ROADSIDE ENVIRONMENTS AND THE EFFECTS OF ROADSIDE MANAGEMENT PRACTICES ON MILKWEEDS AND MONARCHS

By

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Abstract: The potential of roadsides as habitat for wildlife has gained interest in recent years, with research suggesting positive effects for several taxa. Roadsides cover a vast area in the United States, and are actively managed by state and federal agencies. However, not much is known about the potential of roadsides to provide habitat for the monarch butterfly (*Danaus plexippus*). Therefore, I assessed milkweed densities on roadsides and in adjacent lands, the effects of different mowing times in roadsides on milkweed density, number of stems per plant, plant height (cm), and latex production, and mortality rates for monarch butterflies on roadsides oriented in a north/south and east/west direction during fall migration. My results show *Asclepias viridis* and *A. asperula* milkweed densities are higher on roadsides than adjacent lands in Oklahoma; mowing roadsides lowers counts of *A. viridis* milkweed but those lower counts are limited to the actual times of mowing; monarch mortality during fall migration did not differ with highway orientation. Although additional research is needed, roadsides have the potential to provide monarch butterfly habitat and should be considered when assessing conservation strategies.

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CHAPTER I

INTRODUCTION

Habitat loss is a serious threat to biological communities around the world. It leads to a decrease in biodiversity and homogenization of biological communities (Lapiedra et al. 2016). Much habitat loss is driven by habitat transformation for agricultural purposes and urbanization and this trend of habitat loss will continue to increase in the future. The potential value of roadsides as habitat has been a topic of research interest, particularly for butterflies (Munguira and Thomas 1992; Ries et al. 2001; Mueller 2013; Mueller and Baum 2014; Shahani et al. 2015; Kasten et al. 2016; Leston and Koper 2017). While several studies have shown the potential for roadsides to provide monarch butterfly habitat (e.g., Mueller 2013; Mueller and Baum 2014; Shahani et al. 2015; Kasten et al. 2016), additional information is needed to evaluate if roadsides can support monarch butterflies during their migration and spring and summer breeding periods.

For roadsides to be considered habitat for monarch butterflies, roadsides need to provide host plants for developing larvae during the spring and summer breeding periods and nectar sources for adults during the spring and fall migrations. Milkweeds (*Asclepias* spp) are the sole host plant for monarchs, and development from egg to larva to adult takes approximately one month to complete (Zalucki 1982). Milkweeds can also serve as a nectar source along with other plants although monarchs can use other plants as nectar sources (Beall 1948; Brower et al. 2006). Monarch butterflies migrate to Mexico during late summer through fall where they overwinter, and then migrate northwards as far as southern Canada during their spring and summer breeding periods (Urquhart and Urquhart 1979; Brower et al. 2006). Roadsides could negatively impact adult monarchs during migration if they cause high mortality rates. Therefore, it is also important to evaluate adult monarch mortality along roadsides.

This study focused on evaluating the potential of roadsides as suitable habitat for monarch butterflies in Oklahoma. I evaluated milkweed densities on roadsides, milkweed responses to different mowing regimes, and monarch mortality rates associated with roadsides. The first objective was to document milkweed densities of *A. viridis* and *A. asperula* throughout Oklahoma, excluding the panhandle, along roadsides and adjacent lands and to compare milkweed density between these two areas. Documenting the distribution and densities of milkweed is needed to assess habitat availability for monarch butterflies. The second objective was to evaluate the influence of different mowing times and frequencies in roadsides on *A. viridis* density, number of stems per plant, plant height (cm), and latex production. Roadside mowing is necessary for motorist visibility and safety but it is possible that mowing times could be altered to provide suitable habitat for milkweeds. Mowing has been shown to generate *A. viridis* and *A. syriaca* milkweed regrowth during times when it was not otherwise as available in similar sites that were not mowed (Baum and Mueller 2015; Fischer et al. 2015). However, mowing could also modify number of stems per plant, plant height (cm), and/or latex production, which could influence the amount of plant tissue available for monarch larvae and the ability of larvae to eat the tissue. The third objective was to survey north/south and east/west oriented roadsides for monarch butterfly behaviors and mortality as road orientation could influence monarch butterfly behavior and mortality rates during their southward fall migration. If roadsides are to be utilized as habitat for monarch butterflies, identifying how different factors influence mortality is important for developing strategies that reduce the risk of collisions with vehicles.

The results of my project provide information on the potential for roadsides to serve as habitat for monarch butterflies. Managed roadsides could provide monarch habitat and also benefit other organisms, such as bees (Hopwood 2008), flowering plants (Halbritter et al. 2015), and birds (Camp and Best 1994).

CHAPTER II

MILKWEED DENSITIES IN ROADSIDES AND ADJACENT LANDS

INTRODUCTION

The monarch butterfly (*Danaus plexippus*) is one of the most well-known insects in North America because it is easily identified and makes a unique annual migration from southern Canada to central Mexico (Scudder 1893; Urquhart and Urquhart 1979; Gustafsson et al. 2015). The monarch population has declined over the past fifteen to twenty years based on estimates of the size of the population on the overwintering grounds in Mexico (Brower et al. 2011; Rendón-Salinas et al. 2011; World Wildlife Fund Mexico 2017). Although concerns initially focused on deforestation on the overwintering grounds (Wells et al. 1983; Flockhart et al. 2015), recent research suggests the loss of breeding habitat in the United States could negatively affect the monarch population more than loss of overwintering grounds habitat (Flockhart et al. 2015; Inamine et al. 2016). Other threats to the eastern monarch butterfly migration include decreased nectar availability (Brower et al. 2006; Inamine et al. 2016), parasitoids, predators, disease (Oberhauser et al. 2015), and insecticides (Krischik et al. 2015).

Based on stable hydrogen and carbon isotopes, an average of 38% of overwintering monarchs over a 38-year period (from 1976 to 2014) developed in the Upper Midwest where they likely fed on *A. syriaca* as caterpillars (Flockhart et al. 2017), with the highest percent from the Upper Midwest (>50%) observed during the 1996-97 overwintering season (Wassenaar and Hobson 1998; Flockhart et al. 2017). However, herbicides and herbicide resistant crops have removed a large amount of milkweed stems from agricultural fields of corn and soybeans in the Upper Midwest (Pleasants and Oberhauser 2013; Pleasants 2017; Zaya et al. 2017). Monarch densities per milkweed plant are higher in agricultural areas than non-agricultural areas, possibly due to fewer predators in a less diverse habitat, therefore increasing the importance of milkweed loss in agricultural fields for monarchs (Oberhauser et al. 2001). If herbicide use in agricultural fields continues, it is likely that milkweed densities will remain low in agricultural fields even if seeding or planting of milkweed were to occur. Furthermore, there will likely not be support for adding milkweed to crop fields since it is viewed as a nuisance by farmers (Cramer and Burnside 1982; Yenish et al. 1987).

In Iowa, *A. syriaca* occurrence was reduced by 81% in corn and soybean fields from 1999 to 2009, corresponding to a decline in milkweed density from 52 m^2 ha^{-1} of milkweed cover to 5 m^2 ha^{-1} (Hartzler 2010). Similar reductions have likely occurred in other regions that predominantly grow corn and soybeans, including parts of North and South Dakota, Nebraska, Kansas, Missouri, Minnesota, Wisconsin, Michigan, and Ohio, and most of Illinois and Indiana, where 80% of the corn and soybeans in the US are grown (USDA National Agricultural Statistics Service 2011). But in more natural areas of the Midwest, including grassland and wetland habitats, milkweed abundance has not decreased (Zaya et al. 2017). However, less is known about trends in milkweed abundance in the south-central United States.

Conservation efforts are focused on increasing milkweed density throughout the monarch's breeding range. One of the areas proposed for increasing milkweed populations is along roadsides. There are more than 250,000 kilometers of roads in the United States (U.S. Department of Transportation 2014) that could be used for monarch habitat by planting and/or managing existing milkweed and nectar plants. However this estimate includes the safety zone, which requires frequent management to maintain motorist safety, and not all roadsides are suitable as monarch habitat since roadsides need to be maintained to ensure driver safety through maintaining sight distance, minimizing roadside vegetation hazards, and keeping traffic signs visible (Montgomery et al. 2010). Highway vegetation is also managed to decrease erosion, increase biodiversity, improve water quality and wildlife habitat, and limit the spread and growth of invasive plants such as Johnsongrass (*Sorghum halepense*) and musk thistle (*Carduus nutans*) (Montgomery et al. 2010).

Providing monarch habitat on roadsides includes managing for native plants. Roadsides with native plants have been shown to support greater native bee species richness and abundance compared to roadsides dominated by nonnative grasses and nonnative flowering forbs (Hopwood 2008), as well as increased butterfly diversity (Munguira and Thomas 1992; Ries et al. 2001). Roadsides with invasive plants have low levels of plant biodiversity, and invasive plants tend to displace native ones (Daehler

2003). Integrated roadside management programs that include restricting the use of herbicides and mowing can promote native plants on roadsides (Ries et al. 2001; Hopwood 2008), as well as seeding roadsides with native prairie forbs and grasses (Munguira and Thomas 1992; Hopwood 2008).

If roadsides support milkweed populations, they could serve as habitat for monarch butterflies. However, the distribution and density of milkweeds in roadsides throughout Oklahoma is not well documented. Monarchs migrate through and reproduce in Oklahoma during both the spring and fall. *Asclepias viridis* and *A. asperula* are suggested as being common milkweed species in the spring (NAMCP 2008; Xerces Society 2013) in the southern Great Plains. Therefore, the goals of this project were to document the distribution and densities of *A. viridis* and *A. asperula* along roadsides and in adjacent lands in Oklahoma and to compare milkweed densities between these two areas.

Methods

Study Species and Study Region

The study area encompasses the state of Oklahoma, excluding the panhandle, with six ecoregions as classified by the Oklahoma Department of Wildlife Conservation (ODWC). The shortgrass prairie, mixed-grass prairie, and tallgrass prairie ecoregions can be found from central to western Oklahoma and include many grasses as the main vegetation (ODWC 2005; Woods et al. 2005). The crosstimbers ecoregion is roughly the central one-third of Oklahoma and includes both grasses and oak trees as the main vegetation, (ODWC 2005; Woods et al. 2005). The Ozark and Ouachita Mountains ecoregions cover the east and west boundaries of Oklahoma and have oak, hickory, and pine trees in their forests (ODWC 2005; Woods et al. 2005).

The milkweed species selected for this study were *A. viridis* and *A. asperula*. *Asclepias viridis* is found in sixty-four of seventy-seven counties in Oklahoma that stretch from eastern Oklahoma to almost the western edge (Oklahoma Biological Survey 2005). *Asclepias asperula* is found in forty-four counties in Oklahoma from the panhandle to central Oklahoma (Oklahoma Biological Survey 2005). Blooming periods for *A. asperula* can occur from March through July (Woodson 1954). Blooming periods for *A. viridis* can occur from April through August (Woodson 1954). With blooming periods occurring during my survey time, I focused on assessing the distributions and densities of *A. viridis* and *A. asperula* on roadsides and adjacent lands throughout Oklahoma, excluding the panhandle.

Milkweed Density Estimates for A. viridis *and* A. asperula

Roadsides were surveyed in May and June of 2016 prior to when some stems of *A. viridis* and *A. asperula* start to senesce in July and August (Mueller 2013; Baum and Mueller 2015; Borders and Lee-Mäder 2015). I collected data once every ten miles on selected roadsides (see Figure 1) by walking along a 5 m x 50 m transect parallel to the highway, placed approximately in the middle of the roadside between the safety zone and fence line, and recording counts of *A. viridis* and/or *A. asperula* plants. I estimated milkweed density on land adjacent to each roadside transect using distance sampling, which takes into account detectability using a detection function that describes the probability of detecting an object given that it is at a certain distance from the observation point (Buckland et al. 2001; Thomas et al. 2010). The distance sampling procedure involved measuring the perpendicular distance (m from the fence line) to each visible *A. viridis* or *A. asperula* plant using a laser rangefinder, and using the program Distance (version 6.2; http://distancesampling.org/) to convert the recorded distances for each transect into a density estimate using the conventional distance sampling (CDS) engine (Buckland et al. 1993; Oleyar 2007; Thomas et al. 2010). The CDS method assumes that objects on the line or point are detected with certainty, and this assumption is met with immotile populations such as plants (Anderson et al. 1979; Thomas et al. 2010). The density estimate is calculated using the estimation of encounter rate and detection function calculated by the CDS engine using the measured perpendicular distances (Anderson et al. 1979; Thomas et al. 2010) and gives an output of plants per hectare similar to transect sampling.

I also recorded land use/cover type of the adjacent land as grassland, crop field, or if trees blocked the view of the land and no estimate of milkweed density could be made. Universal Transverse Mercator (UTM) coordinates were recorded at either the north or east end of each transect, depending on the orientation of the road. Roadside width (m) was also recorded. When safety conditions did not allow for a transect to be conducted (e.g., narrow or steep shoulder, located on a turn), the transect was conducted at the next safest stopping point.

Analyses

Milkweed density estimates for roadsides were converted from number of plants per transect (within $250m^2$) to number of plants per hectare (multiplying by 40 to extrapolate to $10,000m^2$ or 1 ha); estimates for adjacent land sites were calculated to number of plants per hectare within the Distance program. No data were collected for adjacent lands with trees along the fenceline blocking the view, so these sites were excluded from mean and standard error calculations.

Paired t-tests were used to compare milkweed density estimates between roadside transects and adjacent land transects to test the null hypothesis that there was no difference in collective milkweed densities (both *A. viridis* and *A. asperula*) between roadsides and adjacent lands. An ANOVA was used to compare milkweed densities in different adjacent land types with their roadside densities to test the null hypothesis that there was no difference in milkweed densities between roadsides and adjacent lands based on land use type and Tukey HSD tests were used for post hoc testing. Two t-tests were used to compare milkweed densities between crop fields and grasslands: one compared roadside densities adjacent to crop fields with roadside densities adjacent to grasslands and the other compared adjacent land densities in crop fields to adjacent land densities in grasslands to test the null hypotheses that there was no difference in milkweed densities between crop fields and grasslands among roadsides and adjacent lands. Significance was set at an alpha level of 0.05.

Results

Densities of Milkweed between Roadsides and Adjacent Lands

A total of 284 transects were sampled, of which 40% of roadside transects (115) had at least one *A. viridis* or *A. asperula* present (Figure 2); 26% of adjacent land transects (74) had at least one *A. viridis* or *A. asperula* present (Figure 3). Roadside milkweed density ranged from a single milkweed plant per transect (40 plants/ha) to 62 plants per transect (2,480 plants/ha), and averaged 164 ± 27 (SE) plants/ha (Figure 4). Milkweed densities for adjacent lands ranged from 2 plants/ha to 1,015 plants/ha, and averaged 28 ± 7 (SE) plants/ha (Figure 4). There was more collective *A. viridis* and *A. asperula* along roadsides than in adjacent lands ($t = 4.909$, $df = 204$, $P < 0.001$).

Densities of Milkweed between Roadsides and Adjacent Lands by Land Use Type

Roadside milkweed density adjacent to crop fields ranged from one *A. viridis* or *A. asperula* per transect (40 plants/ha) to 62 plants per transect (2,480 plants/ha), and averaged 144 ± 52 (SE) plants/ha (Figure 7). The crop fields had densities that ranged from 10 plants/ha to 250 plants/ha, and averaged 6 ± 4 (SE) plants/ha (Figure 7). Roadside milkweed density adjacent to grasslands ranged from one *A. viridis* or *A. asperula* per transect (40 plants/ha) to 50 plants per transect (2,000 plants/ha), and averaged 186 ± 34 (SE) plants/ha (Figure 7). The grasslands had densities that ranged from 2 plants/ha to 1,015 plants/ha, and averaged 40 ± 11 (SE) plants/ha (Figure 7). Milkweed densities differed significantly between roadsides and adjacent lands grouped by land use type ($F_{(3,416)} = 10.282$, $P < 0.001$; Figures 5 and 6). Roadsides next to grasslands had more *A. viridis* and/or *A. asperula* than both adjacent grasslands and adjacent crop fields (both P < 0.01) while roadsides next to crop fields had more *A. viridis* and/or *A. asperula* than adjacent crop fields (P < 0.01).

When roadsides are grouped by adjacent land use type (grasslands or crop fields), there is no difference in the amount of *A. viridis* and *A. asperula* on roadsides (t = 1.519, df = 208, P = 0.070). However, more *A. viridis* and *A. asperula* occurred in the adjacent lands grassland land use type than the adjacent lands crop field land use type (t = 2.723, df = 208, P = 0.004).

Discussion

Milkweed densities were higher along roadsides than in adjacent lands in Oklahoma. The higher densities of *A. viridis* and *A. asperula* in roadsides compared to adjacent lands suggests consideration for effective management of roadsides to maintain populations of existing milkweeds as habitat for monarch butterflies. The presence of milkweeds on roadsides suggests potential to provide breeding habitat for monarch butterflies during the spring as they migrate through Oklahoma from late March through mid-June (Monarch Watch 1998; Journey North 2015a). In 2016, the Oklahoma Department of Transportation (ODOT) altered statewide mowing practices by delaying mowing on highway roadsides until July to provide habitat for monarchs during their spring migration (Oklahoma Department of Transportation 2016). Potential habitat also occurs on lands adjacent to roadsides during the spring. For example, *A. viridis* can occur in densities of 2500 plants/ha in rangeland and managed prairie sites in the spring, although densities in these same sites were much lower in the fall in previous years

(Baum and Mueller 2015). Regrowth of *A. viridis* in the fall has been shown to occur in managed prairie sites when they were mowed, and mowed sites had higher densities of *A. viridis* in the fall compared to sites that were not mowed (Baum and Mueller 2015). With blooming periods for *A. asperula* ranging from March through July and for *A. viridis* ranging from April through August (Woodson 1954), milkweed densities could be more variable during other months, especially later in the year. Milkweed densities could also vary with different roadside characteristics and growing conditions.

Management activities on adjacent lands could positively, negatively, or have no effect on *A. viridis* and *A. asperula* densities in roadsides. Roadsides adjacent to crop fields had more milkweed than the crop fields (Table 1). Crop field management activities, such as field cultivation or herbicide applications that could drift into nearby roadsides, have the potential to negatively influence milkweed densities in adjacent lands. Adjacent grasslands could also experience herbicide applications for control of weeds (Plantureux et al. 2005) possibly contributing to lower milkweed densities observed in grasslands compared to adjacent roadsides. However, those lower milkweed densities were still higher when compared to adjacent lands that consisted of crop fields (Table 1). Additional research is needed to evaluate the influence of adjacent land practices on milkweed densities in roadsides.

When considering where monarch habitat can be supported on roadsides in Oklahoma, average milkweed densities were high in the northeast part of the state (Figures 5 and 6). Roadside *A. viridis* and *A. asperula* average densities were also relatively high in the central part of the state. These areas may be a good option for roadside habitats for monarch butterflies (Figure 5). Roadside width averaged 16m to

18m (personal observation) within these regions; the Oklahoma Department of Transportation maintains the 9m width of roadside adjacent to the road as the safety zone. Therefore, potential roadside habitat in these regions was 7m to 9m wide, and narrower roadsides (9m or less) would not provide habitat for monarchs (i.e., the entire roadside would fall within the safety zone). However, it is not known if mortality risk due to vehicle collisions differs with milkweed densities. Previous research has shown that monarchs use milkweed in roadsides to lay their eggs (Mueller 2013; Mueller and Baum 2014; Kasten et al. 2016), but in the Upper Midwest roadside milkweeds have a lower number of eggs per plant when compared to non-roadside sites monitored by volunteers as part of the Monarch Larva Monitoring Project (Kasten et al. 2016). Additional research is needed to evaluate monarch butterfly use of milkweeds and monarch adult and larval survival in roadsides. My research focused on the spring, and more information is also needed about milkweed density and distribution during late August through early October when fifth generation monarch larvae (the offspring of late/fall breeding monarchs) are present in Oklahoma.

CHAPTER III

EFFECTS OF MOWING ON THE DENSITY, NUMBER OF STEMS PER PLANT, PLANT HEIGHT AND LATEX PRODUCTION OF *ASCLEPIAS VIRIDIS*

INTRODUCTION

As part of the *National Strategy to Promote the Health of Honey Bees and Other Pollinators*, the Pollinator Health Task Force proposed improving roadside areas for bees, monarchs, and other pollinators near the Interstate 35 corridor, which extends from Texas to Minnesota. This habitat improvement would contribute to the goal to increase the size of the overwintering monarch population in Mexico to 225 million by 2020 (Pollinator Health Task Force 2015). A challenge with developing a pollinator corridor is identifying and communicating suitable mowing times for roadsides to provide milkweed and nectar plants during appropriate time periods, including time for regrowth and flowering to complete seed production.

Mowing once a year can allow more plants to produce seeds in roadsides when compared to roadsides mowed twice a year, and species evenness of flowering plants has been shown to be higher in areas mowed once (Jantunen et al. 2007). Mowing once a year can also increase species richness of perennial forbs in areas dominated by exotic grasses (Maron and Jefferies 2001), thus being beneficial for general pollinators. In Oklahoma, delaying mowing until November can help allow nectar sources to be available for monarch butterflies during their winter migration (Monarch Joint Venture 2015). Roadsides mowed once every 3 weeks or 6 weeks had significantly lower plant species richness when compared to non-mowed roadsides; the 3-week treatments showed the lowest species richness (Halbritter et al. 2015).

For monarch butterflies, current recommendations from the Monarch Joint Venture for the northern half of Oklahoma suggest mowing before April 1st, between July 1st and 20th only if necessary. For the southern half of Oklahoma, it is recommended to mow before March 1st, between June 30th and August 10th only if necessary (Monarch Joint Venture 2015). Following these guidelines of mowing before March 1st and April 1st reduces the risk of damaging milkweed that could be present on roadsides when monarchs begin breeding in the spring. However, this does not guarantee the presence of milkweed as absence could be caused by non-favorable growing conditions, herbivory, or disease, among other factors. Mowing during July, when monarchs are not present in Oklahoma, can generate milkweed re-growth for late/fall breeding monarchs that return to Oklahoma in mid-August (Baum and Mueller 2015).

It is not known how important roadsides are as breeding habitat for monarchs. Roadsides are mowed for safety and to maintain appearance, as well as to control invasive plants. Current mowing practices in Oklahoma focus on maintaining safety zones by keeping vegetation less than twelve inches high to maintain sight distances, minimize roadside vegetation hazards, and keep traffic signs visible (Montgomery et al. 2010). Highway vegetation is also controlled to decrease erosion, increase biodiversity, improve water quality and wildlife habitat, and limit the spread and growth of invasive plants (Montgomery et al. 2010). Conservation efforts typically focus on sowing native wildflower seeds along highways and turnpikes, and maintaining those wildflowers and native plants through appropriate management (Color Oklahoma 2012). Wildflower-designated areas are located outside of the safety zone, and mowing delayed until wildflower seeds have matured (usually July) (Oklahoma Department of Transportation 2016).

Milkweed loss has been identified as a factor contributing to the decline of the monarch butterfly (Hartzler and Buhler 2000; Hartzler 2010; Pleasants and Oberhauser 2013; Pleasants 2017). Other threats include decreased nectar availability (Brower et al. 2006; Inamine et al. 2016) that is important for providing lipids and energy for the overwintering and migration periods of monarch butterflies (Brower et al 2006), parasitoids and predators that increase mortality, disease (Oberhauser et al. 2015) that decreases flying speeds and distances of infected monarchs (Bradley and Altizer 2005), and insecticides that increase the risk of mortality (Krischik et al. 2015).

Mowing during the early summer has been shown to generate regrowth of *A. viridis* in the late summer when this species often dies back to the roots in undisturbed sites, providing host plants for fall breeding monarchs (Baum and Mueller 2015); if growing conditions are favorable, *A. viridis* may not senesce in undisturbed sites and regrow at least once during the growing season. Regrowth or new growth consists of young, tender leaves that may lead to increased caterpillar growth compared to mature, tougher leaves (Agrawal and Fishbein 2006). Monarch butterflies also prefer to oviposit on younger milkweed foliage, including regrowth following mowing, and caterpillar survival is higher on regrowth (Takagi and Miyashita 2008; Kawasaki et al. 2009; Fischer et al. 2015). A study in upstate New York found that mowing *A. syriaca* in July generated habitat that was suitable for monarch oviposition and larval growth later in the season, unlike non-mowed controls or late mowing (mid-August) (Fischer et al. 2015). Additional research is needed to evaluate how different mowing regimes, including timing and frequency of mowing, influence milkweed regrowth and recruitment.

It is also not known if mowing influences milkweed defenses and if so, to what extent. Plants have evolved physical and chemical defenses in response to both biotic and abiotic factors. Examples of milkweed defenses against herbivores include cardenolides (Malcolm 1991; Fordyce and Malcolm 2000), latex (Agrawal 2005), leaf toughness (Coley 1983; Kause et al. 1999), and trichome density (Hulley 1988; Mauricio and Rausher 1997). There is evidence showing macroevolutionary trends towards milkweeds (*Asclepias* spp.) favoring regrowth ability over defensive traits as a result of specialist herbivore damage (Agrawal and Fishbein 2008). Continued resource input into growth may reduce the concentration of cardenolides in milkweed because cardenolide synthesis is costly and is a resource trade-off with plant growth (Zust et al. 2015). This could translate into reduced monarch fitness since monarchs sequester cardenolides to use as a defense against predators (Malcolm 1991; Malcolm 1995).

Latex can result in mortality of early monarch instars from gumming their mandibles, and reduce monarch feeding rates by requiring additional actions to reduce latex flows (Dussourd and Eisner 1987; Dussourd 1999; Agrawal and Konno 2009). For example, monarch caterpillars may cut around milkweed laticifers to decrease latex pressure and flow (Dussourd and Eisner 1987; Dussourd 1999). Latex production is positively correlated with plant growth rate, and smaller milkweed plants have less latex (Zust et al. 2015), although it is not known if mowing regimes influence this pattern. Rapid larval growth has been observed on milkweeds with experimentally reduced latex with low- to mid-range cardenolide concentrations (Zalucki et al. 2012; Zust et al. 2015). The goals of this project were to evaluate the influence of different mowing treatments in roadsides on *Asclepias viridis* density, number of stems per plant, plant height (cm), and latex production.

Methods

Mowing Treatment Background

To evaluate if mowing timing and frequency influence *Asclepias viridis* density, number of stems per plant, plant height (cm), or latex production in roadside habitats, I used the following treatments: 1) non-mowed, 2) mowed following current Oklahoma Department of Transportation procedures (i.e., mowed the week before Memorial Day, Fourth of July, Labor Day, and following first frost), and 3) mowed in mid-July only. Treatments were mowed using a rear mounted (3-point hitch) rotary mower at a cutting height of 13 cm. Mowing treatments were administered to the traditional mowed sites on June 7-8, June 28-29, and August 24, 2016. The mid-July sites were mowed July 18-19,

2016. Five replicates of treatments 1, 2, and 3 were located in Payne County along Highway 51 west of Stillwater (36.1140° N and 97.1925° W) to Interstate 35 and along Interstate 35 from Highway 51 north to Deer Ridge Road (E0560 Road) (36.1532° N and 97.3289° W). Each site was at least 15 m wide by 120 m long (large enough for one treatment) and located outside the safety zone that extends from the pavement's edge into the roadside about 9 m. Roadside sites were randomly assigned a treatment group using a random number generator, with the additional requirement that sites adjacent to each other did not receive the same treatment. Some sites were large enough to accommodate more than one treatment. Sites were selected to have an initial milkweed density of at least 25 plants per transect (1,000 plants/ha), and to avoid areas where herbicide applications were anticipated because of the presence of invasive species. The sites were mowed by the Oklahoma Department of Transportation, except in a few cases when transects were mowed by the Department of Horticulture and Landscape Architecture, Oklahoma State University.

Measuring Asclepias viridis *Density, Stems per Plant, and Plant Height*

Milkweed density, number of stems per plant, and plant height (cm) were measured once a week from mid-May through late August by walking along a 5 m x 50 m transect and recording the number of stems per plant and plant height (cm above ground level) of each *A. viridis* encountered within the transect. Individual plants were counted as individual stems that were not growing near other stems, or clusters of above ground stems that originated from the same central point. Number of stems per plant was counted based on above ground separation. Milkweed plants that had begun to senesce in mowing sites were excluded from analyses because these plants would likely not be used by monarchs and including these plants in analyses could confuse the interpretation of roadside suitability as monarch butterfly habitat. Senescing milkweed plants were defined as possessing yellowing leaves (may or may not have fallen off the plant) and stems, with no green leaves or indication of new growth on any stems. However, if green leaves or new stem growth was present among the yellowing stems and leaves, the plant was not categorized as a senescing plant, since it could be used by monarch butterflies, and was included in analyses. Milkweed density, number of stems per plant, and plant height (cm) were collected from the same transect (i.e., the locations of transects within each site did not change).

Measuring Asclepias viridis *Latex Production*

Latex sampling occurred once a month from May through July on sites within the three treatments (see above) to evaluate if mowing regimes affect latex production. Plants were randomly selected by starting at different locations (each ¼ of the plot) during each sampling period (May, June, and July). Fifteen milkweed plants were sampled per plot when possible. To measure latex production, I selected the youngest fully expanded leaf on each plant, cut off the tip (0.5 cm), and soaked up all exuding latex (~10 s) with a pre-weighed 1 cm diameter filter paper disc. The filter paper discs were stored in individual centrifuge tubes in the field, dried at 60°C for twenty minutes, and weighed to the nearest milligram (Van Zandt and Agrawal 2004; Agrawal 2005; Agrawal and Fishbein 2006; Rasmann et al. 2009). I also recorded the phenology, number of stems per plant, and plant height (cm) of each plant sampled for latex (see above). Latex sampling did not occur in July in the mid-July mowing treatment sites because sites had just been mowed and no milkweed was available.

Analyses

Analysis of variance (ANOVA) was used to compare milkweed densities among mowing treatments in May to test the null hypothesis that starting densities of milkweed did not differ among treatments. Repeated measures ANOVA was used to test for mowing treatment effect for the null hypothesis that milkweed density, stems per plant, and plant height did not differ among mowing treatments during the sampling period (twelve total weeks) and Tukey HSD tests were used for post hoc testing. Senescing milkweed plants were not included in the estimates of milkweed density, since these plants would likely not be used by monarchs and including these plants in analyses could confuse the interpretation of roadside suitability as monarch butterfly habitat.

Analysis of covariance (ANCOVA) was used to evaluate whether latex production differed with plant height among the mowing treatments or with number of stems per plant. A relationship between milkweed size (as represented by plant height or number of stems per plant) and latex production could make it difficult to determine if observed patterns are due to plant size or mowing regimes.

Results

Asclepias viridis *Density*

Starting milkweed densities did not differ among treatments in May, indicating initial milkweed abundance was similar among treatments prior to the implementation of the mowing treatments ($F_{(2, 12)} = 0.818$, $P = 0.448$) (Figure 8). Milkweed counts differed among the three mowing treatments ($F_{(2, 12)} = 4.697$, $P = 0.0310$ and among the sampling periods ($F_{(11, 132)} = 10.518$, $P < 0.001$). The non-mowed treatment had a larger count of *A.*

viridis than the traditional treatment (P = 0.045) and the mid-July treatment (P = 0.019). Weeks 1 (May 12-13), 2 (May 18-19), and 3 (May 27-28) had larger counts of *A. viridis* than all the other weeks (P < 0.05) except for Week 5 (June 10-11). However there was no significant interaction for milkweed counts between the mowing treatments and the twelve week sampling period, thus, the differences observed are limited to particular time periods and not found over the sampling period ($F_{(22, 132)}$ = 1.325, P = 0.1668) (Figure 8).

<u>Asclepias viridis *Number of Stems per Plant and Plant Height (cm)*</u>

Plant height differed among the three mowing treatments ($F_{(2, 12)}$ = 14.593, P < 0.001) and among the sampling periods ($F_{(11, 132)}$ = 2.426, P = 0.008). The non-mowed treatment had a higher plant height than the traditional treatment (P < 0.001) and the mid-July treatment (P = 0.038). Week 5 (June 10-11) had a higher plant height when compared to Week 10 (Aug 4) (P = 0.038). However there was no significant interaction for plant height between the mowing treatments and the twelve week sampling period, thus, the differences observed are limited to particular time periods and not found over the sampling period ($F_{(22, 132)}$ = 0.908, P = 0.5844) (Figure 9). The number of stems per plant did not differ among the three mowing treatments ($F_{(2, 12)}$ = 0.879, P = 0.440) or among the sampling weeks ($F_{(11, 132)}$ = 1.032, P = 0.422). There was no significant interaction for number of stems per milkweed plant between the mowing treatments and the twelve week sampling period ($F_{(22, 132)}$ = 0.922, P = 0.5664) (Figure 10).

<u>Asclepias viridis *Latex Production*</u>

The slopes of the regression lines for plant height among mowing treatments were not significantly different ($F_{(2, 12)}$ = 0.52, P = 0.595). The y-intercepts were not significantly different ($F_{(2, 12)}$ = 0.12, P = 0.887); there was no difference in the amount of latex produced when looking at plant height among mowing treatments. The same result was found when looking at number of stems per milkweed plant. The slopes of the regression lines for number of stems per plants among mowing treatments were not significantly different ($F_{(2, 12)}$ = 0.11, P = 0.885). The y-intercepts were not significantly different ($F_{(2, 12)}$ = 0.12, P = 0.887); there was no difference in the amount of latex produced when looking at number of stems among mowing treatments.

Discussion

Milkweed densities were similar among plots at the start of the study, indicating that observed patterns should reflect mowing regimes and not initial variation in milkweed availability among sites. Milkweed densities were higher at the start of the study, as would be expected if milkweed senesces later in the season (Baum and Mueller 2015). Over the duration of the study, milkweed densities were higher in the non-mowed treatment than the mowed treatments, suggesting that mowing decreases *A. viridis* counts but only close to the time of mowing; mowing did not create a change in *A. viridis* counts over the twelve week time span. However, this study was conducted over a single growing season, and it is not known how mowing regimes influence milkweed densities across years.

Asclepias viridis regrowth was observed in Weeks 9-12 (July 22; Aug 4; Aug 8; Aug 17) in traditional and mid-July mowing treatments that was not present in the non-mowed treatments (Figure 8). However, this pattern was not statistically significant at the spatial scale of this study. Other studies in old-field farmland habitats and fields mowed for hay have evidence of *A. viridis* and *A. syriaca* regrowing during August and September at sites that have been mowed in July (Baum and Mueller 2015; Fischer et al. 2015); for those studies, mowing in July was compared to sites that had not been mowed (Baum and Mueller 2015; Fischer et al. 2015) and sites that were mowed in August (Fischer et al. 2015). Thus, mowing in July could provide habitat for late-breeding monarchs in the fall for states south of latitude 38 and east of the Rocky Mountains (Monarch Watch 1998) and would not require as much effort and money as a traditional mowing regime. These late-fall breeding monarchs are also referred to as pre-migrants (Baum and Mueller 2015), and the offspring produced by these monarchs are referred to as the “5th generation”. Recent research suggests the 5th generation may be an important component of the overwintering population in some years (Flockhart et al. 2017). More research on mowing regimes is needed because growing seasons and conditions will vary regionally and from year to year. Additional research is also needed to evaluate if mowing regimes influence the timing of plant senescence.

Milkweed plant height was higher in non-mowed plots than mowed plots, although mowing did not affect number of stems per plant. The interaction between the mowing treatments administered in this study and the sampling period of twelve weeks did not influence either plant height or number of stems per plant. This means that the observed differences in plant height were due to the recent mowings taking place. Milkweed plant height would be expected to differ based on time since mowing. It is not clear why plant height only differed between Week 5 (June 10-11) and Week 10 (Aug 4) in the non-mowed treatment. Other research has shown that *A. syriaca* mowed in early

July can reach the same average height by September as non-mowed *A. syriaca* plants, while mowing in late July or later can cause them to reach only half the average height or less of non-mowed milkweed (Fischer et al. 2015). Number of stems per plant did not differ among treatments or across time for the duration of this study, but it is possible that number of stems could differ over longer time periods if mowing regime influences growth or based on how the plant invests energy in the production of more stems. Another study showed *A. viridis* plants that regrew following mowing in July were shorter, narrower, and had one-third fewer stems per plant than plants at the same sites earlier in the year (Baum and Mueller 2015). Additional information is needed on how mowing regimes and other management practices influence milkweed plant growth and plant structures, such as number of stems and plant height.

Latex production was not affected by the different mowing regimes (Figure 11). There were no difference in latex volume (as measured by weight) among the non-mowed, traditional, and mid-July sites suggesting that mowing does not have a large effect on latex production in *A. viridis*. I initially expected mowing would influence the latex production of newer milkweed growth more than older milkweed growth. Plants with newer growth may invest more in growth and herbivore defense against monarchs that prefer to oviposit on tender new growth (Takagi and Miyashita 2008; Kawasaki et al. 2009; Fischer et al. 2015) than plants with older growth that may invest more in seed production (Wilson and Mahlberg 1980). However, this was not found in my study, possibly due to the method of mechanical damage or species of milkweed that was studied, which could be the subject of future research (Van Zandt and Agrawal 2004).

Mowing regime influenced milkweed density early in the season, but not later in the season and the differences observed were limited to the time periods of recent mowings. Mowing regime did not influence the number of stems per plant, plant height, or latex production of *A. viridis* in roadsides. Milkweed density was low in August across all mowing treatments, although milkweed regrowth was observed in the mid-July and traditional mowing treatments that was not present in the non-mowed sites. It may be possible to modify mowing regimes outside of the safety zone to provide habitat for monarchs in the southern Great Plains during both their spring and fall breeding periods. Additional research is also needed to evaluate if mowing regimes influence nectar resource availability for adult monarch butterflies, and to evaluate the influence of roadsides and roadside management activities on monarch reproduction and survival.

CHAPTER IV

MONARCH BUTTERFLY HIGHWAY MORTALITY

INTRODUCTION

Roads are a ubiquitous component of most landscapes. Roads are known to affect ecological flows, such as surface water and the movement of plants and animals across the landscape (Forman 2000). Roads have been shown to decrease mammal and bird abundance (Benítez-López et al. 2010), amphibian populations (Bouchard et al. 2009), and the abundance and diversity of insects (Muñoz et al. 2015). Despite the negative impacts of roads for many animals, roadsides may serve as habitat for some species. Roadsides with native plants have been shown to support greater native bee species richness and abundance compared to roadsides dominated by nonnative grasses and flowering forbs (Hopwood 2008), as well as increased butterfly diversity (Munguira and Thomas 1992; Ries et al. 2001). The butterfly species of interest in this study is the monarch butterfly (*Danaus plexippus*). The monarch population has declined over the past fifteen to twenty years based on estimates of the size of the population on the overwintering grounds in Mexico (Brower et al. 2011; Rendón-Salinas et al. 2011; World Wildlife Fund Mexico 2017). Factors identified as contributing to the decline of the monarch butterfly include milkweed loss (Pleasants and Oberhauser 2013; Pleasants 2017), decreased nectar availability (Inamine et al. 2016; Brower et al. 2006), parasitoids, predators, disease (Oberhauser et al. 2015), and insecticides (Krischik et al. 2015). Roadsides could provide additional habitat for monarch butterflies, as roadsides that contain milkweed are used by monarch butterflies for nectaring and ovipositing (Mueller 2013; Mueller and Baum 2014; Kasten et al. 2016).

Roadsides have lower densities of monarch eggs compared to more natural areas but these lower egg densities could lead to a higher rate of larval survival (Kasten et al. 2016) if density dependent factors, such as parasitism and competition for resources are reduced (Nail et al. 2015). Monarch parasitism rates do not differ between roadsides and small, managed prairies (Mueller 2013; Mueller and Baum 2014). Other factors could influence larval monarch survival on roadsides, such as increased salt levels in milkweed plants (Snell-Rood et al. 2014).

Although roadsides may provide habitat for monarchs and other pollinators, they may also lead to pollinator mortality from vehicle collisions. One study collected 117,675 dead potential pollinators by roadsides: 95,094 were from the order Diptera, 12,639 were from the order Hymenoptera, and 4,763 were from the order Lepidoptera; the average traffic flow during this study was 9,700 vehicles per day (Baxter-Gilbert et al. 2015). When these roadside mortality numbers are extrapolated to continental North America, an estimated ninety-three billion lepidopterans may die every summer (Baxter-Gilbert et al. 2015). Another road mortality study in Illinois collected 1,824 dead Lepidoptera from roadsides in a period of six weeks; extrapolating these data to the state level, it was estimated that 20,000,000 butterflies and moths died from cars during one week across the state of Illinois and 500,000 of those were expected to be monarch butterflies (Mckenna et al. 2002). The traffic levels for the Illinois study were as high as 26,000 vehicles per day (Mckenna et al. 2002).

Butterfly behavior along roadsides could also influence mortality. Previous research has identified a higher percentage of road crossing behaviors in disturbance-tolerant and habitat-sensitive butterflies in grassy and weedy roadsides compared to prairie roadsides (Ries et al. 2001). Roads may directly affect more mobile and abundant butterfly species by increasing mortality due to increased exposure to vehicles. Roads may also limit movement of sedentary species of butterflies, as sedentary species are less likely to emigrate out of habitat that could be degrading from roads as a result from erosion or runoff pollutants from deicing salt and heavy metals (Forman and Alexander 1998; Ranea et al. 2008). Thus, more information about monarch behavior and mortality along roadsides is needed throughout the monarch’s range to assess the impact of roadsides on the overall monarch population.

Monarchs migrate through Oklahoma from late March through mid-June and again in mid-September through mid-October (Monarch Watch 1998; Journey North 2015a). The fall migration period was the focus of this study because monarchs are more concentrated during this time period, versus spring migration when monarchs are more dispersed and the population is at its smallest following overwintering (Journey North 2015b). I evaluated monarch behavior and mortality in association with roadsides for the fall migration period. Specifically, I compared monarch behavior and mortality between roadsides with an east to west orientation and those with a north to south orientation because road orientation could influence road mortality. Monarch butterflies move to the southwest as they migrate south in the fall to reach their overwintering grounds in Mexico. I predicted monarch mortality would be higher for roadsides oriented east to west and that monarchs would exhibit more road-crossing behavior for east to west oriented highways.

Methods

Monarch Mortality Along Highways

I conducted surveys along six highways selected based on highway orientation, speed limit, and ability to visit each highway within a 5 hour timeframe: three extend east to west (Highway 51, Highway 15, Highway 164) and three extend north to south (Highway 77, Highway 74, and Highway 177). Highway 51 is a four lane divided highway that has a maximum speed limit of 105 km/h. Highways 15 and 164 are two lane undivided highways; Highway 15 has a maximum speed limit of 105 km/h while most of Highway 164 has a maximum of 88.5 km/h. Highways 77, 74, and 177 have two lanes, are not divided, and have a maximum speed limit of 105 km/h. Each highway was surveyed daily from September 18 to October 18, 2016, with surveys conducted from 1000 to 1500 h CDST at three locations located 8 km apart along each highway (Table 2). The starting direction of the route alternated every other day (Skorka et al. 2013; Baxter-Gilbert et al. 2015).

At each location I conducted surveys between 1000 and 1600 hours CDST. During each survey I recorded the number of live monarchs encountered and each individual monarch's behavior by slowly walking along a 100 m transect parallel to the road for five minutes. Monarch behaviors were recorded as: approach (butterfly entered the 1 m of vegetation next to the highway shoulder but did not continue into the road), attempt (butterfly entered the 1 m of vegetation next to the highway shoulder, flew across part of the road or shoulder, but turned back), cross (butterfly entered and crossed the entire road; any butterflies struck by vehicles were noted), flying (butterfly flew parallel with the road), nectaring, ovipositing, and resting (Ries et al. 2001; Mueller 2013). I also searched the highway's shoulder and vegetation adjacent to the pavement along that same 100 m transect and counted and collected any dead monarchs found within the 2-3 m wide search area. The same procedure was repeated for the other side of the road. UTM coordinates, temperature, wind speed, and weather conditions, such as cloud cover and rain, were recorded at the beginning and end of each survey period.

Traffic volume was estimated by counting the number of vehicles that passed on both sides of the highway within a 5-minute survey. These surveys were conducted once per highway every day from September 18 to October 18, 2016 unless all-day rain prevented surveys from occurring.

Analyses

T-tests were performed on the number of dead monarchs, the number of live monarchs, and traffic volume between east-west and north-south highways. Only behaviors that occurred 5 or more times for both of the treatments (highway orientations) were compared, including “cross” and “flying”. Behaviors were compared between east-west and north-south highways with a G-test. A G-test was also used to evaluate whether time of day, separated into two categories of morning (before 1200 hours) and afternoon (after 1200 hours) could be used to predict behavior or the number of live monarchs observed; data were combined from east/west and north/south highways for monarchs flying in the same direction for this analysis. For example, the east/west fly behavior was combined with the north/south cross behavior because these behaviors are moving in the same direction. Data were also combined for “cross” (east/west highways) and “flying” (north/south highways) behaviors to evaluate if time of day affected the number of southward moving behaviors. Despite there being an extra two hours for the afternoon category, there were an equal number of surveys for morning and afternoon on every survey that was included in statistical analyses.

Results

Fourteen dead monarchs were collected over the duration of this study; seven dead monarchs were collected at the east/west highways, and seven were collected at the north/south highways (Table 3). There was no difference in monarch mortality between east/west highways and north/south highways ($t = 0.000$, $df = 4$, $P = 0.500$). Most of the dead monarchs on the east/west highways were collected on Highway 51, and all of the dead monarchs on the north/south highways were collected on Highway 177 (Table 3). Both of these highways had the highest traffic volumes, although there was no difference in traffic volume between east/west highways and north/south highways ($t = 0.102$, $df = 54$, $P = 0.460$; Figure 12).

A total of 184 live monarchs were observed across all of the highway sites during the one-month survey period (Table 4). There was no difference in the number of monarchs observed along east/west highways versus north/south highways ($t = 0.326$, $df = 4$, $P = 0.381$) and no difference in the number of monarchs observed in the morning versus afternoon ($G = 0.00$, $df = 1$, $P = 1$). There was more cross than fly behavior (Table 5) at the east/west highway orientation group ($G = 15.544$, $df = 1$, $P < 0.001$), and there was no difference between the cross and fly behaviors (Table 5) at the north/south highway orientation group ($G = 0.476$, $df = 1$, $P = 0.490$). There was no difference in the morning or the afternoon between flying east/west or crossing north/south and flying north/south or crossing east/west behavior (morning: $G = 1.57$, $df = 1$, $P =0.210$; afternoon: $G = 2.995$, $df = 1$, $P = 0.084$). For southward moving behaviors, there was no difference between the morning or afternoon ($G = 0.229$, $df = 1$, $P = 0.632$).

Discussion

Based on traffic volume and monarch presence, there was an equal opportunity for monarch mortality along east-west and north-south highways. Monarch mortality did not appear to be influenced by highway orientation (Table 3). My mortality sampling is likely an underestimation because there were several collections that could not be positively identified as *D. plexippus* due to the incompleteness of the specimens from vehicular collisions. Only specimens that were more than 50% complete were counted and identified to avoid the possibility of double counting. High winds may have moved dead monarchs away from the side of the highway; wind speeds were 24 kph or higher on ten different days during the survey period. Monarchs may also have remained stuck in vehicle grills, which would mean they would not be recorded by this study. Also, four survey days had temperatures below 16 degrees Celsius, which could have modified monarch behavior by lowering their wingbeat frequency and modifying the likelihood of mortality (Kammer 1970; Masters et al. 1988). However, time of day did not affect the number of monarch butterflies observed or their behaviors.

Monarchs were more likely to cross highways than to fly parallel to them at the east/west highways, suggesting that monarch mortality risk could be higher for these highways. There was no difference in the types of behaviors performed at north/south highways. Flowering plants were only present at about 33% of the highway sites and the amount of "nectaring" behaviors was low for both highway orientations (personal observation). Previous research has found a higher percentage of road crossing behaviors by butterflies in roadsides with more than 20% of non-native legumes compared to roadsides dominated by native prairie grasses and forbs (Ries et al. 2001). However, I did not record the species of flowering plants present as part of this study. The Ries et al. (2001) study was conducted during the breeding season and not during migration, which could have influenced the results.

The response of larval monarchs to roadside environments should also be evaluated to gain a better understanding of the implications of roadside habitats for the overall monarch population. Previous research has shown that monarchs will lay eggs on milkweeds in roadsides (Mueller 2013; Mueller and Baum 2014; Kasten et al. 2016), thus roadsides could be managed for milkweed and other flowering plants as habitat for monarch butterflies. Nectar plant conservation is important for the overwintering and migration periods of monarch butterflies (Brower et al. 2006), but managed prairies could be a better source of nectar plants than roadsides (Mueller 2013). Insecticide use increases the risk of mortality to monarch butterflies (Krischik et al. 2015) and both direct spraying and drift into roadsides could create sink habitats instead of source habitats for monarch butterflies.

This study focused on whether adult monarch mortality differed among highways with different orientations. Although no difference was observed, it is possible that road orientation could be more or less important along different parts of the monarch's migration route, or that other road orientations are important. Monarchs fly southwest and not due south through the study area, so it is possible that differences could be observed for highways oriented northeast to southwest (Schmidt-Koenig 1979). The east/west highway orientation had a higher proportion of "cross" behavior, the north/south highways had no difference in behaviors performed, and there was no difference in the number of dead monarchs and live monarchs observed. Other highway characteristics may also influence monarch mortality, such as traffic volume (McKenna et al. 2001; Rao and Girish 2007; Skorka et al. 2013), pollution (Beyer and Moore 1980; Port and Thompson 1980), and road construction activities.

CHAPTER V

CONCLUSIONS

The results of this research show that roadsides in Oklahoma have higher densities of *A. viridis* and *A. asperula* than adjacent lands. Thus roadsides have the potential to provide breeding habitat for monarch butterflies. Milkweed densities did not vary among mowing treatments with time, and mowing lowered *A. viridis* counts close to the actual times of mowing. Milkweed height differed based on if a site was mowed or not but both number of stems per plant and plant height were not affected by the interaction of different mowing treatments and time. Latex production was not affected by the different mowing treatments. This study also showed that highway orientation did not influence the number of dead monarch butterflies found along roadsides. Monarch butterflies exhibited road-crossing behaviors when they were near roadsides and additional research is needed on monarchs and roadside mortality. Future research should evaluate monarch utilization of milkweed on roadsides compared to adjacent lands, observe the effects that different mowing regimes have on milkweed phenology, and evaluate if monarch butterfly mortality varies with different highway and roadside characteristics.

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APPENDICES

Table 1. Summary statistics of *A. viridis* and *A. asperula* on roadsides and adjacent lands grouped by land use.

	Mean (Milkweed Plants/hectare)	**Standard Error**	**Variance**	**Minimum**	**Maximum**	**Sample Size**
Roadsides Crop Field	126.539	37.167	143,661.7	0	2480	104
Adjacent Land Crop Field	8.135	3.109	1004.974	0	250.03	104
Roadsides Grasslands	208.302	38.895	160,357.1	0	2000	106
Adjacent Land Grasslands	45.919	13.403	19,042.76	0	1015.1	106

Table 2. Coordinates for locations of highway sites for the monarch butterfly mortality and behavior surveys. Coordinates for UTM Zone 14S.

Highway Orientation	Highway Name	Site Number	Latitude (N)	Longitude (W)
East/West	15	1	36.5348	97.5755
	15	2	36.5350	97.4793
	15	3	36.5353	97.4065
	51	1	36.1158	97.1455
	51	2	36.1004	97.2282
	51	3	36.1154	97.3273
	164	1	36.2751	97.3762
	164	2	36.2775	97.4615
	164	3	36.2892	97.5534
North/South	74	1	36.3186	97.5864
	74	2	36.4014	97.5865
	74	3	36.5027	97.5865
	77	1	36.1177	97.3914
	77	2	36.1898	97.3716
	77	3	36.2639	97.3722
	177	1	36.5503	97.0769
	177	2	36.4731	97.0679
	177	3	36.3807	97.0678

Table 3. Total number of dead monarchs collected at each highway from September 18 to October 18, 2016.

Highway Orientation	Highway Name	# Dead Monarchs
	15	0
East/West	51	6
	164	1
	74	0
North/South	77	0
	177	7

Table 4. Total number of live monarchs observed at each highway from September 18 to October 18, 2016.

Highway Orientation	Highway Name	Monarchs Observed
	15	22
East/West	51	31
	164	35
	74	19
North/South	77	33
	177	44

Table 5. Mean number of behaviors (±SE) performed by adult monarch butterflies at each highway orientation group from September 18 to October 18, 2016. *Cross* refers to when a butterfly entered and crossed the entire road and *fly* refers to a butterfly flying parallel with the road.

	Cross	**Fly**
East/West	2.1071 (±0.6179)	0.8929 (±0.2485)
North/South	1.9643 (±0.3866)	1.7143 (±0.5317)
Average Totals	**2.0357 (±0.3612)**	**1.3036 (±0.2960)**

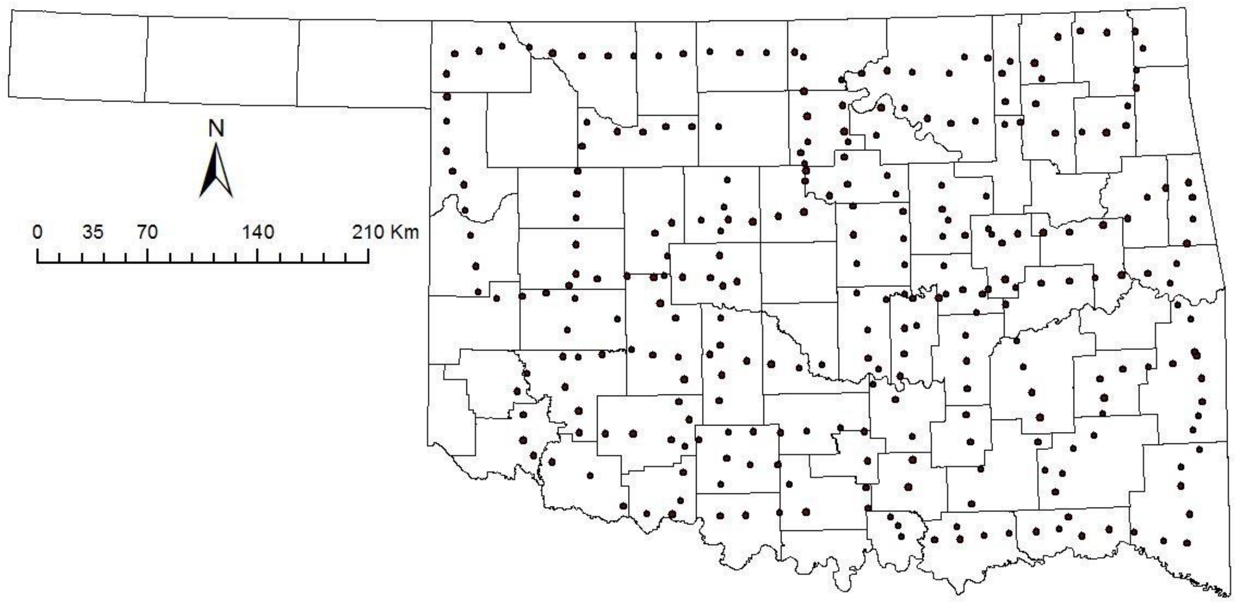

Figure 1. Locations of roadsides and adjacent lands surveyed for *Asclepias viridis* and *Asclepias asperula* density. Highways were selected to effectively cover all of Oklahoma, excluding the panhandle.

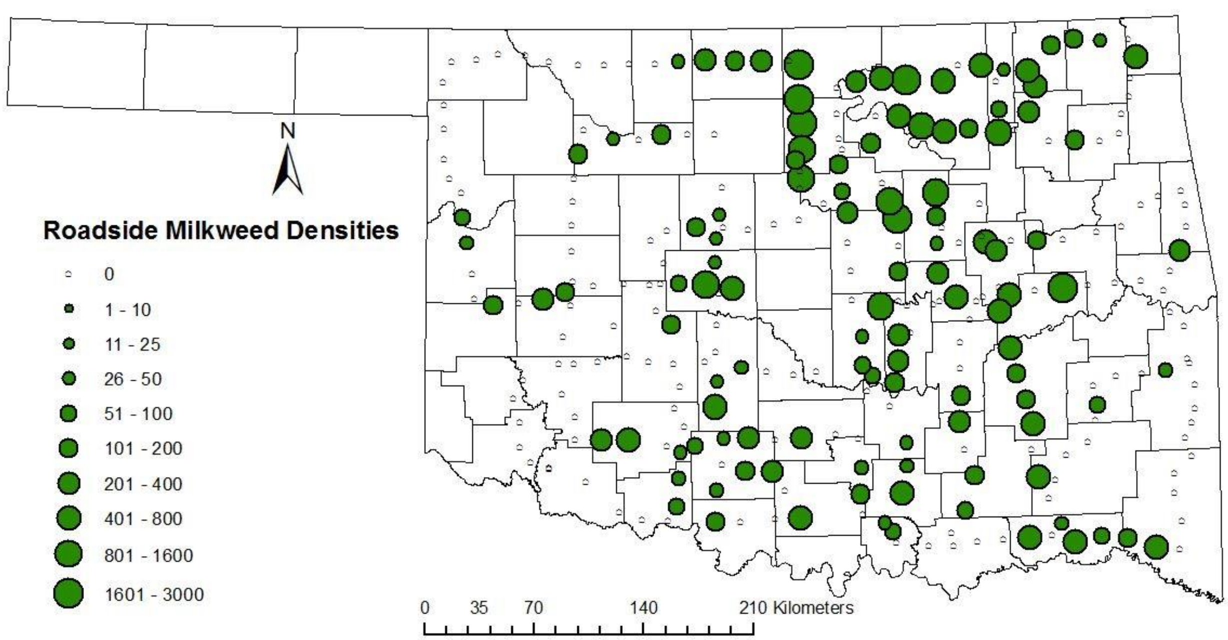

Figure 2. Roadside densities of *Asclepias viridis* and *A. asperula* (plants/hectare) across Oklahoma during May and June of 2016, excluding the panhandle.

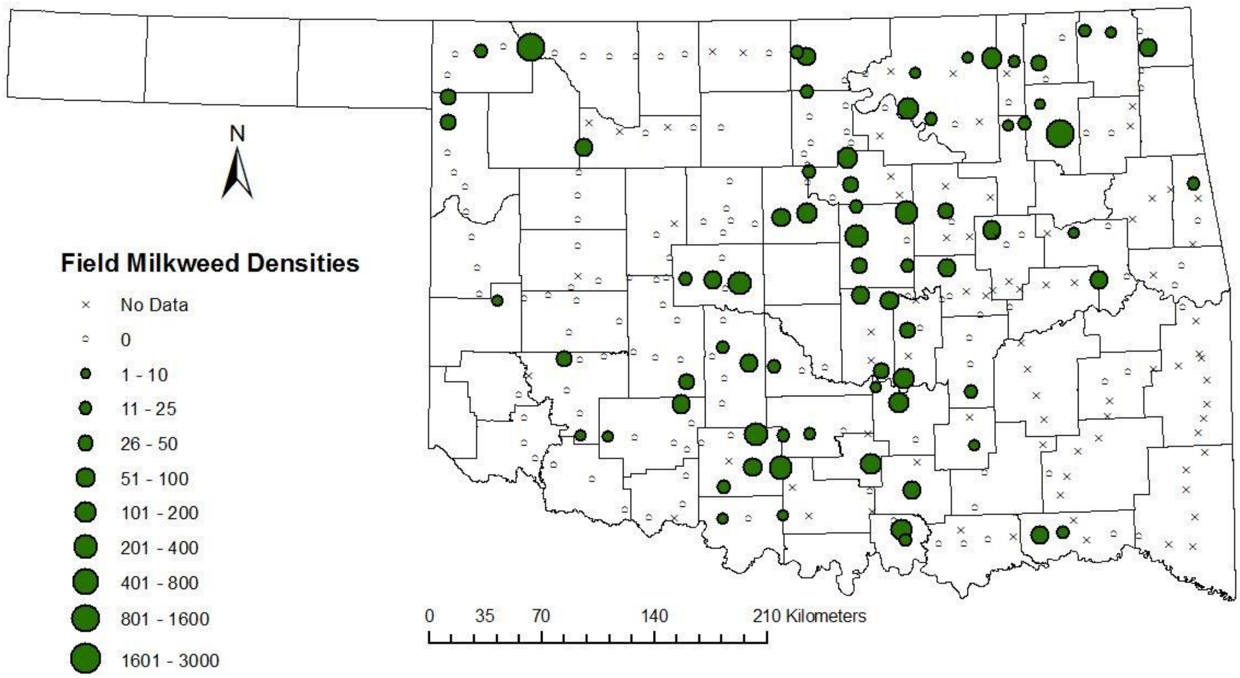

Figure 3. Adjacent land densities of *Asclepias viridis* and *A. asperula* (plants/hectare) across Oklahoma during May and June of 2016, excluding the panhandle. Xs represent transect locations where trees along the fenceline blocked the view of adjacent lands and data were not recorded.

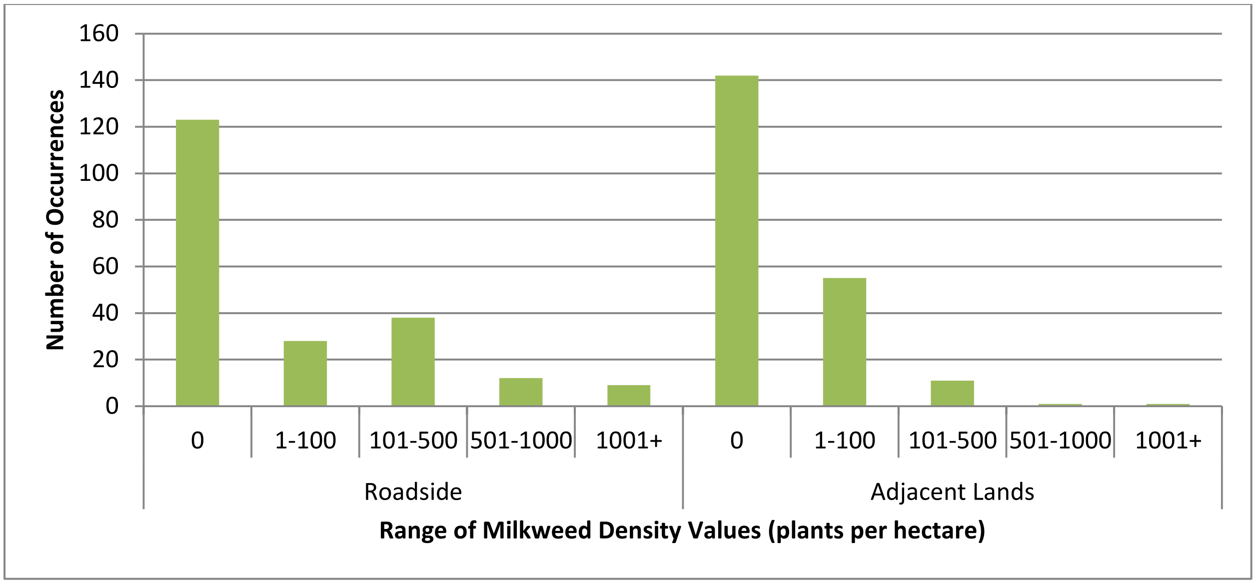

Figure 4. Frequency distribution of *Asclepias viridis* and *A. asperula* plants per hectare across 205 transects in Oklahoma during May and June of 2016, excluding the panhandle. Transects were only included if density estimates were available for both roadsides and adjacent lands. Seventy-nine transects (out of the total of 284) were excluded because trees along the fenceline blocked the view of adjacent lands.

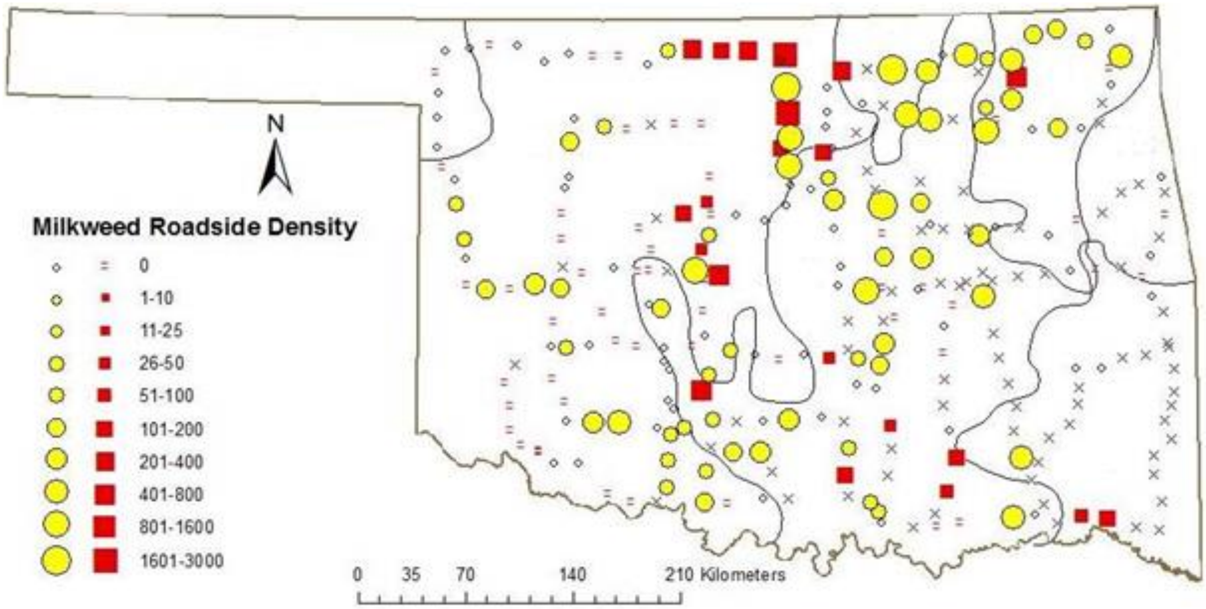

Figure 5. Roadside densities of *Asclepias viridis* and *A. asperula* (plants/hectare) across Oklahoma during May and June of 2016, excluding the panhandle. Xs represent transect locations where trees along the fenceline blocked the view of adjacent lands and data were not included. Locations of crop fields (red squares) and grasslands (yellow circles).

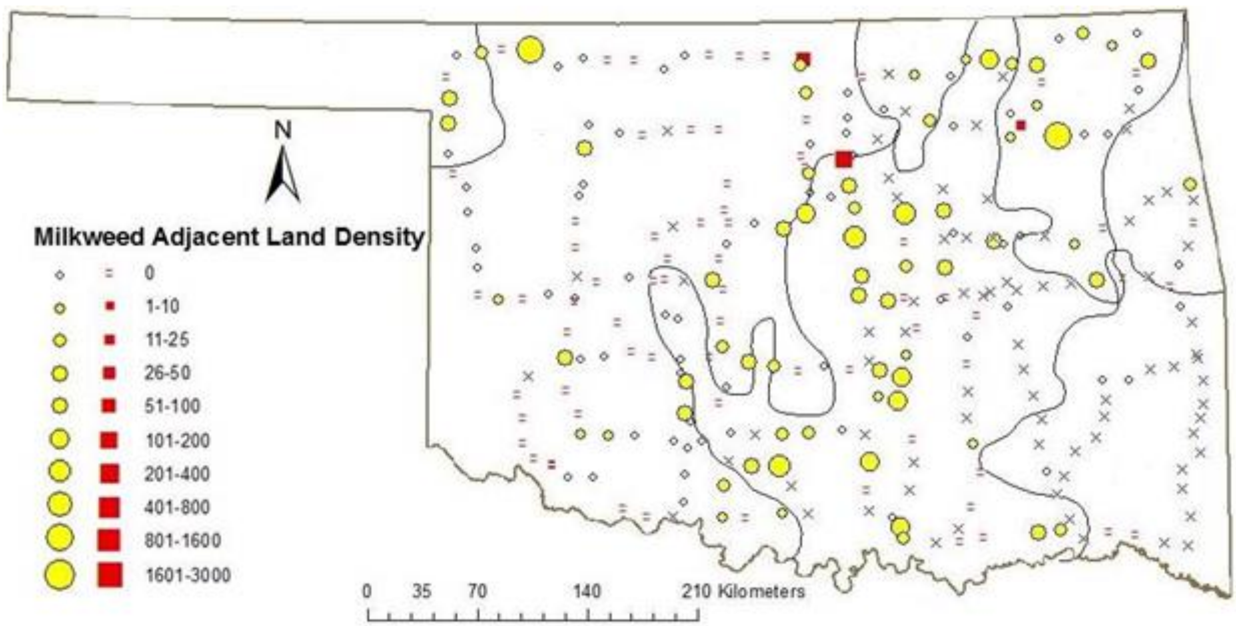

Figure 6. Adjacent land densities of *Asclepias viridis* and *A. asperula* (plants/hectare) across Oklahoma during May and June of 2016, excluding the panhandle. Xs represent transect locations where trees along the fenceline blocked the view of adjacent lands and data were not included. Locations of crop fields (red squares) and grasslands (yellow circles).

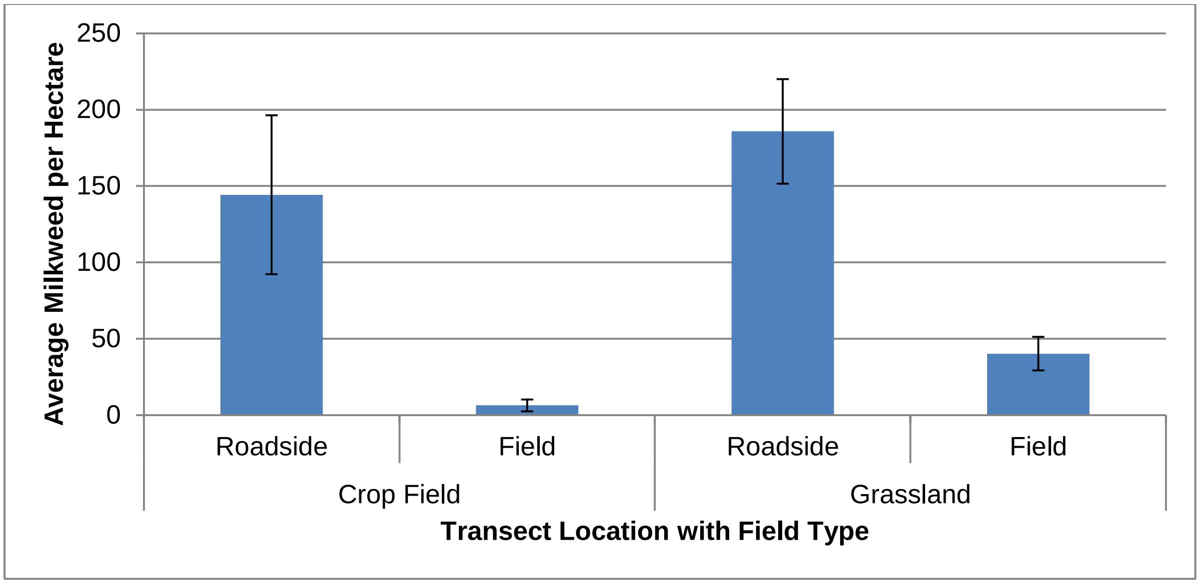

Figure 7. Average number of *Asclepias viridis* and *A. asperula* plants per hectare (±SE) for each land type and its adjacent roadside across 205 transects in Oklahoma during May and June of 2016, excluding the panhandle. Seventy-nine transects (out of the total of 284) were excluded from estimates because trees along the fenceline blocked the view of adjacent lands.

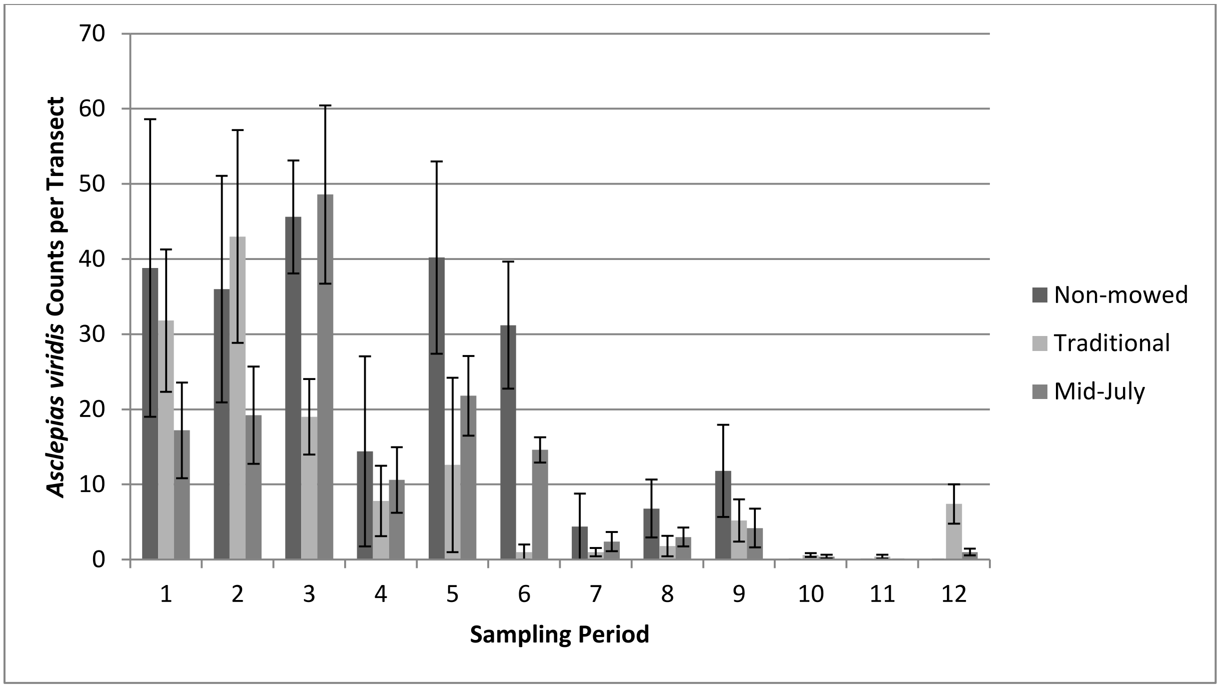

Figure 8. *Asclepias viridis* counts per transect (± SE) across mowing treatments, excluding senescing plants, for weekly sampling periods from Week 1 (May 12-13) through Week 12 (Aug 17). Non-mowed sites (n=5) were not mowed, Mid-July sites (n=5) were mowed in mid-July, and Traditional sites (n=5) were mowed following current Oklahoma Department of Transportation procedures (i.e., June 7-8, June 28-29, and August 24, 2016). There was no significant interaction found between mowing treatments and the sampling period.

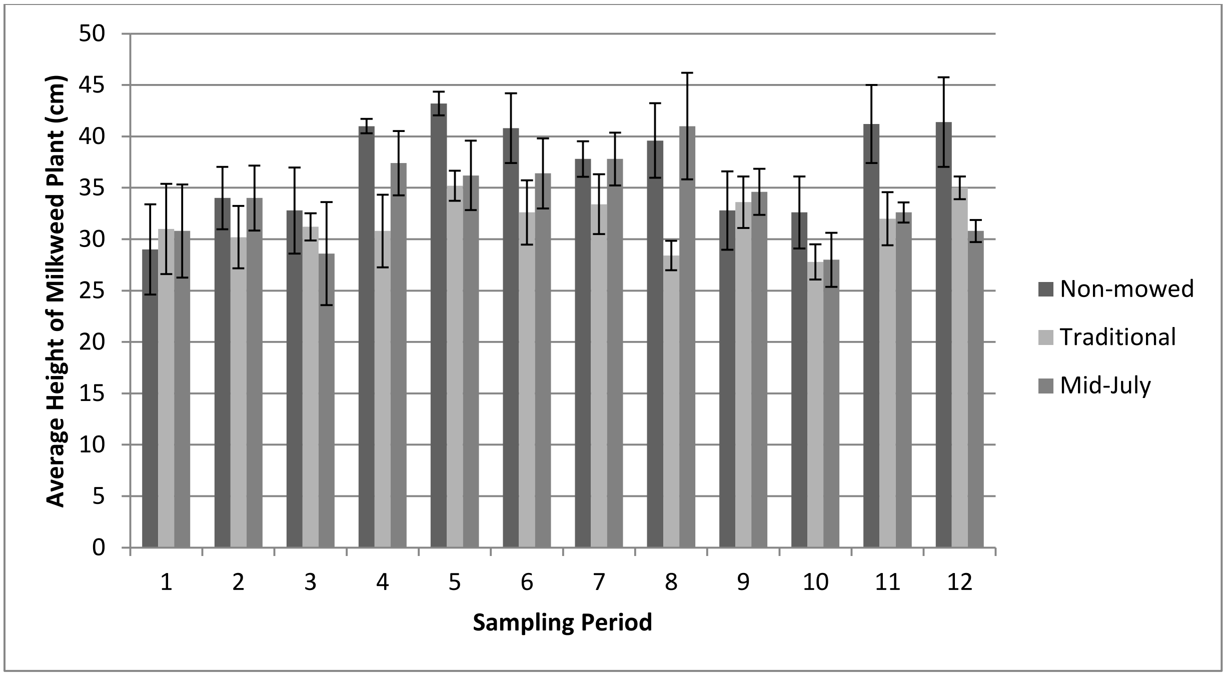

Figure 9. Average height of *Asclepias viridis* (cm) (± SE) across mowing treatments, excluding senescing plants, for weekly sampling periods from Week 1 (May 12-13) through Week 12 (Aug 17). Non-mowed sites (n=5) were not mowed, Mid-July sites (n=5) were mowed in mid-July, and Traditional sites (n=5) were mowed following current Oklahoma Department of Transportation procedures (i.e., June 7-8, June 28-29, and August 24, 2016). There was no significant interaction found between mowing treatments and the sampling period.

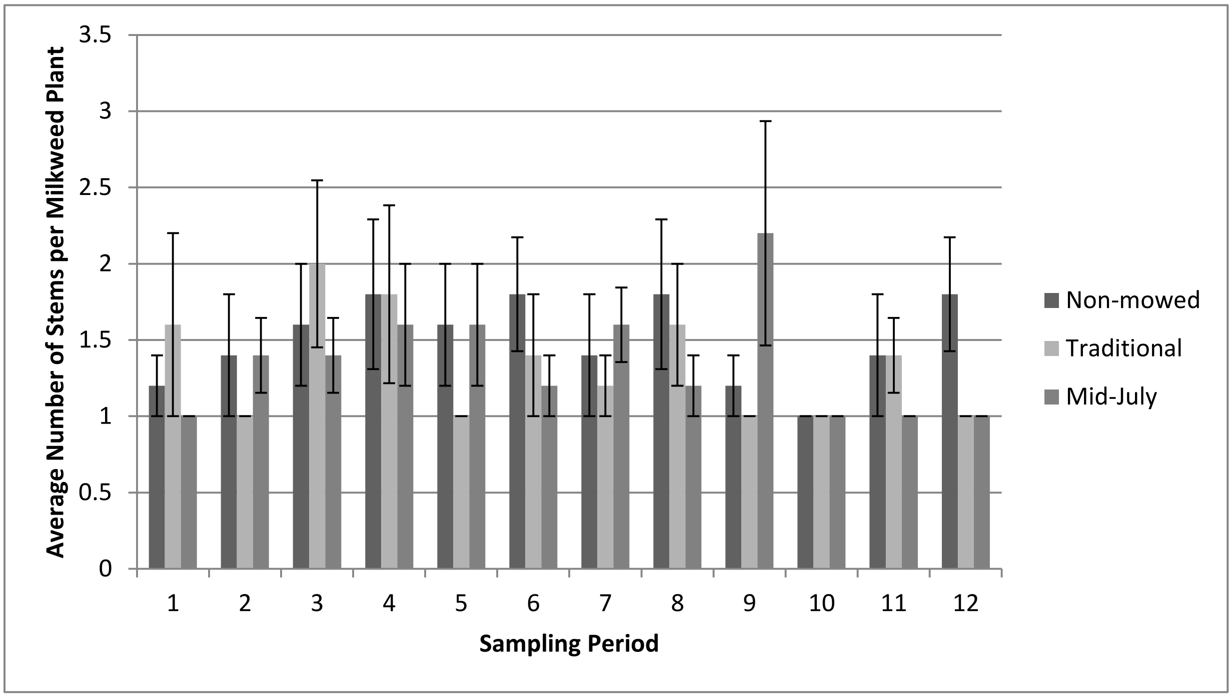

Figure 10. Average number of stems (± SE) per *Asclepias viridis* plant across mowing treatments, excluding senescing plants, for weekly sampling periods from Week 1 (May 12-13) through Week 12 (Aug 17). Non-mowed sites (n=5) were not mowed, Mid-July sites (n=5) were mowed in mid-July, and Traditional sites (n=5) were mowed following current Oklahoma Department of Transportation procedures (i.e., June 7-8, June 28-29, and August 24, 2016). There was no significant interaction found between mowing treatments and the sampling period.

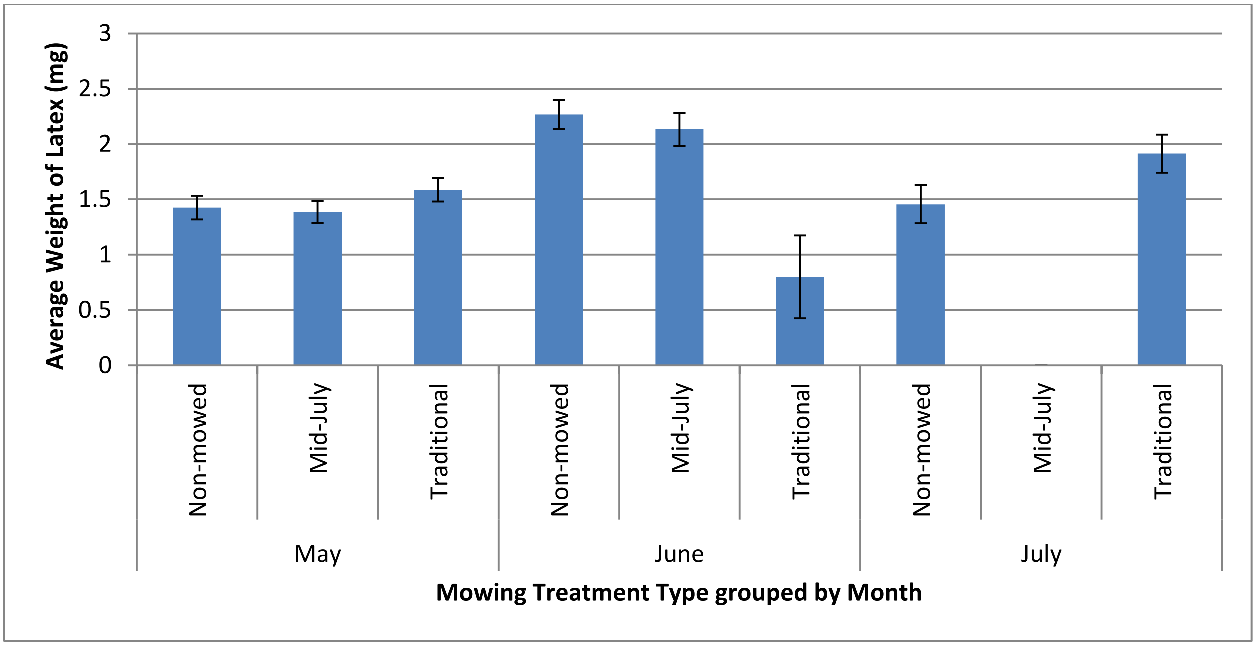

Figure 11. The average latex mass in milligrams (±SE) across mowing treatments, excluding senescing plants, from May through July 2016. Non-mowed sites (n=5) were not mowed, Mid-July sites (n=5) were mowed in mid-July, and Traditional sites (n=5) were mowed following current Oklahoma Department of Transportation procedures (i.e., June 7-8, June 28-29, and August 24, 2016). The monthly target sample size for each treatment was 75 plants (15 per site). The treatments that did not reach the target size were May (n = 57), June (n = 5), July (n = 58). There were no significant differences found among mowing treatments.

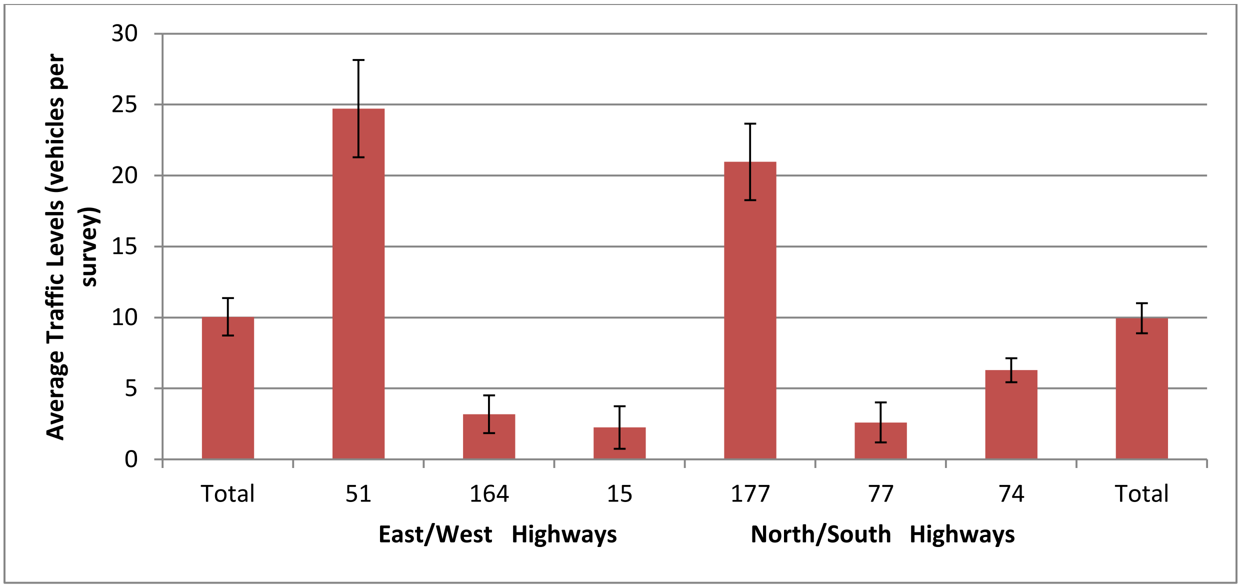

Figure 12. Average traffic levels per day (± SE) for each east/west highway and overall (51, 164, and 15) and each north/south highway and overall (177, 77, and 74) during daily 5-minute surveys from September 18 to October 18, 2016.

VITA

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