2018

Investigations into the function of common milkweed (Asclepias syriaca) in the agricultural landscape

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Investigations into the function of common milkweed (*Asclepias syriaca*) in the agricultural landscape

by

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A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Crop Production and Physiology

Program of Study Committee:
Bob Hartzler, Major Professor
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Micheal Owen

The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University

Ames, IA

2018

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Bob Hartzler, and my committee members, Richard Hellmich and Mike Owen, for their guidance and support throughout the course of this research. This research was partially funded by the USDA, Natural Resources Conservation Service's Conservation Innovation Grant program under Agreement Number 69-3A75-16-006.

Additionally, I would like to thank my mother, Treasa Lizotte and husband, Garrett Hall for their continued encouragement throughout my college career, my friends, colleagues, and the department faculty and staff for making my time at Iowa State University a wonderful experience. I want to also offer my appreciation to those who were willing to participate in my surveys and observations, without whom, this thesis would not have been possible.

ABSTRACT

The monarch butterfly's (*Danaus plexippus* Linnaeus) vibrant orange color with contrasting black veins and white spots make it recognizable to many. Unfortunately, the remarkable migration made by the eastern monarch population is at risk due to the recent decline in this cohort of the species. Many factors are said to have caused the decline, including overwintering habitat destruction, increased dependence on herbicide-tolerant crops, extreme climatic events, predation, loss of breeding habitat, etc.

Two studies were conducted to: 1) determine the impact of sub-lethal herbicides on growth of common milkweed and utilization by monarchs and 2) investigate simple methods for establishing common milkweed and three additional forbs (golden alexanders (*Zizia aurea* L.), wild bergamot (*Monarda fistulosa* L.), and New England aster (*Symphyotrichum novae angliae* L.), into an existing sod landscape.

In the first study, fomesafen caused rapid damage to leaves contacted by the spray, resulting in the loss of many leaves. However, plants recovered rapidly and ovipositing by monarchs was not affected. Additional herbicides commonly used in Iowa crop production were evaluated for their effect on common milkweed in greenhouse experiments. Glufosinate was more injurious to common milkweed than either imazethapyr or mesotrione, but as with fomesafen, treated plants showed signs of recovery from all three herbicides within two weeks of application.

The second study investigated the effects of mowing and sub-lethal rates of glyphosate on the establishment of common milkweed and three forbs in an established stand of smooth brome (*Bromus inermis* Leyss). In general, suppression of smooth brome sod with sub-lethal rates of glyphosate increased recruitment of seedlings, but there was a

low probability of permanent establishment of common milkweed and other forbs. Establishment of golden alexanders and wild bergamot was greater than either common milkweed or New England aster.

CHAPTER 1. GENERAL INTRODUCTION

Background

The monarch butterfly (*Danaus plexippus* Linnaeus) is an icon of the Midwestern United States; however, the population size has declined sharply over the past several decades. Several factors are hypothesized for the decline, including: deforestation of overwintering sites in Mexico (Brower et al., 2002), severe climatic events (Oberhauser and Peterson, 2003; Brower et al., 2004), infection by the protozoan *Ophryocystis elektroscirrha* (*OE*) (Altizer and de Roode, 2015), invertebrate natural enemies (Oberhauser et al., 2015), and loss of summer breeding habitat (Flockhart et al., 2014).

Herbicides have been a primary weed control tactic in United States corn and soybean production for more than 40 years. The majority of herbicides used in these crops are active primarily on annual weeds (Timmons, 2005). Despite the fact that most commonly used herbicides are not lethal to common milkweed, they could impact the quantity and quality of the perennial plant. The sub-lethal effects of herbicides on the suitability for ovipositioning and feeding by monarch butterfly larvae has not been investigated, but is of interest to better understand the importance of the loss of common milkweed in cropland on monarch population dynamics.

Loss of habitat, and therefore essential resources for the monarch, is hypothesized as a cause for the decline of the insect (Flockhart et al., 2014). While the potential to convert large areas to habitat suitable for monarchs is often limited by financial constraints, most farms have small areas of land not utilized for crop production. These small areas of land may be suitable for conversion into monarch summer breeding habitat. Developing low-cost methods of establishing common milkweed and other forbs into an existing sod without complete conversion of the area could increase the participation of rural landowners in converting these small areas into monarch habitat.

Thesis Organization

Chapter 2 is a literature review on the basic biology of the monarch, and pressures impacting its population size. Particular emphasis is placed on the primary host plant of the monarch in the Midwest, common milkweed. Chapter 3 describes field experiments that assess the effects of fomesafen herbicide on common milkweed, and how the herbicide impacts ovipositioning by monarchs. In addition, greenhouse experiments determined the effects of three additional postemergence herbicides (imazethapyr, glufosinate, and mesotrione) representing different sites of action on growth of common milkweed. Chapter 4 evaluates simple methods for establishing common milkweed and other forb species native to the Midwest into an existing perennial sod landscape. This research will provide information about methods for increasing the suitability of the Iowa landscape for monarchs. Chapter 5 summarizes general conclusions of this thesis and connections between successful landscape management practices and establishment of forb species. Impacts from sub-lethal herbicide usage on common milkweed and monarch utilization also will be discussed. Additionally, this chapter will suggest future research options for monarch habitat establishment and fitness of larvae when reared on herbicide injured plants. References are listed at the end of each chapter. An appendix of analyzed data collected throughout both years is provided.

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CHAPTER 2. LITERATURE REVIEW

Introduction

The eastern monarch butterfly (*Danaus plexippus* Linnaeus) population was estimated at approximately one billion butterflies during the winter of 1996-1997. However, since then the population has decreased by as much as 97% (Brower et al., 2014). Numerous factors may have contributed to this decline, including climate change, agricultural practices, lack of nectar and milkweed (*Asclepias syriaca* L.) resources, urbanization, and infection by *Ophryocystis elektroscirrha* (*OE*) (Altizer and de Roode, 2015). As a way to improve the status of pollinators, including the monarch, the White House issued a Presidential Memorandum in 2014 declaring the importance of these organisms (Presidential Memorandum, 2014). This document was an important acknowledgement of the issues facing pollinators; however, much more needs to be done for the monarch species to recover. A petition to list the monarch butterfly under the Endangered Species Act (Brower et al., 2014) was filed because the species qualifies for the five factors described in the ESA (Brower et al., 2014). In North America there are two monarch breeding populations that migrate to avoid the colder winter months in their summer range. The most notable population is found east of the Rockies and makes the migration south each fall to overwinter in Mexico's Oyamel fir forest. The second population lives west of the Rockies, and is much smaller in size, approximately 2.3 million from 1990 to 2000 (Leong et al., 2004). The western population migrates to southern California's coastal areas during winter months. In Florida, a non-migratory population is found and remains year round because of favorable weather conditions. The Florida population is genetically distinct from the other two migratory populations (Zhan et al., 2014). The North American populations make up the largest proportion of worldwide monarchs (Brower et al., 2014). Smaller populations exist outside of North America in tropical and subtropical climates; however, these non-migratory populations would not be able to conserve the monarch species due to lack of genetic diversity. Brower et al. (2014) concludes, loss of the North American populations could make the worldwide monarch population vulnerable to extinction.

Monarch Butterfly Biology and Behavior

The life cycle from egg to adult monarch butterfly takes approximately three to four weeks. Female monarch butterflies oviposit between 300-400 eggs (Oberhauser and Solensky, 2004). The female butterfly soars high above the landscape in search of a suitable host plant, most frequently common milkweed in the Midwestern United States, where it can deposit eggs. In a landscape of green vegetation, the female is able to locate host plants, specific members of the Apocynaceae family, using visual and chemical cues (Floater and Zalucki, 2000; Jactel et al., 2011). Once it lands on an appropriate host, it utilizes sensory organs on its feet to determine the suitability of the plant. To reduce egg predation and ensure adequate food resources for larvae, butterflies do not oviposit all their eggs on a single plant. The female monarch butterfly typically deposits one egg onto the underside of a leaf and then begins searching for another host plant to deposit another egg.

Although many common milkweed plants may be present within the landscape, how a monarch determines whether to deposit eggs on a specific plant is poorly understood. Some of the proposed explanations for preference include cardeneloid plant levels (Zalucki et al., 1990), milkweed patch size (Zalucki and Suzuki, 1987), surrounding vegetation (Floater and Zalucki, 2000; Jactel et al., 2011), and/or plant age (Zaluki and Kitching, 1982a). Pleasants (2015) proposed lower predation risk and/or more suitable microclimate result in higher egg population densities on common milkweed in agricultural fields. Common milkweed in agricultural fields are typically surrounded by a monoculture of vegetation, increasing their visibility to a monarch butterfly (Floater and Zalucki, 2000; Jactel et al., 2001). Zalucki and Kitching (1982a) postulated that common milkweed in crop fields exposed to non-lethal herbicides would produce a flush of new, succulent leaves more attractive to ovipositing females.

A neonate caterpillar hatches from the egg after about four days and proceeds through five instar growth stages. Following the fifth and final instar stage the caterpillar pupates and forms a chrysalis; after approximately ten days it will eclose as the adult monarch butterfly (Oberhauser and Solensky, 2004). The lifespan of the adult monarch varies with generation. There are up to four generations per year. The first three or four generations of adults live from two to five weeks; however, the final generation of the season migrates to overwintering sites in the Oyamel fir forests in Central Mexico's mountains, and can live up to nine months (Oberhauser and Solensky, 2004).

The eastern population of monarchs follows a main "central" flyway until reaching their overwintering habitat located in Central Mexico's oyamel fir forest (Howard and Davis, 2009; Quinn, 2011). The trees where the migrating monarchs cluster are in the mountains of the Trans-Mexican Volcanic Belt. The hibernating colonies are restricted to dense forested areas of high elevations around 3,000 m (Slayback et al., 2007). The butterflies enter a state of diapause and live up to nine months. The forest canopy acts as a blanket or umbrella, shielding butterflies from rain while keeping them warm enough so they do not freeze, but cool enough to not break diapause (Zalucki et al., 2015). Entering diapause conserves lipids that are used as their energy source throughout the winter and for their remigration journey north in the spring (Brower et al., 2011). Monarchs from the overwintering population leave the Oyamel forests during March or April and fly northward to areas of Texas and Oklahoma. Upon arrival the females deposit eggs on milkweed and die soon after. The newly deposited eggs will become the first of the short-lived monarch generations. These generations continue their migration northward to the limits of suitable host plants.

Factors Involved in the Monarch Decline

Greater than 90% mortality occurs as a result of predation, disease, and natural causes during the egg and larval stages, suggesting that prolific egg production is necessary to sustain the population (Borkin, 1982; Zalucki and Kitching, 1982a; Oberhauser et al. 2001; Prysby and Oberhauser, 2004). The *OE* parasite was first identified in the late 1960's, and since has been identified in all monarch populations. During ovipostitioning, an infected female adult scatters spores of the parasite onto egg and the host milkweed (de Roode et al., 2009). Transfer of the protozoa can occur from adult to egg (vertical transfer) or the bacteria can be ingested during larval feeding, but the bacteria is not transferred from larva to larva (Altizer and de Roode, 2015). Heavily infected individuals may die during the pupal stage, not fully emerge from the chrysalis, or develop severe wing deformities (Altizer and de Roode, 2015). Eggs and larvae are also vulnerable to invertebrate predation from ants, spiders, wasp, green lacewing larvae (*Chrysoperla rufilabris* Bermeister), assassin bugs (Family-Reduviidae), and larval parasitism by species of flies and wasps (Oberhauser et al., 2015). Consumption of the cardeneloids present in milkweeds provides larvae some protection from predators. The distasteful and toxic cardenolides help to deter predators from consuming larvae and adult butterflies (Malcolm, 1991 and 1995).

Human activities, such as industrialized agriculture and urbanization, have reduced habitat availability for monarchs (MacDonald et al., 2013). Development decreases habitat suitable for monarchs by converting natural or agricultural habitats to lawns, paved areas, or buildings (Brower et al., 2014). Changes in agricultural practices have reduced the availability of common milkweed growing in agricultural fields (Hartzler, 2010). Oberhauser et al. (2001) stated that common milkweeds growing in agricultural fields are utilized more than non-agricultural common milkweed, thus proposing land involved in crop production is important for the success of the monarch population. Pleasants and Oberhauser (2013) conducted egg and larval counts on common milkweed in agricultural and non-agricultural fields from 2000 to 2003. The authors found 3.9 times more eggs on common milkweed in crop fields than on common milkweed in other areas of the landscape.

An analysis of two decades of citizen science data resulted in Inamine et al. (2016) concluding an unknown factor is reducing the percentage of monarchs that successfully migrates from the summer reproductive range to the overwintering sites in Mexico. Lack of nectar plants for butterflies could play a role in reducing survival of the overwintering monarch populations (Brower et al., 2006). During the fall migration south, adults rely on nectar from plants other than milkweed, thus they stated it is unlikely that the limited milkweed hypothesis is a cause for declining numbers (Inamine et al., 2016). The authors proposed research should focus on reestablishment of the fall migration and wintering habitat (Inamine et al., 2016).

As global climate change progresses, species in the northern hemisphere may escape unfavorable growth conditions by expanding their range northward (Lemoine, 2015). The range of the monarch butterfly is limited by its host plant's geographic range. The northern most range for milkweeds is southern Canada, just above the Great Lakes. Changes in temperature and precipitation alters the growing conditions for all milkweed species, and allows them to spread further north. A MaxEnt model, a software package popularly used for species distribution and environmental niche modeling, predicted that common milkweed growing zones could expand to areas further north in Canada (Lemoine, 2015). The shift in host plant range further north results in a longer migration in the fall. The longer migration could reduce the numbers reaching overwintering sites, and reduce the fitness of those that are successful.

Short-term weather events can also influence the monarch population. Texas and northern Mexico experienced the worst drought on record for the region during 2010 and 2011 (Brower et al., 2015). Monarchs captured at the same location in Texas during 2011 had significantly lower lipid levels than monarchs collected during 1982 and 1994, likely as a result of the drought reducing nectar sources (Brower et al., 2015). Low lipid levels can reduce monarch survival during winter and the spring migration. A second example of an extreme weather event influencing the monarch population is a storm that hit the Mexican Oyamel forest in March 2016 (Brower et al., 2017). The storm was a mix of rain and snow accompanied with powerful winds, followed by freezing temperatures, resulting in tens of thousands of trees being blown over. A field assessment determined the storm killed approximately 40% of the monarch butterflies present at the location. The current low population status reduces the ability of the monarch to recover from these extreme events.

Finally, deforestation of the Oyamel forests also is viewed as a threat to the monarch. The overwintering sites in Mexico were protected in 1986 (Solensky, 2015). Illegal logging reduced suitable overwintering habitat to about 1,620 hectares annually between 2001 to 2009, to approximately 47 hectares in years between 2009 and 2016 (WWF, 2016). However, not all of the overwintering areas are protected. Multiple factors across the broad range of the eastern monarch population impact the success of the species. Increasing the population to a sustainable size will require efforts to mitigate the impact of several of these factors, rather than focusing on a single aspect of the decline.

Common Milkweed Biology and Establishment

Common milkweed (*Asclepias syriaca* L.) is a perennial plant native to United States and Canada east of the Rocky Mountains (Mitich, 1993). It is better adapted to disturbance than other native members of the milkweed family, thus it has increased in prevalence since European settlement of North America. It is commonly found in habitats such as roadsides, crop fields, and restored prairies. Mature plants reach heights up to 2 meters.

The entire plant contains cardenolides that defend against herbivory, with highest concentrations in the plant latex (Agrawal et al., 2012). When damaged, the plant exudes white latex from the wound; this is the reason for its name “milkweed”. Cardenaloids inhibit the Na^+/K^+-ATPase cation pump of susceptible organisms. The gradients created by the cation pump are essential to maintain membrane function and secondary active transport within insects (Jorgensen et al., 2003). The monarch is a specialist to its host plant and is not negatively affected by consumption of the plant. When consumed, the caterpillar sequesters the cardenolides into integument space, protecting the larvae from predation (Agrawal et al., 2012).

The perennial plant completes nine phenological stages of growth during its growing season: emergence, vegetative growth, floral bud stage, umbel emergence, first flowering, full bloom, flower senescence, small seed pod, mature seed pod, and ripe seed pod (Simard et al., 1988). In spring, adventitious rootstocks send up new shoots; once sufficient photosynthetically active foliage develops active root growth occurs (Bhowmik, 1997). Depending on time of emergence and weather patterns, flowering takes place during late June through early August. Plants initiating from seed typically do not flower until their second season (Bhowmik, 1997). Common milkweed seed pods mature and split open in early fall, and seed are wind dispersed via white, hair-like coma (Bhowmik, 1997). Root growth halts mid-August through mid-September when plants senesce.

The lack of host plants in the summer reproductive range is believed to be a contributing factor in the decline of the monarch butterfly (NRCS and USFWS, 2016). The landscape in the reproductive range of the monarch is dominated by agriculture. Land-use changes can result in loss of pollinator habitat needed to sustain a diverse insect community, including the monarch species. Thogmartin et al. (2017) discuss the importance of incorporating monarch/pollinator habitat into agricultural lands of the Cornbelt because they make-up 77% of all of the potential monarch habitat. Because agricultural systems are unlikely to change significantly in the near future, incorporating habitat into small areas of a perennial sod landscape not utilized for agriculture, and recreation, could assist in expanding monarch reproductive habitat as well as provide habitat for other important insects.

Adult monarchs are generalist nectar feeders and feed on an array of flowering plants (Landis and Dumroese, 2015). When enhancing habitat for monarchs, forb species selected as nectar sources should reflect the appropriate growing zone and include a mix of species with different flowering periods to ensure nectar availability throughout the entire monarch season (Tooker et al., 2002). It is especially important to have floral resources available during late summer and early fall for the migrating monarch generation (Dumroese et al., 2016). Nectar, which is high in sugars and, is converted to lipids by monarch adults, thus sustaining the adults during migration and overwintering in Mexico when flowering plants are not available (Alonso-Mejia et al., 1997).

Establishing native forbs and common milkweed in a perennial landscape, often dominated by dense grass sod, broadleaf weeds and/or woody species, is not as simple as simply placing seed into the soil. Successful establishment requires planning and continuous management. Site preparation such as suppressing existing vegetation (e.g., mowing, herbicide) is essential for establishment success for a direct seeding method (Douglas et al., 2007). Established perennial vegetation competes with emerged seedlings and reduces recruitment; therefore, suppression of existing vegetation plays an important role in success of new seedlings (Evans, 1983; Porteous, 1993). Mowing in conjunction with applying an herbicide (e.g., glyphosate) increases seed-soil contact and reduces competition of seedlings with surrounding vegetation (Williams et al., 2007). Douglas et al. (2007) reported plant species that germinate over a short period of time with rapid early-season growth are more likely to succeed in establishing within existing vegetation than plants without these traits. Seed germination of many native species is increased when their seed is scattered during the fall rather than the spring. Fall seeding allows stratification, a requirement for many forb species to break dormancy (Smith et al., 2010).

While suppressing existing vegetation may enhance establishment of desirable forbs, it may also promote invasion of the area by weeds. The majority of seed that enter the seedbank are from annual weeds (Hume and Archibald, 1986; Roberts, 1981). Suppression of the existing vegetation, whether from mowing or herbicide application creates a more favorable environment for recruitment of weed seed within the seedbank. Weed control may be accomplished by spot-treatment with herbicides or with hand weeding (Douglas et al., 2007). Kurtz (1994) explained that mowing throughout the first growing season of a newly seeded prairie increases establishment of seeds scattered. Native forbs in plots that were mowed were taller and produced deeper roots than forbs in control plots (Williams et al., 2007). Research in Iowa by Meissen et al. (2017) concluded that the performance of first year native plantings was greatly increased with routine mowing. Mowing increases light penetration to the soil surface, therefore enhancing forb establishment. Existing perennial vegetation and annual weeds can quickly reoccupy small gap areas, therefore multiple mowings increase resource availability to newly established native species (Bullock et al., 1995; Hitchmough et al., 1996; Rogers and Hartnett, 2001). As the growing season progresses, it is important to raise the height of the mower to minimize the amount of foliage removed from the newly established forbs.

Herbicide Impacts on Common Milkweed

The Cornbelt region of the United States produces approximately half of the overwintering monarch population that migrates to Mexico (Miller et al., 2012). Common milkweed within crop fields is believed to be an important resource for monarchs (Oberhauser et al., 2001; Pleasants and Oberhauser, 2013). While common milkweed is able to survive in crop fields, the impact of production practices, including herbicide use, on the utilization of these plants by monarchs is not known.

Prior to the 1900s, only a few inorganic compounds were used specifically for weed control, as well as animal-powered tillage tools, but hand weeding was a primary component of weed management (Timmons, 1970). Phenoxyacetic herbicides, such as 2,4-D and MCPA (2-methyl-4-chlorophenoxyacetic acid), were discovered in the early 1940s, a time marked as the beginning of the “Chemical Era of Agriculture” (Timmons, 1970). The number of herbicides used in the United States and Canada grew from 15 in 1940 to 25 in 1950 and by 1969 increased to 100. The U.S. Bureau of Census reported 200 million pounds of herbicides were applied in 1962, five years later usage increased to 348 million pounds (Timmons, 2005). Gianessi (1992) reported 57% of corn acres in the U.S. were treated with herbicides in 1966, and increased to 95% by 1982.

During the late 1970s, most herbicides applied to soybean were preemergence products applied at planting (Appleby, 2005). Preemergence herbicides have little effect on common milkweed developing from established rootstocks. In the late 1980’s a large increase in postemergence herbicides was reported in soybean, but herbicide use patterns did not change significantly in corn (Hartzler and Wintersteen, 1991). The herbicides commonly used in corn and soybean in the 1980's and early 1990's would suppress, but not kill common milkweed growing within crop fields (Cramer and Burnside, 1981).

The introduction of glyphosate-resistant crops in the late 1990's dramatically changed weed management programs in corn and soybean (Young, 2006). Hopkins (2017) reported 97% of soybean and 94% of corn planted in 12 North Central states were herbicide-resistant varieties by 2013, with the majority of these being resistant to glyphosate. In 1999 51% of crop fields in Iowa contained common milkweed, but by 2009 only 8% of crop fields were infested with the weed (Hartzler and Buhler, 2000; Hartzler, 2010). Although the surveys were not designed to identify the cause of this decline, the use of glyphosate in conjunction with planting glyphosate-resistant crops likely was a major contributor to the change in infestation level.

Before the introduction of glyphosate-resistant crops, the primary use of glyphosate in agronomic crops involved applications to no-till fields prior to crop planting to control established weeds (Young, 2006). During this period, less than 3 million kg of glyphosate was used in soybean per year in the United States. By 2006, greater than 90% of soybean planted were glyphosate-resistant varieties (Duke, 2018). Glyphosate use in soybean increased to 30 million kg/yr and the number of applications increased from 1.0 to 1.4 per year from 1995 to 2002 (Young, 2006).

The diphenyl ether herbicides were initially introduced in the 1960s and have been commonly used for managing broadleaf weeds since that time (Dayan and Duke, 2010). Aciflourfen was the first herbicide in this family registered for postemergence use in soybean, and was followed by fomesafen and lactofen (Hartzler, personal communication, December 13, 2017). While these herbicides can move within plants via the xylem, there is little translocation of the herbicide following postemergence application. They kill plants by inhibiting protoporphyrinogen oxidase (PPO) (Dayan and Duke, 2010). The enzyme is located in the chloroplast and oxidizes protoporphyrinogen, resulting in protoporphyrin IX. This end product is an essential precursor molecule for heme (required for electron transfer chains) and chlorophyll (required for photosynthesis). Protoporphyrinogen oxidase causes photobleaching and light-dependent desiccation of vegetation. Injury symptoms are chlorotic leaves, which display necrosis 1-3 days post fomesafen application (Johnson et al., 1978). Use of fomesafen in the United States began in 1989 (Gianessi, 1992). The herbicide is primarily active on small, annual broadleaf weeds; it is unlikely it would kill common milkweed plants developing from established rootstocks.

Summary

The loss of common milkweed in Midwest crop fields coincided with the introduction of glyphosate-resistant crops. Herbicides used for weed control prior to widespread use of glyphosate would damage shoots of established common milkweed, but would not kill perennial rootstocks. Determining the impact of this damage on utilization of common milkweed in crop fields by monarchs is needed to understand the importance of these plants and the impact of their loss on the monarch population.

The following thesis consists of two distinct components. The first explores the impact of non-lethal herbicide applications on utilization of common milkweed by monarchs. The second experiment investigates a simple method to enhance the value of under-utilized areas within agricultural landscapes for monarchs.

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CHAPTER 3. EFFECT OF POSTEMERGENCE HERBICIDES ON COMMON MILKWEED (*ASCLEPIAS SYRIACA*) GROWTH AND UTILIZATION BY MONARCHS (*DANAUS PLEXIPPUS*)

Abstract

Field experiments were conducted to investigate impacts of herbicide injury to common milkweed (*Asclepias syriaca* L.) on oviposition preference of monarch butterflies (*Danaus plexippus* Linnaeus). Common milkweed seedlings were transplanted in patches containing five plants spaced 25 cm apart in a no-till soybean field shortly after soybean planting in 2016. A buffer of 3 m East-West and 6 m North-South separated patches. Treatments included an untreated control and 0.14 kg ha^{-1} fomesafen plus 0.5% crop oil concentrate. The experiment was repeated in the same area in 2017 by planting soybean no-till prior to common milkweed emergence. Common milkweed leaves displayed chlorosis and necrotic lesions five days after fomesafen application. Two weeks following application many leaves damaged by fomesafen dehisced, and plants averaged an injury rating of 3.4 (based on a visual scale of 1 = healthy and 5 = dead). Leaves emerging from the apical meristem following fomesafen application appeared normal, and four weeks after application plants averaged an injury rating of 2.6. In 2017, multiple stems emerged from the majority of plants that were established in 2016, and response to fomesafen was similar as in 2016. Dry weight of common milkweed ten weeks after application was not affected by fomesafen in either year. Common milkweed plants were examined for monarch eggs and larval instars weekly from May to August. In 2016, patches averaged 0.6 eggs, whereas in 2017 patches averaged 38.5 eggs. Fomesafen did not affect ovipositing by monarch butterflies. The increased egg densities during 2017 may be due to adult female monarchs being better able to detect the multiple, more vigorous common milkweed ramets emerging from established rootstocks. Additionally, many of the second-year ramets produced flowers that could have attracted monarchs. Larval instars were observed and recorded throughout 2017. Presence and survival of instars were not affected by fomesafen, patches averaged 15.0 and 0.2 first and fifth instars, respectively. Common milkweed response to fomesafen (0.03, 0.07, 0.14 kg ha^{-1} and 0.28 kg ha^{-1} plus 0.5% crop oil concentrate), glufosinate (0.23, 0.47 kg ha^{-1} and 0.91 kg ha^{-1} plus 3.4 kg AMS ha^{-1}), imazethapyr (0.04, 0.07 kg ha^{-1} and 0.14 kg ha^{-1} plus 1.9 kg AMS ha^{-1} + 1.25% crop oil concentrate), and mesotrione (0.05, 0.10 kg ha^{-1} and 0.21 kg ha^{-1} plus 2.4 kg AMS ha^{-1} + 1% crop oil concentrate) was evaluated in greenhouse experiments. The high rate represents 2X the typical use rate in corn or soybean. No herbicide caused plant mortality, and plants showed signs of recovery within two weeks of application. Glufosinate had the greatest negative impact on common milkweed, followed by mesotrione and imazethapyr.

Introduction

The eastern monarch butterfly (*Danaus plexippus* Linnaeus) population was estimated at approximately one billion butterflies during the winter of 1996-1997; however, since then the population has decreased by as much as 97% (Brower et al., 2014). The decrease in common milkweed in agricultural fields due to adoption of glyphosate-resistant crops and resulting use of glyphosate has been one of the proposed causes for the declining population of the monarch butterfly (Pleasants and Oberhauser, 2013). Hartzler (2010) found that common milkweed presence in Iowa crop fields declined by almost 90% between 1999 and 2009. However, there is little information documenting the importance of common milkweeds in crop fields for monarch reproduction prior to the loss of common milkweed from this habitat. Oberhauser et al. (2001) stated that common milkweed growing in agricultural fields are utilized more than non-agricultural common milkweed, thus proposing common milkweeds established in crop fields are important for the success of the monarch population. Pleasants and Oberhauser (2013) conducted egg and larval counts on common milkweed in agricultural and non-agricultural fields from 2000 to 2003. The authors found 3.9 times more eggs on a common milkweed in crop fields than on common milkweed in other areas of the landscape. Pleasants (2015) suggested higher egg count in agricultural fields might be due to lower predation risk and/or more suitable microclimate. Common milkweeds in agricultural fields are typically surrounded by a monoculture of vegetation, likely increasing their visibility to a monarch butterfly (Floater and Zalucki, 2000). Zalucki and Kitching (1982) postulated that common milkweeds in crop fields exposed to non-lethal herbicides would produce a flush of new, succulent leaves more attractive to ovipositing females.

While herbicides used in agronomic crops prior to the introduction of glyphosate-resistant crops usually did not kill perennial milkweed, postemergence herbicides would have caused foliar damage and in some cases killed the above ground shoot (Cramer and Burnside, 1981). In contrast, glyphosate translocates into the common milkweed rootstock, thus eliminating recurring presence of the weed in crop fields. Little information is available regarding the impact of sub-lethal herbicide injury to common milkweed on utilization of the plants by monarchs. The objectives for this experiment were 1) determine the effect of fomesafen on growth of common milkweed and ovipositioning by monarchs, and 2) determine the effects of other herbicides used postemergence in corn and soybean on common milkweed.

Materials and Methods

Effect of Fomesafen on Common Milkweed Utilization

Field research sites were located at the Iowa State University Johnson Farm near Ames, Iowa. The first study began in 2016 within field #23 and was bordered with smooth brome (*Bromus inermis* Leyss) alleyways on three sides. The experiment was repeated in 2017 in the same plots, and a second site was initiated at the ISU Johnson Farm in a field approximately 200 m southeast of the initial site. Poor establishment and growth of plants at the second site resulted in lack of utilization by monarchs, so data are not presented.

Common milkweed seeds were hand collected in September 2015 in Story County, Iowa. Seeds were separated from their pods and coma, then mixed with wet/damp sand and kept in a walk-in cooler at 4° C until planting. Stratified seeds were planted during March 2016 in flats of potting soil in a greenhouse for germination. Individual seedlings were transplanted into 7.6 cm^2 pots 10 days later and kept in the greenhouse until transplanting in the field.

The experiment was a randomized complete block design with two treatments (control and 0.14 kg ha^{-1} fomesafen plus 0.5% crop oil concentrate) and 12 replications. Blocks were 3 m x 6 m each with two 3 m x 3 m subplots. An additional four blocks, referred to as the “harvest-plots”, were created to evaluate the effect of fomesafen on shoot biomass of common milkweed. The harvest plots were established as previously mentioned.

Soybean was planted in 76 cm rows on May 5, 2016 and the field was treated with 43.0 g ha^{-1} flumioxazin, 1.2 kg ha^{-1} pendimethalin, and 1.6 kg ha^{-1} glyphosate. A 0.3 m^{-2} patch of five common milkweed plants approximately 15 cm in height was established in each plot on May 6th. A power drill with a 6.4 cm diameter auger attachment was used to drill holes approximately 20 cm deep holes in a diagonal pattern with 25 cm separating holes. Common milkweed were placed in holes, backfilled with potting soil, and then watered. Patches were placed in the center of the subplots, equidistant between two soybean rows.

Fomesafen was applied with a 1.9 m boom, CO_2-powered sprayer calibrated to apply 186 l ha^{-1} using flat fan nozzles at 300 kPa on June 24, 2016 when common milkweed were approximately 20 cm tall. At time of application, weather conditions were 29° C, sunny, SSE 18 km h^{-1} wind, 51% humidity and 18° C dew point. At the time of fomesafen application, twenty-four common milkweed, twelve plants from the fomesafen treated plots and twelve plants from the control plots, were tagged so that all plants were paired with a plant of the other treatment of similar height and vigor. Before the application of fomesafen, six common milkweed pairs were randomly selected and harvested; the remaining six pairs of common milkweed were harvested ten weeks after fomesafen application. Plants were cut at soil level and put into separate labeled paper bags and kept in a drying oven held at 60° C for seven days. The dry weight of each plant was then recorded.

The experiment was repeated in the same area in 2017, using the milkweed patches established in 2016. The same preemergence herbicides were applied on April 18, and soybean were planted on April 26. Fomesafen was applied on June 15, 2017 when common milkweed was approximately 50 cm tall. At time of application weather conditions were 17° C, calm winds, 41% humidity and 19° C dew point.

Lengths of individual common milkweed stems were recorded weekly from May to August. Beginning one week after fomesafen was applied, injury ratings were taken for all plants on a weekly bases using a 1 to 5 scale (1 = no effect; 2 = < 20% necrosis, minor burning; 3 = > 20% necrosis, < 2 leaves lost; 4 = at least one pair of leaves lost; 5 = dead). Eggs found on the plants during 2016 were recorded and removed weekly to eliminate plant stress from larval feeding. No larvae were present during year 2016 because of egg removal. During 2017 eggs and larvae were allowed to remain on the plants due to the increased vigor of the plants. Egg and larval counts were conducted each week on Monday and Friday. Larval instar growth stages were recorded throughout the season to track survival.

Data from heights and dry weight of common milkweed plants were analyzed using a paired t-test and one-way analysis of variance (ANOVA) to assess significance of height and dry weight, respectively (SAS Institute 1990). Egg and larval counts were analyzed using PROC MEANS (SAS Institute 1990). Common milkweed injury ratings were analyzed both years using TTEST (SAS Institute 1990).

Common Milkweed Response to Postemergence Herbicides

Common milkweed response to representative herbicides from four sites of action was evaluated in a series of greenhouse experiments. Common milkweed was started from seed as previously described. In the first experiment, a randomized complete block design with five replications was used to evaluate the response of common milkweed to five rates of fomesafen (0, 0.03, 0.07, 0.14 kg ha^{-1}, and 0.28 kg ha^{-1}) plus 0.5% crop oil concentrate. The standard field use rate is 0.14 kg ha^{-1}. Herbicides were applied in a laboratory track sprayer calibrated to apply 286 l ha^{-1} with a Tee Jet 80015EVS nozzle at 303 kPa when plants were approximately 20 cm tall. The experiment was repeated in time.

In a second experiment, common milkweed plants were sprayed with three rates of glufosinate (0.23, 0.47 kg ha^{-1} and 0.91 kg ha^{-1} plus 3.4 kg AMS ha^{-1}), imazethapyr (0.04, 0.07 kg ha^{-1} and 0.14 kg ha^{-1} plus 1.9 kg AMS ha^{-1} plus 1.25% crop oil concentrate), and mesotrione (0.05, 0.10 kg ha^{-1} and 0.21 kg ha^{-1} plus 2.4 kg AMS ha^{-1} plus 1% crop oil concentrate) plus a control. The rates represented 0.5, 1.0, and 2.0 times the normal field use rate of each herbicide. The same experimental design and application methods were used as for the fomesafen experiments.

The height of each common milkweed plant was recorded before herbicide application and once a week for two weeks thereafter. Plant injury ratings were also recorded at these times using a scale of 1 to 10 (1 = no effect; 2-6 = level of leaf injury; 7-9 = level of dehiscence and injury; 10 = dead). Two weeks after herbicide application, each common milkweed plant was harvested at the soil surface, placed into paper bags and kept in a drying oven held at 60° C for one week. The dry weight of each plant was then recorded.

Common milkweed dry weights and injury ratings were analyzed using CONTRASTS analysis under PROC GLM procedure in Statistical Analysis Software (SAS) (SAS Institute 1990).

Results and Discussion

Field Experiment

Fomesafen was applied five weeks after soybean planting both years. Common milkweed in 2016 were shorter than in 2017, likely due to a combination of transplanting shock and a dry period following establishment in the field in 2016 (Table 1). In 2017, plants developed from established rootstocks. Reduction in common milkweed height due to fomesafen was evident four and two weeks following application in 2016 and 2017, respectively (Table 1). Eight weeks after application common milkweed height was reduced 13 and 6% in 2016 and 2017, respectively. Visual injury was significant for fomesafen treatment two and four weeks following fomesafen application during both years (Table 1). New leaves from the apical meristem were normal; however by 4 weeks after treatment differences in injury were still observed on older existing leaves. Shoot biomass was not impacted by fomesafen either year (Table 2).

Higher monarch egg densities were found on common milkweed during 2017 than 2016 with an average of 45.2 and 0.8 eggs per patch, respectively; fomesafen did not affect ovipositioning in either year (Table 3). In 2017, common milkweed produced multiple vigorous stems, and many of these stems produced flowers whereas in 2016 common milkweed plants did not produce flowers. Greater biomass and presence of flowers may have attracted more monarch butterflies to visit plots in 2017 compared to 2016. Peak egg densities occurred between July 24 and August 7 with the greatest density of eggs occurring on July 31, 2017 (Fig. 1). The first generation of monarchs typically arrive in Iowa in May to early June, the second generation occurs around mid-June to early July, and the final generation, which migrates to their overwintering site, develops from mid-July through August.

The majority of newly expanded leaves at the time of fomesafen application dehisced within two weeks of application, thus most eggs were laid on leaves that emerged following application or on lower leaves. Monarchs prefer to oviposit onto succulent, lush common milkweed plants, thus it was thought that common milkweed damaged by fomesafen would be unattractive to female monarchs due to foliar injury characteristic of this herbicide. A common milkweed preference experiment by Fisher et al. (2015) revealed that mowed common milkweed plots contained higher egg densities than unmowed common milkweed plants, which consisted of older vegetation. Zalucki and Kitching (1982) proposed herbicide injury to common milkweed in agricultural fields would increase ovipositioning by monarchs. Rapid recovery of plants from fomesafen may minimize any negative impacts on monarch choice for ovipositioning, but this research does not support an increased preference.

Monarch eggs were allowed to hatch and develop through the instar growth stages in 2017. Approximately eight first instar caterpillars were found per patch over the course of the study; however, less than one fifth instar per patch was observed (Table 4). Fomesafen did not affect larval survival rates. The number of first instars represent 17% of the eggs, and there was greater than 95% loss of instars from the first to fifth stage. The high mortality observed in this experiment is typical for monarchs; studies have shown $< 10\%$ of monarchs complete a full lifecycle as a result of biotic and abiotic factors (Borkin, 1982; Zalucki and Kitching, 1982; Oberhauser et al., 2001; Prysby and Oberhauser, 2004; Nail et al., 2015). The results for this experiment do not support our hypothesis that sublethal herbicide injury to common milkweed plants negatively affects monarch utilization. No eggs were found on common milkweed plants in the replicate site established in 2017. Common milkweed plants in the replicate site struggled to establish quickly, thus the plants were much shorter in height and by July were mostly covered by the soybean canopy. The canopy of the soybean may have hindered visibility by adult monarchs.

Common Milkweed Response to Postemergence Herbicides

The response of common milkweed to several herbicides was evaluated in the greenhouse. Contrasts showed that all rates of fomesafen caused significant visual injury and reduced common milkweed biomass compared to the control two weeks after application (Table 5 and 6). Visual injury increased with increasing rate, but this was not observed for biomass. Similar to field observations, leaves contacted by fomesafen developed extensive necrosis and many dehisced; however, new growth from the apical and lateral buds suggested that plants would recover over time.

Three additional herbicides with different sites of action were evaluated for impacts on common milkweed. Contrasts indicated significance when comparing the three rates of all herbicides to the control for both visual injury and biomass (Tables 7 and 8). Based on injury ratings, glufosinate > mesotrione >imazethapyr, whereas with biomass reduction glufosinate > mesotrione = imazethapyr than both mesotrione and imazethapyr; mesotrione was greater than imazethapyr (Table 7). Glufosinate is a non-selective broad-spectrum herbicide that inhibits activity of glutamine synthetase and the production of glutamine (Devine et al., 1993; Hinchee et al., 1993). Glufosinate caused rapid necrosis and leaf loss; some common milkweed stems were without leaves except for new leaves that emerged from the apical meristem following application. Imazethapyr did not cause leaf loss, symptoms included slight necrosis of older leaves and cupping. Imazethapyr is a selective broad-spectrum herbicide that controls grass and broadleaf weeds by inhibiting acetolactate acid synthase (ALS). Inhibition of this enzyme disrupts protein synthesis, interfering with DNA synthesis and cell growth (Scarponi et al., 1995). Mesotrione herbicide caused bleaching of leaves; symptoms were greatest in the uppermost leaves. Mesotrione inhibits the enzyme p-hydroxyphenylpyruvate dioxygenase (HPPD). This enzyme is involved in synthesis of carotenoid pigments, which protect chlorophyll from photooxidation (Meazza et al., 2002). Bleaching of foliage is a common symptom of mesotrione and other HPPD inhibiting herbicides. While all herbicides evaluated caused initial severe injury, the presence of new growth two weeks after application indicates that in the field these herbicides probably would not effectively control the perennial common milkweed.

Summary

Damage from fomesafen was ephemeral both years of the experiment and did not affect ovipositioning by monarchs. Fomesafen caused greatest injury to plants two weeks after application; however, normal growth resumed by three weeks following application causing injury ratings to decrease. Although the modes of action and symptomology of the other herbicides were different than those caused by fomesafen, common milkweed in the greenhouse experiment resumed growth within two weeks of application. Based on our results, it is likely that monarchs would utilize plants damaged by herbicides used in this experimental study.

Table 1. Effect of 0.14 kg ha^{-1} fomesafen applied postemergence on common milkweed height and visual injury. Iowa State University, Ames, Iowa.[1, 2]

Treatment	Height			Injury rating	
	Weeks after treatment			Weeks after treatment	
	2	4	8	2	4
	------------------ cm ----------------			------------- (1-5) ----------	
2016					
Control	27.7 (0.9)	39.8 (1.4)	53.6 (2.6)	1.4 (0.1)	1.2 (0.1)
Fomesafen	26.3 (0.8)	35.3* (1.4)	46.6* (2.2)	3.4* (0.1)	2.6* (0.1)
2017					
Control	54.0 (1.6)	96.0 (2.5)	104.0 (3.1)	1.0 (0.01)	1.0 (0.01)
Fomesafen	44.1* (1.5)	85.4* (2.3)	99.0* (2.5)	3.6* (0.1)	2.2* (0.1)

[1]Parentheses indicate standard error.
[2] *indicates fomesafen different from control according to t-test analysis.

Table 2. Effect of 0.14 kg ha^{-1} fomesafen applied postemergence in soybean on common milkweed shoot dry weight. Iowa State University, Ames, Iowa.[1, 2]

Treatment	2016		2017	
	Shoot dry weight (g)		Shoot dry weight (g)	
	0 WAA	10 WAA	0 WAA	10 WAA
	------------------------------- g -------------------------------			
Control	2.2 (0.3)	7.9 (1.2)	10.0 (1.2)	32.1 (6.8)
Fomesafen	2.3 (0.3)	5.2 (1.4)	10.7 (1.0)	34.2 (5.3)

[1]Fomesafen did not affect common milkweed shoot dry weight in either year.
[2]Parentheses indicate standard error.

Table 3. Effect of fomesafen applied to common milkweed on ovipositioning of the monarch butterfly. Iowa State University, Ames, Iowa.[1, 2, 3]

Treatment	2016	2017
	------------- number of eggs per patch	--------------
control	0.6 (0.3)	43.1 (18.3)
fomesafen	1.0 (0.3)	47.3 (19.8)

[1]Common milkweed was planted in 0.3 m^{-2} patches within herbicide plots.
[2]No significance was found in egg numbers between treatments.
[3]Parentheses indicate standard error.

Table 4. Effect of 0.14 kg ha^{-1} fomesafen applied postemergence to common milkweed on monarch larvae survival during 2017. Iowa State University, Ames, Iowa.[1, 2, 3]

	Instar Stage				
Treatment	1	2	3	4	5
	---------------------- number of instars per patch ----------------------				
Control	7.3 (2.7)	3.8 (1.2)	0.6 (0.3)	0.2 (0.1)	0.1 (0.1)
Fomesafen	7.8 (2.9)	3.1 (1.0)	0.8 (0.3)	0.1 (0.1)	0.1 (0.1)

[1]Common milkweed was planted in 0.3 m^{-2} patches within herbicide plots.
[2]No significance was found in larvae numbers between treatments.
[3]Parentheses indicate standard error.

Table 5. Contrasts analyses for fomesafen applied to common milkweed two weeks after application. Iowa State University, Ames, Iowa.

Fomesafen[1]	Injury rating	Shoot dry weight
$kg\ ha^{-1}$	---------------------- P – value ----------------------	
0 – 1	< 0.0001	0.0128
0 – 2	< 0.0001	0.0023
0 – 3	< 0.0001	0.0002
0 – 4	< 0.0001	< 0.0001
1 – 2	0.0152	0.5223
2 – 3	0.2665	0.3582
3 – 4	0.0075	0.3582

[1]0 = 0.0 $kg\ ha^{-1}$; 1 = 0.035 $kg\ ha^{-1}$; 2 = 0.07 $kg\ ha^{-1}$; 3 = 0.14 $kg\ ha^{-1}$; 4 = 0.28 $kg\ ha^{-1}$.

Table 6. Effect of fomesafen on milkweed injury and shoot dry weight two weeks after application. Iowa State University, Ames, Iowa.[1, 2]

Fomesafen	Injury rating	Shoot dry weight
$kg\ ha^{-1}$	----------- (1-10) -----------	----------- (g) -----------
0.0	1.0 (0.3)	1.8 (0.2)
1.04	3.2 (0.3)	1.3 (0.2)
0.07	4.1 (0.3)	1.1 (0.2)
0.14	4.5 (0.3)	0.9 (0.2)
0.28	5.9 (0.3)	0.7 (0.2)

[1]Greenhouse experiment implemented during 2016.
[2]Parentheses indicate standard error.

Table 7. Contrasts analysis of three broad-spectrum herbicides on common milkweed two weeks after herbicide exposure.

Comparison[1]	Injury rating	Shoot dry weight
	------------------------------ P – value	------------------------------
C – G	< 0.0001	< 0.0001
C – M	< 0.0001	< 0.0001
C – I	< 0.0001	< 0.0001
G – M	< 0.0001	< 0.0001
G – I	< 0.0001	< 0.0001
M - I	0.0060	0.0885

[1]C = control; G = glufosinate; M = mesotrione; I = imazethapyr.

Table 8. Common milkweed response to three broad-spectrum herbicides two weeks after application.[1, 2, 3]

Herbicide	Injury Rating	Shoot Dry Weight
	(1 – 10)	% Control
Control	1 (0.2)	100 (0.5)
Glufosinate	7.3 (0.2)	33.0 (0.5)
Imazethapyr	4.0 (0.2)	78.5 (0.5)
Mesotrione	5.2 (0.2)	67.4 (0.5)

[1]Data were pooled for the three herbicide rates.
[2]Treatments were significant when contrasting the control to all rates for each herbicide ($P < 0.05$).
[3]Parentheses indicate standard error.

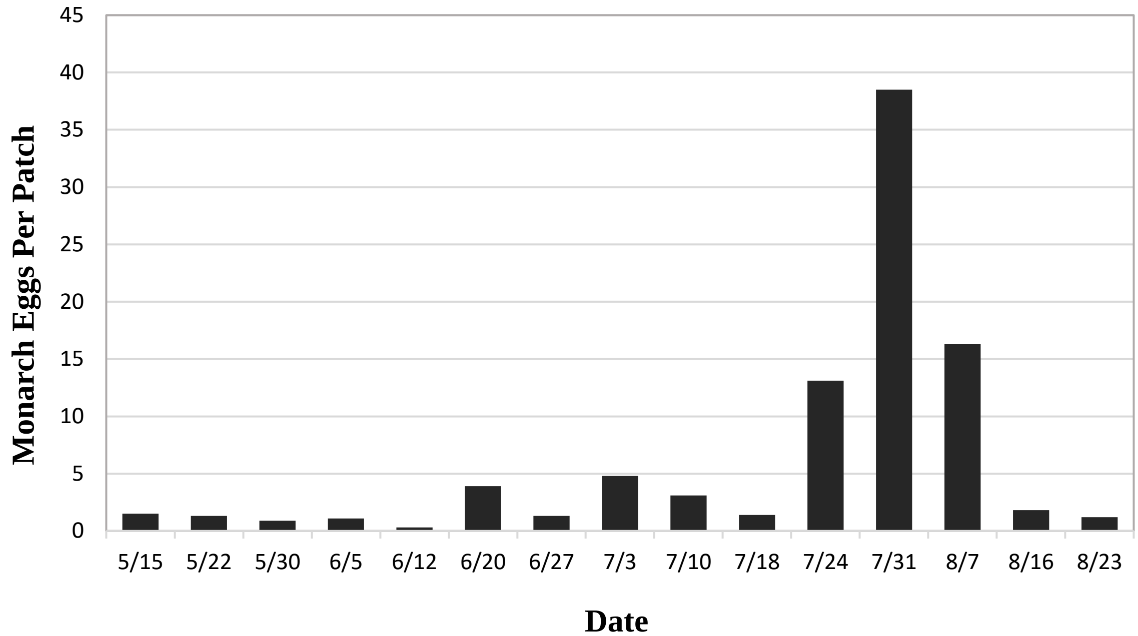

Figure 1. Monarch egg counts per patch in 2017 in a no-till soybean field. Data represent means of fomesafen and control plots. Iowa State University, Ames, Iowa.

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CHAPTER 4. ESTABLISMENT OF COMMON MILKWEED (*ASCLEPIAS SYRIACA)* AND THREE OTHER FORBS IN EXISTING PERENNIAL SOD.

Abstract

As agricultural practices have intensified, much of the land devoted to native species has been lost. There is increased interest by some landowners and the public in installing habitat that provides resources to pollinators and other organisms, including the monarch butterfly. The purpose of this study is to investigate a simple method for establishing common milkweed (*Asclepias syriaca* L.) and three other native forb species into existing sod landscapes. We hypothesize that suppression of smooth brome (*Bromus inermis* Leyss) will reduce interspecific competition, allowing for increased establishment of common milkweed, wild bergamot (*Monarda fistulosa* L.), golden alexanders (*Zizia aurea* L.), and New England aster (*Symphyotrichum novae-angliae* L.). A factorial experiment was used to evaluate contributions of mowing and sub-lethal rates of glyphosate (0.25 and 0.50 kg ha^{-1}) in establishing the four species in established smooth brome sod. Three seeding treatments were used for common milkweed (100 pure live seed (pls) m^{-2}, 100 pls m^{-2} + mid-June mowing, and 2000 pls m^{-2}), whereas a single seeding rate of 150 pls m^{-2} was used for the other forbs. Suppression of the sod prior to sowing seed increased recruitment of common milkweed, but mowing prior to glyphosate application did not affect recruitment. Although glyphosate increased the number of seedlings, these plants eventually succumbed to competition. At the end of two years there was no evidence of permanent establishment of common milkweed in the smooth brome sod. Of the three other forbs evaluated, the success of New England aster was less than golden alexanders and wild bergamot. Establishment of golden alexander and wild bergamot seedlings was similar to common milkweed, but these species developed

sufficiently to potentially persist in the sod. Suppression of a perennial sod with sub-lethal rates of glyphosate can increase recruitment of seedlings, but there is a low probability of permanently establishing common milkweed and other forbs. More intense disturbance may increase establishment, but could increase invasion of the area by weeds.

Introduction

Lack of resources, both nectar and larval host plants, in the summer reproductive range are a contributing factor in the decline of the monarch butterfly (*Danaus plexippus* Linnaeus) (Brower 2006). Most Iowa farms have small areas of land not utilized for crop production, recreation, etc. In these areas the most common species used as cover is smooth brome (*Bromus inermis* Leyss). We hypothesize that suppression of smooth brome will reduce interspecific competition, allowing for increased establishment of common milkweed (*Asclepias syriaca* L.) and three other forb species, wild bergamot (*Monarda fistulosa* L.), golden alexanders (*Zizia aurea* L.), and New England aster (*Symphyotrichum novae-angliae* L.). Common milkweed serves as both a nectar source for adult butterflies and as a food source for larvae. The three additional forbs chosen for this experiment provide floral nectar resources throughout the summer and early fall months for adult monarchs and other pollinators.

Common milkweed, a member of the dogbane family (Apocynaceae), is a perennial plant that grows to a height of 0.6 to 2.0 meters, and flowers late June through early August (Mitich 1993). The stem and leaves are pubescent, and when damaged exude white latex from the wound; this is the source of the name "milkweed". The entire plant contains cardenolides that defend against herbivory, with highest concentrations in the plant latex (Agrawal et al. 2012). Leaves are opposite and oval in shape with a rounded base and tip, typically 11.4 cm by 6.4 cm in size (Christiansen & Müller 1999). The inflorescence consists of many-flowered umbels developing at the upper leaf axils and the stem tip. Flowers are pinkish-purple in color with 0.6 cm long petals and hoods 0.6 cm tall with protruding horns (Christiansen & Müller 1999). Common milkweed, one of more than 70 *Asclepias* species in the United States, is the most prevalent food source for monarch larvae in the Midwestern United States, and also provides nectar to the adult monarch butterfly. Hartzler (2010) reported common milkweed was reduced in crop fields by approximately 90% between 1999 and 2009 due to changes in crop management practices.

In addition to milkweeds, adult monarchs require nectar producing plants as a nutrient source. A mix of species is required to provide resources throughout the growing season. Wild bergamot, a member of the mint family (Lamiaceae), is a sturdy perennial plant that grows 0.6 to 1.5 meters in height and blooms July through September (Runkel & Roosa 2009). Typically branched, this plant has a square stem and opposite leaves that are oval in shape, but narrow to a point. Leaves and stems are greenish-grey with a slight tinged purple hue. Individual flowers can be pink and lavender in color and are slender inch-long tubes with a distinct lobe (Runkel & Roosa 2009). The slender individual tube-like flowers are clustered together in dense ragged heads, usually 3.8 cm in diameter (Runkel & Roosa 2009).

Golden alexanders, a member of the parsley family (Apiaceae), is a perennial plant that grows 0.3 to 0.9 meters in height and blooms April through June (Runkel & Roosa 2009). It has a smooth stem and alternate leaves that contain three leaflets, and sometimes these three leaflets can be subdivided further into three parts (Runkel & Roosa

2009). Leaflets vary in shape from lance-shaped with toothed margins and are usually 2.5 to 5.0 cm long. Petiole length decreases on leaves closer to the top of the plant. A common point on the stem gives rise to several branched flower heads, each of with has 10 to 20 branches. Each individual flower is deep yellow in color and less than 3.2 mm wide. Golden alexanders resembles wild parsnip; however, golden alexanders is smaller in size, and the center flower in each umbel cluster of golden alexander has no stalk whereas the center flower of wild parsnips has a stalk (Runkel & Roosa 2009). The stem of wild parsnip also has vertical grooves running full length and its leaflets are egg-shaped rather than lanceolate.

New England aster, a member of the aster family (Asteraceae), is a perennial plant that grows 0.8 to 1.2 meters in height and blooms from late August through late September (Christiansen & Müller 1999). Its stem is pubescent and unbranched below its inflorescence. Leaves are alternate and without petioles, instead the base of each leaf wraps around the stem (chordate) of the plant (Christiansen & Müller 1999). Inflorescences are branched with heads that have purple rays and yellow center disk flowers. Each flower head is about 2.5 cm in diameter (Christiansen & Müller 1999).

By 1910, 11 million hectares of Iowa tallgrass prairie was destroyed by crop farming or overgrazing (Kurtz 2013). Today, less than 2% of Iowa is maintained in tallgrass prairie. As research has revealed the importance of diverse landscapes, many property owners are becoming interested in establishing native forbs in their own landscapes.

Kurtz (2013) states planting in late October to mid-November of forbs is ideal as this timing provides a period for cold-wet stratification to increase seed germination.

Stratification involves seed being exposed to cold temperatures, typically 30 to 90 days, in order to break dormancy (Kurtz 2013). Existing vegetation needs to be managed to reduce competition with new seedlings. Depending on the nature of the vegetation, mowing, tillage or herbicides can be used to create a favorable environment for seeding (Kurtz 2013). The initial years after establishing native forbs often results in a mix of native forbs and grasses, and weeds. Weeds from previous plant communities reside within the seed bank, or emerge from perennial structures. Throughout the following summer it is recommended that the area is mowed to a height of 7.6 to 10.2 cm to control weed growth (Kurtz 2013). Successful renovation of existing sites requires permanent establishment of desired species without increasing the presence of invasive plants.

The objective of this research is to evaluate a simple method for establishing common milkweed and other forbs into existing sod by suppressing smooth brome without enhancing the invasion of these areas by weeds.

Materials and Methods

Field experiments were conducted from 2015 to 2017 at the Iowa State University Swine Farm and East Curtiss Farm near Ames, Iowa. The Swine Farm experiment was initiated during fall 2015 in a landscape with a Clarion loam soil and was dominated by smooth brome. The Swine Farm research plots received full sun. The experiment was repeated at the East Curtiss Farm in the fall of 2016 in a landscape with Clarion loam and Belview loam soils with a mix of smooth brome and Kentucky bluegrass (*Poa pratensis* L.). The Curtiss Farm research plots were adjacent to a row of trees on the east and a crop field along the west side. These plots received full sun during afternoon hours only. Both sites had low population densities of biennial and perennial broadleaf weeds, with the East Curtiss Farm site being more heavily infested than the Swine Farm. Neither site had been mowed for at least one year prior to initiating the experiments.

Common milkweed seed were collected during October from Story County, Iowa. A tetrazolium test indicated 62% viability. The other forbs were obtained from a local commercial producer of native plants. The experiment design was a factorial, split-split plot design with four replicates. The main plots were mowed versus unmowed, subplots were glyphosate application, and sub-subplots were seeding treatments. Mowing involved a single-mowing at a height of approximately 11 cm in August 2015. Subplots consisted of three glyphosate treatments (0.0, 0.25, or 0.50 kg ha^{-1} glyphosate plus 10 g $^{-1}$ ammonium sulfate) applied during mid-September. Glyphosate was applied with a 1.9 m boom while walking at 4.8 km h^{-1}. At application, weather conditions were 16° C, sunny, SSE 10 km h^{-1} winds, 79% humidity, and 17° C dew point. Each subplot contained four sub-subplots. Three of the sub-subplots were seeded with common milkweed (100 pure live seed (pls) m^{-2}, 100 pls m^{-2} + a single mid-June mowing, and 2,000 pls m^{-2}). Mid-June mowing was done using a handheld string trimmer at a height of approximately 10 to 15 cm. Clippings were removed from the sub-subplot after mowing. The fourth sub-subplot was scattered with a mix of three forb species, wild bergamot, golden alexanders, and New England aster, each at 150 pls m^{-2}. Main plots measured 9.1 m by 10.3 m, subplots were 3.0 m by 10.3 m, and sub-subplots were 1.2 m by 1.2 m. To ensure seed were scattered evenly within the sub-subplots, seed were mixed with wood shavings and spread by hand in early December. The same protocol was used at the East Curtiss location, with mowing, glyphosate application, and seeding for establishing the plots completed during 2016.

Vigor of the perennial sod was visually evaluated by determining approximate percentage of sod that was brown or green in color during the spring and summer of the first year. Census of common milkweed and forb establishment began in June during each year of the study. A 1 m^2 quadrat was placed in the center of each sub-subplot to count emerged forbs. Seedling counts were recorded once a week for each site and ended in August. At the end of August, sub-subplots were evaluated for the invasion of weeds. Vegetation samples were taken from the center of each of the common milkweed sub-subplots using a 30.5 cm^2 quadrat at soil level and separated into smooth brome, weeds, and common milkweed. Samples were put into paper bags and kept in an oven for one week at 60^o C and weighed.

Suppression of smooth brome data were analyzed using PROC MIXED model in Statistical Analysis Software (SAS). Common milkweed, golden alexanders, wild bergamot and New England aster seedling counts were analyzed with PROC MIXED model on a log + 1 scale due to the presence of many sub-subplots with counts of zero. Data were back-transformed for presentation.

Results and Discussion

In April 2016, eight months following applications, 0.25 and 0.50 kg ha^{-1} glyphosate provided approximately 50 and 70% suppression of smooth brome, respectively (Table 1). Glyphosate caused delayed development of smooth brome in the spring following application and resulted in chlorosis. Smooth brome recovered from glyphosate and no visible injury was evident by July (data not presented). Mowing prior to glyphosate application did not affect smooth brome, thus data were pooled across the mowing treatments (Table 1). There was no interaction between mowing and glyphosate in smooth brome suppression.

Glyphosate and seeding rate affected common milkweed recruitment, whereas mowing did not (Table 2). Suppression of smooth brome with glyphosate increased common milkweed seedling counts from less than one plant m^{-2} in plots without suppression to approximately eight plants m^{-2} at either rate of glyphosate in June 2016 at the low common milkweed seed density (Table 3). The high seeding rate increased June seedling counts by nearly ten-fold in glyphosate treated plots compared to the low seeding rate, but mid-June mowing had no effect. Similar treatment effects on common milkweed establishment were observed in August 2016 as in June 2016 (Tables 2 and 3). Weekly common milkweed seedling counts indicated there was continual turnover of seedlings within plots, thus common milkweed present in August were mid-summer emerging plants rather than plants that emerged at the start of the growing season (data not presented). Common milkweed seedlings reached heights of approximately 2.5 – 7.6 cm before succumbing to competition from the perennial sod.

Common milkweed were present during 2017; however, these plants were new seedlings rather than plants emerging from established rootstocks (Table 3). Suppressing smooth brome with glyphosate resulted in an increase in the presence of common milkweed at the end of Year 2 (2017) ($P = 0.0720$) (Table 2); however, as in August 2016, these plants were less than 7.6 cm in height and unlikely to successfully overwinter.

Mowing prior to glyphosate application did not influence common milkweed establishment at the East Curtiss Farm, but suppression of the perennial sod with glyphosate increased common milkweed establishment (Table 4). Similar to the Swine Farm experiment, there was no interaction between mowing and glyphosate, but the seeding treatment affected common milkweed establishment.

Smooth brome suppression increased common milkweed counts in the 100 pls m^{-2} treatments from approximately one plant m^{-2} in plots without suppression to approximately five plants m^{-2} at either rate of glyphosate in June 2017 (Table 5). The high seeding rate increased June seedling counts by almost twenty-fold in glyphosate treated plots compared to the low seeding rate, but mid-June mowing had no effect. Similar treatment effects on common milkweed counts were observed in August 2017 as in June 2017 (Tables 4 and 5). There was continual turnover of seedlings within plots, just as at the Swine Farm experiment. Suppressing smooth brome with glyphosate resulted in an increase in the presence of common milkweed at the end of 2017 ($P = 0.0002$) (Table 4). Seedlings were less than 7.6 cm in height and unlikely to successfully overwinter.

For successful establishment of common milkweed, it is imperative to have a suitable ecological niche available. Jarchow and Liebman (2011) stated that establishing forbs is difficult because during establishment, most energy is put towards producing roots rather than above ground shoots. The lack of photosynthetic tissue results in many small seedlings being outcompeted by surrounding vegetation (Jarchow & Liebman 2011). Mowing smooth brome during the summer prior to sowing or in early summer following seeding emergence did not increase establishment of common milkweed at either location.

Mowing prior to glyphosate application increased golden alexanders and wild bergamot establishment at the Swine Farm during 2016; however, mowing was not significant for any of the three forbs species during 2017 (Table 7). Suppression of the perennial sod with glyphosate increased wild bergamot and golden alexanders recruitment during both years, whereas New England aster was not affected by glyphosate. There was a significant interaction between mowing and glyphosate for golden alexanders and wild bergamot in 2016, but not in 2017.

A combination of mowing and glyphosate resulted in a more than two fold increase in golden alexanders in 2016 than glyphosate alone and numbers were similar at the two glyphosate rates (Table 7). Wild bergamot averaged approximately one plant m^{-2} at both glyphosate rates in mowed plots, but was only present at the high glyphosate rate in unmowed plots. The interaction between mowing and glyphosate might be due to mowing reducing the amount of residue, therefore increasing seed soil contact. Another explanation could be that mowing increased the activity of glyphosate due to the presence of new growth on the smooth brome. However, visual rating of injury to the smooth brome did not detect a mowing effect (Table 1). At the end of 2017, glyphosate was significant for both wild bergamot and golden alexanders (Table 6). Unlike common milkweed, many of the wild bergamot and golden alexanders flowered, suggesting sufficient development to allow permanent establishment.

Mowing prior to glyphosate application did not influence establishment of the three forb species at the end of 2017 at the East Curtiss Farm (Table 8). Suppression of perennial sod with glyphosate increased establishment of golden alexanders and wild bergamot, but not New England aster. Golden alexanders and wild bergamot seedling counts increased from less than one plant m^{-2} in plots without sod suppression to approximately four plants m^{-2} for golden alexanders and wild bergamot when sod was suppressed. New England aster counts were not different than control seeding plots (Table 9). Many of the golden alexanders and wild bergamot grew to a height of up to 30.5 cm, suggesting sufficient resources to overwinter and remain in the landscape the following season.

Composition of the vegetation at the two sites was sampled in August, 2017 (Table 10). At the Swine Farm experiment, the biomass of perennial grasses, weeds, or common milkweed was not affected by any treatment. At the East Curtiss Farm experiment, glyphosate reduced sod biomass while increasing the presence of weeds. There are two possible reasons suggested for the different responses at the two locations. The Swine Farm experiment was sampled two years after establishment of the trial. In the first year, there was an increase in weeds such as giant foxtail (*Setaria faberi* Herm.), common lambsquarters (*Chenopodium album* L.), wild parsnip (*Pastinaca sativa* L.), and Canada thistle (*Cirsium arvense* L.). Smooth brome recovered in the second season and reduced the presence of the weedy species. At the East Curtiss Farm experiment, sod biomass was sampled the first year following suppression, so the sod did not have time to recover. In addition, the sod at East Curtiss Farm was a mix of smooth brome and Kentucky bluegrass (*Poa pratensis* L.). Kentucky bluegrass is much more sensitive to glyphosate than smooth brome, so the vigor of the sod at this site would have been reduced more than at the Swine Farm experiment.

Suppression of a perennial sod with sub-lethal rates of glyphosate increased recruitment of common milkweed seedlings, but there was a low probability of permanently establishing common milkweed (Tables 3 and 5). Numerous seedlings emerged throughout the growing season; however, as the surrounding vegetation's canopy increased, light and other resources were reduced and most seedlings succumbed to competition and died. Golden alexanders and wild bergamot appeared more successful at advancing from seedlings to mature plants than common milkweed (Tables 7 and 9). Smooth bromegrass is a vigorous perennial with creeping rootstocks and after about three years can form a dense, strong sod (Lamson-Scribner 1899). Peltzer and Köchy (2001) suggests that grasses are strong competitors for resources within the soil (i.e., water and nutrients) and for light. The intense competition from the perennial sod did not prevent the emergence of forb seedlings, but it did greatly reduce establishment. Higher rates of glyphosate should provide greater suppression of the sod, and therefore increase the success in establishing forbs. However, there would be a good likelihood that this would also increase the invasion of the area by weeds. Repeated mowing during the seeding year is a standard practice in new seedlings of forbs to reduce competition with annual weeds (Minnesota Board of Water and Soil Resources 2017), and could be beneficial when attempting to establish forbs within an established sod.

Table 1. Smooth brome suppression with mowing and glyphosate eight months following glyphosate application. Iowa State University Swine Farm, Ames, Iowa.[1, 2]

Glyphosate	Mowed	Unmowed
kg ha^{-1}	------------ Smooth brome suppression (%) -------------	
0	0.0 (0.0)	0.0 (0.0)
0.25	57.5 (10.3)	42.5 (4.8)
0.50	72.5 (6.3)	62.5 (2.5)

[1]Glyphosate P < 0.0001; Mowing: P = 0.0750; Glyphosate * mowing: P = 0.3874.
[2]Parentheses indicate standard error.

Table 2. Common milkweed establishment ANOVA analysis. Iowa State University Swine Farm, Ames, Iowa.[1]

Factor	June 2016	August 2016	June 2017	August 2017
	----------------------------- P – value -----------------------------			
Mow	0.1201	0.4943	0.5413	0.4872
Glyphosate	< 0.0001	< 0.0001	0.0003	0.0720
Mow*Gly[2]	0.0840	0.2221	0.3323	0.3827
Seeding	< 0.0001	< 0.0001	< 0.0001	0.0027
Gly[2]*Seeding	< 0.0001	< 0.0001	0.0095	0.1829

[1]Mow, glyphosate and seeding were implemented during 2015.
[2]Gly = glyphosate.

Table 3. Effect of glyphosate and seeding rate on establishment of common milkweed in smooth brome. Iowa State University Swine Farm, Ames, Iowa.[1, 2, 3]

	Glyphosate (kg ha^{-1})		
Seeding treatment[4, 5]	0	0.25	0.50
	----------------------- Stems m^{-2} -----------------------		
June 2016			
0 pls	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
100 pls	0.1 (0.1)	7.9 (3.2)	7.1 (2.1)
100 pls + mow	1.3 (0.3)	7.8 (2.5)	8.8 (2.2)
2,000 pls	2.4 (1.0)	62.3 (13.0)	67.3 (13.0)
August 2016			
0 pls	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
100 pls	0.0 (0.0)	1.3 (0.8)	1.5 (0.7)
100 pls + mow	0.0 (0.0)	1.4 (0.6)	3.4 (1.3)
2,000 pls	0.1 (0.4)	7.3 (2.9)	17.1 (8.5)
June 2017			
0 pls	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
100 pls	0.5 (0.5)	2.0 (0.9)	2.9 (0.9)
100 pls + mow	0.1 (0.1)	1.6 (0.7)	3.8 (1.4)
2,000 pls	0.1 (0.1)	11.8 (5.2)	17.6 (10.6)
August 2017			
0 pls	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
100 pls	0.1 (0.1)	0.3 (0.2)	0.1 (0.1)
100 pls + mow	0.0 (0.0)	0.6 (0.4)	0.5 (0.3)
2,000 pls	0.0 (0.0)	1.0 (0.4)	0.5 (0.2)

[1]Mowing, glyphosate and seeding were implemented during 2015. Data are pooled across mowing treatment due to lack of effect.
[2]Glyphosate was significant for June 2016 and August 2016 ($P < 0.0001$), and June 2017 ($P = 0.0003$), but not for August 2017; seeding treatment was significant throughout both years of the experiment ($P < 0.05$); glyphosate by seeding treatment interaction was significant for June 2016, August 2016 ($P < 0.0001$), and June 2017 ($P = 0.0095$), but not for August 2017.
[3]Parentheses indicate standard error.

Table 4. Common milkweed establishment ANOVA analysis. Iowa State University East Curtiss Farm, Ames, Iowa.[1]

Factor	June 2017	August 2017
	---------------------- P – value ----------------------	
Mow	0.6556	0.7915
Glyphosate	< 0.0001	0.0002
Mow*Gly[2]	0.9098	0.7644
Seeding	< 0.0001	< 0.0001
Gly[2]*Seeding	0.0006	< 0.0001

[1]Mow, glyphosate and seeding were implemented during 2016.
[2]Gly = glyphosate.

Table 5. Effect of glyphosate and seeding rate on establishment of common milkweed in smooth brome. Iowa State University East Curtiss Farm, Ames, Iowa.[1, 2, 3]

	Glyphosate rate (kg ha^{-1})		
Seeding treatment	0	0.25	0.50
	------------------------- Stems m^{-2} -------------------------		
June 2017			
0 pls	0.0	0.0	0.0
100 pls	0.9 (0.5)	4.6 (2.1)	5.8 (1.5)
100 pls + mow	1.3 (0.7)	5.3 (2.1)	7.5 (1.7)
2,000 pls	21.8 (17.0)	76.3 (18.1)	120.8 (30.3)
Aug 2017			
0 pls	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
100 pls	0.0 (0.0)	1.8 (0.8)	0.9 (0.4)
100 pls + mow	0.5 (0.5)	1.6 (0.7)	4.6 (2.3)
2,000 pls	1.3 (0.8)	29.3 (12.7)	74.3 (23.1)

[1]Mowing, glyphosate and seeding were implemented during 2016. Data pooled over mowing treatment due to lack of effect.
[2]Glyphosate was significant throughout Year 1 ($P < 0.05$); seeding treatment was significant throughout Year 1 ($P < 0.0001$); glyphosate by seeding treatment interaction was significant throughout Year 1 ($P < 0.05$).
[3]Parentheses indicate standard error.

Table 6. Forb establishment ANOVA analysis during August 2016 and 2017. Iowa State University Swine Farm, Ames, Iowa.[1]

Factor	2016			2017		
	Species[2]			Species[2]		
	WB	NEA	GA	WB	NEA	GA
	---------------------------- P – value ----------------------------					
Mowing	0.0192	0.1249	0.0192	0.3370	0.2185	0.2416
Glyphosate	< 0.0001	0.1060	< 0.0001	< 0.0001	0.2060	0.0091
Mow*Gly[3]	0.0210	0.1060	0.0210	0.3966	0.2060	0.1148

[1]Mowing, glyphosate and seeding were implemented during 2015.
[2]WB = wild bergamot; NEA = New England aster; GA = golden alexanders.
[3]Gly = glyphosate.

Table 7. Effect of mowing and glyphosate on forb establishment in smooth brome. Iowa State University Swine Farm, Ames, Iowa.[1]

Species[5]	Mowed[2, 3]			Unmowed[2, 3]		
	Glyphosate (kg ha^{-1})			Glyphosate (kg ha^{-1})		
	0	0.25	0.50	0	0.25	0.50
	Stems m^{-2}					
August 2016[4]						
control	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
g. alexanders	0.0 (0.0)	8.8 (1.4)	8.5 (1.2)	0.0 (0.0)	1.5 (0.6)	2.5 (1.6)
N.E. aster	0.0 (0.0)	0.0 (0.0)	0.1 (0.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
w. bergamot	0.0 (0.0)	8.8 (1.4)	8.5 (1.2)	0.0 (0.0)	1.5 (0.6)	2.5 (1.6)
August 2017						
control	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
g. alexanders	0.3 (0.3)	0.0 (0.0)	3.0 (1.2)	0.0 (0.0)	0.0 (0.0)	0.5 (0.5)
N.E. aster	0.0 (0.0)	0.0 (0.0)	1.0 (0.4)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
w. bergamot	0.0 (0.0)	1.0 (0.0)	1.3 (0.3)	0.0 (0.0)	1.0 (0.0)	1.0 (0.0)

[1]Mowing, glyphosate and seeding were implemented during 2015.
[2]Mowing treatment was significant during Year 1 for golden alexanders ($P = 0.0192$) and wild bergamot ($P = 0.0192$), but not for New England aster; glyphosate treatment was significant for golden alexanders and wild bergamot both years ($P < 0.05$), but not for New England aster in either year; mowing by glyphosate interaction was significant for all forb species during 2016 only ($P < 0.05$).
[3]Parentheses indicate standard error.
[4]Abbreviations: g. alexanders = golden alexanders; N.E. aster = New England aster; w. bergamot = wild bergamot.

Table 8. Forb establishment ANOVA analysis. Iowa State University East Curtiss Farm, Ames, Iowa.[1]

Factor	2017		
	Species[2]		
	WB	NEA	GA
	------------------------- P – value -------------------------		
Mowing	0.8266	0.1571	0.4056
Glyphosate	0.0051	0.1450	0.0003
Mow*Gly[3]	0.9547	0.1450	0.3006

[1]Mowing, glyphosate and seeding were implemented during 2016.
[2]WB = wild bergamot; NEA = New England aster; GA = golden alexanders.
[3]Gly = glyphosate.

Table 9. Effect of glyphosate on establishment of three forbs in smooth brome, August 2017. Iowa State University East Curtiss Farm, Ames, Iowa.[1]

Species[2]	Glyphosate rate (kg ha^{-1})[3, 4]		
	0	0.25	0.50
	------------------------------ Stems m^{-2} ------------------------------		
control	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
g. alexanders	0.3 (0.3)	3.6 (1.2)	4.1 (1.0)
N.E. aster	0.6 (0.5)	0.0 (0.0)	0.0 (0.0)
w. bergamot	0.0 (0.0)	2.5 (1.1)	4.3 (1.5)

[1]Mowing, glyphosate and seeding were implemented during 2016. Data are pooled over mowing treatments due to lack of effect.
[2]Abbreviations: g. alexanders = golden alexanders; N.E. aster = New England aster; w. bergamot = wild bergamot.
[3]Glyphosate was significant for golden alexanders (P = 0.0003) and wild bergamot (P = 0.0019), but was not significant for New England aster.
[4]Parentheses indicate standard error.

Table 10. ANOVA analysis of vegetation biomass composition at Swine Farm and East Curtiss Farm establishment trials. August, 2017. Ames, Iowa.[1]

Factor	Sod	Weeds	Milkweed
Swine Farm			
Glyphosate	0.5552	0.1418	0.1231
Mow	0.9234	0.8529	0.4316
Mow*Gly[2]	0.5531	0.9715	0.6744
East Curtiss Farm			
Glyphosate	0.0006	0.0016	0.2954
Mow	0.2785	0.1027	0.3180
Mow*Gly[2]	0.3114	0.2234	0.6405

[1]Mowing and glyphosate treatments implemented during 2015 growing season at the Swine Farm, and the 2016 growing season at 2016. Biomass harvested at end of second growing season after initiation of experiment at Swine Farm and after first season at East Curtiss Farm.

[2]Gly = glyphosate.

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CHAPTER 5. GENERAL SUMMARY

Based on the findings from Chapter 3, we concluded that application of fomesafen did not reduce end-of-season aboveground biomass or utilization of common milkweed by ovipositing monarchs. Furthermore, in greenhouse experiments common milkweed growth resumed growth within two weeks of postemergence application of fomesafen, glyfosinate, imazethapyr, and mesotrione, herbicides commonly used in corn and soybean. Future efforts should investigate the fitness of monarch larvae when reared on herbicide-treated plants.

We concluded, based on the results from Chapter 4, that mowing smooth brome prior to a late-season application of sub-lethal doses of glyphosate did not increase establishment of common milkweed, golden alexanders, New England aster, or wild bergamot. Our results indicate that suppression of a perennial sod with glyphosate can increase recruitment of milkweed and forb seedlings, but there is a low probability of permanently establishing the forbs. More intense disturbance may increase milkweed and forb establishment, but is also likely to increase invasion of the area by weedy species. Suppression of a perennial sod with glyphosate benefits establishment of milkweed and forb seedlings, but plots will need to be followed in the future to determine if plants become a permanent component of the vegetation. The experiments described in Chapter 4 provide information on the importance of landscape management to ensure successful establishment of forbs for pollinator species.