2021

Effects of Dicamba on monarch oviposition and larval growth and development

Sahar Saghi
Iowa State University

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Effects of Dicamba on monarch oviposition and larval growth and development

by

Sahar Saghi

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTERS OF SCIENCE

Major: Sustainable Agriculture

Program of Study Committee:
Robert G. Hartzler, Major Professor
Richard L. Hellmich
Mary H. Wiedenhoeft

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, Iowa
2021

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>iv</td>
</tr>
<tr>
<td>CHAPTER 1. GENERAL INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Thesis Organization</td>
<td>2</td>
</tr>
<tr>
<td>References</td>
<td>3</td>
</tr>
<tr>
<td>CHAPTER 2. LITERATURE REVIEW</td>
<td>5</td>
</tr>
<tr>
<td>Introduction</td>
<td>5</td>
</tr>
<tr>
<td>Monarch biology</td>
<td>7</td>
</tr>
<tr>
<td>Factors contributing to monarch population decline</td>
<td>10</td>
</tr>
<tr>
<td>Dicamba, milkweed, and monarchs</td>
<td>12</td>
</tr>
<tr>
<td>Direct herbicidal effects</td>
<td>13</td>
</tr>
<tr>
<td>Indirect herbicidal effects</td>
<td>14</td>
</tr>
<tr>
<td>Summary</td>
<td>15</td>
</tr>
<tr>
<td>References</td>
<td>16</td>
</tr>
<tr>
<td>CHAPTER 3. DICAMBA EFFECT ON MONARCH OVIPOSITION PREFERENCE AND</td>
<td>22</td>
</tr>
<tr>
<td>LARVAL DEVELOPMENT</td>
<td>22</td>
</tr>
<tr>
<td>Abstract</td>
<td>22</td>
</tr>
<tr>
<td>Introduction</td>
<td>22</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>24</td>
</tr>
<tr>
<td>Preparation of common milkweed plants</td>
<td>24</td>
</tr>
<tr>
<td>Oviposition studies</td>
<td>26</td>
</tr>
<tr>
<td>Feeding studies</td>
<td>26</td>
</tr>
<tr>
<td>Results and Discussion</td>
<td>28</td>
</tr>
<tr>
<td>References</td>
<td>35</td>
</tr>
<tr>
<td>CHAPTER 4. GENERAL CONCLUSIONS</td>
<td>38</td>
</tr>
</tbody>
</table>
ACKNOWLEDGMENTS

I would like to thank my committee chair, Bob Hartzler, and my committee members, Richard Hellmich and Marry Wiedenhoeft, for their guidance and support throughout the course of this research.

In addition, I would also like to thank my husband, Hamed Babaei, my friends and 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

Eastern monarch butterflies (Danaus plexippus L.) are iconic creatures that migrate thousands of kilometers every year to overwinter in Mexico’s Oyamel fir forest. The eastern monarch butterfly population has decreased as much as 84% between 1997 and 2015 (Thogmartin et al., 2017), and it comprises a large proportion of the global monarch population (Brower, 2014). Many factors contribute to this decline, including habitat destruction, lack of host plants, agricultural practices, climate change, and infection by the parasite Ophryocystis elektroscirrha (OE). Herbicide use in crop fields across monarch butterflies breeding range may impact host plants' availability and quality within and adjacent to fields. The rise in herbicide-resistant weeds has resulted in changes in herbicide use patterns. One of these changes is an increase in dicamba use following the introduction of dicamba-resistant soybean and cotton varieties. The increase in dicamba use has raised concerns because of its ability to injure plants outside of the treated field. Thus, experiments were conducted during 2019 and 2020 to determine if dicamba injury on common milkweed (Asclepias syriaca) affected monarch (Danaus plexippus) oviposition preference and larval development. Common milkweed plants in a no-till soybean field were sprayed with 5 g a.e. ha⁻¹ dicamba or left unsprayed as untreated controls. Common milkweed leaves that emerged within two weeks after dicamba application were malformed, with leaf cupping typical of dicamba injury. Plants were examined for monarch eggs twice a week for 11 weeks. In 2019, 35% fewer eggs were found on dicamba-treated plants than on control plants, but no effect on oviposition was observed in 2020. Caterpillars were fed leaves harvested 27 days after dicamba application. Dicamba treatment did not affect monarch larval weight, pupa weight, adult forewing length, or sex ratio. Larvae fed leaves near the apical
bud had greater weight gain than larvae fed older leaves lower on stems, likely due to the higher nutritional quality of young leaves compared to older leaves.
CHAPTER 1. GENERAL INTRODUCTION

Eastern monarch butterflies (*Danaus plexippus* L.) are iconic creatures that migrate thousands of kilometers every year to overwinter in Mexico's Oyamel fir forest. Another population lives west of the Rocky Mountains and is much smaller in size, approximately 2.3 million adults from 1990 to 2000 (Leong et al., 2004). The western population migrates to southern California during winter. A non-migratory population is found in Florida, and other populations reside in Hawaii and Australia, and other locations.

The eastern monarch butterfly population has decreased as much as 84% between 1997 and 2015 (Thogmartin et al., 2017), and it comprises most of the global monarch population (Brower, 2014). Many factors contribute to this decline, including habitat destruction, lack of host plants, agricultural practices, climate change, and infection by the parasite *Ophryocystis elektroscirrha* (OE) (Altizer and de Roode, 2015). The only host plants for oviposition and larval development are milkweeds in the Apocynaceae family; however, the milkweed populations in the Midwest have declined dramatically (Pleasants and Oberhauser, 2013). For example, common milkweed abundance (*Asclepias syriaca*) declined in Iowa by about 58% from 1999 to 2010, likely due to herbicide use related to increased planting of genetically engineered (GE) glyphosate-resistant corn and soybean (Hartzler, 2010). Insecticide use is another potential threat to monarch butterflies; exposure to neonicotinoid insecticides caused delayed development, smaller body size, elevated mortality rates, and behavioral changes (Godfray et al. 2014; Woodcock et al., 2016; Krishnan et al., 2020). The decline in the monarch population is complicated and is particularly concerning because of its remarkably high societal, cultural (Diffendorfer, 2014), and educational value (Young-Isebrand et al., 2015).
Approximately 90% of monarchs migrating to Mexico use common milkweed as their larval food source (Malcolm et al., 1993), and female monarchs lay more eggs on common milkweed in and near agricultural fields than milkweeds in non-agricultural areas (Oberhauser et al., 2001). Herbicide use in crop fields across monarch butterflies breeding range may impact the availability and quality of host plants within and adjacent to fields. The rise in herbicide-resistant weeds has resulted in changes in herbicide use patterns; one of these changes is increased dicamba use (Behrens et al., 2007; Heap 2019) following the introduction of dicamba-resistant soybean and cotton varieties (Jones et al., 2019). Dicamba is a synthetic auxin herbicide historically used to control broadleaf weeds in corn, small grains, turf, and other grass crops for over 50 years (Jones et al., 2019). The increase in dicamba use has raised concerns because of its propensity to injure plants outside of the treated field due to particle and vapor drift. Since dicamba herbicides' approval on genetically engineered (GE) cotton and soybean, there has been a large increase in off-target injury to susceptible plants (Jones et al., 2019). Thus, the Center for Biological Diversity proposed that dicamba is an escalating threat to monarch populations (Donley, 2018).

Investigating the effects of dicamba on the suitability of common milkweed for monarchs can help us evaluate the risk an increase in dicamba use poses to the monarch population.

**Thesis Organization**

Chapter 2 is a literature review on monarchs' biology and the reasons underlying monarch population decline. A focus is the potential impacts of the increased use of dicamba on milkweed resources on the monarch population. Chapter 3 describes the field and laboratory experiments that have been done to determine the effects of dicamba injured common milkweed plants on female monarch oviposition preference and larvae fitness and development. This chapter will be
submitted for publication in Crop Protection. Chapter 4 includes a summary of the studies and our conclusion based on the observations and results derived from the data. All chapters except chapter 4, follow with related references.

References


CHAPTER 2. LITERATURE REVIEW

Introduction

The eastern North American population of monarch butterflies is unique in its long-distance migration. Every fall, these butterflies migrate from their breeding range in the Midwest and Northeast United States and southern Canada to their overwintering sites in Mexico’s Oyamele forests. Other populations of North American monarchs are found in coastal areas of California and Florida. The eastern monarch population is the largest population of this butterfly (Brower, 2014).

The eastern population of monarch butterflies has decreased by more than 84% over the last decade (Thogmartin et al., 2017). Several factors have contributed to the monarch decline, including habitat destruction in Mexican overwintering sites, loss of host and nectar plants, climate change, and Ophryocystis elektroscirrha (OE) infection (Altizer and de Roode, 2015; Thogmartin et al., 2017). Plants of the Apocynaceae family are the only food source for the monarch caterpillars (Pleasants 2017), whereas adult monarchs use nectar from a variety of plants (Landis and Dumroese, 2015). Monarchs use a variety of milkweed species (Pocius et al., 2017), but cardenolide fingerprinting shows that the larvae of 90% of monarchs migrating to Mexico used common milkweed Asclepias syriaca as their food source (Malcolm et al., 1993). Hartzler (2010) reported that common milkweed infestations declined dramatically in corn and soybean fields from 1999 to 2009 (Hartzler, 2010). This is a concern since monarchs prefer to lay eggs on milkweed plants in agricultural fields compared to plants in non-agricultural areas (Oberhauser et al., 2001).
Although milkweeds are essential for monarch reproduction, common milkweed is considered a weed by many farmers and others due to its propensity to establish dense colonies via vegetative spread and wind-dispersed seed. Weed control is critical for crop production, and herbicides are the primary control strategy used by the majority of farmers in the United States. The increase in herbicide-resistant weeds has resulted in shifts in herbicides used, including the widespread adoption of genetically engineered (GE) herbicide-resistant crops in the past 20 years (Bohnenblust et al., 2013; Mortensen et al., 2012).

Dicamba is a synthetic auxin herbicide historically used to control broadleaf weeds in corn, small grains, turf, and other grass crops for over 50 years (Jones et al., 2019). The use of dicamba has increased with the introduction of dicamba-resistant soybean and cotton varieties. Although the new crops provide farmers additional herbicide options, dicamba can affect non-target plants due to particle and vapor drift (Bohnenblust et al., 2013). Dicamba is a volatile compound, and non-target plant injury increased after introducing dicamba-resistant cotton and soybean (Bradley, 2017; Gray, 2017).

Off-target herbicide movement can indirectly affect herbivorous species and pollinators by reducing plant quality and floral quantity (Bohnenblust et al., 2013; Bohnenblust et al., 2016). Synthetic auxin herbicides cause a range of injury symptoms, including twisting or epinasty of stems and cupping of leaves (Egan et al., 2014b). Similar symptoms have been observed on common milkweed sprayed with sub-lethal doses of dicamba (Hoey et al., 2016). Herbicide injury to milkweed could change monarch butterfly egg-laying behavior and also change the feeding behavior of monarch larvae. Dicamba drift levels also could alter the nutritional value of susceptible plants because of the changes in plant development (Grossmann, 2010).
Monarch biology

A neonate or first instar caterpillar hatches from the egg about four days after the egg is laid then goes through second, third, fourth, fifth instars, and pupation to make the chrysalis. The time it takes for each instar is temperature-dependent; 1-3 days for first, second, third, and fourth instars, and 3-5 days for fifth instars. After approximately ten days as a chrysalis, it emerges as an adult monarch butterfly (Oberhauser and Solensky, 2004). The lifespan of adult monarchs is two to five weeks for non-migratory generations and up to nine months for the generation that migrates to Central Mexico Oyamel fir forests to overwinter (Oberhauser and Solensky, 2004). These forests act as an umbrella to moderate temperatures, allowing monarchs to survive while maintaining their diapause (Zalucki et al., 2015). The nine-month diapause helps preserve lipid energy reserves throughout the winter (Brower et al., 2011). The overwintering monarchs return to their breeding range during March or April, and upon arrival, the females lay eggs on milkweeds and soon afterward die.

The overwintering generation produces the first short-lived generation of monarchs in northern Mexico and the southern United States. The migration of subsequent generations continues as far as southern Canada. A female monarch butterfly can deposit between 300-400 eggs during her lifetime (Oberhauser and Solensky, 2004). Female monarch butterflies fly over landscapes in search of milkweed plants to lay their eggs. Monarchs find milkweed plants using chemical and visual cues (Floater and Zalucki, 2000; Jactel et al., 2011). After they land on the host plant, they use sensory organs on their feet to determine the plant's suitability (Dixon et al., 1978). They usually lay a single egg on the underside of a leaf and then search for other host plants to lay the remaining eggs.

Milkweeds contain toxins called cardenolides that serve as a defense mechanism against herbivores. The compounds within monarchs reduce predation since most organisms are either
killed or sickened by the cardenolides (Agrawal, 2017). Specific physical and chemical attributes of host plants determine the oviposition preference of monarchs. Quercetin glycosides and cardenolides are essential factors in determining whether a milkweed plant is a suitable host for monarch larvae (Agrawal, 2017). Two quercetin glycosides, C$_{21}$ H$_{20}$ O$_{12}$ and C$_{26}$ H$_{28}$ O$_{16}$, stimulate monarch oviposition. Cardenolides are steroids with highly variable structures and have evolved in over twelve plant families but are most common in the Apocynaceae family, including milkweed and dogbane (Agrawal, 2017). Cardenolides exist in all parts of plants, from roots to flowers, with the highest concentrations in the plant latex (Agrawal et al., 2012). These cardenolides are toxic to most organisms, but monarchs have a high tolerance to them (Agrawal, 2017). Female monarchs sense these compounds by sensilla on their legs and antennae (Agrawal, 2017).

Monarchs can sequester over 0.25 mg of cardenolides to protect themselves against carnivorous enemies (Agrawal, 2017). The concentration of cardenolides in adult monarchs seems to have a logarithmic relationship to cardenolide concentration in plants (Nelson et al., 1990). Ingesting milkweed leaves with high cardenolide levels may protect the monarch from enemies through sequestration. Yet, it comes at a cost to monarchs manifested as lower quality flight phenotypes: rounder, thinner wings with lower wing loading values (Decker, 2019).

Female monarchs are very selective in finding plants for egg placement (Dethier, 1959; Rothschild and Schoonhoven, 1977). Host plant quality can affect insect size, fecundity, and reproductive strategy (Awmack and Leather, 2002). Zalucki et al. (1990) reported that female monarchs lay eggs on plants with intermediate cardenolide levels, between 2 and 4 mg per g of dry leaf mass. The number of eggs per plant increases with plant height but decreases with plant age since females prefer young plants or plants with fresh regrowth of leaves (Zalucki and
Kitching, 1982). Schoonhoven et al. (2005) concluded that young leaves are more nutritious, but at the same time, often contain higher levels of toxic secondary metabolites than mature leaves. Thus, higher amounts of quercetin glycosides in younger leaves (Agrawal, 2017), along with higher nutritional value, can be reasons for higher oviposition preference for these leaves over older ones. A recent study reported that burning the Oklahoma prairie in summer leads to the growth of milkweed resprouts that may be beneficial for monarch oviposition later in the summer (Agrawal, 2017). Practices that result in new growth on milkweed plants, such as mowing during summer, increases utilization by female monarchs (Knight et al., 2019).

Lavoile and Oberhauser (2004) evaluated the effects of nitrogen fertilization on common milkweed on monarch butterfly growth and development. High nitrogen fertilization resulted in increased leaf nitrogen and plant height, but also higher disease and pest levels on plants. Monarchs feeding on plants with low nitrogen showed higher relative consumption rates than larvae feeding on high fertility plants. They observed those monarch larvae compensate for lower plant quality by consuming more; increased consumption is costly because of increased exposure to natural enemies, or increased expenditure on consuming and processing low nitrogen host plants may result in decreased fitness.

Monarchs seem to prefer some milkweed plants for oviposition over others within a landscape. Cardenolide plant levels (Zalucki et al., 1990), milkweed patch size (Zalucki and Suzuki, 1987), surrounding vegetation (Floater and Zalucki, 2000; Jactel et al., 2011), plant age (Zalucki and Kitching, 1982), and predation risk are some factors believed to influence egg-laying preference (Pleasants, 2015). More suitable microclimate and lower predation risk may lead to higher egg population densities on common milkweeds in agricultural fields (Pleasants, 2015). Common milkweeds in agricultural fields are surrounded by monoculture vegetation,
increasing the chance milkweeds in this habitat will be found by a monarch butterfly (Floater and Zalucki, 2000; Jactel et al., 2001). Common milkweeds in agricultural fields exposed to non-lethal herbicides could produce a flush of new growth that is more favorable to monarchs (Zalucki and Kitching, 1982).

**Factors contributing to monarch population decline**

Habitat loss in Mexico and North America is one of the important factors in monarch decline. Logging in Mexico and industrialized agriculture and urbanization have reduced habitat suitable for both overwintering and breeding (MacDonald et al., 2013). Monarch overwintering habitats in the Michoacan State have lost more than 94 ha due to climate change and 38 ha due to illegal logging from 2012 to 2018 (Flores-Martinez, 2019). Loss of milkweed, the monarch's only host plant, and nectar plants in the Upper Midwestern United States especially pose risks to monarch butterflies (Pleasants, 2013). Milkweed numbers in agricultural fields have decreased significantly in the last two decades (Hartzler, 2010). Pleasants and Oberhauser (2013) observed that female monarchs lay 3.9 times more eggs on common milkweed plants in crop fields than on common milkweed in non-agricultural areas. In Iowa, for example, 75% of the land is involved in agricultural production, and thus the majority of milkweed plants occur in crop fields (Hartzler, 2010). Thus, weed management strategies can impact monarchs indirectly via their impact on the presence of milkweed in agricultural landscapes (Oberhauser et al., 2001). With the introduction of herbicide-resistant crops, herbicide use patterns have changed significantly. A high percentage of crops such as soybeans, corn, and cotton are tolerant to glyphosate (Benbrook, 2016). Concurrently, many weeds have evolved resistance to glyphosate, therefore increasing the use of alternative herbicides (Behrens et al., 2007).

*Ophryocystis elektroscirrha* (OE) is a protozoan parasite that reduces monarch survival, mass, flight speed, and endurance (Bartel et al., 2011; Altizer and Oberhauser, 1999; Bradley and
Altizer, 2005). An infected female monarch can transfer spores of the parasite onto the egg and the host plant (de Roode et al., 2009). Protozoa transfer can occur from adult to egg, or the parasite can be ingested during larval feeding, but the infection is not transferred from larva to larva (Leong et al. 1997). Death during the pupal stage, failure to fully emerge from the chrysalis, or severe wing deformations can occur with infection (Altizer and de Roode, 2015). Monarchs infected by OE and reared on milkweeds with high cardenolide concentrations have less parasitic load and longer lifespan compared to infected monarchs reared on milkweeds with low cardenolide concentrations (De Roode et al., 2008; Sternberg et al., 2012; Gowler et al., 2015; Tao et al., 2015). Monarchs also are at risk of predation by spiders, ants, green lacewing larvae (*Chrysoperla rufilabris* Bermeister), wasps, and parasitism by some species of flies and wasps (Hermann et al., 2019; Oberhauser et al., 2015).

Climate change, including extreme weather events, is another factor involved in monarch population decline (Thogmartin et al., 2017). It has been observed that monarchs are shifting their niche north during the breeding season, and this leads to longer migration times and an increased risk of mortality (Lemoine, 2015). Climate change also may make the overwintering habitat unsuitable (Oberhauser and Peterson, 2003), and extreme weather events like winter storms have caused high mortality in the overwintering population (García-Serrano et al., 2004). Northern Mexico and Texas had the worst drought in recorded history in 2010 and 2011 (Brower et al., 2015); monarchs captured in these areas during 2011 had significantly lower lipid levels than those captured during 1982 and 1994 (Brower et al., 2015). Lipid reservoirs are essential for monarchs to have the energy to complete their migration and survive the winter.
**Dicamba, milkweed, and monarchs**

Auxin, indole-3-acetic acid (IAA), is a key phytohormone that controls plant growth and development (Grossmann, 2010). Auxin has been referred to as a 'master hormone' because of its interactions with other hormones (Grossmann 2010, Ross et al. 2002). Vascular tissue and floral meristem differentiation, leaf initiation, senescence, phyllotaxy, and root formation are all developmental processes controlled by auxin. In the 1940s, synthetic auxin hormones were produced for use as plant growth regulators and as herbicides to manage weeds (Grossmann, 2010). These herbicides simulate auxin by binding to auxin receptors, but are more stable in plants. When used at high concentrations the herbicides can destroy natural regulation of growth processes and cause damage and death of plants (Grossmann, 2010). These systemic herbicides are used primarily to control dicot weeds in grass crops. Several chemical families have been discovered that mimic auxin, including phenoxyacetic acids, benzoic acids, pyridinecarboxylic acids, aromatic carboxymethyl derivatives and quinolinecarboxylic acids. The effectiveness of these compounds depends upon concentration within plants, tissue sensitivity, physiological stage, and plant species (Grossmann, 2010).

Dicamba herbicide was first described in 1958 and registered for use in 1962 (Hartzler, 2017). For more than 50 years, it has been used for the control of broadleaf weeds in corn, small grains, and pastures (Jones et al., 2019), and recently its use has increased with the introduction of dicamba-resistant crops. Dicamba is a volatile compound, meaning it can evaporate from surfaces and move into the atmosphere following application (Behrens and Lueschen, 1979). It is capable of causing abnormal plant responses at fractions of the herbicide use rate (Bhatti et al., 1996; Everitt and Keeling, 2009; Solomon and Bradley, 2014). Dicamba spray drift, movement of spray droplets by wind currents, is another source of off-target movement and injury to non-
target plants (Egan and Mortensen, 2012). In 2017, when dicamba was approved for postemergence use in dicamba-resistant crops, damage to susceptible crops was reported on about 1.3 million acres of farmland (Bradley, 2017). Although the new formulations of dicamba used on dicamba-resistant crops are claimed to be less volatile, research has shown that concentrations of vapors capable of causing plant injury may leave treated areas (Jones et al., 2019; Norsworthy et al., 2017).

In the past 30 years, weeds have evolved resistance to herbicides more quickly than new herbicides have been discovered (Heap 2019). In response to this, companies have genetically engineered crops for resistance to existing herbicides. The epitome of this approach was glyphosate-resistant crops, including soybean, corn, cotton, canola, and others, introduced in the late 1990s (Green, 2009). More recently, several crops have been modified for resistance to the auxinic herbicides 2,4-D and dicamba. The development of 2,4-D- (Enlist®) and dicamba-resistant crops (Xtend®) has expanded the use of these herbicides in quantity, time, and space (Donley, 2018). Prior to the development of dicamba-resistant crops, dicamba was primarily used early in the growing season, typically before mid-May in Iowa. However, with the introduction of dicamba-resistant crops, dicamba could be used from before planting and throughout June and later in the season (Donley, 2018). Monsanto estimated that dicamba use would increase by 25.1 million pounds per year, and The Center for Biological Diversity estimated dicamba use at 57.2 million pounds by 2019 (Donley, 2018).

Direct herbicidal effects

Dicamba may affect insects directly via oral or cuticular exposure. Freydier and Lundgren (2016) reported sublethal doses of dicamba (120 mg/µl) reduced lady beetle (Coleomegilla maculate) survival and body weight. The DMA commercial formulation of dicamba at 250 µl/ml also reduced the proportion of males in the lady beetle population.
However, dicamba did not affect two lepidopteran species exposed to sublethal rates of dicamba (0.0056 to 561 g/ha) (Bohnenblust et al., 2013). In another study, researchers found that 2,4-D was toxic to larvae of spotted lady beetle (*Coleomegilla maculate* DeGeer) and convergent lady beetle (*Hippodamia convergens* Guérin-Meneville) when applied directly at concentrations corresponding to recommended field rates (1.17 L/ha). Topical application of 2,4-D at the same rate resulted in 25% and 60% mortality in the two species, respectively, and *C. maculata* developmental time increased by 5% (Michaud and Vargas, 2010). However, dicamba was not toxic to honey bees (Morton and Moffett 1972), and the mixture of dicamba and triasulfuron at 280 gm/ha did not affect two coccinellid species (Michaud and Vargas, 2010).

**Indirect herbicidal effects**

Exposure of non-target plants to dicamba can alter plant growth and consequently affect the food web. Bohnnenblust et al. (2016) found that sublethal doses of dicamba decreased or delayed flowering of some plant species, leading to reduced pollinator visitation. Hoey et al. (2016) observed that dicamba injury to common milkweed did not affect monarch oviposition preference. Egan et al. (2014a) observed that 1% of the field application rate (561 g ha) of dicamba decreased forb cover in plots and also reduced flowering of *Monarda fistulosa* L. The size of *Vanessa cardui* L. caterpillars and pupa developing on *Carduus nutans* L. damaged by concentrations of dicamba associated with drift was reduced compared to those feeding on healthy plants (Bohnenblust et al., 2013). It is likely that smaller caterpillars and pupa would result in smaller adults, which may negatively affect mate competition and fecundity (Boggs and Freeman 2005; Russell and Schultz 2010). The nitrogen content of *Carduus* thistle declined with increasing dicamba rates (Bohnenblust et al., 2013). Nitrogen is required for protein production, and host plants with higher nitrogen levels have been shown to increase development rates of some species of Lepidoptera (Slansky and Feeny, 1977; Tabashnik, 1982; Taylor, 1984), and
increase larval survival (Myers and Post, 1981; Myers, 1985; Clancy, 1992). Oyeyele and Zalucki (1990) observed that oviposition in monarch butterflies was not affected by *Asclepias fruticosa*, L. nitrogen concentration, and females preferred plants with average cardenolide levels of 0.2-0.3 mg/0.1 g dry weight.

Herbicides can affect nutrient concentrations and hormone pathways used in defense, which may influence plant susceptibility to herbivores (Bohnenblust et al., 2013; Grossmann et al., 2004). Therefore, when low doses of herbicides affect plant community structure and function, they also can affect insects because of the reduced availability and suitability of host plants (Egan et al., 2014). Food deprivation of monarch larvae may result in adult monarchs with stunted wings. This could ultimately lead to lower migration success (Johnson et al., 2014) since migratory monarchs tend to have larger, more elongated wings than non-migratory monarchs (Altizer and Davis, 2010). Monarch migration success depends highly on their flight ability and especially the wing attributes (Johnson et al., 2014).

**Summary**

The decrease in milkweed numbers and changes in herbicide use patterns are threats to the monarch population. Some herbicides, such as dicamba, are particularly concerning because of their ability to induce responses in non-target plants at very low doses. Herbicide injury to non-target plants may affect insects if it changes the quality and quantity of host plants. Determining the impact of dicamba damage on milkweed plants to monarch oviposition and monarch larvae development is critical and helps us to understand factors underlying their decline. The following chapter focuses on the effects of sub-lethal dicamba doses on monarch egg-laying preference and monarch larval development.
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CHAPTER 3. DICAMBA EFFECT ON MONARCH OVIPOSITION PREFERENCE AND LARVAL DEVELOPMENT

Sahar Saghi, Robert G. Hartzler

Iowa State University, Department of Agronomy, Ames, IA, 50011, USA

Modified from a manuscript to be submitted to Crop Protection

Abstract

Experiments were conducted during 2019 and 2020 to determine if dicamba injury on common milkweed (Asclepias syriaca L.) affected monarch (Danaus plexippus L.) oviposition preference and larval development. Common milkweed plants in a no-till soybean field were sprayed with 5 g a.e. ha⁻¹ dicamba or left unsprayed as untreated controls. Common milkweed leaves that emerged within two weeks after dicamba application were malformed, with leaf cupping typical of dicamba injury. Plants were examined for monarch eggs twice a week for 11 weeks. In 2019 35% fewer eggs were found on dicamba-treated plants than on control plants, but no effect on oviposition was observed in 2020. Caterpillars were fed leaves harvested 27 days after dicamba application. Dicamba treatment did not affect monarch larval weight, pupa weight, adult forewing length, or sex ratio. Larvae fed leaves near the apical bud had greater weight gain than larvae fed older leaves lower on stems, likely due to the higher nutritional quality of young leaves compared to older leaves.

Introduction

The monarch butterfly (Danaus plexippus L.) population in the eastern United States, the largest monarch population in the world (Brower et al., 2014), has decreased by more than 84% since 1997 (Thogmartin et al., 2017). The only host plants for female oviposition and larval development are milkweeds in the Apocynaceae family (Thogmartin et al., 2017). One proposed cause of the decline in monarchs is a decrease in common milkweed since the adoption of
glyphosate-resistant crops and the resulting use of glyphosate herbicide (Pleasants and Oberhauser, 2013). Oberhauser et al. (2001) reported that common milkweed growing in agricultural fields are utilized more than common milkweed in non-agricultural settings; therefore, suggesting common milkweed infesting 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. They found 3.9 times more eggs on common milkweed in crop fields than on common milkweed in other areas of the landscape.

Due to the increase in herbicide-resistant weeds, companies have developed crops that are resistant to synthetic auxin herbicides, 2,4-D and dicamba (Behrens et al., 2007). Historically these herbicides have been used to control broadleaf weeds in grass crops (Jones et al., 2019). The development of 2,4-D- and dicamba-resistant crops have expanded the use of these herbicides in quantity, time, and space, and The Center for Biological Diversity estimated dicamba use at 57.2 million pounds per year by 2019 (Donley, 2018).

Dicamba is prone to vapor and particle drift (Behrens and Lueschen, 1979; Boerboom, 2004; Sciumbato et al., 2004; Auch and Arnold, 1978), causing a range of injury symptoms, including twisting or epinasty of stems and cupping of the leaves (Egan et al., 2014b). Since the introduction of dicamba-resistant crops in 2017, there has been an increase in off-target injury to susceptible plants (Jones et al., 2019). Similar injury has been observed on common milkweed sprayed with sub-lethal doses of dicamba herbicide (Hoey et al., 2016).

Sub-lethal doses of dicamba (120 mg/µl) reduced lady beetle (Coleomegilla maculata) survival and body weight, and a commercial formulation of dicamba at 250 µl/ml reduced the proportion of males in the lady beetle population (Freydier and Lundgren, 2016). Sub-lethal
doses of dicamba negatively affected non-target plants (Bohnenblust et al., 2016; Bohnenblust et al., 2013; Egan et al., 2014a; Hoey et al., 2016; Behrens and Lueschen, 1979). For instance, 1% of the field application rate (561 g/ha) of dicamba reduced flowering of alfalfa (*Medicago sativa* L.) and *Eupatorium perfoliatum* L., and decreased pollinator visits (Bohnenblust et al., 2016).

Dicamba drift may change the nutritional value of susceptible plants because it disrupts growth processes and the vascular system (Grossmann, 2010), consequently changing the host plants’ availability and suitability (Egan et al., 2014a).

Since the landscape in Iowa and surrounding states is dominated by agricultural fields, the majority of milkweeds are near agricultural areas and may be exposed to dicamba drift. We hypothesize that concentrations of dicamba associated with drift will reduce the suitability of common milkweed for monarch oviposition and larval development.

**Materials and Methods**

**Preparation of common milkweed plants**

Plants used in these experiments were from a field historically maintained in corn and soybean production where common milkweed plants had been established in 2016 (Lizotte-Hall and Hartzler, 2018). The field was located at the Iowa State University Johnson Research Farm south of Ames, IA. In 2019, a combination of 1.1 kg ha\(^{-1}\) glyphosate, 28 g ha\(^{-1}\) flumioxain, and 36 g ha\(^{-1}\) pyroxasulfone was applied on May 30 before the emergence of milkweed plants to control emerged weeds and provide residual weed control. Soybean was planted in 76-cm rows using a no-till planter on June 23. Common milkweed had emerged from perennial rootstocks at the time of planting. Prior to the dicamba application, 40 common milkweed plants of similar height and vigor were selected for the studies (20 for dicamba exposure, 20 for an untreated
control). Using a CO₂-pressurized backpack sprayer, 5 g a.e. ha⁻¹ of dicamba¹ was applied in 187 l ha⁻¹ on June 26 when plants were 20 to 25 cm in height. Weather conditions were 29°C, 42% humidity, and 10.2 kmph wind at the time of application. The number of nodes present on stems was counted, and plant heights were measured after spraying. Three weeks after dicamba application, plants were rated for injury using a scale of 0 to 5 (0 = no effect; 1 = one node shows necrosis or cupping; 2 = two nodes show necrosis; 3 = three nodes show necrosis or cupping, 4 = four nodes show necrosis or cupping; 5 = five nodes or more show necrosis or cupping).

The experiment was repeated in 2020 using plants in the same field. A combination of 0.18 kg ha⁻¹ sulfentrazone, 0.18 kg ha⁻¹ flumioxazin, 1.12 kg ha⁻¹ glyphosate, and 0.56 kg ha⁻¹ 2,4-D was sprayed on April 27 to control emerged weeds and provide residual control. Soybean was planted on May 10. Forty-four pairs of common milkweeds in the 25 to 30 cm height range were chosen for the experiment, one plant per pair as a control and the other for dicamba treatment. Plants were treated with 5 g a.e. ha⁻¹ dicamba using a CO₂-pressurized backpack sprayer in 187 l ha⁻¹ on June 2. At the time of application, the conditions were 23°C, sunny, 70% relative humidity, and 13 km h⁻¹ wind. Plants were rated for injury three weeks after application as in 2019.

In 2020, thirty greenhouse-grown common milkweed plants were used for one feeding experiment. The plants were sprayed on April 4, with dicamba (control and 5 g a.e. ha⁻¹ dicamba) herbicide using a CO₂-pressurized backpack sprayer in 187 l ha⁻¹ with conditions of 17°C, 72% relative humidity, and 24 km h⁻¹ wind. Spraying was conducted in the field; both

¹ Engenia; N, N-Bis-(3-aminopropyl) methylamine salt; 600 g dicamba a.e.l⁻¹; BASF Corportion, Research Triangle Park, NC.
treated and control plants were taken to the field for this operation. All the plants were fertilized with 5 ml of 18-6-12 slow-release fertilizer 4 days after herbicide application.

Leaf samples from three plants at two positions within the canopy (middle and top) from each treatment for the first and second four-day trials were submitted to a commercial lab for nutritional analysis².

**Oviposition studies**

Plants in the field were used to evaluate the effect of dicamba injury on oviposition. The experiment was a completely randomized design with two treatments (control and 5 g a.e. ha⁻¹ dicamba) and 12 replications. Starting on June 26, 2019, the number of eggs on plants were recorded twice a week for 11 weeks. Eggs were removed after recording to eliminate plant stress from larval feeding. The experiment was repeated in 2020 in the same field with a similar protocol, eggs were counted from June 9 to August 25 (12 weeks).

The number of eggs and plant heights for each treatment were analyzed using a two-sample t-test (SAS Institute 9.4). Common milkweed injury ratings were analyzed using TTEST, and for correlation between injury ratings and the number of eggs per plant, a simple linear regression was used (SAS Institute 9.4).

**Feeding studies**

**Four-day study**

Feeding experiments were conducted to determine the effect of simulated dicamba drift on the suitability of common milkweed for monarch larvae. The experiments were initiated three weeks after dicamba application in 2019 and 2020. Leaves were collected randomly from three positions within the canopy (bottom, middle and top), placed in individual plastic bags, put in a

² Minnesota Valley Testing Laboratories, Inc. Nevada, IA.
cooler, and transferred to the lab. The top leaves emerged after the dicamba application, the middle leaves were expanding at the time of application, and the bottom leaves were fully expanded at the time of application. After harvest for each experimental unit, a single leaf was placed in a Petri dish (100 ×15 mm) containing 12 ml of 2% water agar, and one neonate was placed on the leaf. The weight of a subsample of the neonates was determined at the start of experiments in each feeding trial. There were six treatments (two dicamba treatments by three leaf positions). Petri dishes were placed in an incubator maintained at 26°C, 65% relative humidity with a 16:8 h light: dark setting. At the end of four days, larval weight, developmental stage, and survival were determined. The amount of leaf area consumed was determined by scanning pictures of the leaf using ImageJ software. There were 32 and 20 replications for the first and second trials, respectively.

Life-cycle study

Life cycle experiments were initiated 23 days after the dicamba application. Leaves were harvested from milkweed plants using the same method as in the four-day feeding trial. Petri plates were prepared using the same method as above, and a single leaf was placed in each Petri plate. One neonate was placed in each plate using a small paintbrush sterilized with axin and deionized water before picking the neonates to prevent the spread of bacteria. The first life-cycle experiment used greenhouse-grown plants, while the second experiment used field-grown plants. Both experiments had 6 treatments and 15 replications. The ninety plates were placed in trays and maintained under the same conditions as the four-day study. Larvae were monitored daily, and leaves were provided ad libitum or if they desiccated. On day 10, larval weight was determined, then all larvae were fed non-dicamba treated common milkweed leaves until pupation. Pupa weight, adult wingspan, sex, and leaf area consumed by larvae were determined.
Larval weight from all feeding studies was analyzed using the TTEST and the effect of treatments on monarch larvae and pupa weight, adult forewing length and sex ratios were analyzed using the Proc GLM and Proc MIXED. Sex ratios in the dicamba treatment were compared with the control treatment using a one-sample t-test (Pilorget et al. 2010).

**Results and Discussion**

The simulated drift of dicamba on common milkweed resulted in low levels of injury typical of growth regulator herbicides (Table 1). Leaves present at the time of application did not exhibit symptoms but leaves emerging within two weeks after exposure were malformed. Typical symptoms were elongation of affected leaves with distorted venation; symptoms were limited to one to three leaf nodes. Later emerging leaves did not display visible symptoms, and dicamba did not affect plant height or date of flowering (data not presented).

The number of eggs laid on control plants was higher than on dicamba injured plants in 2019, but not in 2020 (Table 1, Figure 1). In 2019 dicamba was applied two days prior to first eggs being recorded, whereas in 2020 dicamba was applied 15 days before the appearance of eggs. No relationship was found between the visible appearance (injury ratings) of individual plants and the number of eggs laid (data not shown). The lack of correlation between egg laying and visible injury ratings could indicate the reduction in egg-laying on dicamba-treated plants in 2019 was due to factors such as leaf quality or quantity of cardenolides rather than visual appearance.

Researchers have reported that monarchs prefer to lay eggs on newly emerged leaves rather than older leaves (Zalucki and Kitching, 1982; Schoonhoven et al., 2005; Agrawal 2017), probably because young leaves are more nutritious and have less fiber and lignin. In our experiments, peak egg-laying occurred in mid-August in 2019 and the last week of July in 2020 (Figure 1). In most years in Iowa, the majority of dicamba is applied from mid-May through
mid-June. Common milkweed near crop fields affected by dicamba drift would have resumed normal growth during peak monarch egg-laying; thus, the upper leaves would not exhibit dicamba symptoms. However, common milkweed plants contacted by dicamba would produce abnormal foliage during the time frame when earlier generations of monarchs are active. The first generation of monarchs normally arrive in Iowa in May to early June, the second generation appears around mid-June to early July, and the final generation occurs in mid-July through August, after which monarchs migrate to their overwintering sites in Mexico (Lizzotte-Hall and Hartzler, 2019).

Neonate weight gain in the four-day study was affected by leaf position, but not by dicamba nor the interaction between the two factors (Table 3). Data from the two four-day experiments were pooled since there was no experiment effect. Larvae feeding on top leaves had the highest weight gain, whereas larvae feeding on lower leaves had the lowest weight gain. Survival rates were not affected by dicamba or leaf position (data not presented). Dicamba symptoms were expressed primarily on the middle leaves, but the weight gain of neonates feeding on these leaves was the same as on untreated plants. This indicates that low levels of dicamba injury did not affect the quality of common milkweed for larvae.

Leaf area consumed by larvae was determined using image analysis (Table 4). Dicamba did not affect consumption in either year, but in 2019 larvae consumed more leaf area when feeding on upper leaves. Dicamba did not affect the protein or fiber content of common milkweed leaves (Table 5). The data show that dicamba did not have any significant effects on the nutritional value of the leaves.

Larval weight was not affected by dicamba in the life-cycle experiments (Table 3). Leaf position affected weight gain in the second trial, but not the first. This may due to the use of
greenhouse-grown plants in Trial 1, which were not actively growing at the time of dicamba application and thus did not produce new leaves following application. The second trial used field-grown plants that were actively growing. Neither dicamba nor leaf position affected pupal weight, adult wingspan, and sex ratios in either trial (Table 6).

Dicamba treatment did not affect larval survival rates in either feeding study. The difference in survival rates between the first experiment (greenhouse-grown plants) and the second experiment (field-grown plants) of the life-cycle trials was likely due to the quality of the plants (data not presented). The survival rate for the first instar through adult was 18% with greenhouse-grown plants and 81% with field-grown plants; survival from neonate through day 4 was 89% for the first and 95% for the second four-day experiments. The typical survival rate for monarch butterflies to complete their full life cycle in laboratory settings is about 75% (Krishnan, 2020).

The dicamba rate used in these experiments was selected to mimic doses associated with particle drift (Bohnenblust et al., 2016) and caused minor injury on the leaves that emerged following application. Common milkweed plants started to show injury about two weeks after application, and typically, after three to four weeks, plants generated new growth, which did not show symptoms. Dicamba injury affected oviposition in the first year, but it did not affect it in the second year. Dicamba injury did not impact monarch larval development or adult morphology. The level of injury to common milkweed evaluated in these experiments would be limited to plants within a few m of areas sprayed with dicamba, thus representing a small percentage of the total common milkweed population. Thus, our results suggest that the common milkweed plants injured by drift levels of dicamba have minimal impact on monarch butterfly reproduction.
### Table 1. Common milkweed response to simulated drift of dicamba.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2019</th>
<th>2020</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 g ha(^{-1}) dicamba</td>
<td>2.3 (0.4)</td>
<td>1.0 (0.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2019</th>
<th>2020</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
</tbody>
</table>

1Parentheses indicate standard error.
2\(0\) = no effect; 1 = one node with necrosis or cupping; 2 = two nodes with necrosis or cupping; 3 = three nodes with necrosis or cupping, 4 = four nodes with necrosis or cupping; 5= five nodes with necrosis or cupping.

### Table 2. Effect of dicamba injury on oviposition of monarch butterflies on common milkweed. Iowa State University, Ames, Iowa.\(^1\)\(^2\)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2019</th>
<th>2020</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 g ha(^{-1}) dicamba</td>
<td>3.9 (0.4)*</td>
<td>10.0 (1.3)</td>
</tr>
<tr>
<td>Control</td>
<td>6.0 (0.8)</td>
<td>9.0 (1.2)</td>
</tr>
</tbody>
</table>

1Parentheses indicate standard error.
2*indicates dicamba different from control according to t-test.
Figure 1. Oviposition of monarch butterfly throughout the growing season, Ames, IA.
Table 3. Effect of dicamba injury and common milkweed leaf position on monarch neonate biomass. 1, 2, 3

<table>
<thead>
<tr>
<th>Factor</th>
<th>4-day 2</th>
<th>10-day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Greenhouse experiment</td>
</tr>
<tr>
<td>Dicamba</td>
<td></td>
<td>----------------------</td>
</tr>
<tr>
<td>Control</td>
<td>31.9 (1.6)</td>
<td>813.4 (0.1)</td>
</tr>
<tr>
<td>5 g ha⁻¹</td>
<td>35.9 (1.6)</td>
<td>894.2 (0.1)</td>
</tr>
<tr>
<td>Leaf position</td>
<td></td>
<td>----------------------</td>
</tr>
<tr>
<td>Top</td>
<td>44.5 (2.0)</td>
<td>819.9 (102.9)</td>
</tr>
<tr>
<td>Middle</td>
<td>33.5 (1.8)</td>
<td>830.0 (110.8)</td>
</tr>
<tr>
<td>Bottom</td>
<td>23.7 (1.9)</td>
<td>919.7 (102.9)</td>
</tr>
<tr>
<td>ANOVA</td>
<td></td>
<td>P-Value</td>
</tr>
<tr>
<td>Dicamba</td>
<td>0.2891</td>
<td>0.786</td>
</tr>
<tr>
<td>Leaf position</td>
<td>0.0018</td>
<td>0.761</td>
</tr>
<tr>
<td>D*LP</td>
<td>0.5356</td>
<td>0.661</td>
</tr>
</tbody>
</table>

1 Parentheses indicate standard error.  
2 Neonates allowed to feed for four days and then weighed. Data represent the means of two trials.  
3 Neonates allowed to feed for ten days on treated leaves, then transferred to untreated leaves to complete life cycle.
Table 4. Effect of common milkweed leaf position on monarch neonate consumption for four days.¹ ²

<table>
<thead>
<tr>
<th>Position</th>
<th>2019</th>
<th>2020</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top</td>
<td>3.0 (0.3)</td>
<td>6.2 (0.3)</td>
</tr>
<tr>
<td>Middle</td>
<td>2.5 (0.2)</td>
<td>4.8 (1.3)</td>
</tr>
<tr>
<td>Bottom</td>
<td>1.2 (0.2)</td>
<td>4.4 (0.2)</td>
</tr>
</tbody>
</table>

¹Parentheses indicate standard error.
²The effect of leaf position on diet was significant in the first experiment, but not for the second experiment.

Table 5. Effect of dicamba injury on protein and fiber content of common milkweed leaves.¹ ²

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaf Quality</th>
<th>Protein (%)</th>
<th>Fiber (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.8 (0.2)</td>
<td>3.0 (0.3)</td>
<td></td>
</tr>
<tr>
<td>5 g ha⁻¹ dicamba</td>
<td>4.1 (0.1)</td>
<td>2.9 (0.1)</td>
<td></td>
</tr>
</tbody>
</table>

¹Parentheses indicate standard error.
²Dicamba did not affect nutritional value of the leaves.

Table 6. Effect of dicamba injury to common milkweed on monarch pupal weight, adult forewing length, and sex ratio. Iowa State University, Ames, Iowa.¹ ²

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Life cycle trial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pupal weight (g)</td>
</tr>
<tr>
<td>Control</td>
<td>1.18 (0.02)</td>
</tr>
<tr>
<td>Dicamba</td>
<td>1.23 (0.03)</td>
</tr>
</tbody>
</table>

¹Data were pooled for the first run and second run of the life-cycle trials.
²Parentheses indicate standard error.
References


CHAPTER 4. GENERAL CONCLUSIONS

Due to the increase in herbicide resistance weeds and the introduction of herbicide-resistant crops, herbicide use patterns have changed considerably. One change is an increase in the use of dicamba in the breeding range of the monarch butterfly. This is concerning because dicamba can affect non-target plants at very low doses. Since the landscape in Iowa and surrounding states is dominated by agricultural fields, the majority of milkweeds are near agricultural areas and may be exposed to dicamba drift. We hypothesize that concentrations of dicamba associated with drift will reduce the suitability of common milkweed for monarch oviposition and larval development.

Thus, oviposition and feeding experiments were conducted during 2019 and 2020 to determine if dicamba injury on common milkweed (*Asclepias syriaca*) affected monarch (*Danaus plexippus* L.) oviposition preference and larval development. The dicamba rate used in these experiments was selected to mimic doses associated with particle drift (Bohenenblust et al., 2016) that would cause minor injury on the leaves that emerged following application. Common milkweed plants started to show injuries about two weeks after application, and typically after three to four weeks, plants generated new growth that did not show any injuries. Oviposition on plants damaged by dicamba was reduced by 35% in one year, but no differences in egg-laying on common milkweed were observed between treated and control plants a second year. Dicamba injury did not affect monarch larval development or adult morphology. Our results suggest that low levels of dicamba injury on common milkweed growing adjacent to treated fields do not have a large impact on the utilization and suitability of milkweed plants and has minimal impact on monarch butterfly reproduction.