has been widely used to study the feeding behavior of several species of aphids on many host plants (Van Helden and Tjallingii 2000), no studies have documented aphid feeding behavior on switchgrass. Further, no attempt has been made to document the presence of antixenosis within switchgrass populations to potential insect pests. Therefore, the specific objectives in this research were to characterize the expression of antixenosis among select switchgrass populations to *S. graminum* and *S. flava*, and compare *S. graminum* feeding behavior on resistant and susceptible switchgrasses using the EPG technique.

**Materials and Methods**

**Plant material.** Choice studies and EPGs were performed among two switchgrass cultivars, (populations), Kanlow and Summer and one experimental strain, KxS. Kanlow is a lowland-tetraploid population that originated from switchgrass collected near Wetumka, OK (Alderson and Sharp 1994, Vogel and Mitchell 2008). Summer is an upland-tetraploid population, derived from plants collected near Nebraska City, NE (Alderson and Sharp 1994, Vogel and Mitchell 2008). The experimental strain, KxS (HP1 C1 High Yield strain), was produced by reciprocal matings between Kanlow and Summer plants, followed by selection among the F1 progeny for winter survival and
vigor, and followed by two generations of random mating to stabilize the populations and then one generation of selection for seedling vigor at six weeks after planting and high biomass yields. The experimental strain, was developed by Dr. Kenneth Vogel, USDA-ARS, Lincoln, NE who also provided seed of the cultivars.

**Insect colonies.** Choice studies, to assess aphid preference among switchgrass populations, were conducted with *S. graminum* (biotype I) and *S. flava*. In addition, EPGs to assess aphid feeding behavior were performed for *S. graminum* (biotype I). Colonies for both aphid species were 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. However, the *S. flava* colony could not successfully be kept in a growth chamber, thus the colony was maintained in the greenhouse at 25 ± 7°C and 16:8 (L:D) h on a continuous supply of ‘Haxby’ barley plants within clear plastic cages, approximately 12.5 cm diameter and ventilated with organdy fabric.

**Antixenosis studies.** Choice studies were performed for both *S. graminum* and *S. flava* to assess aphid preference among the three switchgrass populations. 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 population of switchgrass (Kanlow, Summer, and KxS) was planted near the perimeter of the pot. Within a pot, seeds for each population were equally spaced from each other and 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 at 25 ± 7°C with the lighting augmented by 400-W Metal Halide lamps to produce a photoperiod of
16:8 (L:D) h until the plants reached the V2 developmental stage, as described by Moore et al. (1991). Plants were fertilized every two weeks with a soluble (20:10:20 N-P-K) fertilizer.

Each individual pot functioned as a test arena, where one plant of each switchgrass population was represented within each pot. Prior to introduction, aphids were placed in a petri dish and starved for approximately one hour. 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 (20 inches in length by 14 inches in width by 3 inches in depth) filled with water to prevent aphids from moving between pots. The number of aphids was visually documented by counting the number of aphids on each switchgrass population at 1, 2, 4, 8, 24, 48, and 72 h after aphid introduction. Experiments were conducted in a controlled laboratory setting at 23 ± 5°C with continuous light. The experimental design was a randomized complete block design with 10 replications per experiment.

Statistical analysis. Choice studies were analyzed as a repeated measures design using generalized linear mixed model analyses (PROC GLIMMIX, SAS Institute 2011) to identify differences in aphid preference for resistant and susceptible switchgrass populations. Where appropriate, means were separated using Fisher’s least significant difference (LSD) procedure (α = 0.05).

EPG recording. For the feeding behavior study, 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), and were maintained as previously described for the choice study. After
emergence, plants were thinned to one plant per container. Switchgrass 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 (23 ± 5°C), and allowed to acclimate for approximately 2 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 recording.

Adult, apterus *S. graminum* were preconditioned on the susceptible KxS
population for 24 hours prior to all recordings. Aphids were placed in a petri dish and
denied food 1 h prior to 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 length) (Sigmund
Cohn Corp., Mount Vernon, NY) attached to the dorsum of the aphid by a 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 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 individual was able to move freely on the leaf surface. 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 three switchgrass populations represented in each recording. The feeding behavior of *S. graminum* was recorded for 15 h with 20 replications per switchgrass population. Recordings began mid-afternoon and were maintained under continuous fluorescent light.

**Feeding Behavior Parameters and Experimental Design.** Electrical penetration graph 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 are grouped into three main behavioral phases: pathway phase, xylem, and phloem or 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, Alvarez et al. 2006). Waveforms F (stylet penetration problems) were not common in the recordings and were included in the 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 active drinking of water from xylem elements (Spiller et al. 1990, Tjallingii 1990). Xylem sap generally provides fewer nutrients than the phloem sap (Powell and Hardie 2002) and more commonly occurs with water deprived aphids (Spiller et al. 1990). The sieve element phase reflects salivation secretions (waveforms E1) and ingestion of phloem sap (waveforms E2). Waveforms E1 and E2 can be difficult to distinguish, therefore the two 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 the 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 aphids with sustained phloem ingestion (E > 10 min).

Statistical Analysis. EPG files were annotated by waveform and the duration of
each was calculated in Microsoft Excel Workbook. Data were combined, separated by switchgrass population and 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 Institute 2011). 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 Fisher’s least significant difference (LSD) test ($\alpha = 0.05$). Normality was assessed for all parameters using graphical analysis of the residuals and a Shapiro-Wilk test (Shapiro and Francia 1972). A log transformation was performed for data that did not follow a normal distribution. Transformed data were reconverted to the original scale for summarization in all tables.

**Results**

**Antixenosis studies.** *Antixenosis study 1.* A significant interaction between switchgrass population and evaluation time was detected (Figure 4.1) ($F = 1.87$; df = 12, 180; $P = 0.04$). Due to the significant interaction between switchgrasses and evaluation time, simple effects were used to determine if differences existed among treatment means. There were no significant differences between switchgrass populations tested at 1, 2, 4, and 8 h after *S. graminum* introduction. At 24 h after introduction, KxS had significantly more aphids than Summer; however despite numerical differences, there were no significant differences between KxS and Kanlow. Likewise, at 48 h after aphid introduction, KxS had significantly more *S. graminum* than both Summer and Kanlow. The greatest difference in *S. graminum* preference was observed at 72 h after aphid introduction, where KxS had significantly more aphids than Summer and Kanlow. At 72
h after introduction, KxS had over a 3-fold higher mean aphid number (± SEM) than Summer, and over 4-fold higher mean aphid number than Kanlow (7.8 ± 2.0, 2.1 ± 0.5, and 1.6 ± 0.8, respectively). No significant differences were detected between Summer and Kanlow at any time point.

Antixenosis study 2. No significant differences were detected for overall S. flava preference between switchgrass populations (Figure 4.2) ($F = 0.10; \text{df} = 2, 180; P = 0.91$), or for S. flava preference within a given evaluation time (switchgrass population by evaluation time interaction) ($F = 1.25; \text{df} = 12, 180; P = 0.25$). The overall trend was the same for all switchgrass populations, showing a steady decline in S. flava over time. At 48 h after aphid introduction, only KxS had any aphids remaining, with a mean S. flava number (± SEM) of 0.3 ± 0.2. This demonstrated that all three switchgrass populations had a similar behavioral effect on S. flava.

EPG study. Parameters for time and duration of pattern segments (Table 4.1 & Figure 4.3). Analysis of variance determined that switchgrass effects were not significant for time to first probe ($F = 0.24; \text{df} = 2, 57; P = 0.78$) or time to first sieve element phase ($F = 0.55; \text{df} = 2, 54; P = 0.58$) from the start of the EPG recording. Further, after feeding was initiated, no significant differences were found between switchgrasses for time from the first probe to first sieve element phase ($F = 0.70; \text{df} = 2, 54; P = 0.50$). Additionally, significant differences were not detected between switchgrass populations for parameters in the mean duration of pathway phases ($F = 0.34; \text{df} = 2, 57; P = 0.71$), xylem phases ($F = 0.30; \text{df} = 2, 53; P = 0.74$), first probe ($F = 0.17; \text{df} = 2, 57; P = 0.85$), and first sieve element phase ($F = 0.57; \text{df} = 2, 54; P = 0.57$). However, significant differences were detected for mean total duration of sieve element phases ($F = 7.87; \text{df} = 2, 54; P = 0.001$)
and non-probing events \((F = 8.43; \text{df} = 2, 57; P = 0.0006)\). In the mean duration of sieve element phases and non-probing events, Kanlow was significantly different from all other switchgrasses. Specifically, aphids spent significantly less time overall in phloem sieve elements and significantly more time in non-probing than when feeding on both KxS and Summer. However, no differences were observed between KxS and Summer in either of those parameters.

**Parameters linked to stylet pathway and xylem ingestion activities** (Table 4.2).

Significant differences were recorded between switchgrasses in mean number of pathway phases \((F = 4.10; \text{df} = 2, 57; P = 0.022)\) and non-probing events \((F = 4.41; \text{df} = 2, 57; P = 0.017)\). In mean number of pathway phases, KxS \((15.6 \pm 1.9)\) had significantly fewer than both Summer \((23.4 \pm 2.8)\) and Kanlow \((26.0 \pm 3.2)\). Again, KxS had significantly fewer non-probing events \((8.9 \pm 1.5)\) than Kanlow \((17.8 \pm 2.8)\); however, Summer \((13.7 \pm 2.2)\) was not significantly different from either group. However, no significant differences were detected among switchgrasses in the number of potential drops \((F = 0.93; \text{df} = 2, 57; P = 0.40)\), xylem phases \((F = 0.30; \text{df} = 2, 57; P = 0.74)\), and probes after the first sieve element phase \((F = 1.74; \text{df} = 2, 46; P = 0.19)\).

**Parameters linked to sieve element phases** (Table 4.2). The number of sieve element phases was not significant among switchgrass populations \((F = 1.62; \text{df} = 2, 57; P = 0.21)\). However, an analysis of variance detected significant differences in the potential phloem ingestion index (PPII) \((F = 9.40; \text{df} = 2, 54; P = 0.0003)\) and percentage of *S. graminum* showing sustained phloem ingestion \((E > 10 \text{ min.})\) \((F = 5.67; \text{df} = 2, 57; P = 0.0057)\). The potential phloem ingestion index (PPII) was significantly lower for Kanlow \((12.1 \pm 5.6)\) than all other switchgrasses, while KxS \((47.6 \pm 9.1)\) and Summer
(44.4 ± 7.4) were not significantly different from each other. Similarly, Kanlow had significantly fewer aphids that demonstrated sustained phloem ingestion than both KxS and Summer, with only 35% of *S. graminum* able to sustain phloem ingestion for more than 10 minutes on Kanlow.

**Discussion and Conclusions**

Choice studies for *S. graminum* revealed a clear preference for the susceptible switchgrass, KxS, 24 h after aphid introduction, relative to all other switchgrasses. Initially, *S. graminum* colonization was similar among all switchgrasses, however, at 24 h after aphid introduction, there was a clear movement of *S. graminum* from the resistant switchgrasses, while aphid numbers remained relatively high on KxS. This may indicate that external plant cues, such as plant volatiles, do not play a prominent role in the preference of switchgrasses for *S. graminum*, since aphids seemed to settle similarly on all switchgrasses. Accordingly, only at 24 h after aphid introduction did differences in *S. graminum* preference become apparent, suggesting the lack of attractant or repellent factors on the plant surface. This was also corroborated by the lack of significant differences in the EPG parameter for time to first probe, which found no delay in aphid probing among resistant and susceptible switchgrasses. The mean time (± SEM) for *S. graminum* to initiate probing on the susceptible KxS was 21.4 ± 7.6 minutes, versus 26.5 ± 14.9 minutes on the resistant Kanlow. Indeed, the statistically similar time needed to begin probing on all switchgrasses suggests that no deterrent factors are located on the plant surface.

Further, EPG parameters indicate that the resistance factors in the selected switchgrasses are not located in the peripheral layers of the plant tissue. Resistance
factors in the epidermis and mesophyll may be indicated by a large number of test probes and an increased time to reach the first phloem sieve element phase (Alvarez et al. 2006). Although, significant differences were detected for the mean number of pathway phases between KxS (15.6 ± 1.9) and both Summer (23.4 ± 2.8) and Kanlow (26.0 ± 3.2), no differences were recorded among any of the switchgrasses for time to reach the first sieve element phase. Aphids were also quicker to reach the sieve elements on the resistant Kanlow than any other population. This indicates that aphids did not encounter physical barriers along the peripheral tissues, which would challenge phloem access by the aphids. Similarly, aphids had a statistically similar time to first sieve element phase from first probe for all switchgrass populations. Time to first sieve element phase from first probe is sometimes considered more a meaningful parameter in localizing plant resistance since it corrects for potential differences in time to reach the first sieve element phase due to delayed probing as the result of epidermal factors. Accordingly, a lack of differences for this parameter demonstrates that phloem is not harder to reach or to locate, due to mechanical barriers or chemical differences, respectively (Van Helden and Tjallingii 2000).

However, several of the parameters tested indicate that resistance factors may be associated with phloem sieve elements. Although no differences were recorded in aphid access to phloem sieve elements, S. graminum were unable to spend as much time feeding in the sieve elements on Kanlow, spending over three-fold more time in the sieve elements on KxS and Summer, relative to Kanlow. In addition, Kanlow had a PPII value (± SEM) of 12.1 ± 5.6, which was significantly lower than both KxS (47.6 ± 9.1) and Summer (44.4 ± 7.4). The PPII parameter is a corrected index used to determine the
acceptability of phloem (Van Helden and Tjallingii 2000). The potential phloem ingestion index (PPII) measures 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). Correspondingly, 70 and 95 percent of aphids were able to feed in phloem sieve elements for sustained periods (i.e. longer than 10 minutes) on KxS and Summer, respectively, while only 35 percent of aphids tested on Kanlow were able to achieve sustained phloem feeding. Collectively, these data demonstrate that Kanlow does have a significant impact on *S. graminum* feeding behavior, and indicates that resistant factors are likely located in the phloem sieve elements. Furthermore, the differences in phloem acceptability likely explain the significant increase in duration and number of non-probing events as well as the number of pathway phases in Kanlow relative to KxS. Because each phase is mutually exclusive, *S. graminum* feeding on the susceptible KxS would have less time available for other phases, such as pathway and non-probing, since more time was spent in the sieve element phase (Van Helden and Tjallingii 2000). However, aphids feeding on resistant plants may continue probing, searching for a suitable feeding site and leading to a greater number of probes and pathway phases. Similar findings have been reported with the potato aphid, *Macrosiphum euphorbiae* (Thomas), on tomatoes lines, with increased non-probing on resistant lines resulting from phloem-based resistance (Kaloshian et al. 2000). However, no clear explanation is available for why Summer had significantly more pathway phases, since it was statistically similar to KxS in all other parameters.

Phloem-based mechanisms of resistance to aphids have previously been reported in a number of EPG studies for plant resistance in other systems, including resistance in
melon genotypes (*Cucumis melo* L.) to the cotton melon aphid, *A. gossypii*, (Garzo et al. 2002). The underlying mechanisms for resistance located in phloem tissue may be chemical (i.e., deterrent compounds in sieve tubes) or physical (i.e., difficulty overcoming phloem wound response) (Tjallingii 2006, Le Roux et al. 2008). Phloem feeding is particularly important for aphids, providing them with the necessary nutrients not available in xylem sap (Powell and Hardie 2002); limiting the nutrient uptake by the aphids may also negatively effect aphid demographics. Indeed, the antibiosis and antixenosis categories of resistance often overlap and may be difficult to distinguish, since a strong deterrent effect may initiate aberrant behavior in an insect, resulting in a weakened physiological condition which could produce an antibiotic effect (Smith 2005).

Previous work on the categories of resistance in no-choice studies with the same selected switchgrass populations suggested that Kanlow possesses high levels of antibiosis to both *S. graminum* and *S. flava* (Chapter 3). However, this study provides evidence that antixenotic resistant factors are important in Kanlow to *S. graminum*. Although we could not determine in this study if some combination of antixenosis and antibiosis categories to *S. graminum* work concurrently in Kanlow, combinations of resistance categories are often reported, including many examples of antibiosis and antixenosis together (Castro et al. 1996, Garzo et al. 2002, Hawley et al. 2003, Heng-Moss et al. 2003, Diaz-Montano et al. 2007, Eickhoff et al. 2008, Le Roux et al. 2008).

Choice studies for *S. flava* revealed no clear preference for the aphid among the three selected switchgrass populations. Previous work investigating the categories of resistance to *S. flava* in switchgrass has shown that Kanlow possesses high levels of antibiosis, while KxS possesses low levels of antibiosis with potentially low levels of
tolerance (Chapter 3). However, this study designed to assess the presence of antixenosis among the selected switchgrass populations to *S. flava* indicates that superficial plant characteristics do not appear to play an important role in the switchgrasses, influencing aphid settling and feeding behavior. In the choice study for *S. flava*, aphids settled on the plants at the onset of the experiment and then subsequently declined in numbers similarly for all switchgrasses. Several plant properties may act as repellants or attractants. For example, plant volatiles emitted in the air layer close to the plant surface may act as repellants or attractants for aphids (Smith 2005, Le Roux et al. 2008). Additional physical attributes of the plants surface, such as trichome density or morphology, texture, or color may also influence aphid behavior (Powell et al. 2006, Le Roux et al. 2008). However, *S. flava* responded similarly between all switchgrasses in this study, indicating that superficial plant characteristics may not be important in switchgrass for resistance to *S. flava*.

This research provides the first detailed documentation of the feeding behavior of any aphid on selected switchgrass populations. The results indicate that the resistant switchgrass population Kanlow markedly altered the probing behavior and sieve element acceptance of *S. graminum*, relative to susceptible switchgrasses tested. Previous work identified Kanlow with high levels of antibiosis, and Summer with moderate levels of tolerance and possibly low levels of antibiosis to *S. graminum*. However, data provided in this study show that Kanlow possesses high levels of antixenosis to *S. graminum* and further localized important resistance mechanisms to the phloem tissue of Kanlow. This also means that Kanlow may possess both antibiosis and antixenosis categories of resistance to *S. graminum*. The combination of multiple categories and mechanisms of
resistance may lower the probability or at least delay aphid populations from overcoming resistant switchgrasses; therefore, Kanlow should be of considerable interest for any switchgrass breeding program for improved biomass feedstocks. Moreover, prior work has shown Kanlow possesses high levels of antibiosis to *S. flava*, in addition to *S. graminum*. Multi-species resistance in combination with the potential of multiple resistance categories is a very important finding and should not be understated. However, while Kanlow possesses high levels of resistance to *S. flava* and *S. graminum*, it does not imply that the resistance mechanisms are the same for both aphid species. Resistance to aphids is generally very species-specific (Tjallingii 2006), thus future work should focus on detailing *S. flava* feeding behavior on switchgrasses to determine the possible mechanisms and location of resistance to *S. flava*. Identification of resistance mechanisms is of great importance, in order to provide effective integrated pest management strategies and possibly informing foresight for resistance management (i.e., managing insect countermeasures to host resistance). Therefore, future research should also concentrate on improving our understanding of specific resistant mechanisms.
Table 4.1. Comparison of EPG parameters (mean ± SEM) for time and duration of pattern segments for 15 h of *Schizaphis graminum* feeding on switchgrass populations (2nd leaf stage).

<table>
<thead>
<tr>
<th>Feeding Variable</th>
<th>Mean ± SEM(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KxS</td>
</tr>
<tr>
<td>Time to 1(^{st}) probe(^b)</td>
<td>21.4 ± 7.6a</td>
</tr>
<tr>
<td>Time to 1(^{st}) SE(^1) phase</td>
<td>181.9 ± 30.6a</td>
</tr>
<tr>
<td>Time from 1(^{st}) probe to 1(^{st}) SE phase</td>
<td>159.5 ± 30.6a</td>
</tr>
<tr>
<td>Duration of pathway phases(^b)</td>
<td>401.5 ± 51.4a</td>
</tr>
<tr>
<td>Duration of xylem phases</td>
<td>56.9 ± 11.9a</td>
</tr>
<tr>
<td>Duration of SE phases</td>
<td>339.9 ± 67.5a</td>
</tr>
<tr>
<td>Duration of NP events</td>
<td>119.2 ± 32.3b</td>
</tr>
<tr>
<td>Duration of 1(^{st}) probe</td>
<td>85.0 ± 45.2a</td>
</tr>
<tr>
<td>Duration of 1(^{st}) SE phase</td>
<td>81.8 ± 50.3a</td>
</tr>
</tbody>
</table>

\(^a\) Treatment means within the same row followed by the same letter indicate no significant differences (P ≤ 0.05), LSD test.

\(^b\) Time and duration calculated in minutes

\(^1\) Sieve element

\(^2\) Non-probing
Table 4.2. Comparison of EPG parameters (mean ± SEM) for stylet activities for 15 h of *Schizaphis graminum* feeding on switchgrass populations (2\textsuperscript{nd} leaf stage).

<table>
<thead>
<tr>
<th>Feeding Variable</th>
<th>KxS</th>
<th>Summer</th>
<th>Kanlow</th>
</tr>
</thead>
<tbody>
<tr>
<td>potential drops</td>
<td>152.1 ± 21.1a</td>
<td>154.7 ± 21.8a</td>
<td>183.9 ± 21.4a</td>
</tr>
<tr>
<td>pathway phases</td>
<td>15.6 ± 1.9b</td>
<td>23.4 ± 2.8a</td>
<td>26.0 ± 3.2a</td>
</tr>
<tr>
<td>xylem phases</td>
<td>2.4 ± 0.4a</td>
<td>2.7 ± 0.4a</td>
<td>2.9 ± 0.4a</td>
</tr>
<tr>
<td>SE(^1) phases</td>
<td>4.8 ± 0.9a</td>
<td>7.2 ± 1.1a</td>
<td>5.7 ± 0.9a</td>
</tr>
<tr>
<td>NP(^2) events</td>
<td>8.9 ± 1.5b</td>
<td>13.7 ± 2.2ab</td>
<td>17.8 ± 2.8a</td>
</tr>
<tr>
<td>probes after 1(^st) SE phase</td>
<td>6.9 ± 1.6a</td>
<td>9.7 ± 2.4a</td>
<td>11.3 ± 2.3a</td>
</tr>
<tr>
<td>Potential phloem ingestion index (PPII)</td>
<td>47.6 ± 9.1a</td>
<td>44.4 ± 7.4a</td>
<td>12.1 ± 5.6b</td>
</tr>
<tr>
<td>% of aphids showing sustained ingestion (E &gt; 10 min.)</td>
<td>70 (14/20)a</td>
<td>95 (19/20)a</td>
<td>35 (7/20)b</td>
</tr>
</tbody>
</table>

\(^a\) Treatment means within the same row followed by the same letter indicate no significant differences (P ≤ 0.05), LSD test.

\(^1\) Sieve element

\(^2\) Non-probing
Figure 4.1. Comparison of *Schizaphis graminum* preference among Summer, KxS and Kanlow. * Denotes significant differences (P ≤ 0.05), LSD test.
Figure 4.2. Comparison of *Sipha flava* preference among Summer, KxS and Kanlow.
Figure 4.3. Comparison of EPG parameters for time (mean ± SEM) to 1st probe and 1st SE phase, and duration of SE phases and NP events for 15 h of *Schizaphis graminum* feeding among switchgrass population. Bars with the same letter within a column are not significantly different (P > 0.05), LSD test.
REFERENCES CITED


Painter, R. H. 1951. Insect resistance in crop plants, University of Kansas Press, Lawrence.


