THE ECOLOGY OF GRASSLAND LEAFHOPPERS

(HEMIPTERA: CICADELLIDAE) WITH EMPHASIS ON

THE GENUS *BALCLUTHA*

By

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Major Field: ENTOMOLOGY AND PLANT PATHOLOGY

Abstract: Leafhoppers (Hemiptera: Cicadellidae) inhabit a broad range of ecological niches, including grassland ecosystems. These insects feed on vascular tissues and many species are economically important vectors of plant pathogens. The invasive red streaked leafhopper, *Balclutha rubrostriata* (Melichar), was first documented in Texas on King Ranch bluestem, *Bothriochloa ischaemum*, and has since been found in Louisiana and Oklahoma. As part of an effort to document range expansion of *B. rubrostriata* and the seasonal abundance of key vector species, optimal time of day for leafhopper sampling, leafhopper assemblages on mixed grasses with and without dominant *B. ischaemum*, and seasonal presence/absence of *B. rubrostriata on B. ischaemum* was determined. After repeated observations of *Balclutha* leafhoppers on seed heads, preference of the *B. rubrostriata* congener, *B. neglecta,* for specific *B. ischaemum* tissues was also tested. Sweep samples were collected at morning, midday, and evening at the Oklahoma State Botanic Garden to study sampling efficiency, and weekly sweep samples were collected from grass patches throughout Payne County, with the captured leafhoppers identified to genus. The presence or absence of *B. rubrostriata* in central Oklahoma was monitored, and *B. ischaemum* tissues were stained to quantify salivary sheaths left by *B. neglecta* and *Exitianus exitiosus*. More leafhoppers were captured during midday, but insignificantly so. Furthermore, leafhopper populations fluctuated diurnally and seasonally and this varied by species. Leafhoppers were more abundant in mixed grasses without dominant *B. ischaemum*, but there were no significant differences in richness or diversity. Key vector species that were consistently collected were *E. exitiosus*, *Endria inimica*, and *Macrosteles quadrilineatus*. *Balclutha rubrostriata* was absent during spring and summer months, but present in the adult stage throughout central Oklahoma in late fall, suggesting vegetational or migratory movement from southern regions. Finally, this study documents leafhopper probing in grass seed heads, but no specific preference for them over stem tissue by *B. neglecta* or *E. exitiosus*. The high proportion of leafhopper vectors present in multiple habitat types has potential implications for the movement of pathogens from mixed grasses to cultivated grasses. Knowing when target species are most abundant could facilitate future grassland health or crop system research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PROLOGUE

Leafhoppers belong to the family Cicadellidae within the order Hemiptera, and they

inhabit a broad range of ecological niches, including grassland ecosystems. The genus

Balclutha contains 111 species that can be found feeding on grasses worldwide.

Balclutha rubrostriata (Melichar), the red streaked leafhopper, is approximately 3.5 mm

in length as an adult and can be distinguished from other species by its red tinted

forewings with straw-colored veins (Zahniser et al. 2010). Native to India and Sri Lanka,

it has spread to Africa, the Mediterranean, Southeast Asia, the Asian Islands, Australia,

Japan, Hawaii, Central America, the Virgin Islands, and Puerto Rico (Zahniser et al.

2010; Knight 1987). In recent years, it has also been documented in Texas, Louisiana,

and Oklahoma (Morgan et al. 2013; Andreason et al. 2015). Although it is not known to

transmit any plant pathogens (Andreason et al. 2015), *B. rubrostriata* is closely related to

the vector of Maize streak mastrevirus, a detrimental maize pathogen in Africa (DeLong

1948). Thus, from an agricultural biosecurity standpoint, potential vectors of List A

agents such as MSV (USDA APHIS OPIS) should be carefully monitored.

Balclutha rubrostriata was first collected in Kleberg County, Texas in 1991, and in 2006

and 2008, the species was also reported in Kerr County and Travis County (Zahniser et

al. 2010). Zahniser et al. (2010) also documented *B. rubrostriata* in Bexar County, where

over 15,000 individuals were collected, composing almost 85% of the survey samples.

Morgan et al. (2013) further substantiated the occurrence of *B. rubrostriata* in 15 Texas counties and one Louisiana parrish. Moreover, the vast majority of individuals were collected from *Bothriochloa ischaemum*, or King Ranch bluestem, where *B. rubrostriata* was always present. Native to the north Asian steppes and Mediterranean Europe, *B. ischaemum* is an invasive grass that was originally introduced into Texas due to its drought tolerance and palatable, nutritional characteristics which made it favorable for livestock (Harlan 1951; Correll and Johnson 1970; Dwyer et al. 1964). By the 1950s, it had been sown throughout the Edwards Plateau and into Oklahoma (Riskind and Diamond 1988; White and Dewald 1966). The co-occurrence of *B. rubrostriata* with *B. ischaemum* suggests that this may be a desirable host plant, and the expansion of King Ranch bluestem across Texas could potentially enable the spread of *B. rubrostriata* into new areas. Moreover, the distribution of *B. rubrostriata* in Texas mirrors the gradient of King Ranch bluestem (Morgan et al. 2013). In 2013 and 2014, Andreason et al. (2015) documented the presence of *B. rubrostriata* in 10 *B. ischaemum* patches throughout central Oklahoma. However, it remains unknown if *B. rubrostriata* is indeed expanding its range north by true migration or by traveling along the paths of *B. ischaemum* that extend north into Oklahoma and Kansas from Texas.

The impact of *B. rubrostriata* on native leafhopper communities is additionally undetermined. Zahniser et al. (2010) noted almost complete displacement of other leafhopper species by *B. rubrostriata* in *B. ischaemum* patches. The last surveys of leafhopper fauna in Oklahoma were conducted over 30 years ago in the tallgrass prairie ecosystem (Cwickla and Blocker 1981). If *B. rubrostriata* continues to move north and becomes established in Oklahoma, it will be important to know what endemic species are

already in place. From an epidemiological perspective, leafhopper phytopathogen vector populations may be impacted by any invasion of *Balclutha* (or other) species, thus periodic surveillance of leafhopper habitats establishes a baseline for documentation of leafhopper invasion.

Sampling for leafhoppers can be problematic because they are thought to move up and down the plant canopy as ambient temperatures fluctuate. In some species, migrations to different microhabitats within plant canopies have been correlated with diurnal cycles (Larsen and Whalon 1987; Andersen et al. 1992). In cotton, jassid leafhoppers move up into the canopy in the morning, and down lower toward noon and evening, presumably to escape extreme heat in subtropic India (Simwat and Dhawan 1995). In order to obtain accurate measurements of leafhopper diversity for assessing grassland health, it is necessary to know the optimal time of day when leafhoppers are most abundant.

Finally, observations of leafhoppers in their natural habitat and on plants in colonies indicate that preferred feeding sites are along stems and abaxial sides of leaves (Wayadande, personal observation). However, both *B. rubrostriata* and its congener *B. neglecta* have been observed on the seed heads of *B. ischaemum*, but their purpose for choosing that location and if they prefer it over other parts of the plant are unknown, and there are no reports of leafhoppers feeding on grass seed heads. Therefore, the objectives of my project are to:

1. Determine the relative abundance of leafhoppers throughout the day.
2. Determine if leafhopper assemblages differ between mixed grasses with and without dominant *Bothriochloa ischaemum*.

3. Determine the seasonal presence of *Balclutha rubrostriata* in central Oklahoma on *Bothriochloa ischaemum*.

4. Determine if *Balclutha neglecta* prefers probing on specific *Bothriochloa ischaemum* tissues.

CHAPTER I

REVIEW OF LITERATURE

Agricultural Biosecurity

Biosecurity was initially defined as an approach that precludes or reduces the spread of infectious diseases in crops and livestock. Agricultural diseases, such as karnal bunt fungus, soybean rust, and foot-and-mouth disease, are thus additionally classified as potential biological weapons. In more recent years, biosecurity has been broadened to include endeavors that prevent harm from deliberate or accidental introductions of organisms to the environment, agricultural industries, and human health and infrastructure. The current invasion rate of detrimental organisms, or the amount and diversity of invaders, is higher than it has ever been in the U.S. Termed "invasive alien species," these nonnative organisms are harmful, or potentially harmful, to the economy, environment, or human health, and can include pests of agricultural or natural habitats, infectious diseases, or bioterrorism agents that originated in foreign countries. Invasive alien species have increasingly become a chief cause of environmental change and economic hardship throughout the world and one of the most critical ecological and economic issues for the U.S in the 21st century. Introduced diseases of crops, livestock, and humans cost an estimated $41 billion dollars each year, and invasive plants may grow on over 100 million U.S. acres. The risks of biological harm could be further

amplified due to globalization, changes in climate and land usage, and technological advancements. In addition, detrimental organisms may be exchanged purposely or may "hitchhike" on other wares and thus have the chance to establish and propagate. However, predicting the invasiveness of introduced species is extremely complicated as numerous complex factors, such as globalization, global changes in climate and land use, and technological advancement, are at play (Meyerson and Reaser 2002).

In order to understand an invasive species impact upon the native inhabitants within an ecosystem, it is important to know what species are present and how their biologies interact with each other and with the invader. Background surveillance of such populations is necessary to know the geographic and temporal boundaries of potential invasive species (Gormley et al. 2011).

Invasive Species

An invasive species is defined as a nonnative species whose introduction causes or is likely to cause harm to the economy, environment, or human health (Clinton 1999). The effects of invasive species are extensive and varied, impacting native biodiversity, complicating ecosystem management worldwide, and causing billions in economic damage (Strayer et al. 2006). In the U.S. alone, it is estimated that invasive species cost over $120 billion each year while being the second leading cause of species endangerment and extinction (Crowl et al. 2008). They control ecological dynamics on numerous spatial and organizational levels, altering species genetics and populations, community composition and diversity, natural disturbance patterns, and biogeochemical cycles, and causing extinctions of native organisms and communities both locally and

regionally (Crowl et al. 2008; Strayer et al. 2006). When an invasive species enters a new area, it adapts to better match the environment, thereby increasing its population size, range, and impacts. Furthermore, through feeding and engineering behaviors, invasive species modify abiotic ecosystem components, affecting the levels, forms, and fluctuations of various chemicals as well as the physical structure of the environment. These changes may be cumulative and take many years to fully transpire, and the consequences of displaced native species can take even longer to fade (Strayer et al. 2006). Ecosystem engineers are defined as organisms that directly or indirectly control resource availability to other species through physical alterations in biotic or abiotic materials. By doing this, they ultimately change, maintain, and/or generate new habitats (Jones et al. 1994). One such example of an ecosystem engineer with enduring effects is the prairie dog (*Cynomys* spp.). Initially, the burrowing behavior of this animal causes constant and intense ground disturbance and results in soil mounds, yet physical and chemical changes occur in the soil that can span hundreds and even thousands of years (Whicker and Detling 1988).

The red imported fire ant, *Solenopsis invicta*, is native to South America and was accidentally introduced into Alabama via ballast soil on ships in the early 1900s (Vinson 1997). Since that time, it has spread rapidly, with its range expanding in the southern U.S. and expected to extend northward. Fire ants can kill livestock, impact human health and the mutualisms associated with pollination, and cause increased pesticide usage. In disturbed areas, fire ants reduce invertebrate diversity and successful nesting of birds. These invasive ants additionally affect ecosystems by displacing native ants that usually build deep, long-lasting nests which are rich in organic material (Crowl et al. 2008).

Native to Asia, the emerald ash borer, *Agrilus planipennis*, was detected in Detroit, Michigan and Windsor, Ontario in 2002. It was likely introduced through solid wood packing material used in international trade and its establishment was facilitated due to the abundance of host ash trees. Furthermore, North American ash species are more susceptible to the beetle than Asian species, and within two years, approximately 15 million ash trees in forested and urban locations were dead or dying. Over 8 billion ash trees grow in the U.S. and at least 16 endemic ash species are threatened by the emerald ash borer's expanding range. Moreover, at least six ash species are commercially significant as their wood is utilized in numerous products. The undiscounted compensatory worth of U.S. forest ash is estimated at over $280 billion, and the undiscounted loss of U.S. urban ash is estimated at $20-60 billion, which includes removal expenses but not those of replacement (Poland and McCullough 2006).

The Asian citrus psyllid, *Diaphorina citri*, is a pest of citrus and its close relatives, and it is a vector of the pathogen that causes the economically devastating citrus greening disease, Huanglongbing. Found in Asia, Afghanistan, Saudi Arabia, Reunion, Mauritius, South and Central America, Mexico, and the Caribbean, the psyllid was intercepted 170 times at U.S. ports on plant material from 1985 to 2003 (French et al. 2001; Grafton-Cardwell 2005). It was first reported in Palm Beach County, Florida in 1998, and by 2001, it had spread to 31 Florida counties on infested nursery stock, as well as into the Rio Grande Valley of Texas (Halbert et al. 2002; French et al. 2001). Furthermore, citrus greening disease, which is prevalent throughout Asia, the Indian subcontinent and nearby islands, and the Saudi Arabian peninsula, was detected in Florida in 2005 (Grafton-Cardwell 2005).

Native to Mediterranean Europe and the north Asian steppes, *Bothriochloa ischaemum*, or King Ranch bluestem, is an invasive grass that was originally introduced due to its ability to establish easily, maximize superior fodder, and endure despite grazing. In the 1930s, it was planted in Texas as a livestock grass because it was drought tolerant and a palatable, nutritional grass for herds. King Ranch bluestem also aids in enhancing rangelands, revegetating tilled or graded terrain, and minimizing erosion. By the 1950s, it was sown throughout the Edwards Plateau and introduced into Oklahoma (Gabbard and Fowler 2007; Morgan et al. 2013). *Bothriochloa ischaemum* occurs in a wide range of habitats throughout the lower half of the United States, with the exception of densely wooded canopies, and it is often found growing along highways (Gabbard and Fowler 2007).

Pathogen Transmission Biology of Hemipterans

Plant pathogens can be transmitted by insects, including aphids, leafhoppers, whiteflies, and other hemipteran taxa. These pathogens include bacteria and viruses. A virus is defined as protein-coated nucleic acid molecules that can only replicate within the cells of a suitable host (Matthews 2012). Plant viruses can be acquired through noncirculative, circulative, propagative, or nonpropagative transmission via an insect vector (Nault 1997). Vector competence, or the ability of an insect to transmit a virus, is determined by the ability of the virus to invade or adhere to critical tissues of the vector. Viruses may thus infect a potential vector, but then be unable to be transmitted, possibly due to a midgut escape barrier or a salivary gland infection barrier (Gray and Bannerjee 1999). The vast majority of insect vectors of plant pathogens are found in the order Hemiptera, of which leafhoppers (family Cicadellidae) account for the greatest proportion of

members, with over 22,000 species (Nault 1997; Zahniser and Dietrich 2008). Like all hemipterans, leafhoppers possess piercing-sucking mouthparts that penetrate the cell wall of plants, causing physical damage despite their hollow, needlelike qualities. This is beneficial to viruses that require living plant cells in order to reproduce. Upon the initial penetration, leafhoppers will perform a series of quick probes into several epidermal cells to assess if the plant is a suitable host or food source. Once a plant is accepted, prolonged feeding is likely to begin. This can occur in the preferred carbohydrate-rich phloem as well as in epidermal or mesophyll cells, and results in the inoculation of noncirculative and circulative viruses (Nault 1997; Gray and Bannerjee 1999). Thus, numerous plant viruses reside in the phloem, such as *Tobacco ringspot virus*, *Tobacco rattle virus*, *Tomato bushy stunt virus*, *Carrot red leaf virus*, *Potato leaf roll virus*, *Potato mop-top virus*, *Beet western yellows virus*, *Cucumber mosaic virus*, and *Brome mosaic virus* (Nault 1997; Wan et al. 2015).

Leafhoppers spread viruses through noncirculative, circulative, propagative, or nonpropagative transmission. In noncirculative transmission, the virus is retained in either the stylets or the foregut and it remains viable there for a few hours to days. In circulative transmission, the virus must be internalized for successful transmission. Following ingestion, the virus is absorbed by the epithelial cells of the midgut and hindgut and then released into the hemocoel and hemolymph. In time, it passes into the salivary glands where it spreads to the salivary ducts, ultimately allowing the leafhopper to inoculate plant hosts as it salivates during prolonged feeding (Nault 1997; Gray and Bannerjee 1999). The latent period between ingestion and salivary incorporation can range from hours to weeks and is also temperature dependent. Circulative viruses can be

propagative or nonpropagative, and those that are propagative proliferate in their vectors and persist despite ecdysis, allowing them to be transmitted for much of the vector's life. Some propagative viruses can be passed transovarially from females to offspring. Transmission efficiency is typically highest in vectors that acquired the virus as nymphs instead of adults, and while transmission efficiency often decreases over time, it can be restored when the virus is reacquired due to the vector feeding on infected plants (Nault 1997).

Leafhopper Vectors

Knowledge of hemipteran vectors is limited to viruses, mollicutes, and vector species that occur on primarily on annual crops in temperate North America, Europe, Australia, and Japan (Nault 1997), but this is rapidly expanding. Within the Hemiptera, the family Cicadellidae contains over 22,000 described leafhopper species with thousands more still undescribed (Zahniser and Dietrich 2008). Despite the great abundance of leafhoppers and their status as pests of grain, row, and tree crops, only a fraction of species have been confirmed as virus vectors (Nault 1997). The vast majority of these economically important vectors comprise the subfamily Deltocephalinae, which contains 117 of the world's recognized 151 virus-transmitting leafhopper species and subspecies. Herein, the monophyletic tribe Macrostelini includes such genera as *Macrosteles*, *Dalbulus*, *Cicadulina*, *Nesoclutha*, and *Baldulus*, all of which contain species that are able to transmit viruses (Knight 1987; Zahniser and Dietrich 2008; Zahniser and Dietrich 2013). *Macrosteles quadrilineatus* is a vector of American and European aster yellows phytoplasma in numerous plants, and a plethora of pathogens are transmitted by other *Macrosteles* species. *Dalbulus maidis* and *D. elimatus* are vectors of corn stunt

11

spiroplasma, maize bushy stunt phytoplasma, and maize rayado fino virus in Mexico and Central and South America, and *Cicadulina mbila* transmits maize streak virus in sub-Saharan Africa. *Balclutha*, a little-studied genus within Deltocephalinae and Macrostelini, is monophyletic with both *Macrosteles* and *Dalbulus* (Zahniser and Dietrich 2013) and is not known to include pathogen vectors.

Balclutha Leafhoppers

The cosmopolitan genus *Balclutha* contains 111 species of widespread yet morphologically uniform leafhoppers which are common and even highly abundant in grasslands (Knight 1987; Zahniser et al. 2010). Host plants are typically grasses and sedges, and the vast distribution of certain taxa has caused confusion with the identification of numerous species and subspecies (Blocker 1967). Furthermore, while *Balclutha* itself is easily distinguished from other genera due to its distinct combination of size, head shape, wing venation, and coloration, its species are much more difficult to tell apart without an examination of the male genitalia (Knight 1987). *Balclutha incisa*, for example, is a major pest of groundnut in India and the most prevalent leafhopper on rice in Egypt. It possesses similar external characteristics to *Balclutha rubrostriata*, but the two species can be differentiated by the appearance of the aedeagus (Zahniser et al. 2010; Khatri et al. 2011).

Balclutha rubrostriata, the red streaked leafhopper, is native to southeast Asia and highly invasive, spreading to Japan, Australia, the eastern Mediterranean, and several countries in Africa as well as Puerto Rico, the Virgin Islands, Central America, and Hawaii (Morgan et al. 2013). Measuring 3.6-4.7 mm in length, it can be distinguished from many

other *Balclutha* species by the bright red coloration of its forewings (Knight 1987). In fact, only *Balclutha rosacea* has similar wing pigmentation, yet the color of its body and wing veins, in addition to the males' genitalia, are dissimilar from *B. rubrostriata*.

In 2006 and 2008, *B. rubrostriata* was documented in Kerr County and Travis County, Texas, and a single museum specimen, collected in Kleberg County, Texas in 1971, was also found. Sampling conducted in Bexar County, Texas yielded over 15,000 *B. rubrostriata* individuals, which comprised 85% of all invertebrates collected. While it remains unknown how *B. rubrostriata* became introduced, long distance dispersion has been recorded in another *Balclutha* species, *B. pauxila* (Zahniser et al. 2010). The dominance of *B. rubrostriata* in Bexar County suggests that it is capable of changing the invertebrate community structure in grasslands, and its overwhelming abundance indicates that species diversity has already been impacted (Zahniser et al. 2010; Andreason et al. 2015). In November 2012, Morgan et al. (2013) documented *B. rubrostriata* in Lousiana and noted that the majority of individuals were collected from *Bothriochloa ischaemum*, which suggests that this may be a desirable host plant for it. Furthermore, *B. rubrostriata* mirrors the gradient of *B. ischaemum* in Texas (Morgan et al. 2013). In November 2013 and October and November 2014, *B. rubrostriata* was collected from 10 *B. ischaemum* patches throughout central Oklahoma. If *B. rubrostriata* becomes established and transitions to other grasses, it could outcompete native leafhoppers in prairies or become a pest in agronomically important grasses (Andreason et al. 2015). Moreover, while it is unknown if *B. rubrostriata* can transmit any plant pathogens, 30% of individuals tested in Thailand carried the phytoplasma that causes the economically devastating sugarcane white leaf disease (Hanboonsong et al. 2006). The

impact of *B. rubrostriata* on native leafhopper communities, as well as its vector status in the U.S., is undetermined. Furthermore, if *B. rubrostriata* is expanding its range northward by traveling with the *B. ischaemum* that grows along the highways extending from Texas, it is unknown if *B. rubrostriata* has established in Oklahoma. In the absence of *B. rubrostriata*, *Balclutha neglecta* may be studied as it is a congener that similarly inhabits *B. ischaemum* in Oklahoma and Texas and is likely to behave similarly to *B. rubrostriata*. Closely related congener leafhoppers often use the same plant hosts and feed very similarly (Wayadande and Nault 1996).

Ecology of Grassland Leafhoppers

Leafhoppers have a significant dual purpose in ecosystems, serving as herbivores and as food for predators (Everwand et al. 2014). Several studies have demonstrated that herbivore diversity increases with plant quantity (Siemann et al. 1998; Scherber et al. 2010). Rösch et al. (2013) noted that more leafhopper species are able to inhabit a location when more plant species are present because suitable host plants are provided for a wider range of leafhoppers. The richness and diversity of leafhoppers increases with plant richness, and the composition of leafhopper communities varies among different plant communities (Rowe and Holland 2013). In croplands, the species richness of leafhoppers decreases as habitats become more isolated. This outcome is led by generalist species and does not occur in complex landscapes (Rösch et al. 2013).

The composition and diversity of leafhopper assemblages in grasslands are directly and indirectly influenced by a number of interrelated habitat components, such as area; climate; site/soil nutrients, moisture, and disturbance; vegetation structure, density,

composition, and diversity; and the intensity of land use and management (Biedermann et al. 2005). Everwand et al. (2014) further noted that grassland leafhopper species richness and abundance is driven both directly and indirectly by plant composition and diversity, the availability and heterogeneity of food resources, disturbance frequencies, and management regimes. In a long-term study conducted in eastern Germany, Schuch et al. (2012) reported that, although the species richness of leafhoppers did not decline between 1963 and 2010 in protected grasslands, species composition was altered due to the presence of novel leafhoppers and there was a strong decrease in leafhopper abundance overall. Throughout the central U.S., agriculture has replaced the vast majority of the tallgrass prairie, such that less than 1% of this diverse habitat remains (Sampson and Knoppf 1996). Furthermore, this remnant prairie is extremely fragmented and in danger of degradation (Collinge 2000; Cully et al. 2003; Rowe and Holland 2013). Leafhoppers can be classified as generalist or dependent species, with the latter depending on the tallgrass prairie habitat and rarely inhabiting weedy locales (Panzer et al. 1995).

Leafhopper species collected from the central Nebraska tallgrass prairie in early June through mid-August by Nemec et al. (2008) included *Amplicephalus inimicus* (only in restored TGP), *A. kansiensis*, *Athysanus argentarius* (only in native TGP), *Chlorotettix spatulatus* (only in restored TGP), *Cicadula ciliata*, *Commellus comma* (only in restored TGP), *Flexamia albida*, *F. prairiana*, *F. reflexa*, *Graminella mohri*, *Laevicephalus minimus*, *L. unicoloratus*, *Limotettix osborni* (only in restored TGP), *Mesamia straminea*, *Paraphlepsius nebulosus*, *Pendarus magnus*, *Polyamia dilata*, *Psammotettix lividellus* (only in native TGP), *Scaphytopius cinereus* (only in restored TGP), and *Xerophloea peltata*. Plant species present included *Agrostis stolonifera*, *Andropogon gerardii*,

Bromus inermis, *Calamagrostis* spp., *Carex* spp., *Desmanthus illinoensis*, *Eleocharis elliptica*, *Elymus canadensis*, *Equisetum* spp., *Helianthus maximilianii*, *Hypoxis hirsuta*, *Liatris pycnostachya*, *Panicum virgatum*, *Silphium integrifolium*, *Sorghastrum nutans*, and *Spartina pectinata* (Nemec and Bragg 2008). A bluestem prairie with minimal grasses and forbs, the Flint Hills of Kansas and Oklahoma are characterized by such grass species as *Andropogon scaparius*, *A. gerardi*, *Bouteloua curipencula*, *Poa pratensis*, *Sorghastrum nutans*, and *Panicum virgatum* (Cwikla and Blocker 1981). Cwikla and Blocker (1981) reported over 110 leafhopper species from 54 genera and 12 subfamilies. The most abundantly occurring species in the Flint Hills were *Draeculacephala mollipes*, *Graphocephala hieroglyphica*, *Aceratagallia uhleri*, *Empoasca* spp., *Athysanella texana*, *Balclutha neglecta*, *Chlorotettix spatulatus*, *Endria inimica*, *Exitianus exitiosus*, *Flexamia prairiana*, *Graminella mohri*, *Macrosteles fascifrons*, and *Stirellus bicolor*. The abundance and diversity of leafhopper species have not been studied in habitats with multiple grass species versus habitats that are dominated by a single grass species and thus resemble monocultures.

Diurnal Activity of Leafhoppers

Leafhopper species inhabit varying strata within the vertical plant structure (Denno 1980), and this distribution shifts both seasonally (Andrzejewska 1965) and diurnally (Romney 1945). Cicadellidae were reported to be most active between 11 AM and 8 PM during summer months in Ontario (Dondale et al. 1972). *Eutettix tenellus* exhibits vertical repositioning throughout the day on beet plants (Romney 1945), but this behavior has not been studied extensively in other leafhopper species (Stewart 2002). The flight activity of *Scaphytopius magdalensis* peaks at dawn and dusk and is significantly influenced by

16

plant height. During midday, movement is correlated with high temperature and low

humidity (Meyer and Colvin 1985). *Graminella nigrifrons* has a similar peak in flight

activity during dusk while interplant movement within the canopy decreases. However,

this varies by sex and age and can further be impacted by temperature (Lopes et al. 1995;

Rodriguez et al. 1992). No studies have been done to document the diurnal activity of

leafhopper communities as a whole or how activity varies among the species present and

throughout the sampling seasons. This information would enable greater sampling

efficiency and enhanced accuracy in leafhopper surveillance.

Leafhopper Feeding Site Preferences

Leafhoppers, like most Homoptera, prefer sheltered parts of plants such as abaxial sides

of leaves, whorls, and stem tissues (Litsinger et al. 2007). This is thought to be due to

greater protection from predators and greater access to vascular tissues from which they

feed (Backus et al. 1990; Naito and Masaki 1967). There are no reports of leafhoppers

feeding on the seedheads or seeds, suggesting that leafhoppers do not feed on seeds.

Interestingly, both *B. rubrostriata* and *B. neglecta* are commonly observed on the seed

heads of *B. ischaemum* (Wayadande and Gahm, unpublished observations). One

explanation for these observations is that these leafhoppers are capable of feeding on

seeds. Many leafhoppers feed by piercing plant tissues down to the phloem and xylem

vasculature with their stylets (Wayadande and Nault 1996). As the stylets penetrate the

plant, they are surrounded by a lipoproteinaceous salivary sheath that remains after

feeding ceases (Backus et al. 1988). These salivary sheaths, including the external

salivary flanges on the plant surface, can be stained and counted to quantify leafhopper

feeding activities and preferences (Bowling 1979; Viator et al. 1983; Marion-Poll et al.

17

1987; Backus et al. 1988). It is possible that *B. rubrostriata* and *B. neglecta* are feeding on the seed head tissues of *B. ischaemum*, but their purpose for being there and whether or not they prefer to feed on certain plant tissues are unknown.

Thus, several gaps exist in the current understanding of grassland leafhopper ecology and these can be filled through my research studies. After assessing the best time of day to survey leafhopper assemblages, I can use that information to more effectively sample Cicadellidae communities in mixed grasses versus a monoculture grass. Data can also be gathered to support or refute the overwintering of *B. rubrostriata* in Oklahoma and to document its presence or absence and potential interstate movement. Finally, based upon personal observations, the feeding activity and preferences of *B. neglecta* for the seed heads versus the stems of *B. ischaemum* can be tested. Therefore, my objectives are to:

1. Determine the relative abundance of leafhoppers throughout the day.
2. Determine if leafhopper assemblages differ between mixed grasses with and without dominant *Bothriochloa ischaemum*.
3. Determine the seasonal presence of *Balclutha rubrostriata* in central Oklahoma on *Bothriochloa ischaemum*.
4. Determine if *Balclutha neglecta* prefers probing on specific *Bothriochloa ischaemum* tissues.

CHAPTER II

METHODOLOGY

Growth and maintenance of food plants:

Certified organic Conlon barley (*Hordeum vulgare*), certified organic common oats,

(*Avena sativa*) certified organic Glenn hard red spring wheat (*Triticum aestivum*), and

certified organic double standard corn (*Zea mays*) (Johnny's Selected Seeds, Winslow,

ME) were planted in pots 10.16 cm diam. x 8.89 cm in height containing Sun Gro®

Horticulture Professional Growing Mix soil and grown in a greenhouse under 16:8 L:D

photoperiod. Approximately 1 TBSP per gallon of water of Miracle-Gro® Water Soluble

Tomato Plant Food 18-18-21 ($N:P_2O_5:K_2O$) fertilizer was applied once a week. When

young plants were approximately 15 cm tall, they were placed in colony cages weekly or

as needed.

Rearing of leafhopper colonies:

Adults of *Exitianus exitiosus* and *B. neglecta* were collected from patches of *Cynodon*

dactylon (bermudagrass) and *B. ischaemum* respectively via sweep netting and placed

into mesh covered aluminum cages measuring 49x25x45.5 cm. They were provided

barley, oats, wheat, and maize that had been grown in the greenhouse.

Objective 1: Determine the relative abundance of leafhoppers throughout the day.

19

Leafhoppers are highly mobile insects that move rapidly up and around plant foliage (Wayadande, personal observation), presumably to select optimal feeding sites. This dispersal behavior is probably temperature dependent (Simwat and Dhawan 1995), but it has never been tested in temperate regions of the US. To determine the relative abundance of leafhoppers and if there is an optimal time of day for sampling in Oklahoma that will result in the highest variety of captured species possible, three sampling times will be tested for maximum leafhopper catch. This will ensure that sampling efforts for Objectives 2 and 3 provide accurate representations of leafhopper assemblages present.

H_0: There is no difference in leafhopper numbers present in upper foliage throughout the day.

H_A: There is a difference in leafhopper numbers present in upper foliage throughout the day.

Methodology:

Leafhoppers were sampled at the same location at three different times during the day. The study site was the Oklahoma State Botanic Garden in Stillwater, OK (Figure 1). Between May 16, 2014 and August 22, 2014, leafhoppers were collected with a 38 cm diam. sweep net at 08:00, 13:00, and 20:00 twice per week, weather permitting. One hundred pendular sweeps per time block were made along a 40 meter transect within the study site. Temperature and humidity were recorded for each sample using www.accuweather.com. Sweep net contents were emptied into a labeled Ziploc® bag and frozen for at least 24 hours. Adult leafhoppers were then sorted out of each collection

sample, preserved in 70% ethanol in corresponding labelled vials, and tentatively identified to genus level, and to species level if possible, by comparing head shape and markings, wing coloration and venation, and genital structure to published keys (DeLong 1948; Medler 1942; Osborn 1928) and internet resources (www.americaninsects.net; www.bugguide.net). American Insects and Bugguide leafhopper identifications were regularly confirmed by Dr. Andy Hamilton, a retired leafhopper taxonomist. To determine the influence of time on capture rate, the abundance of the most commonly captured species were statistically analyzed using a Kruskal-Wallis non-parametric ANOVA (SigmaPlot 11.0).

Objective 2: Determine if leafhopper assemblages differ between mixed grasses with and without dominant *Bothriochloa ischaemum*. Insect abundance and diversity are usually positively correlated with plant species richness. Mixed grass plantings generally support more leafhopper species than do grasslands with fewer plant species (Mitchell and Litt 2016; Rowe and Holland 2013), but this is not always so. Significantly more Cicadellidae were collected from areas in Tennessee forests with the exotic and invasive *Microstegium vimineum* grass present than in areas without *M. vimineum* (Marshall and Buckley 2009). In 2010, Zahniser et al. observed that leafhopper species richness was lower in stands of *B. ischaemum*, but this was not statistically substantiated at the time of that study. To more thoroughly answer this question, the following study compared leafhopper species richness between two types of grassland habitats: mixed grasses with and without a predominant *B. ischaemum* presence.

H₀: Leafhopper assemblages captured in mixed grasses dominated by *B. ischaemum* do not differ from those captured in mixed grasses without a dominant *B. ischaemum* presence.

Hₐ: Leafhopper assemblages in mixed grasses with dominant *B. ischaemum* differ from those captured in mixed grasses without *B. ischaemum*.

Methodology:

Several mixed grass field sites were previously sampled for leafhopper populations in a previous study and it was from those sites that the mixed grass sites were selected for this study based upon plant community structure. Predominant plant species were noted in some, but not all, sampling sites, and although the sites were similar, they were not identical.

Weekly sweep net samples were collected from patches of mixed grasses with dominant *B. ischaemum* (three in 2015 and four in 2016) and patches of mixed grasses without dominant *B. ischaemum* (three in 2015 and four in 2016) in Payne County, Oklahoma from May through August 2015 and 2016 (Figures 2-3). Individual patch sizes are listed in Table 1. At each patch, three transects of 30 sweeps each were performed between 10:30 AM and 1 PM. Samples were placed into Ziploc® storage bags, labelled, and frozen for at least 24 hrs before all immature and adult leafhoppers were separated out. Adults were identified to genus via keys (DeLong 1948; Medler 1942; Osborn 1928) and internet resources (www.americaninsects.net; www.bugguide.net), catalogued, and counted to determine dominant genera, overall abundance, and species richness. The leafhopper counts were statistically analyzed using Student's t-test (SigmaPlot 11.0) and

any p values ≤ 0.05 were considered statistically significant. Shannon's index, a measure of community species diversity that incorporates abundance and evenness, was also calculated for the combined mixed grass sites and combined *B. ischaemum* sites in 2015 and 2016 (Beals et al. 2000).

In 2016, an additional sampling area in the Tallgrass Prairie ecosystem north of Pawhuska, OK was added for additional information on species richness in the absence of *B. ischaemum*. Early surveys of the Tallgrass Prairie Preserve indicated that *B. ischaemum* was largely absent, except for two discreet patches located along the main road. Previously, five study sites were identified and analyzed for presence of cryptic plant viruses and putative insect vectors (Thapa et al. 2015). Within these study sites, no *B. ischaemum* was noted (Wayadande, unpublished observations). Weekly trips to the Tallgrass Prairie were made from early June through mid-July, weather permitting. Three sites with mixed grasses and forbs and dominated by prairie grasses were subjected to three sets of 30 pendular sweeps as described above. Leafhoppers were selected and identified as described above. These totals were not subject to statistical analysis, but annotated and compared to lists previously generated by Cwickla and Blocker (1981). Shannon's index was calculated for the combined Tallgrass Prairie sites as well.

Objective 3: Determine the seasonal presence of *Balclutha rubrostriata* in central Oklahoma on *Bothriochloa ischaemum*. This serves to document how *B. rubrostriata* is occurring in Oklahoma. It is unknown if adults exhibit northward movement from Texas and other southern locations or if there are overwintering populations of *B. rubrostriata* in Oklahoma.

H$_0$: Adult or immature *Balclutha rubrostriata* are present during all spring and summer months in Oklahoma.

H$_A$: Adult or immature *Balclutha rubrostriata* are not present during all spring and summer months in Oklahoma.

Methodology:

Long distance migrating leafhoppers are not uncommon. Well-described leafhopper migrators include *Macrosteles quadrilineatus* (Hoy et al. 1992), *Dalbulus maidis* (Taylor et al. 1993), and *Circulifer tenellus* (Lawson et al. 1951). Usually, migrants are the second generation females generated from populations of overwintering eggs or nymphs in mid to late spring (Meade and Peterson 1964). Thus, in overwintering areas, it is the nymphs that are detected first, and adults that are detected in emigrant areas further north (Meade and Peterson 1964; Taylor et al. 1993). If *B. rubrostriata* was overwintering in Oklahoma on *B. ischaemum*, one would expect to see nymphs emerging from oviposition sites/plants, and later observe male and female adults on similar plants mixed with nymphs. To monitor for overwintering adults and nymphs of *B. rubrostriata*, yellow 12.5x7.5 cm sticky cards were placed in *B. ischaemum* patches throughout Payne County, Oklahoma in the early spring to document the presence of *B. rubrostriata* nymphs. Adult *B. rubrostriata* were previously caught in most of these patches (Andreason et al. 2015). Sticky cards were removed every two weeks, placed into Ziploc® sandwich bags labeled with the location and date, and replaced with new sticky cards. This continued through the early summer. An absence of nymphs would suggest that *B. rubrostriata* does not overwinter in Payne County and instead enters as adults that travel up from Texas via

vegetational movement on *B. ischaemum*. Sweep net samples of *B. ischaemum* patches

for Objective 2 also served to monitor for *B. rubrostriata* adults and these extended into

November. In addition, collecting trips were made in October and November to detect *B.

rubrostriata* in northern Texas and southern Oklahoma and to monitor for northward

progression. Approximately 10-20 sites were sampled in 2015 and 2016.

**Objective 4: Determine if *Balclutha neglecta* prefers probing on specific

Bothriochloa ischaemum tissues.** An interesting observation was made during early

leafhopper monitoring efforts in large swaths of *B. ischaemum*: *Balclutha* leafhoppers (*B.

neglecta*, *B. incisa*, and later *B. rubrostriata)* were observed in close association with the

developing seed heads of *B. ischaemum* (Andreason et al. 2015) (Figure 4).

Deltocephaline leafhoppers are sheath feeders, usually feeding from vascular tissues. No

leafhoppers have been documented to feed on seeds. To determine which tissues the

Balclutha leafhoppers are feeding upon when associated with the seed heads, a

comparison of feeding sites was performed on *B. ischaemum* using two leafhoppers, *B.

neglecta,* and a known stem feeder (positive control), *E. exitiosus*, the gray lawn

leafhopper.

H$_0$: *Balclutha neglecta* probes randomly on all *B. ischaemum* plant tissues.

H$_A$: *Balclutha neglecta* does not probe randomly on specific *B. ischaemum* plant tissues.

Methodology:

Native and introduced grasses generally produce seed during summer months, although

some of the cultivated grasses like wheat and corn head out in late spring. Newly

emerged seed heads of *B. ischaemum* start appearing in September to October. This

roughly corresponds to peaks in *Balclutha* leafhopper numbers. In this study, field-grown *B. ischaemum* in natural stands were used to test feeding site preferences of two leafhoppers, *B. neglecta* and *E. exitiosus*. Natural stands of *B. ischaemum* were used because the seeds heads were all uniform in development, unlike those grown under greenhouse conditions which flowered unpredictably.

At three locations of *B. ischaemum* in Payne County, Oklahoma, 13 test plants were identified and covered with 30 x 20 cm sheer white organza bags (ATCG Storefront, www.amazon.com) (Figures 5-8) for leafhopper introduction: five bags for *Balclutha*, three bags for *Exitianus*, and five bags for the negative control (no leafhoppers). For each bag, 10 *B. neglecta* adults or 10 *E. exitiosus* adults were taken unsexed from colonies and placed on the bagged test plants. Each test plant consisted of one seed head and three stems to create approximately equal seed head-to-stem surface area. Leafhoppers were allowed to feed for 48 hrs after which test plants were removed from the field and brought to the lab for processing. Leafhoppers were returned to colonies.

The test plants were cut into two sections – seed head versus stem – and submerged in McBride's (1936) staining solution which consisted of 0.2% acid fuchsin in 95% ethanol and glacial acetic acid (1:1 vol/vol). The plant tissues remained there for 24 hrs while the stain permeated and then they were transferred to beakers that contained a clearing solution consisting of distilled water, 99% glycerine, and 85% lactic acid (1:1:1 vol/vol/vol). Beakers were labelled by location and treatment (*Balclutha*, *Exitianus*, or control), covered with aluminum foil, autoclaved for 20 minutes, and cooled (Backus et al. 1988). The plant tissues were then removed and placed in fresh clearing solution, and salivary sheaths were counted under a microscope (Olympus SZX10, Tokyo, Japan) for

each plant section. Results were statistically analyzed using the One-way ANOVA and

Tukey test (SigmaPlot 11.0) and any p values ≤ 0.05 were considered significant.

Table 1. Site name and size of patches of mixed grasses (MG) with and without *B. ischaemum* that were sampled in 2015 and 2016.

Site and Year	Patch Size
Botanic Garden (2015) – MG	61 m x 31 m
Cross Country Field MG (2015) – MG	183 m x 183 m
Edward Jones (2015) – MG	213 m x 122 m
Cross Country Field (2015) – *B. isch.*	183 m x 91 m
Kaw Lake (2015) – *B. isch.*	335 m x 30 m
Taylor's RV (2015) – *B. isch.*	213 m x 31 m
19th and Western (2016) – MG	335 m x 46 m
Boomer MG (2016) – MG	61 m x 61 m
Botanic Garden (2016) – MG	61 m x 31 m
Edward Jones (2016) – MG	213 m x 122 m
Cross Country (2016) – *B. isch.*	213 m x 61 m
Insect Zoo (2016) – *B. isch.*	640 m x 91 m
Rodco (2016) – *B. isch.*	213 m x 31 m
Western Petroleum (2016) – *B. isch.*	152 m x 122 m

Figure 1. The Oklahoma State Botanic Garden optimal sampling time study site, with sampling location circled in red (Google Maps 2017).

Figure 2. Sampling locations in 2015. Red circles denote sites dominated by *B. ischaemum*, and yellow circles denote mixed grass sites (Google Maps 2016). Kaw Lake location is shown in (a), Stillwater locations are shown in (b), and Perkins location is shown in (c).
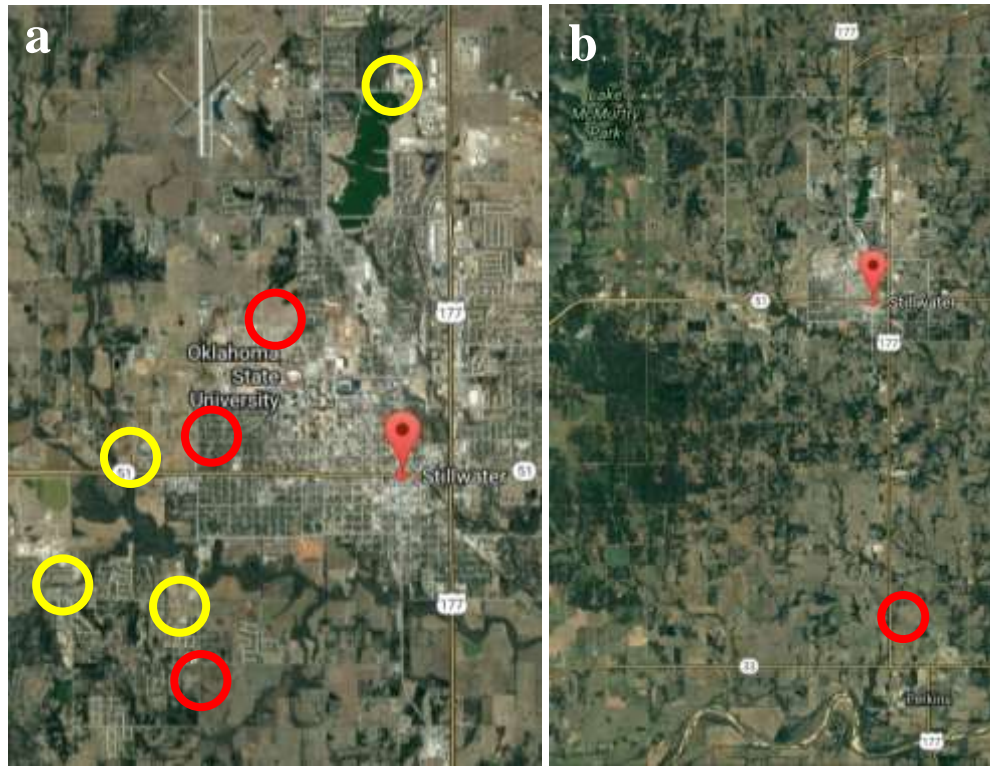
Figure 3. Sampling locations in 2016. Red circles denote sites dominated by *B. ischaemum*, and yellow circles denote mixed grass sites (Google Maps 2016). Stillwater locations are shown in (a) and Perkins location is shown in (b).

Figure 4. Observation of *B. rubrostriata* on *B. ischaemum* seed head.

Figure 5. Locations of mature *B. ischaemum* patches used for feeding experiments (Google Maps 2016). Numbers indicate site order.

Figure 6. Set-up of the Site 1 feeding experiment on October 21, 2016.

Figure 7. Set-up of the Site 2 feeding experiment on October 28, 2016.
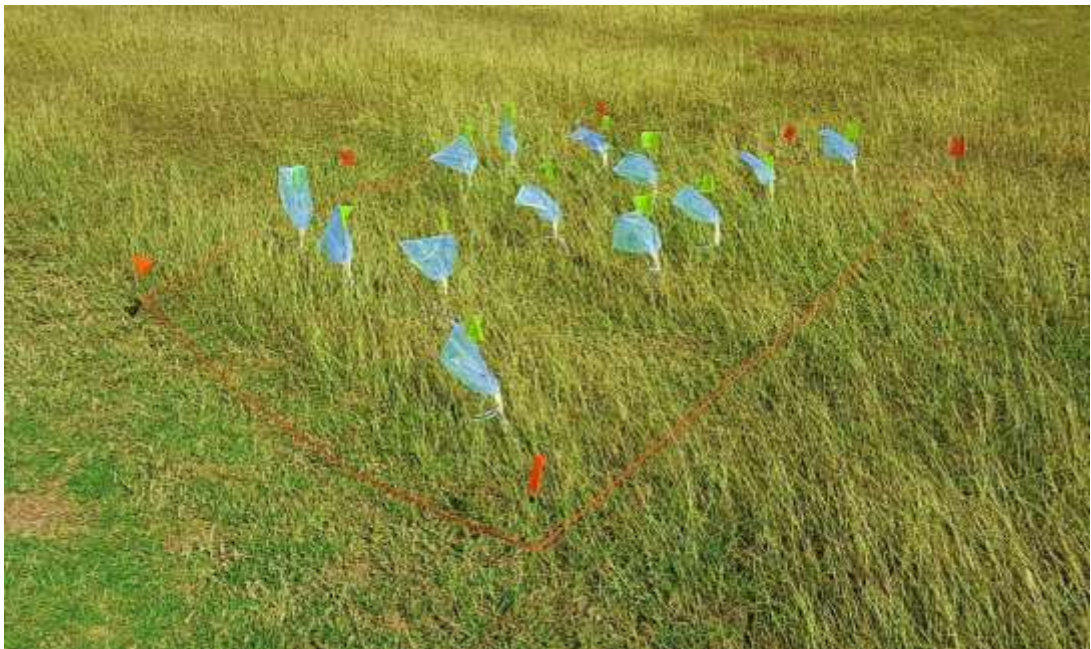
Figure 8. Set-up of the Site 3 feeding experiment on October 31, 2016.

CHAPTER III

RESULTS

Objective 1: Determine the relative abundance of leafhoppers throughout the day.

A total of 48 samples were collected for 16 sampling days over a period of three months.
All adult leafhoppers were identified to genus/species through the use of keys and
websites. The average temperature and humidity at 08:00 was 69.2°F and 80.3%
respectively, 83.3°F and 49.7% at 13:00, and 83.4°F and 56.7% at 20:00. There were no
statistically significant differences in capture rate by time of day (*Aceratagallia*:
H=4.243, P=0.120; *Balclutha*: H=2.824, P=0.244; *Exitianus*: H=4.243; P=0.120;
Polyamia: H=0.164; P=0.921; *Torpedo*: H=1.629, P=0.443), but there were some clear
trends. The highest number of leafhopper genera and vector species were captured in
June (Figure 9). In May, a total of 707 adult leafhoppers were collected for 5 sampling
days. In June, a total of 1119 adult leafhoppers were collected for 8 sampling days. In
August, a total of 1161 leafhoppers were collected for 3 sampling days. Many of these
leafhoppers were captured in low numbers, but there were several species that were
captured more frequently or in higher numbers. *Aceratagallia* species were most
abundant in May and June yet absent in August, while *Balclutha* species were absent in
May and June but were captured in great abundance in August (Figure 10). *Aceratagallia*
species and *E. exitiosus* had the highest number of individuals present at midday,

33

while *Polyamia* species had the most individuals present in the evening (Figure 11).

Objective 2: Determine if leafhopper assemblages differ between mixed grasses with and without dominant *Bothriochloa ischaemum*.

Plant communities within test sites: In earlier, non-quantitative surveys of the plant communities in some of the monitored field sites, several plant species dominated at different times throughout the spring and summer months. All sites were a mix of annual and perennial grasses and forbs, but dominated by grasses. Species consistently found at most sites included downy brome (*Bromus tectorum*), clovers (*Trefolium* spp.), ryegrass (*Lolium* spp.), bermudagrass (*Cynodon dactylon*), wild geranium (*Geranium maculatum*), johnsongrass (*Sorghum halepense*), little bluestem (*Schizachyrium scoparium*), and others. Many of these species, in particular downy brome, were dominant early in the year, then died or became inconspicuous in summer/fall. At sites that were considered dominated by *B. ischaemum*, other plant species were more prevalent early in the season and then succeeded by the maturing *B. ischaemum*. Towards the end of the summer/fall, *B. ischaemum* sites appeared to have only this species present. The additional Tallgrass Prairie location was dominated by forbs throughout the sampling months.

Cicadellidae abundance was nearly twice as high in mixed grasses than in *B. ischaemum*-dominant grasses in both 2015 and 2016 (Figures 12-13). In mixed grasses, Shannon's H=2.07 for all identified adult Cicadellidae in 2015. In 2016, Shannon's H=2.369 for all identified adult Cicadellidae. In *B. ischaemum*-dominant grasses in 2015, Shannon's H=2.03. In 2016, Shannon's H=2.296. At the Tallgrass Prairie, Shannon's H=2.996 in 2016. In 2015 and 2016, leafhopper assemblages, including the number of vector genera,

were similar in both mixed grasses with and without dominant *B. ischaemum* (Table 2). When individual sites were compared, the tallgrass prairie exceeded any mixed grass or *B. ischaemum*-dominated location for the most genera represented and the most unique genera overall (Table 3). While leafhopper populations fluctuated throughout the spring and summer, this varied by genus (Figures 14-15). The populations of four vector genera exhibited similar genus-specific trends in 2015 and 2016 despite having only a month of overlap in the sampling dates, and all four vector genera had population peaks in June of both years (Figures 16-19).

Objective 3: Determine the seasonal presence of *Balclutha rubrostriata* in central Oklahoma on *Bothriochloa ischaemum*.

In 2015, yellow sticky card traps yielded zero *B. rubrostriata* nymphs in Payne County. In October 2015, the northernmost occurrence of *B. rubrostriata* adults was White Settlement, Texas. In November 2015, *B. rubrostriata* adults were collected from *B. ischaemum* in Maud, Sulphur, and Ardmore, Oklahoma (Figure 20). Numbers were low, with approximately 10 adults captured from the Ardmore sweep site, approximately 5 adults from the Sulphur site, and approximately 2 adults from the Maud site. In October 2016, *B. rubrostriata* adults were collected from *B. ischaemum* throughout central Oklahoma, ranging as far south as Marietta to the Tallgrass Prairie Preserve in northern Oklahoma (Figure 21). However, numbers followed the previous year's trend, with more *B. rubrostriata* adults collected in locations closer to the Texas border (e.g. approximately 10 adults captured in Marietta) and fewer adults collected in the northernmost locations (e.g. approximately 2 adults captured in the Tallgrass Prairie

Preserve). No statistical analyses were done on the data as only presence/absence was monitored.

From 2014 to 2016, large numbers (100-150 adults) of *B. rubrostriata* were captured by Astri Wayadande in late October to late November in southern Payne County by sweeping large swaths of *B. ischaemum* along Highway 177 near the Rodco sampling site. These insects were brought back to the lab for the purposes of initiating a breeding colony. During identification, the gender of each individual was noted; 99% of the captured insects were female. In 2015, only three males were captured and in 2016, only a single male was captured. No offspring were observed in the colony cage and the establishment of a *B. rubrostriata* colony failed.

Objective 4: Determine if *Balclutha neglecta* prefers probing on specific *Bothriochloa ischaemum* tissues.

Three replicates of the experiment comparing salivary sheath numbers deposited on *B. ischaemum* by *B. neglecta* and *E. exitiosus* were completed in October 2016. For the October 21, 2016 experiment, the average high temperature over 48 hours was 76.5°F and the average low temperature was 40°F. Winds averaged 6.5 MPH and humidity averaged 57%. For the October 28, 2016 experiment, the average high temperature over 48 hours was 87°F and the average low temperature was 63°F. Winds averaged 11 MPH and humidity averaged 61%. For the October 31, 2016 experiment, the average high temperature over 48 hours was 85.5°F and the average low temperature was 59.5°F. Winds averaged 9.5 MPH and humidity averaged 70.5% (Weather Underground). Examination of the seed heads (glumes, racemes, and awns) and stems revealed a very

high number of recognizable sheaths (Figure 22). *Balclutha neglecta* left an average of

18.953 salivary sheaths on stem tissue per insect versus 17.533 sheaths on seed heads per

insect, whereas *E. exitiosus* left 27.667 sheaths on stem tissue per insect versus 18.144

sheaths on seed heads per insect. Due to the high variation in the mean number of

salivary sheaths overall for all three treatments, there were no statistically significant

differences between the stem and the seed head (Table 4). For the seed head components,

only the racemes fed upon by *B. neglecta* had significantly more mean salivary sheaths

than the negative control raceme (P=0.013); however, the racemes fed upon by *E.*

exitiosus nearly had significantly more mean salivary sheaths than the control raceme as

well (P=0.053) (Table 5). As the *B. ischaemum* matured in each successive trial, the mean

number of salivary sheaths in both the stem and the seed head tissues increased for all

treatments (Figure 23-24).

Table 2. Leafhopper assemblages in mixed grasses with and without dominant *B. ischaemum* are compared, with vector species in bold. An asterisk denotes genera that were unique to that grass type and year.

2015 Mixed Grass	2015 *Bothriochloa*	2016 Mixed Grass	2016 *Bothriochloa*	2016 Tallgrass Prairie
		Aceratagallia	***Aceratagallia***	
		Agallia	***Agallia***	***Aceratagallia***
		Amblysellus	***Amblysellus***	***Agallia***
Aceratagallia	***Aceratagallia***	***Chlorotettix***	***Chlorotettix***	***Amblysellus***
Agallia	***Agallia***	***Ciminius***	***Ciminius***	***Chlorotettix***
Chlorotettix	***Chlorotettix***	***Cuerna***	***Cuerna***	***Ciminius***
Ciminius	***Ciminius***	***Deltocephalus***	***Deltocephalus***	***Cuerna***
Cuerna	***Cuerna***	***Draeculacephala***	***Draeculacephala***	***Deltocephalus***
Deltocephalus	***Deltocephalus***	***Endria***	***Endria***	***Endria***
Draeculacephala	***Draeculacephala***	***Exitianus***	***Exitianus***	***Exitianus***
Endria	***Endria***	***Graminella***	***Graminella***	***Graminella***
Exitianus	***Exitianus***	***Gyponana***	***Gyponana***	***Gyponana***
Graminella	***Graminella***	***Macrosteles***	***Macrosteles***	***Macrosteles***
Graphocephala*	***Gyponana***	***Paraphlepsius***	***Norvellina****	***Scaphytopius***
Gyponana	***Macrosteles***	***Scaphytopius***	***Paraphlepsius***	***Stirellus***
Macrosteles	***Norvellina****	***Stirellus***	***Stirellus***	***Xerophloea***
Paraphlepsius	***Paraphlepsius***	***Xerophloea***	***Xerophloea***	***Xestocephalus***
Scaphytopius	***Scaphytopius***	***Xyphon***	***Xestocephalus***	*Balclutha*
Stirellus	***Stirellus***	*Acinopterus*	***Xyphon***	*Commellus*
Xerophloea	***Xerophloea***	*Balclutha*	*Balclutha*	*Deltanus**
Balclutha	*Balclutha*	*Dikraneura*	*Commellus*	*Dikraneura*
*Dikraneura**	*Doratura**	*Doratura*	*Dikraneura*	*Driotura**
Empoasca	*Empoasca*	*Empoasca*	*Doratura*	*Empoasca*
Extrusanus	*Extrusanus*	*Extrusanus**	*Empoasca*	*Eratoneura**
Flexamia	*Flexamia*	*Flexamia*	*Flexamia*	*Flexamia*
Laevicephalus	*Laevicephalus*	*Laevicephalus*	*Laevicephalus*	*Laevicephalus*
Polyamia	*Polyamia*	*Memnonia*	*Memnonia*	*Memnonia*
		*Paraulacizes**	*Polyamia*	*Spangbergiella*
		Polyamia	*Prairiana*	
		Prairiana	*Spangbergiella*	

38

Table 3. The total number of leafhoppers, genera, and unique genera for all 2016 sampling sites (top four are mixed grasses without dominant *B. ischaemum*, next four are mixed grasses with dominant *B. ischaemum*, bottom is separate Tallgrass Prairie location).

Site	Total Leafhoppers	Total Genera	Total Unique for Grass Type	Total Unique Overall
19th and Western	983	22	0	0
Boomer MG	2605	25	1	0
Botanic Garden	2137	26	3	2
Edward Jones	1332	21	0	0
Cross Country	1104	24	0	0
Insect Zoo	1279	23	0	0
Rodco	220	15	1	1
Western Petroleum	341	22	5	0
Tallgrass Prairie	509	27	---	3

Table 4. Mean number of salivary sheaths per leafhopper treatment with standard error (in parentheses) for *B. ischaemum* stem and seed head.

Plant Tissue	Control	*Balclutha*	*Exitianus*
Stem	11.667 (S.E. 6.379)	189.533 (S.E. 61.217)	276.667 (S.E. 88.266)
Seed head	36.933 (S.E. 13.482)	175.333 (S.E. 6.755)	181.444 (S.E. 68.876)

Table 5. Mean number of salivary sheaths per leafhopper treatment with standard error (in parentheses) for *B. ischaemum* racemes, glumes, and awns (all seed head tissues).

Seed Head Tissue	Control	*Balclutha*	*Exitianus*
Raceme	1.467 (S.E. 0.176)	29.667 (S.E. 8.027)	21.667 (S.E. 1.644)
Glume	33.067 (S.E. 12.887)	129.400 (S.E. 10.046)	153.333 (S.E. 64.748)
Awn	2.400 (S.E. 1.217)	16.267 (S.E. 5.780)	6.444 (S.E. 2.724)
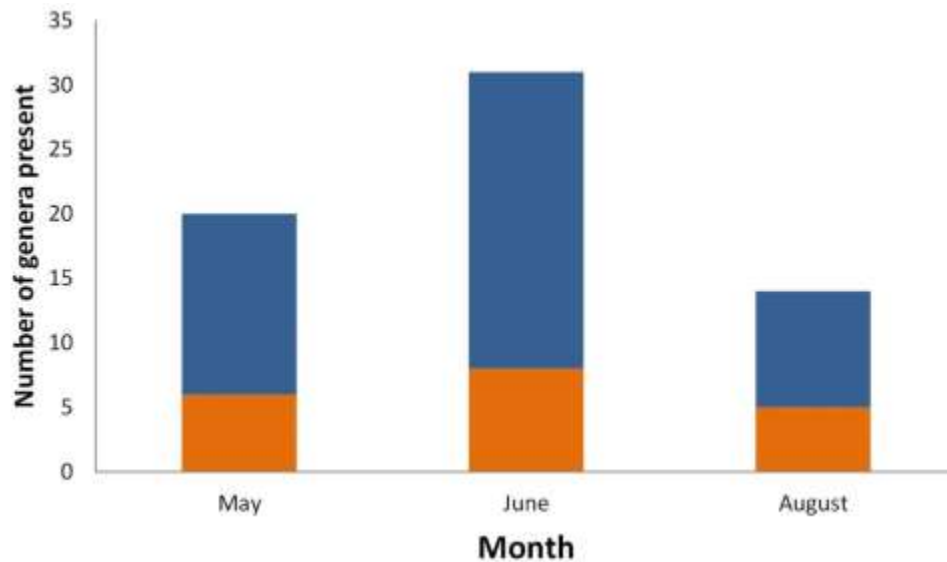
Figure 9. The number of Cicadellidae genera collected by month in 2014. The orange region represents the portion that are known vectors of plant pathogens.
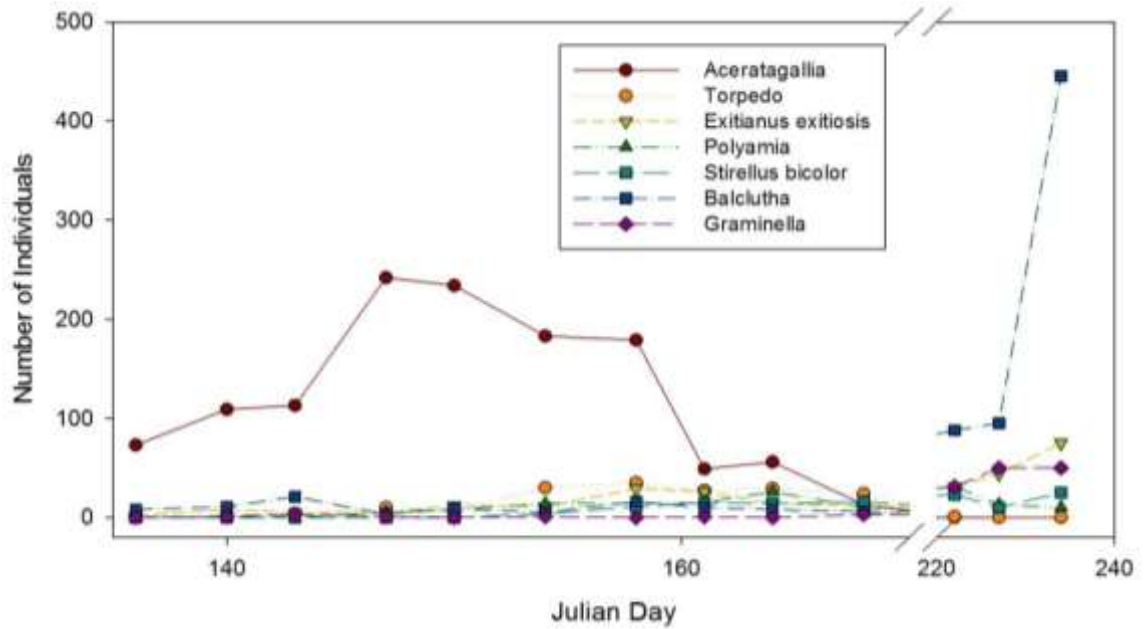
Figure 10. The number of individuals present from seven leafhopper genera/species as they varied by Julian day from May 16 through August 22, 2014.
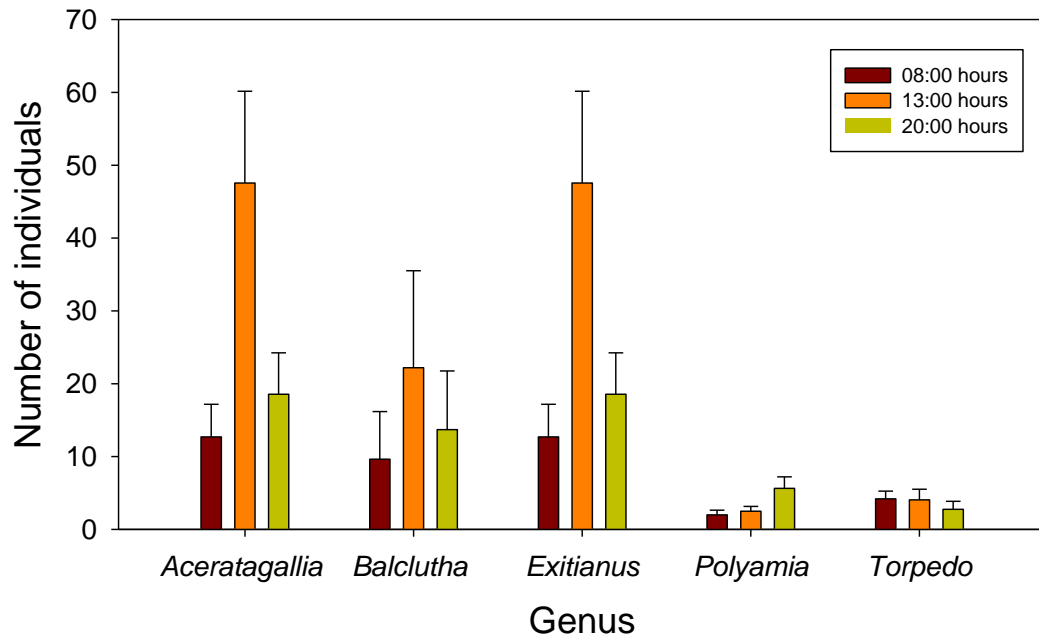
Figure 11. Mean (±1 S.E.) number of captured individuals from five leafhopper genera by time-of-day. No significant differences were detected although mid-day captures were more than 2x higher than other periods for three genera.
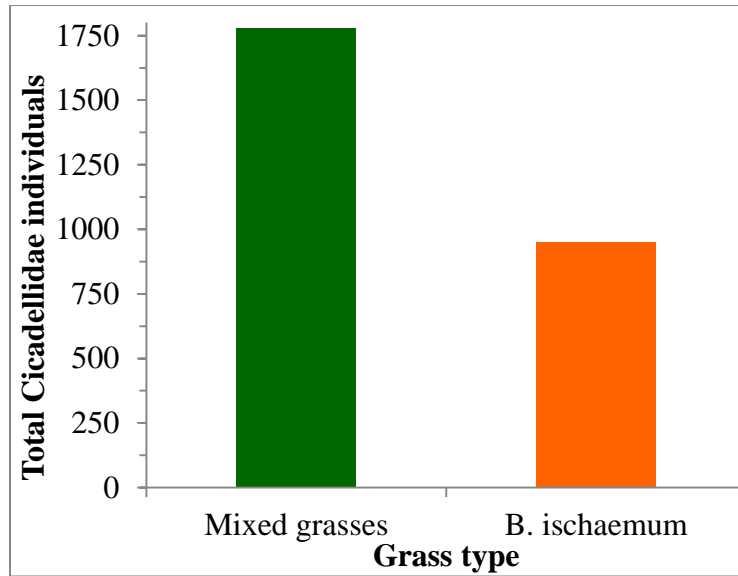
Figure 12. The total number of Cicadellidae individuals, both immatures and adults, collected in mixed grasses and in *B. ischaemum*-dominant grasses from April 12 through June 26, 2015.
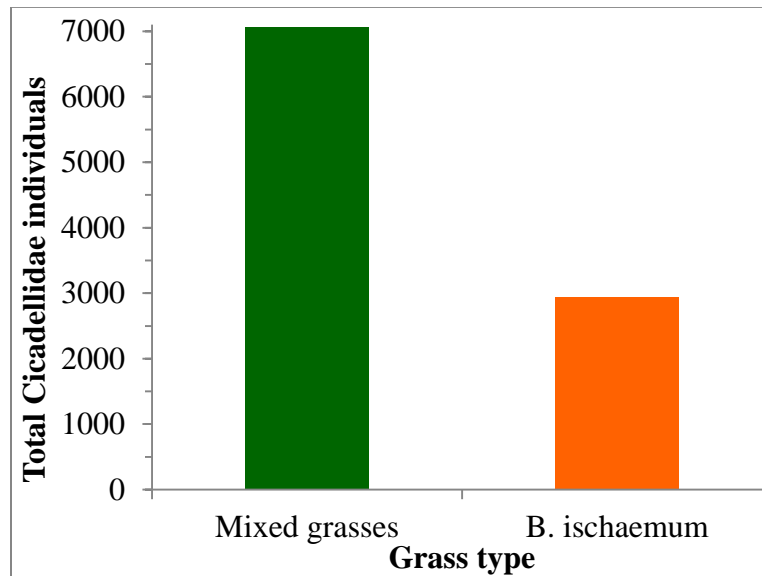
Figure 13. The total number of Cicadellidae individuals, both immatures and adults, collected in mixed grasses and in *B. ischaemum*-dominant grasses from May 26 through August 1, 2016.
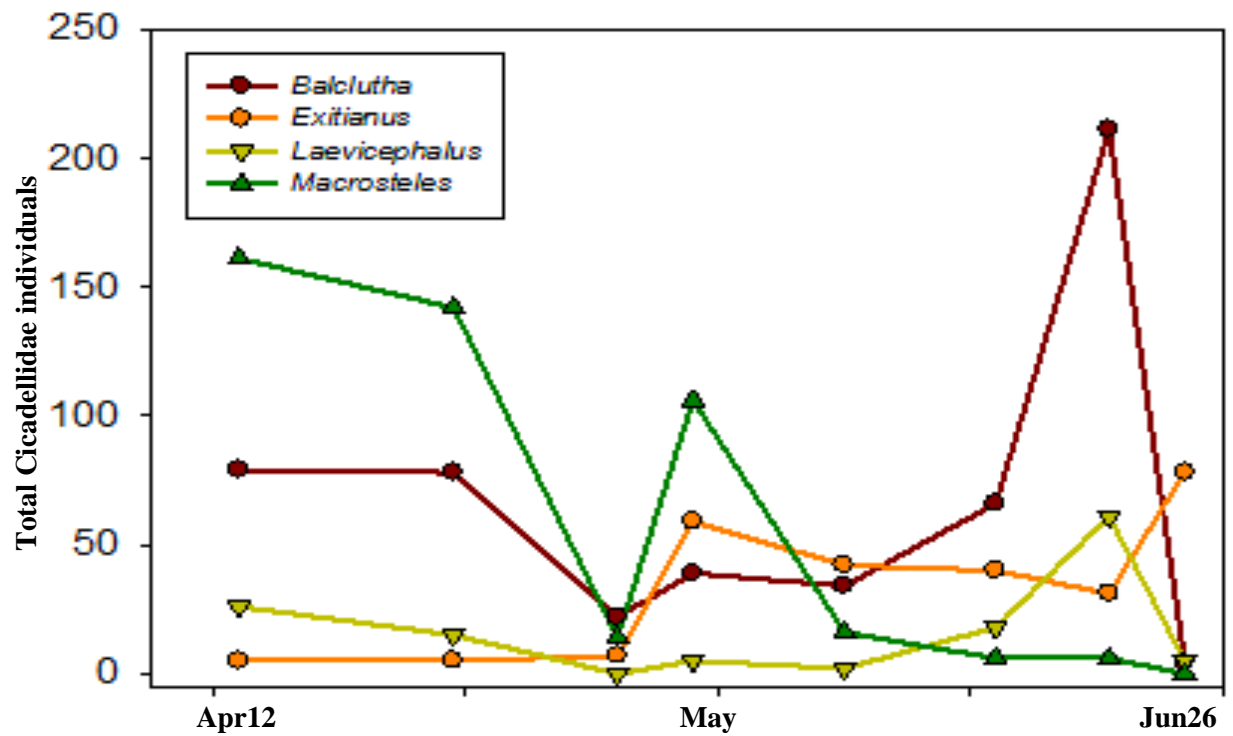
Figure 14. Fluctuations in the populations of the four most abundant genera from April

12 through June 26, 2015. Rain events in late April and mid-May caused sharp declines

in some leafhopper populations.
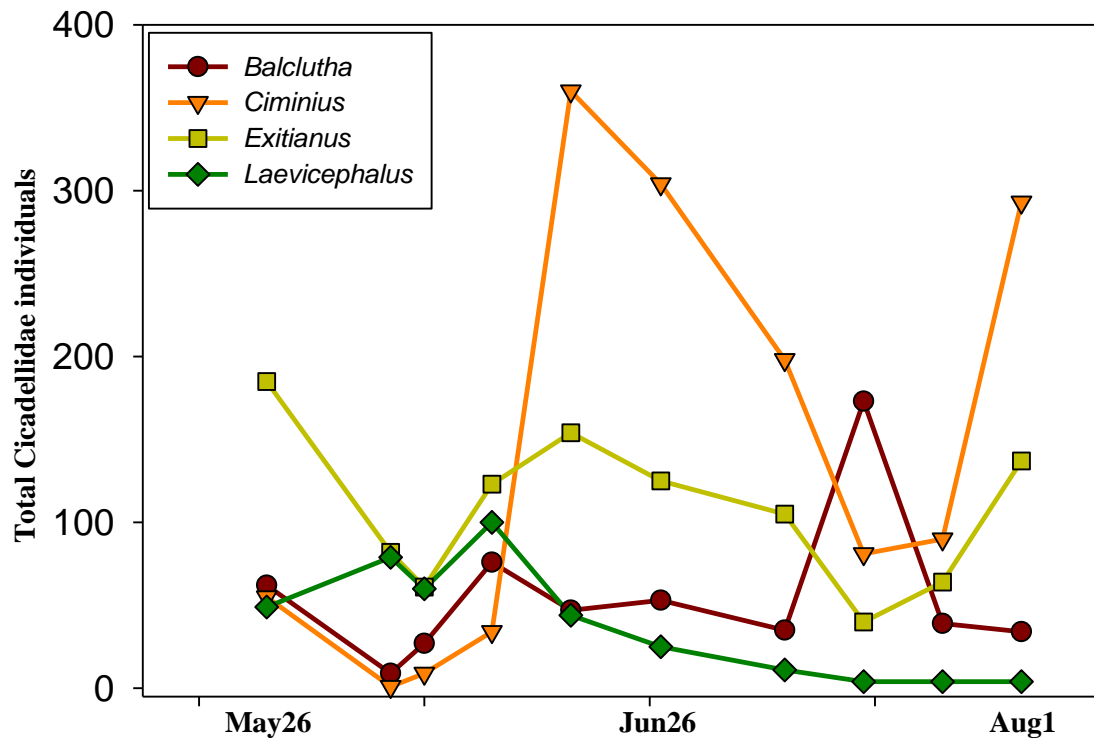
Figure 15. Fluctuations in the populations of the four most abundant genera from May 26

through August 1, 2016.
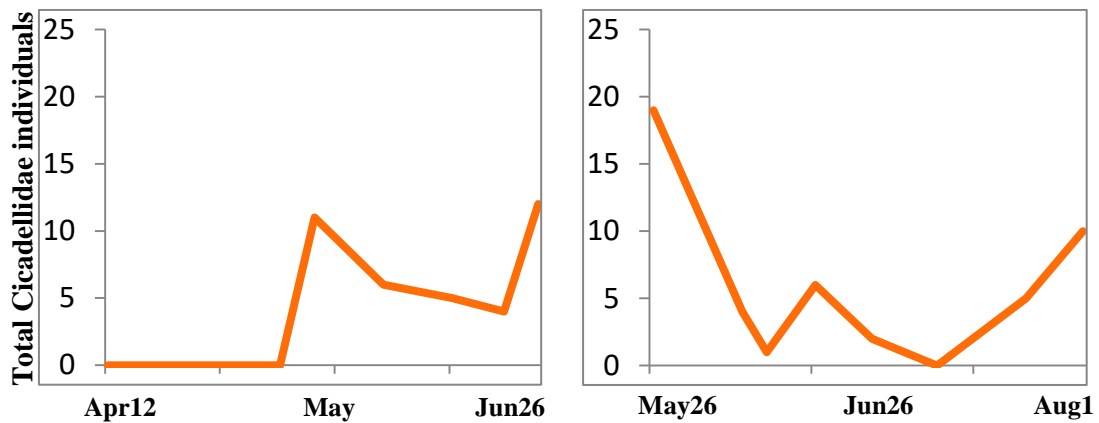
Figure 16. Fluctuations in the populations of the vector *Deltocephalus* from April 12

through June 26, 2015 (left) and May 26 through August 1, 2016 (right).
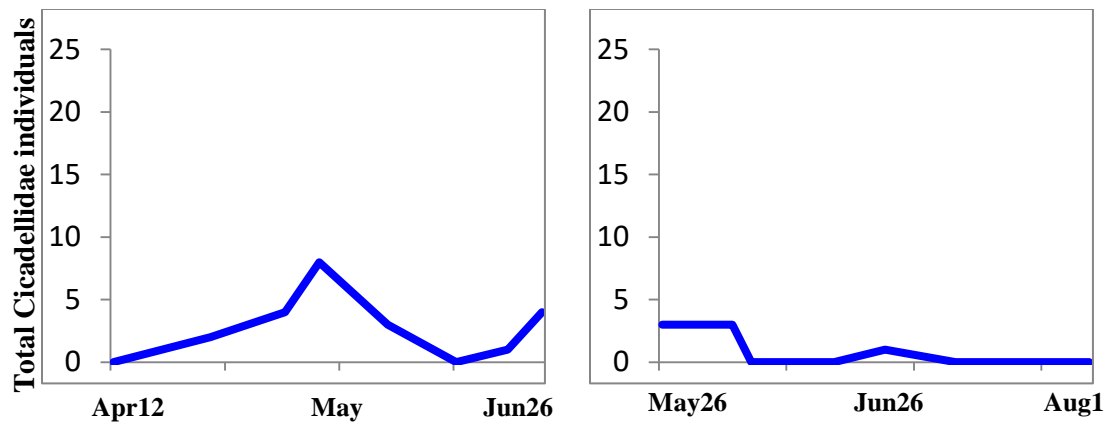
Figure 17. Fluctuations in the populations of the vector *Endria inimica* from April 12

through June 26, 2015 (left) and May 26 through August 1, 2016 (right).
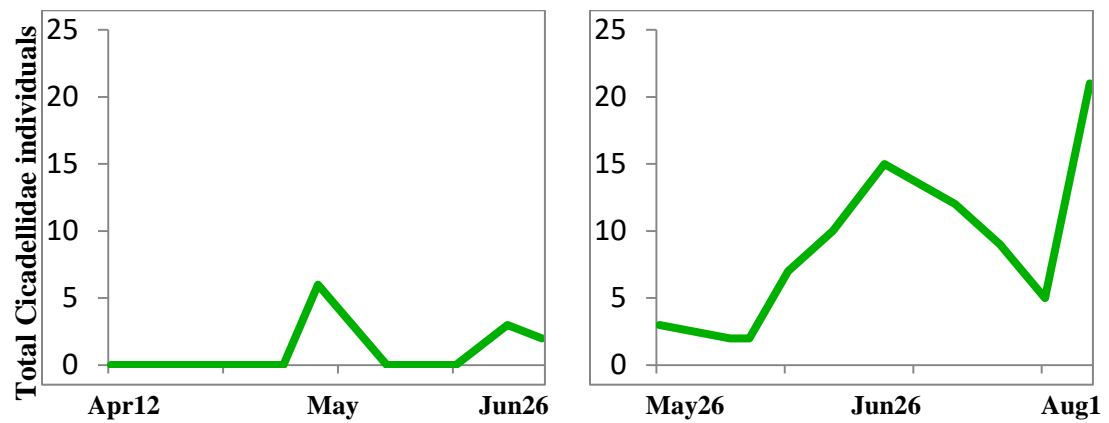
Figure 18. Fluctuations in the populations of the vector *Graminella* from April 12

through June 26, 2015 (left) and May 26 through August 1, 2016 (right).

45
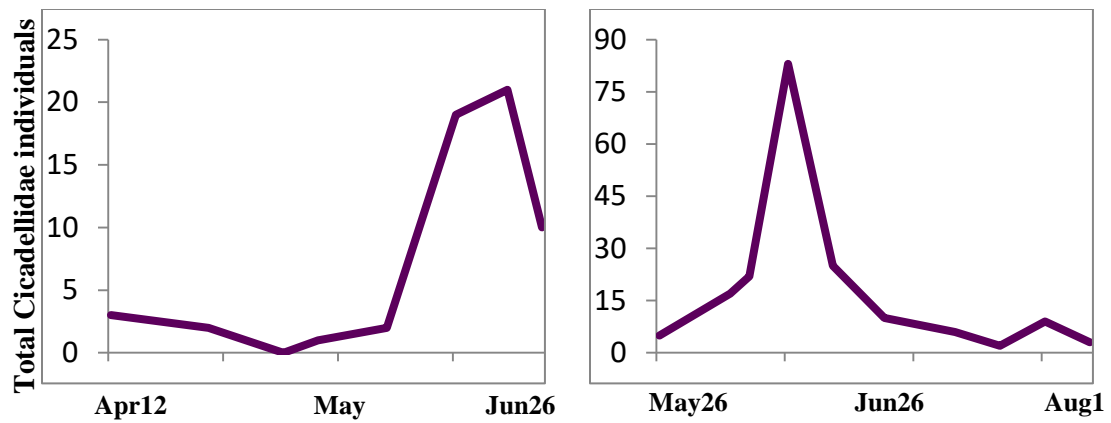
Figure 19. Fluctuations in the populations of the vector *Stirellus bicolor* from April 12 through June 26, 2015 (left) and May 26 through August 1, 2016 (right).

Figure 20. Locations of *B. rubrostriata* in October (blue circle) and November (green circle) 2015 (Google Maps 2015).
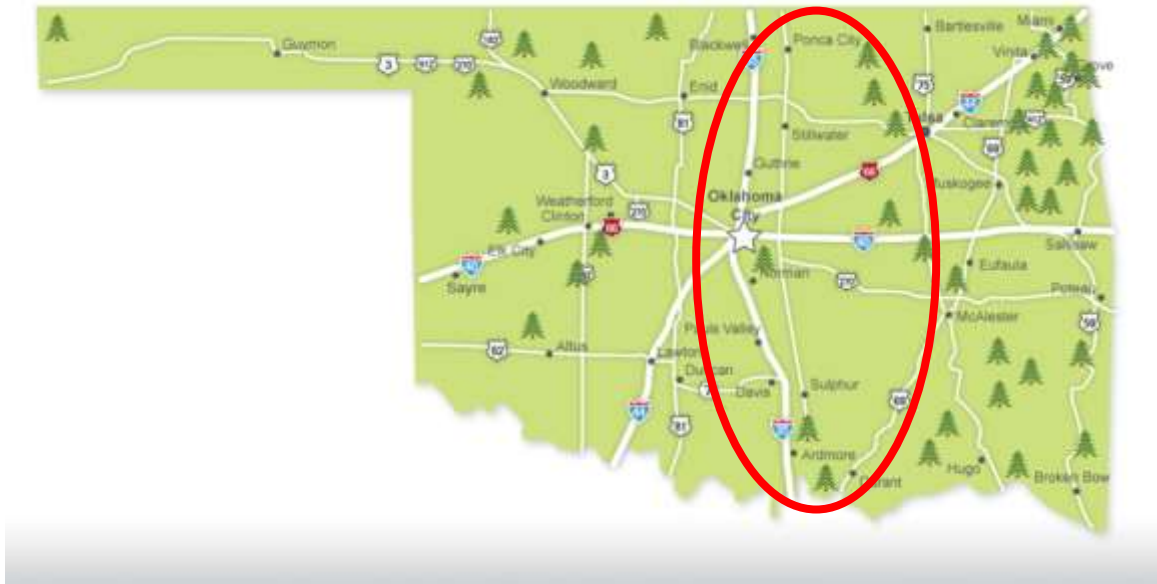
Figure 21. Locations of *B. rubrostriata* in October 2016

(http://www.travelok.com/maps/view.parks).

Figure 22. Salivary sheaths left by *E. exitiosus* in *B. ischaemum* stem tissue.
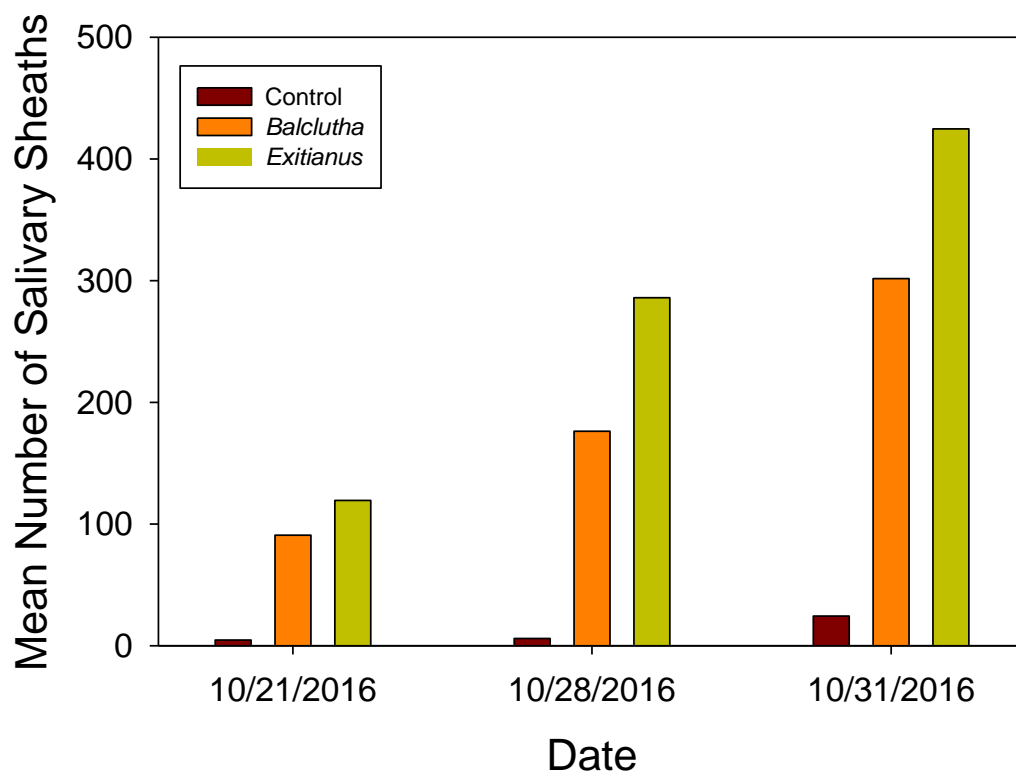
Figure 23. Mean number of salivary sheaths on *B. ischaemum* stems for each treatment by date. For each progressive date, the standard error for the control treatments is 0.568, 0.594, and 1.393 respectively, for the *Balclutha* treatments is 8.211, 14.019, and 19.312 respectively, and for the *Exitianus* treatments is 11.107, 11.262, and 25.767 respectively.
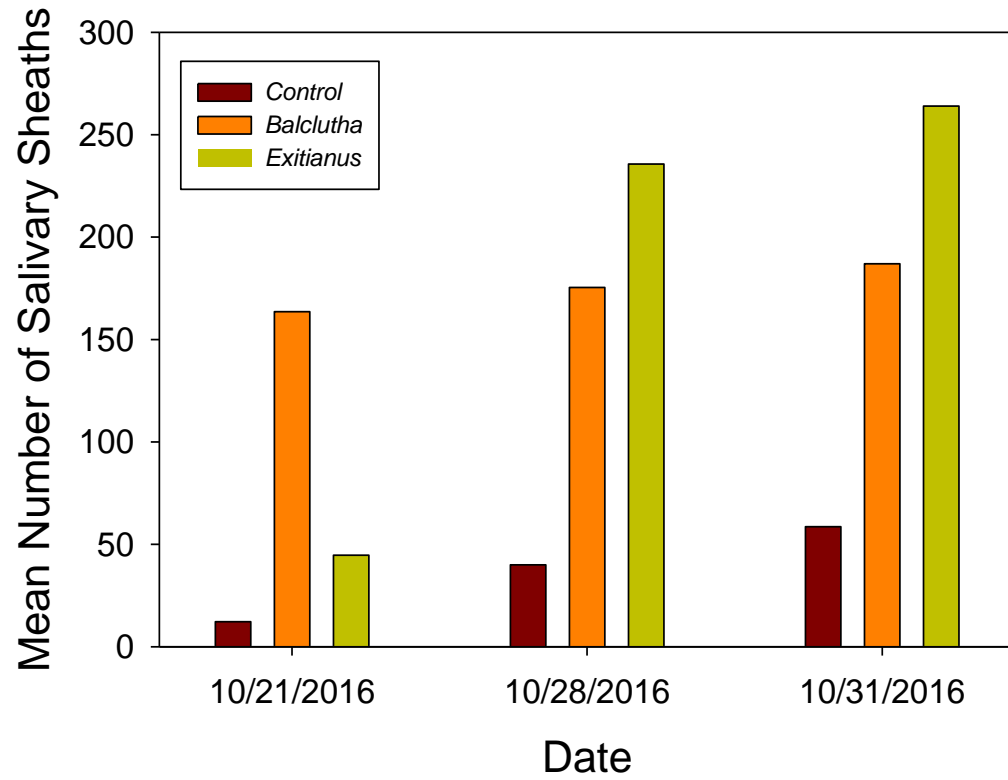
Figure 24. Mean number of salivary sheaths on *B. ischaemum* seed heads for each

treatment by date. For each progressive date, the standard error for the control treatments

is 3.121, 9.317, and 10.768 respectively, for the *Balclutha* treatments is 21.565, 39.625,

and 27.317 respectively, and for the *Exitianus* treatments is 4.702, 82.649, and 85.106

respectively.

CHAPTER IV

DISCUSSION

Relative Abundance of Leafhoppers and Optimal Sampling Time by Sweeping

In general, leafhoppers were most abundant at midday and this could therefore be concluded as the optimal time to sample. However, sampling efficiency at any time was impacted by taxon, daily environmental factors like temperature and moisture, and seasonal progression. While there were no statistically significant differences in the overall leafhopper capture rate throughout the day, activity varied by species and changed throughout the sampling season. Although June sweep samples had the most genera and vector species present, leafhoppers were most abundant in August despite fewer sampling days. However, species richness was low as samples were dominated by late-season genera like *Balclutha*.

Sampling in the early morning would not be recommended as the dew caused immense sweep net wetness and reduced sampling efficiency. Not only did it take longer to remove wet contents from a soaked net, Cicadellidae stuck to the wet fabric and ring of the net and had to be painstakingly removed with delicate forceps in the hope of maintaining accurate assemblage counts. Although temperatures at midday and dusk were similar, leafhoppers could be more abundant (and thus more efficiently sampled) at

midday for several reasons. Just as Cicadellidae are hindered by a wet sweep net, the early morning dew (or water droplets from any source of wetness) on grasses also restricts their movement. The midday heat eliminates this wetness and it may additionally deter potential predators (thereby creating a safer environment for leafhoppers) as well. Water transpiration increases during the middle part of the day and there may thus be more nutrients in the upper parts of plants for leafhoppers to feed upon. DeGooyer (1997) also noted that dew affected sweep sampling and that wet samples could potentially bias population estimates. Moreover, not only is dew present in the morning prior to evaporation but it also begins to form again in the evening, further making midday the optimal time for leafhopper sampling.

Sweep net sampling is an effective technique for accurately measuring leafhopper densities, although it can be problematic due to its variability (DeGooyer 1997). Weather conditions, time of day, dew, vegetation characteristics, sampling regime, and the person doing the sampling all create variation among sweep net samples. Other techniques, like vacuum-net sampling, can gauge leafhopper density with great accuracy, but are too costly, rigorous, or unfeasible. In contrast, methods like various trap types are unreliable for density estimates (DeGooyer 1997).

While *Balclutha* species were collected in great abundance, the invasive *B. rubrostriata* was absent from all samples despite being present in the nearby vegetation by late October. This is because even invasive species of Cicadellidae can be host specialists. Bess, O'Neill, and Kemp (2004) found that the composition of leafhopper assemblages is more similar on noncontiguous sites with the same vegetation than on contiguous sites with different vegetation. Knowing when target species of leafhoppers are most abundant

both seasonally and during the day could facilitate future grassland health or crop system research, and future studies should seek to track the activity of those Cicadellidae diurnally and throughout the appropriate sampling months.

Differences in Leafhopper Assemblages in Mixed Grasses With and Without Dominant *Bothriochloa ischaemum*

It is generally accepted that complex plant communities support greater herbivore species numbers; more insect species are found in habitats with more plant diversity (Nagel 1979; Tscharntke and Greiler 1995). The data from this study supported this as well, that the abundance and diversity of leafhoppers is higher in mixed grass patches than in patches dominated by *B. ischaemum*. Interestingly, individual species appeared and disappeared from the landscape as the season progressed. Because several of these species are economically important vectors of plant pathogens, this seasonality data could be important for the monitoring and management of target pest insects. While *M. quadrilineatus*, a vector of *Candidatus Phytoplasma asteris*, was numerous in the springtime mixed grasses, it was absent by the summer months. This leafhopper is a known migrator, overwintering in the southern states and flying north as the temperatures warm up (Meade and Peterson 1964). In both 2015 and 2016, *M. quadrilineatus* numbers peaked in late May to early June, then dropped dramatically by the end of June. It is likely that this is when migrating females that overwintered as eggs left this part of central Oklahoma to migrate to northern regions, including Minnesota, North Dakota, Ohio, and Canada (Hoy et al. 1992; Solomon 1986), using jetstream winds that carried leafhoppers north and east (Solomon 1986).

In contrast, *E. exitiosus* is abundant year round and seemed to prefer *B. ischaemum* - dominant grass communities despite being a habitat generalist (Cho et al. 2012). Because this leafhopper is also a vector of *Spiroplasma kunkelii* (Pitre and Hepner 1967) and Maize chlorotic dwarf virus (Nault and Madden 1989), and other members of the genus are known phytoplasma vectors (Weintraub et al. 2004; Arocha et al. 2009; Rao et al. 2014), the risk of plant pathogen transmission goes up as the population rises. Indeed, although Aster Yellows incidence is usually tied to *Macrosteles* population incursions (Meade and Peterson 1964), some years see low *Macrosteles* numbers but high aster yellows incidence (Wayadande, personal communication). It is possible that undocumented vectors of Aster Yellows, possibly other *Exitianus* species, may be contributing to the incidence of Aster Yellows, but this needs to be tested.

Cicadellidae in the genus *Balclutha* dominated both early-season mixed grasses and late-season *B. ischaemum*. Their ability to exploit a wide range of resources, including less attractive monocultures, could have implications for agriculture, especially if members of the genus *Balclutha* are found to transmit plant-infecting microbes. *Macrosteles*, *Exitianus*, and *Balclutha* all belong to the subfamily Deltocephalinae which contains 117 of the world's recognized 151 virus-transmitting Cicadellidae species and subspecies. *Macrosteles* and *Balclutha* are even more closely related as they both are in the tribe Macrostelini (Zahniser and Dietrich 2008; Zahniser and Dietrich 2013). A high proportion of Cicadellidae in both of the studied habitat types were known vectors, and this has potential implications for the movement of pathogens from mixed grasses to cultivated grasses. Pathogens can function as a bridge connecting wild and agricultural host plants (Burdon and Thrall 2008). Burdon et al. (1992) documented gene flow

between wild and cultivated oats and showed how it can affect resistance genes employed for disease control. Both weeds and native plants can further impact the occurrence of agricultural diseases by serving as reservoirs for crop pathogens (Wisler and Norris 2005). Barberry (*Berberis* spp.) or buckthorn (*Rhamnus* spp.) bushes that border cereal crops can create substantial variation in the pathogens that cause wheat stem and oat crown rust (Burdon and Thrall 2008).

Although sampling in true prairie ecosystems was not originally part of this study, the opportunity to sample leafhoppers in a very stable landscape (no mowing, plowing, or shifting land use) provided some interesting information in 2016. Despite fewer samples being taken from the Tallgrass Prairie due to rain events, the location had higher leafhopper species richness and diversity than any of the mixed grass or *B. ischaemum*-dominant patches from 2015 or 2016. Increased mowing frequency, which reduces the heterogeneity of food sources and increases disturbance, directly decreases the species richness of leafhoppers (Everwand et al. 2014). In contrast, increased sward height can positively influence species richness (Kőrösi et al. 2012). While Buri et al. (2016) did not find statistically significant differences among mowing regimes and Auchenorrhynchan richness or diversity, they did document a significant and abruptly negative effect of mowing on the density of Auchenorrhynchans. Meadows in which cutting was delayed until late July and occurred only once or twice a year supported Auchenorrhynchan densities that were on average 3.5 times higher than meadows with earlier and more frequent mowing (Buri et al. 2016). Everwand et al. (2014) noted that, although forb-dominated areas lack nutrients, because they provide more plant diversity and thus more heterogeneity of food resources, they indirectly support a greater richness

of leafhopper species. In contrast, increased graminoid cover in extremely productive areas with reduced plant diversity directly leads to higher leafhopper species richness due to the increased accessibility of graminoids as a desirable food resource for numerous Cicadellidae species (Everwand et al. 2014).

The Tallgrass Prairie also yielded several unique leafhopper species that were not present in mixed grasses or *B. ischaemum*-dominant grass communities. One possible explanation for this is host specificity, which is the direct relationship between leafhopper distributions and host plants. Both *Driotura gammaroides*, which was unique to the tallgrass prairie, and *Commellus comma*, which was present only in the tallgrass prairie and one *B. ischaemum*-dominant site, are highly host specific to *Solidago* spp. and *Elymus* spp. respectively (Nemec and Bragg 2008). *Driotura gammaroides* and *C. comma* were reported as uncommon in the Flint Hills of Kansas and Oklahoma by Cwikla and Blocker (1981), and only *C. comma* was found in Oklahoma, in Osage County (Cwikla and Blocker 1981). This is the first documentation of *C. comma* as far south as Payne County, Oklahoma.

Because some leafhoppers are vectors of important plant pathogenic viruses and bacteria that are still outside US borders (e.g. *Cicadulina mbila* transmitted maize streak virus), another underlying goal of this leafhopper sampling study was surveillance for exotic leafhopper species. Although almost 50 percent of the identified genera contained known pathogen vector taxa, no recently introduced exotic leafhopper genera were recovered and identified. Previously introduced species like *B. rubrostriata* and *E. inimica*, have become established in the US and are now considered endemic.

Given the diversity of ecosystems that exist in Oklahoma's range of contrasting biogeographic regions, future studies should further document leafhopper assemblages throughout the state (Webb 1950). Knowing which rare species inhabit locations like the Tallgrass Prairie or other protected parks could help to promote the conservation of these valuable locations. In addition, Cicadellidae communities in and around Oklahoma's vast monoculture croplands can be surveyed to further determine the span of leafhopper diversity and better understand how it is impacted by various polycultures.

Seasonal Presence of *Balclutha rubrostriata* in Central Oklahoma on *Bothriochloa ischaemum*

While cooler winters may consistently prevent *B. rubrostriata* from overwintering in Oklahoma, its variable northward movement into the state from Texas each year may depend on favorable annual temperatures and precipitation. When *B. rubrostriata* was first detected in central Oklahoma in 2013, the annual temperature for the state was below normal while the annual precipitation was above normal. The occurrence of several violent tornados in late May may also be of note as leafhoppers are known to travel long distances via jet stream winds (National Climatic Data Center; Drake and Chapman 1965; Chiykowski and Chapman 1965). *Balclutha rubrostriata* was again present in 2014 in Oklahoma, which saw both below average temperature and precipitation for the year (National Climatic Data Center). In 2015, when *B. rubrostriata* was largely absent from much of the state, both Oklahoma and Texas had above average annual temperatures and record high annual precipitation (National Climatic Data Center). Finally, *B. rubrostriata* was present once more throughout central Oklahoma in 2016, and the annual temperature was much above average while the annual precipitation

was near average (National Climatic Data Center). Interestingly, Oklahoma had more tornadoes in 2015 (a total of 111) than it did in 2013, 2014, or 2016 (totals of 82, 16, and 57, respectively) when *B. rubrostriata* was documented throughout the central region of the state (National Weather Service). While abundant populations of *B. rubrostriata* seem to thrive in southern Texas (Zahniser et al. 2010), the lack of consistent correlation between its presence in Oklahoma and the annual temperature, precipitation, and tornadic activity warrants continued surveillance coupled with the monitoring of both abiotic and biotic environmental factors.

Andreason et al. (2015) suggested that *B. rubrostriata* may have an established population at Kaw Lake due to the collection of a large number of individuals from the sampling site in 2014, but this was not supported by the 2015 data as no nymphs or adults were found there. Perhaps, then, *B. rubrostriata* is travelling into Oklahoma along the vast swaths of *B. ischaemum* because it is effectively exploiting its preferred food resource. It is possible that *B. ischaemum* matures and expires sooner in the Texas climate so *B. rubrostriata* must continually move northward in order to utilize sufficient food plants. Similarly, the overwhelming abundance of *B. rubrostriata* feeding on Texas *B. ischaemum* could deplete the plant's nutritional value so the leafhoppers are drawn northward as they follow the lesser utilized, more nutritive *B. ischaemum*. Taylor et al. (1993) noted that the activity of *Dalbulus maidis* females is directly associated with the condition of host plants, while the activity of males is tied to light level, and that a change in sex ratio may be indicative of migration. This could explain why the vast majority of *B. rubrostriata* adults collected from Oklahoma *B. ischaemum* are female, but further studies are needed to determine if true migration is occurring.

Circulifer tenellus, the beet leafhopper, migrates using low-level wind jets in California, traveling from overwintering and spring breeding locations to summer oviposition locations. This is similarly indicated by influxes of gravid females with below-average fat content (Lawson et al. 1951; Cook 1967). *Melanaphis sacchari*, the sugarcane aphid, also utilizes low-level jets to achieve local and long distance dispersal. Other aphid species, such as *Rhopalosiphum maidis* and *Schizaphis graminum*, engage in this type of movement as well (Bowling et al. 2016; Irwin and Thresh 1988). Diminished plant quality due to high aphid density and windy, arid conditions can cause substantial production of alates and subsequent dissemination via winds (Singh et al. 2004). The rapid range expansion of *M. sacchari* put over 90 percent of North American sorghum-producing areas at risk of damage within two years. Low-level jets originating in Texas occur frequently and enable migratory species like *M. sacchari* to inhabit more northerly locations in a matter of weeks and even days (Bowling et al. 2016). If *B. rubrostriata* was similarly dispersing via wind-aided movement, it would be present readily and regularly throughout central Oklahoma, given its abundance in Texas. Its varied presence and absence suggest that this leafhopper may not be traveling on low-level jets.

Annual monitoring of *B. rubrostriata*, as well as determining its biology and vector competence, is necessary to better understand its potential impact on native ecosystems and croplands. Future research should seek to ascertain *B. rubrostriata*'s overwintering capabilities, biological requirements, and what plant pathogens it carries versus what it successfully transmits. If *B. rubrostriata* is not a vector, the barrier that prevents this (either within the leafhopper or within the plant) should be determined.

Probing Preferences of *Balclutha neglecta* on *Bothriochloa ischaemum* Tissues

When *B. rubrostriata* was first detected on *B. ischaemum* in Oklahoma, it was observed on the seed heads of mature plants. This is unusual because seed feeding has not documented for leafhoppers, which feed primarily from vascular or mesophyll tissues on stems and leaves (Tonkyn and Whitcomb 1989). Because *B. rubrostriata* did not survive and reproduce in laboratory colonies, its congener, *B. neglecta*, was used to study feeding site preference on *B. ischaemum*. Although the data suggest that the *B. ischaemum* seed head is not a more desirable feeding location for *B. neglecta* or *E. exitiosus* than the stem, further studies are needed to determine if these results are consistently true or an anomaly. *Exitianus exitiosus* was selected as a positive control due to its reputation as a known stem feeder, so the high number of salivary sheaths left in seed head tissues by these leafhoppers cannot be explained from this study alone. Since *E. exitiosus* is a generalist feeder, it could have adapted its probing behaviors to better utilize the *B. ischaemum* plant. It is possible that *E. exitiosus* can feed from various plant tissues even though it prefers stem sites.

Wayadande and Nault (1996) first noted that leafhoppers may have feeding site preferences despite the ability to ingest from multiple plant tissues, and that these preferences could vary by species. While *Graminella nigrifrons*, *G. oquaka*, and *Amblysellus grex* preferred phloem over other tissues, *D. maidis* did not exhibit this preference. Furthermore, although *D. maidis* had a low proportion of probe-to-phloem contact, the probability of prolonged ingestion was high, which suggests that this species may take longer to settle before feeding (Wayadande and Nault 1996). It is possible, then, that the higher proportion of *E. exitiosus* salivary sheaths left in both stem and seed head tissues of *B. ischaemum* could be due to longer settling and ingestion times in *B.*

59

neglecta. Vector competence may also play a role in probing behavior and feeding site preference (Wayadande and Nault 1996), and while the vector status of *B. neglecta* is undetermined, *E. exitiosus* is a well-known vector (Pitre and Hepner 1967; Nault and Madden 1989). This could further explain the differences in location and number of salivary sheaths, but the ability of *B. neglecta* to acquire and transmit phytoplasmas needs to be ascertained. Electrical penetration graph studies could additionally help to elucidate the probing activities and feeding preferences of *B. neglecta* and *E. exitiosus*.

Just as leafhoppers can move to the apex of grasses during the day in order to follow the path of water transpiration and subsequent nutrient flow, this may also be why they choose to reside and feed upon seed head tissues. Yet while seeds offer concentrated nutrition in relation to other plant tissues, they may be surrounded by a hardened coat that could possibly be difficult for leafhopper stylets to penetrate (Slansky and Scriber 1985; Souza and Marcos-Filho 2001). In contrast, *B. ischaemum* has a potentially more accessible seed unit that consists of the external glumes, encased florets, and elongated awn (Harms). This accessibility and nutrient richness may make *B. ischaemum* seed heads more desirable to leafhoppers.

It is also possible that the seed head of *B. ischaemum* provides other benefits beyond feeding. Its structure, with multiple branches covered in layered glumes, as well as its coloration when mature, could allow for effective camouflage from predators. *Balclutha rubrostriata*, with its red-streaked wings, particularly blends in well with the purple inflorescence, so perhaps predator protection is the primary reason why this species is frequently observed on the seed head and feeding merely occurs out of convenience. Future research could assess *B. rubrostriata*'s attraction to the pigmentation of mature *B.*

ischaemum by altering the color or by testing the attraction to colors like purple versus green (representative of young *B. ischaemum*) or brown (representative of older *B. ischaemum*).

In addition, further studies on the feeding preferences of *Balclutha* species are needed to better ascertain if seed head tissues are selected for over stem tissues. This research should take place entirely in a laboratory setting with greenhouse-grown *B. ischaemum* as well as another grass with a seed head, like *Setaria* species, and a grass which lacks a distinct seed head, like *Stenotaphrum secundatum*. Future experiments also need to employ more replications and greater numbers of test plants and leafhoppers (with sex, age, and reproductive status accounted for).

EPILOGUE

My research has sought to fill some of the gaps that exist in our knowledge of

Cicadellidae ecology. I determined that, although leafhopper activity and abundance

fluctuates diurnally, seasonally, and by genera (if not species), it may generally be

optimal to sample Cicadellidae assemblages in the middle part of the day in order to

obtain the most accurate representations of diversity present. Next, I determined that

leafhopper communities can be quite similar between true plant polycultures and those

that are closer to monocultures. I also monitored for the non-native *B. rubrostriata* in

Oklahoma and documented the absence of nymphs (which suggests that overwintering is

not occurring) as well as the delayed appearance of adults on *B. ischaemum* in late fall,

nearest to the Texas border. This suggests that *B. rubrostriata* may indeed be traveling

north into Oklahoma via vegetational movement, but the extent of this behavior varies by

year and may be influenced by shifts in annual temperatures and precipitation. Finally, I

determined that *B. neglecta* and *E. exitiosus* do feed on the seed head tissues of *B.*

ischaemum in addition to feeding on the stem. This is the first record of leafhoppers

probing on grass seed heads, and while no leafhopper is known to feed on seeds, the

documentation of these species leaving salivary flanges and sheaths on *B. ischaemum*'s

penetrable seed unit could stimulate interest in some exciting potential studies.

Future research should expand upon the progress that I have made in Cicadellidae

ecology. Targeted leafhopper species, such as those of economic importance, invasive threat, or conservation concern, should be extensively sampled to fully comprehend how their activity and abundance fluctuates diurnally and seasonally and how they are affected by external factors like weather and anthropogenic disturbance. Knowing this will enable better management and prevention or conservation practices. Oklahoma's range of ecosystems should be surveyed to determine the scope of leafhopper diversity within the state and to promote continued protection of parks and preserves like the Tallgrass Prairie. The invasive *B. rubrostriata* requires continued annual surveillance so we may better understand its biology, vector status, vegetational movement, and ecological impact. Lastly, the behavior of *B. rubrostriata* should be explored to determine why it resides upon the seed head of *B. ischaemum* and if it is attracted to the inflorescence for camouflage benefits or if it is employing the seed unit as a preferential food source.

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APPENDICES

Leafhopper genera and abundance by sampling date in the Tallgrass Prairie

June 3, 2016	
Genera	**Total Leafhoppers**
Chlorotettix	1
Deltocephalus	1
Dikraneura	8
Empoasca	14
Endria	1
Gyponana	1
Laevicephalus	5
Macrosteles	22
Scaphytopius	1
Spangbergiella	1
Stirellus	1

June 10, 2016	
Genera	**Total Leafhoppers**
Aceratagallia	1
Balclutha	5
Dikraneura	1
Driotura	1
Empoasca	1
Exitianus	1
Flexamia	2
Graminella	2
Gyponana	1
Laevicephalus	1
Macrosteles	2
Stirellus	2
Xestocephalus	2

June 17, 2016	
Genera	**Total Leafhoppers**
Aceratagallia	2
Agallia	14
Amblysellus	8
Balclutha	15
Commellus	6
Cuerna	8
Dikraneura	5
Empoasca	9
Eratoneura	1
Flexamia	3
Gyponana	5
Laevicephalus	1
Macrosteles	3
Memnonia	1
Scaphytopius	1
Spangbergiella	1
Stirellus	5
Xestocephalus	1

June 24, 2016	
Genera	**Total Leafhoppers**
Agallia	6
Amblysellus	3
Chlorotettix	1
Ciminius	1
Deltanus	1
Dikraneura	1
Driotura	1
Exitianus	4
Flexamia	2
Graminella	3
Gyponana	3
Laevicephalus	1
Memnonia	1
Scaphytopius	3
Spangbergiella	1
Stirellus	2
Xerophloea	1

July 1, 2016	
Genera	**Total Leafhoppers**
Aceratagallia	1
Agallia	4
Chlorotettix	2
Commellus	4
Endria	5
Eratoneura	1
Exitianus	4
Flexamia	2
Graminella	1
Laevicephalus	3
Memnonia	1
Scaphytopius	5
Stirellus	5

July 7, 2016	
Genera	**Total Leafhoppers**
Agallia	1
Empoasca	2
Flexamia	1
Laevicephalus	3
Scaphytopius	1

July 22, 2016	
Genera	**Total Leafhoppers**
Agallia	4
Amblysellus	1
Chlorotettix	5
Cuerna	1
Deltanus	3
Driotura	3
Empoasca	2
Flexamia	2
Laevicephalus	3
Scaphytopius	2
Xestocephalus	1

VITA

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