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Challenges and Opportunities for Weed Control in Nebraska Popcorn

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CHALLENGES AND OPPORTUNITIES FOR WEED CONTROL IN NEBRASKA POPCORN

by

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CHALLENGES AND OPPORTUNITES FOR WEED CONTROL IN NEBRASKA POPCORN

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Weed control in popcorn is challenging with limited herbicide options and popcorn's perceived sensitivity to herbicides. Understanding the impact of weeds maximizes yield and profit. New herbicide-resistant crops increase chances of drift or misapplication into popcorn, which doesn't have herbicide-resistant traits. Herbicides that are labeled in popcorn are often only conditionally labeled with reduce rates, warnings, or limited popcorn types. Dent-sterility in popcorn is contingent on the Ga1 gene (Ga1-s), but this system is at risk from Ga1-m field corn introduced from Mexico because it overcomes dent-sterility. This risk is under-assessed as Ga1-m carriers are undocumented and Mexican germplasm usage is increasing for genetic diversity. Experiments conducted 2017-2019 are assessing weed control, herbicide sensitivity, and popcorn purity risk.

Chapter 1 outlines the history of popcorn in the United States, current production practices, agronomic challenges, herbicide use in popcorn, and a strategic plan for improving popcorn production. Chapter 2 determines the critical time for weed removal in popcorn produced with and without atrazine/S-metolachlor applied pre-emergence (PRE). Chapter 3 determines weed control options and crop injury potential of five herbicide programs on eight popcorn hybrids. Chapter 4 evaluates the efficacy and crop safety of labeled post-emergence (POST) herbicides for controlling velvetleaf that survived S-metolachlor/atrazine applied PRE in Nebraska popcorn and determines the

effect of velvetleaf growth stage on POST herbicide efficacy, popcorn injury, and yield. Chapter 5 examines the effects from drift or misapplication of herbicides to white and yellow popcorn. Chapter 6 models the cross-pollination of popcorn by field corn and investigates the factors influencing contamination and isolation distance.

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CHAPTER 1 BACKGROUND

1.1 INTRODUCTION

History. Popcorn (*Zea mays* L. var. everta) is a popular healthy snack food in the United States and is increasing popularity worldwide (Karababa 2006, Lago et al. 2013). Popcorn is a recommended snack to meet daily ChooseMyPlate.gov whole grain consumption guidelines (USDA ARS 2015; 2017). This is because popcorn contains high fiber, no cholesterol, and low fat and several vitamins, minerals, and polyphenols (USDA ARS 2016; 2019). Popcorn was first commercialized in the United States in the 1880's (Ziegler 2001). It gain popularity in the early 1900's and by 1912 commercial production had increased to 7,700 ha mostly in Iowa ((D'Croz-Mason and Waldren 1978; Eldredge and Thomas 1959; Ziegler 2001). The first commercial hybrid for the Northern U.S. Corn Belt was released in 1934 from the University of Minnesota and in 1940 from Indiana University and Kansas State University for the Central U.S. Corn Belt (Ziegler 2001). Popcorn sales increased globally from 160 million kg in 1970 to nearly 519 million kg in 2016 (Figure 1-1; Popcorn Board 2019). Popcorn consumption has historically been tied to movie theater attendance (Ziegler 2001). Home television viewing increased in popularity and movie attendance decreased in the late 1940's and early 1950's leading to the first time the popcorn industry experienced a significant decrease in sales (Popcorn Board 2019; Ziegler 2001). In response, the Popcorn Institute, Coca-Cola, and Morton Salt merged advertising efforts to convince consumers that salted popcorn and Coca-Cola had a place in the American home (Ziegler 2001). In the 1980's microwave popcorn was developed (Popcorn Board 2019; Ziegler 2001). Today, Americans consume 14 billion quarts of popped popcorn each year (Popcorn Board 2019).

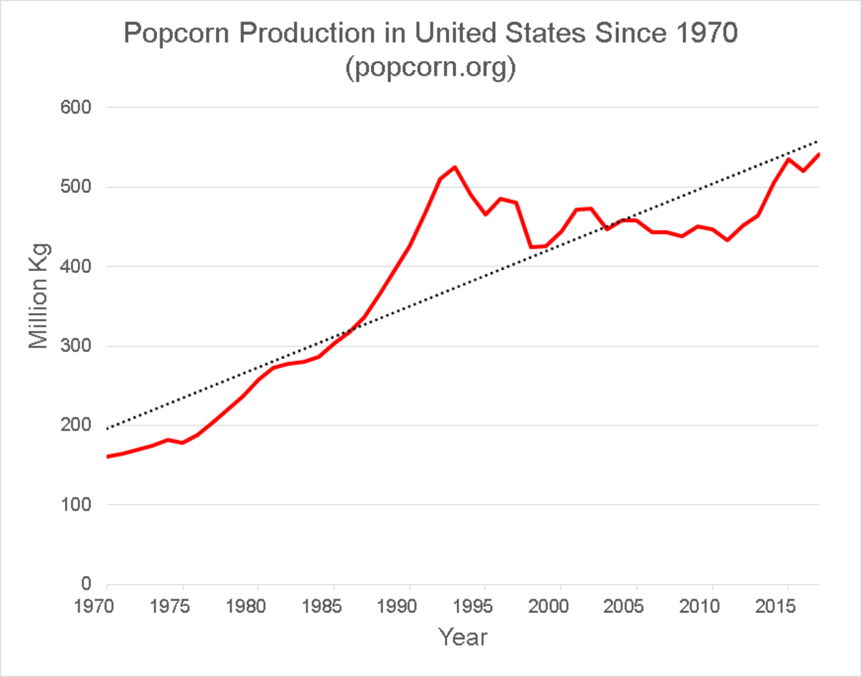

Figure 1-1. Popcorn production since 1970 in the United States. Data provided by the Popcorn Board available at popcorn.org.

Current Production. Popcorn is an important field crop to many producers in the Midwestern United States. Popcorn production is predominantly in Illinois, Indiana, Iowa, Kansas, Kentucky, Michigan, Nebraska, and Ohio (USDA NASS 2019). Additionally, aforementioned states are also among the eight largest dent corn [*Zea mays* (L.) var. indentata] producing states (USDA NASS 2019). Therefore, popcorn and dent corn are grown in proximity with usually similar planting and flowering time. The United States produced 486 million kg of popcorn in 2017 of which Nebraska is the leading state, producing 167 million kg annually or 34% of the total popcorn production in the United States on 25,949 ha (Figure 1-2; USDA NASS 2019). Nebraska has often produced more than any other state since 1970's because the high availability of irrigation (D'Croz-Mason and Waldren 1978). Popcorn is commonly grown in rotation with soybean [Glycine max (L.) Merr]/dent corn and produced under contracts with the private company/manufacturer which specify the hybrids and area to be planted (D'Croz-

Mason and Waldren 1978; Ziegler 2001). Producers tend to financially benefit from the contract production of popcorn on their farms primarily in recent years where corn, soybean, and wheat (*Triticum aestivum* L.) price is low (Edleman 2004; 2006).

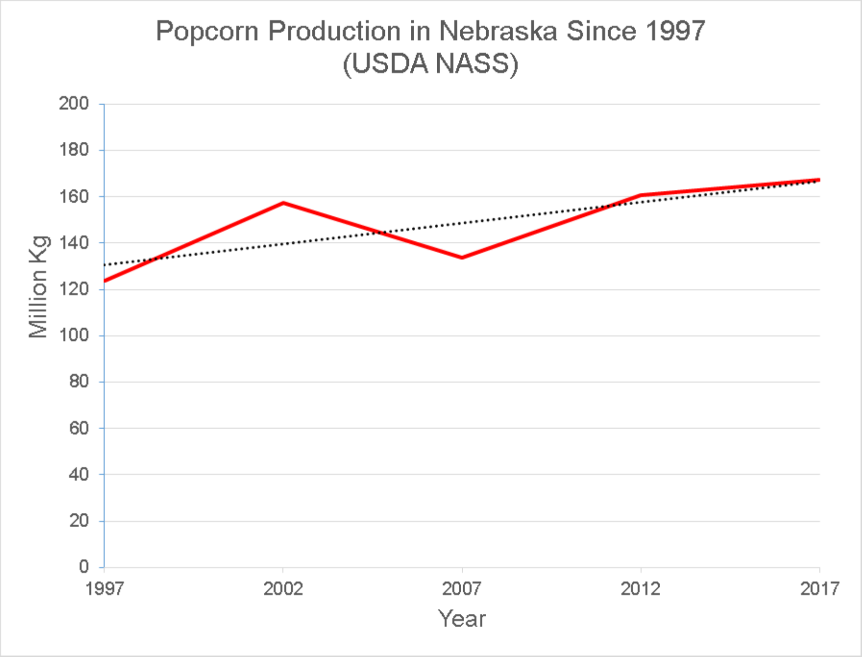

Figure 1-2. Popcorn production since 1997 in Nebraska. Data provided by USDA NASS

Agronomics. The majority of popcorn is produced under conservation tillage systems (Pike et al. 2002). Popcorn varies agronomically from field corn [*Zea mays* (L.) var. indentata] in several ways. Popcorn emerges slower, produces narrower and more upright leaves, and tends to have shorter and thinner stalks compared to field corn (*Zea mays* L. var. indentata) (Ziegler 2001). Popcorn has a larger tassel that produces more pollen than dent corn (Ziegler 2001). Popcorn is prolific and generally produces two ears per plant but has smaller ear shoots than dent corn (Ziegler 2001). For these reasons, growers depend on herbicides for weed control in popcorn production but herbicide options in popcorn are limited compared to field corn (Pike et al. 2002; Ziegler 2001). Popcorn is generally more susceptible than field corn to yield loss due to weed competition and herbicide injury. Additionally, white popcorn is said to be more sensitive to herbicides than yellow popcorn (Loux et al. 2017). Despite the general perception that white popcorn hybrids are less tolerant to herbicides, evidence has not been published.

Herbicides in Popcorn: While 92% of dent corn planted in the United States is genetically-modified (GM), there is no GM popcorn commercially available in the market place; therefore, popcorn is not resistant to commonly used POST herbicides such as glyphosate or glufosinate used in much of the field corn grown in the United States (Fernandez-Cornejo et al. 2014; Ziegler 2001). Herbicide-resistant field corn represents 80% of the field corn in the United States and 84% of Nebraska field corn in 2018 (USDA NASS 2019). Many herbicides labeled for field corn are not labeled for popcorn, such as premixes of isoxaflutole plus thiencarbazone (Corvus; Bayer CropScience 2016), and tembotrione plus thiencarbazone (Capreno; Bayer CropScience 2012. Additionally, certain pre-mixtures such as acetochlor plus clopyralid plus mesotrione (Resicore; Corteva Agriscience 2017) and atrazine plus bicyclopyrone plus mesotrione plus *S*-metolachlor (Acuron; Syngenta Crop Protection 2017) are labeled for PRE and POST application for field corn, but are not labeled for POST application for popcorn. Lack of inclusion could be due to sensitivity of popcorn or lack of research and interest by the herbicide industry to register these herbicides in a minor crop. Some herbicides that are labeled for field corn, sweet corn (*Zea mays* L. var. saccharata), and yellow popcorn specifically exclude white popcorn from the label. Herbicide labels often have statements indicating the selectivity of the herbicide on popcorn is unknown and to test in a small area or contact supplier or University specialist to verify sensitivity.

Dent Sterility. Popcorn relies on *gametophyte factor 1* (*ga1*) for protection against pollen mediated gene flow from dent corn (Kermicle et al. 2006; Ziegler 2001). The *ga1* system is the only utilized genetic system for preventing gene flow from dent/sweet corn to popcorn (Jones and Goodman 2016). The *Ga1-m* allele is cross neutral which can pollinate and accept pollen from any *gametophyte factor 1* allele including *Ga1-s* (Jones and Goodman 2016, Jimenez and Nelson 1993). *Ga1-m* is not prevalent in the United States but is being unintentionally introduced from Mexican and Central American germplasm to increase the genetic base of dent corn (Jones et al. 2015, Jones and Goodman 2016). Popcorn relies on *Ga1-s* to maintain genetic purity; however, the introduction of *Ga1-m* puts the popcorn production system at risk from dent corn cross-pollination and genetic impurity that can affect popcorn export market (Jones et al. 2015, Jones and Goodman 2016). Pollen-mediated gene flow (PMGF) is the largest potential biological source of on farm mixing of genetic material in corn (Palaudelmàs et al. 2012). Isolation distances of 200 m and 300 m are typically recommended to maintain 99% and 99.5% purity standards in dent corn (Ingram 2000; Luna et al. 2001; National Research Council 2000). Complete isolation of dent corn fields planted at the same time require 1,600 m (Bech 2003). Due to presence of dent-sterility in popcorn, a physical isolation from dent corn is not required to prevent cross-pollination (Ziegler 2001). However, dent corn with the *Ga1-m* allele can cross-pollinate with popcorn and genetic contamination of popcorn breeding or production programs from *Ga1-m* dent corn would have significant impacts.

Strategic Plan. Given the aforementioned production dynamics and current and future potential challenges with popcorn production, a pest management strategic plan was generated following a workshop by key players of the popcorn industry, including popcorn growers, agronomists, university faculty and agricultural professionals, and popcorn breeders to communicate pest, pesticides, and pest management practices available in popcorn and to identify the challenges associated with pest management in popcorn (Pike et al. 2002). This strategic plan was based on the information gathered in the crop profile for popcorn (Bertalmio et al. 2003). The pest management strategic plan for popcorn prioritized regularity, research, and education priorities for enhancing popcorn production and production efficiency (Pike et al. 2002). The strategic plan outlined the importance of educating custom applicators about the lack of herbicide-resistant traits in popcorn compared to field corn and the necessity of checking herbicide labels for special instructions or reduced rates in regard to popcorn when herbicides are labeled for both crops. The top regulatory priority outlined in the plan was to identify and expand the number of herbicides with distinct sites of action for weed control and crop safety. Another regulatory priority in the strategic plan is the critical issue of hybrid sensitivity to new herbicides as they are being registered for use in corn/soybean with the potential of drift or carryover injury to popcorn. The top research priority outlined in the strategic plan is to determine hybrid sensitivity to herbicides.

1.2 OBJECTIVES

Objective 1. The critical time of weed removal (CTWR) in field corn has been well studied but is not available for popcorn in North America. Understanding the CPWC is important for developing an integrated weed management plan and to decide timing of a POST herbicide application (Knezevic and Datta 2015). The CTWR can be delayed by the application of a PRE herbicide, allowing for a longer application window for a POST herbicide (Knezevic et al. 2013). The objective of this study was to determine the CTWR in popcorn produced with and without atrazine/*S*-metolachlor applied PRE. We hypothesized that atrazine/*S*-metolachlor applied PRE would delay CTWR compared with no herbicide applied due to early season weed control.

Objective 2. Research has been conducted to determine the sensitivity of sweet corn to a number of herbicides labeled for field corn (Bollman et al. 2008; Meyer et al. 2010; Nordby et al. 2008; Williams and Pataky 2010; Williams et al. 2008); however, scientific literature does not exist regarding weed control and response of commercially-grown yellow and white popcorn hybrids to herbicides. The objectives of this research were to evaluate weed control and crop growth and yield response in Nebraska commercially available yellow and white popcorn hybrids treated with PRE- and POST-emergence herbicides labeled in yellow popcorn.

Objective 3. Scientific literature is not available about control of velvetleaf in popcorn with POST herbicides. The objectives of this research were (1) to evaluate the efficacy and crop safety of labeled POST herbicides for controlling velvetleaf that survived *S*-metolachlor/atrazine applied PRE in Nebraska popcorn and (2) to determine effect of velvetleaf growth stage on POST herbicide efficacy, popcorn injury, and yield.

Objective 4. Herbicides applied POST in corn/soybean such as glyphosate, 2,4-D choline, or dicamba may result in off target movement and popcorn injury. Misapplication, drift, and tank contamination risk of glyphosate, 2,4-D choline/glyphosate, or dicamba to popcorn fields has not been assessed. The objective of this research was to determine the effect of simulated drift rates of glyphosate, 2,4-D choline/glyphosate, or dicamba on the injury, above ground biomass, height reduction, and yield loss of yellow and white popcorn hybrids at two growth stages.

Objective 5. Scientific information is not available in the literature about PMGF from dent corn to popcorn. The objectives of this research were to model the frequency of PMGF from a homozygous *Ga1-m* dent corn to homozygous *Ga1-s* popcorn under field conditions and to evaluate the role of wild speed and direction.

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CHAPTER 2 PRE-EMERGENCE HERBICIDE DELAYS THE CRITICAL TIME OF WEED REMOVAL IN NEBRASKA POPCORN (*ZEA MAYS* VAR. EVERTA)

2.1 ABSTRACT

Understanding the critical time of weed removal (CTWR) is necessary for designing effective weed management programs in popcorn production that do not result in yield reduction. The objective of this study was to determine the CTWR in popcorn with and without a premix of atrazine and *S*-metolachlor applied PRE. A field experiment was conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE in 2017 and 2018. The experiment was laid out in a spilt-plot design with PRE herbicide as the main plot and weed removal timing as the subplot. Main plots included no herbicide or atrazine/*S*-metolachlor applied PRE. Subplot treatments included weed-free control, non-treated control, and weed removal timing at V3, V6, V9, V15, and R1 popcorn growth stages and then kept weed-free throughout the season. A four-parameter log-logistic function was fitted to popcorn yield loss (%) and growing degree days (GDD) separately to each main plot. Growing degree days when 5% yield loss was achieved was extracted from the model and compared between main plots. The CTWR was from the V4 to V5 popcorn growth stage in absence of PRE herbicide. With atrazine/*S*-metolachlor applied PRE, the CTWR was delayed until V10 to V15. It is concluded that weeds must be controlled before the V4 popcorn growth stage when no PRE herbicide is applied to avoid yield loss and PRE herbicide, such as atrazine/*S*-metolachlor in this study, can delay the CTWR until the V10 growth stage.

2.2 INTRODUCTION

Herbicide options in popcorn are limited compared to field corn (Pike 2002; Ziegler 2001). For example, thiencarbazone/isoxaflutole is labeled in field corn but not in popcorn (Anonymous 2016). This is because popcorn is more sensitive to a number of herbicides compared with field corn and seed corn. Atrazine and *S*-metolachlor are the most commonly used herbicides in popcorn due to their crop safety. For example, Bertalmio (2003) reported that atrazine (PRE and/or POST) was applied on 99% and *S*-metolachlor (PRE) on 11% of popcorn production fields in 1999 in the USA. Additionally, a premix of atrazine and *S*-metolachlor can provide broad spectrum weed control compared to either herbicide applied alone (Geier et al. 2009; Grichar et al. 2003; Steele et al. 2005). As of 2018, there is no herbicide-resistant popcorn hybrid commercially available; therefore, the use of POST herbicides, such as glyphosate, glufosinate, or 2,4-D choline used in herbicide-resistant field corn, are not options for weed control in popcorn (ISAAA's GM Approval Database 2018; Pike 2002). The use of a PRE herbicide is very important in popcorn production because of limited effective POST herbicides. Pre-emergence herbicides with multiple effective sites of action in corn production often results in reduced weed densities and weed biomass and leads to greater yields (Nurse et al. 2006; Schuster and Smeda 2007). Although PRE herbicides are important to corn production, they usually do not provide season-long control of certain weed species with wide emergence patterns such as common waterhemp [*Amaranthus tuberculatus* (Moq.) J. D. Sauer] and Palmer amaranth (*Amaranthus palmeri* S. Watson) unless followed by a POST herbicide (Chahal et al. 2018a, 2018b; Steckel et al. 2002; Nolte and Young 2002).

The critical period of weed control (CPWC) is the period during the life cycle of a crop when weeds must be controlled to avoid unacceptable yield losses (Zimdahl 1988). The CPWC consists of the critical time for weed removal (CTWR), which determines the earliest point in the life cycle of a crop that weeds must be removed to prevent unacceptable yield losses (Knezevic et al. 2002). Knowledge gained from understanding the CPWC aids in determining the need for and timing of weed control (Knezevic et al. 2002). The CTWR has been determined in cotton (*Gossypium* L. spp.) (Buken 2004), dry beans (*Phaseolus vulgaris* L.) (Burnside et al. 1998), peanut (*Arachis hypogaea* L.) (Everman et al. 2008), field corn (Hall et al. 1992), soybean [*Glycine max* (L.) Merr.] (Van Acker et al. 1993), spring canola (*Brassica napus* L.) (Martin et al. 2000), sunflower (*Helianthus annuus* L.) (Knezevic et al. 2013), and winter wheat (*Triticum aestivum*, cv. Mercia) (Welsh et al. 1999). The CTWR in popcorn has been reported in Turkey to be at crop emergence (VE) (Tursun et al. 2016). The CTWR in field corn has been reported in several studies in North America. In Ontario, Canada, Hall et al. (1992) reported the CTWR to vary from the 3-leaf stage (V3) to the 14-leaf stage (V14) in non-GMO field corn, whereas Halford et al. (2001) reported it to be at V6 in no-till field corn. In Nebraska, Evans et al. (2003a) reported the CTWR in field corn from V4 to V7 under ideal nitrogen fertilizer. Norsworthy and Oliveira (2004) found that the CTWR in field corn was variable between locations in South Carolina such as V1 to V2 at one location and V5 to V6 at another research site regardless of narrow- (48 cm) or wide-row spacings (97 cm). Although the CTWR in field corn has been well studied, this information is not available for popcorn in North America. Understanding the CPWC is a major requirement for developing an integrated weed management plan for a crop (Knezevic and Datta 2015). The CTWR can be delayed by the application of a PRE herbicide, allowing for a longer application window for a POST herbicide (Knezevic et al. 2013). The objective of this study was to determine the CTWR in popcorn produced with and without atrazine/*S*-metolachlor applied PRE. We hypothesized that atrazine/*S*-metolachlor applied PRE would delay CTWR compared with no herbicide applied due to early season weed control.

2.3 MATERIALS AND METHODS

Site Description. Field experiments were conducted at the University of Nebraska-Lincoln, South Central Agricultural Laboratory near Clay Center, NE (40.5752˚N, 98.1428˚W) in 2017 and 2018. The most common weed species at the experimental site were common lambsquarters (*Chenopodium album* L.), common waterhemp, Palmer amaranth, velvetleaf (*Abutilon theophrasti* Medik.), and foxtail species, consisting of green foxtail [*Setaria viridis* (L.) P. Beauv.] and yellow foxtail [*Setaria pumila* (Poir.) Roem. & Schult.], which have been grouped and will be referred to as foxtails. The soil texture at the experimental site was Crete silt loam (montmorillonitic, mesic, Pachic Argiustolls; 17% sand, 58% silt, and 25% clay) with a pH of 6.5, and 3% organic matter. The experimental site was disked with a tandem disk to a depth of 10 cm and fertilized with 202 kg ha^{-1} of nitrogen in the form of anhydrous ammonia (82-0-0) in early spring.

Treatments and Experimental Design. The study was arranged in a split-plot design with main plots arranged in a randomized complete block with four replications. The main plot treatments consisted of 1) atrazine/*S*-metolachlor (Bicep II Magnum; Syngenta Crop Protection, Greensboro, NC 27419) applied PRE at 2,470 g ai ha^{-1}; and 2) no PRE herbicide applied. Atrazine/*S*-metolachlor was selected to represent the PRE herbicide treatment because it is used on 61% of commercial popcorn production fields in the United States (Bertalmio et al. 2003). Sub-plot treatments consisted of a non-treated control, weed-free control, and five weed removal timings including V3 (weeds removed at the 3-leaf growth stage of popcorn), V6, V9, V15, and R1 (popcorn reproductive silking growth stage). Sub-plot dimensions were 9 m long by 3 m wide. A yellow popcorn hybrid (VYP315; Conagra Brands 222 W. Merchandise Mart Plaza Chicago, IL 60654) was planted on April 27, 2017 and April 30, 2018 in rows spaced 76 cm apart at 4 cm depth at planting density of 89,000 seeds ha^{-1}. Starter fertilizer was applied as ammonium polyphosphate (APP; 10-34-0) in-furrow at 6 kg ha^{-1} of nitrogen plus K_2O during planting. Atrazine/*S*-metolachlor was applied on April 27, 2017 and May 2, 2018 using a handheld CO_2-pressurized backpack sprayer equipped with four AIXR 110015 flat-fan nozzles (TeeJet® Technologies, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60187) spaced 60 cm apart and calibrated to deliver 140 L ha^{-1} at 276 kPa at a constant speed of 4.8 km h^{-1}. Popcorn emergence was observed on May 18, 2017 and May 14, 2018. Observable weed emergence in the plots without PRE herbicide was noted on May 13, 2017 and May 14, 2018. Weeds were removed by hand or with a hoe from the entire plot area after weed removal timings and kept weed-free by hand weeding until harvest.

Data Collection. Temperature and rainfall data for 2017 and 2018 growing seasons were obtained from the nearest High Plains Regional Climate Center. Temperatures were converted to Celsius growing degree days (GDD_c) using the equation (Gilmore and Rodgers 1958):

$$GDD_c = \sum[\{T_{max} + T_{min}/2\} - T_{base}] \qquad [1]$$

where T_{max} and T_{min} are the daily maximum and minimum air temperatures (°C), respectively, and T_{base} is the base temperature (10 °C; Gilmore and Rodgers 1958).

During each removal timing, a randomly placed 1-m^2 quadrat was used to collect weed species composition information from each plot including density, height, and biomass. Weed biomass of each species was measured by clipping weeds in the quadrat at the soil surface, placing them into paper bags, and drying them at 65° C for 10 d to constant mass and weighing the samples. Popcorn vegetative area index (VAI) was measured indirectly using a LAI-2200C Plant Canopy Analyzer (LI-COR Biosciences, 4647 Superior Street, Lincoln, NE 68504) after the R1 removal timing from every treatment excluding the non-treated control. Eight LAI-2200C readings in each plot were taken from the center two rows using the 45˚ sensor view cap in two diagonal transects as described in the manual (LI-COR Biosciences 2016).

Popcorn grain yield components, including plant number, ear number, 100-seed weight, and total seed weight, were measured from 1 m of row subsample from the center two rows. Popcorn seed was dried at 65° C for 10 d prior to weighing and measuring 100-seed weights. Three random ears from the 1 m subsample were selected and kernel number was counted. Popcorn yield was harvested from the center two rows of 9 m using a plot combine on October 23, 2017 and September 28, 2018. Popcorn seed yield was corrected to 14% moisture. Yield loss was calculated as:

$$YL = 100 \times (1 - P/C) \qquad [2]$$

where YL is the yield loss relative to the weed-free control, P is the treatment plot yield, and C is the yield of the weed-free control plot.

Statistical Analysis. Statistical analysis was performed in R (R Core Team 2018) utilizing the base packages and the *drc: Analysis of Dose-Response Curves* package (Ritz et al. 2015). Data were subjected to ANOVA to test for significance of fixed effects (treatments) and random effects (replications nested in years). Data were analyzed using the four-parameter log-logistic model (Knezevic et al. 2007).

$$Y = c + (d - c)/\{1 + \exp[b(\log x - \log e)]\} \qquad [3]$$

where Y is the dependent variable [yield (kg ha^{-1}), plants m^{-1} row, ears plant^{-1}, seeds ear^{-1}, 100 seed weight (g), or yield loss (YL)], c is the lower limit, d is the upper limit, x is time expressed in GDD$_c$ that correspond with weed removal timings and controls (weed-free control, V3, V6, V9, V15, R1, and non-treated control), e is the ED$_{50}$ (GDD$_c$ where 50% response between lower and upper limit occurs; inflection point), and b is the slope of the line at the inflection point. The CTWR in this study was determined based on 5% YL. Yield loss data were regressed using a four-parameter log-logistic model (equation 3) where x is VAI of the weeded plots at the R1 stage to determine how well VAI described popcorn YL.

Root mean square error (RMSE) and modeling efficiency (ME) were calculated to evaluate goodness of fit for popcorn yield, popcorn YL, and VAI models (Barnes et al. 2018; Roman et al. 2000; Sarangi and Jhala 2018). The RMSE was calculated using:

$$\mathrm{RMSE} = [1/n \textstyle\sum_{\mathrm{i}=1}^{n} (Pi - Oi)^2]^{1/2} \qquad [4]$$

where Pi and Oi are the predicted and observed values, respectively, and n is the total number of comparisons. The smaller the RMSE, the closer the model predicted values are to the observed values. The ME was calculated using (Barnes et al. 2017; Mayer and Butler 1993):

$$\mathrm{ME} = 1 - [\textstyle\sum_{\mathrm{i}=1}^{\mathrm{n}} (Oi - Pi)^2 / \sum_{\mathrm{i}=1}^{n} (Oi - \bar{O}i)^2] \qquad [5]$$

where $\bar{O}i$ is the mean observed value and all other parameters are the same as equation 4. Modeling efficiency differs from R^2 only in not having a lower limit. ME values closest to 1 indicate the most accurate predictions (Sarangi et al. 2015).

2.4 RESULTS AND DISCUSSION

Temperature and Precipitation. Near average temperatures were observed during 2017 and 2018 growing seasons at the research site (Table 2-1). Monthly precipitation varied from the 30-yr average throughout the study but resulted in near average seasonal precipitation. Irrigation was applied with pivot irrigation as needed (Table 2-1).

Table 2-1. Average air temperature, total precipitation, and irrigation during 2017 and 2018 growing seasons and the 30 year average at the University of Nebraska–Lincoln South Central Agricultural Laboratory near Clay Center, NE

	Average temperature °C			Total precipitation (mm)			Total Irrigation (mm)	
Timing	2017	2018	30 yr average	2017	2018	30 yr average	2017	2018
April	11	6	10	77	27	62	0	0
May	16	20	16	201	74	135	0	0
June	24	25	22	41	145	101	65	28
July	26	24	24	51	134	109	118	75
August	21	23	23	92	113	96	38	39
September	20	20	19	61	137	60	0	0
Season	20	17	19	75	90	80	221	142

[a] Air temperature and precipitation data were obtained from the nearest High Plains Regional Climate Center

Weed Density and Species Composition, Biomass, and Height. Common lambsquarters, *Amaranthus* species, and velvetleaf were the dominant weed species at the research site. In absence of PRE herbicide, common lambsquarters averaged 78 plants m^{-2} accounting for 43% and 84 plants m^{-2} accounting for 37% of the species composition by density in 2017 and 2018, respectively (Table 2-2). Common waterhemp averaged 77 plants m^{-2} (43%) in 2017 and Palmer amaranth averaged 103 plants m^{-2} (45%) in 2018. To that point, Palmer amaranth in 2017 and common waterhemp in 2018 were not major contributors to total weed density. Velvetleaf averaged 21 (11%) and 16 plants m^{-2} (7%) in 2017 and 2018, respectively. Foxtails comprised of only 3 and 6% of the total weed density in 2017 and 2018, respectively.

When atrazine/*S*-metolachlor was applied PRE, the weed composition shifted to velvetleaf as the dominant species with 16 plants m^{-2} (60%) and 18 plants m^{-2} (45%) in 2017 and 2018, respectively. This was likely due to the lack of control atrazine/*S*-metolachlor provides for velvetleaf (Anonymous 2014; Taylor-Lovell and Wax 2001). Common waterhemp was reduced to 6 plants m^{-2} and 23% of the total density in 2017 and Palmer amaranth was reduced to 15 plants m^{-2} and 38% of the total density in 2018. The total weed density was reduced from 180 plants m^{-2} with no PRE herbicide to 26 plants m^{-2} with atrazine/*S*-metolachlor in 2017. Similarly, in 2018, total weed density was reduced from 228 plants m^{-2} with no PRE herbicide to 40 plants m^{-2} when atrazine/*S*-metolachlor was applied.

*Table 2-2. Weed composition and average density with and without atrazine/*S*-metolachlor applied PRE (2,470 g ai ha^{-1}) in a field experiment conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE in 2017 and 2018.*

PRE treatment	Weed species	2017	2018
		Weed density and (% of total)	
		plants m^{-2}	
no herbicide	velvetleaf	21 (11%)	16 (7%)
	common lambsquarter	78 (43%)	84 (37%)
	common waterhemp	77 (43%)	11 (5%)
	Palmer amaranth	0 (0%)	103 (45%)
	foxtails	5 (3%)	14 (6%)
	Total	180 (±20)	228 (±7)
atrazine/*S*-metolachlor	velvetleaf	16 (60%)	18 (45%)
	common lambsquarter	4 (15%)	1 (1%)
	common waterhemp	6 (23%)	1 (3%)
	Palmer amaranth	0 (0%)	15 (38%)
	foxtails	1 (3%)	5 (13%)
	Total	26 (±3)	40 (±2)

Atrazine/*S*-metolachlor applied PRE reduced weed biomass at the R1 popcorn growth stage from 1,034 g m^{-2} with no herbicide to 738 g m^{-2} in 2017 and from 867 g m^{-2} with no PRE to 195 g m^{-2} in 2018 (Table 2-3). Biomass of common lambsquarters and common waterhemp in 2017, and common lambsquarters, Palmer amaranth, and foxtails in 2018 were reduced by atrazine/*S*-metolachlor. Velvetleaf contributed more to total weed biomass when atrazine/*S*-metolachlor was applied because of limited control in 2017.

*Table 2-3. Total weed biomass, individual species contribution to total weed biomass, and individual species height at the R1 popcorn growth stage with and without atrazine/*S*-metolachlor applied PRE (2,470 g ai ha^{-1}) in a field experiment conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE in 2017 and 2018.*

			velvetleaf		common lambsquarters		common waterhemp		Palmer amaranth		foxtails	
Year	PRE treatment	Total weed biomass	biomass	height	biomass	height	biomass	height	biomass	height	biomass	height
		g m^{-2}	%	cm	%	cm	%	cm	%	cm	%	cm
2017	no herbicide	1,034 (±231)	20	99	27	152	54	152	0	---	0	---
	atrazine/ *S*-metolachlor	734 (±83)	84	175	1	91	15	163	0	---	0	---
2018	no herbicide	867 (±119)	2	47	32	97	0	---	49	130	17	76
	atrazine/ *S*-metolachlor	195 (±42)	3	76	12	91	0	---	85	105	0	---

Velvetleaf was taller in the plots where atrazine/*S*-metolachlor was applied in both 2017 and 2018 in the R1 removal timing (Table 2-3). Furthermore, velvetleaf was taller in 2017 than in 2018. On average, weeds in 2017 were taller than in 2018 except for common lambsquarters where atrazine/*S*-metolachlor was applied (91 cm) as well as foxtails that were only present in 2018 in plots without PRE herbicide. Overall, there was greater weed densities in 2018 but greater weed biomass and height in 2017 (Table 2-2, 2-3). Weed density and biomass response to atrazine/*S*-metolachlor applied PRE was similar to those reported in the literature (Steele et al. 2005; Swanton et al. 2007; Taylor-Lovell and Wax 2001; Whaley et al. 2009).

Popcorn Yield. Popcorn yields varied between years, so data were analyzed separately for each year. Popcorn yield in weed-free treatments was greater in 2017 (7,045 kg ha^{-1}) than 2018 (6,497 kg ha^{-1}) (Figure 2-1; Table 2-4). Popcorn yield in non-treated control plots without PRE herbicide were 384 and 1,036 kg ha^{-1} in 2017 and 2018, respectively, compared with 1,677 and 4,069 kg ha^{-1} with atrazine/*S*-metolachlor applied PRE in 2017 and 2018, respectively. This was because the herbicide was effective in controlling the majority of weeds, except velvetleaf, and thus reduced weed interference (Table 2-2; Table 2-3). Whaley et al. (2009) reported variable field corn yield (3,870 to 9,080 kg ha^{-1}) with atrazine/*S*-metolachlor applied PRE without a POST herbicide in a three-yr study in Virginia. Yield reduction was attributed to reduction in crop yield components (Adigun et al. 2014; Eaton et al. 1976; Elezovic et al. 2012; Trezzi et al. 2015).

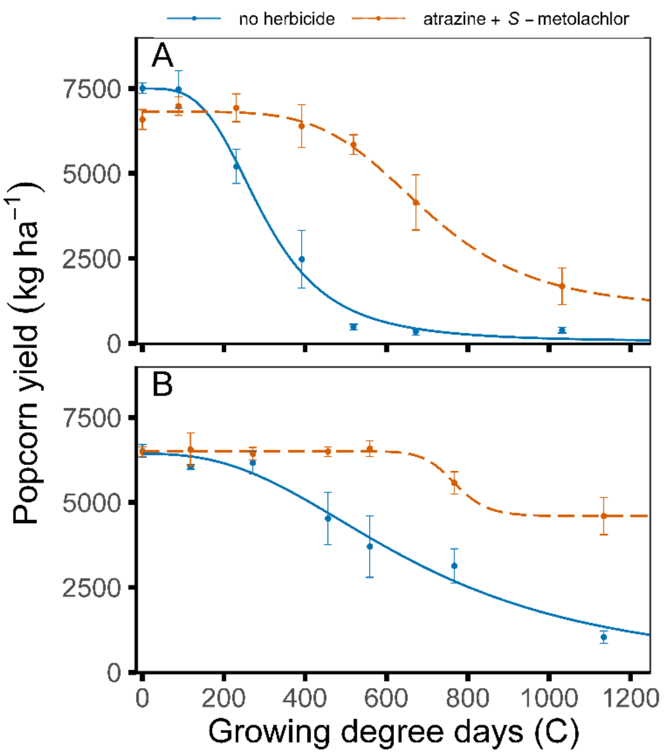

*Figure 2-1. Popcorn (*Zea mays *var.* everta*) yield (kg ha^{-1}) in response to increasing duration of weed interference as represented by growing degree days (GDD after emergence, C) in popcorn with and without atrazine/*S*-metolachlor applied PRE (2,470 g ai ha^{-1}) in (A) 2017 and (B) 2018 in a field experiment conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE. Regression lines represent the fit of a four-parameter log-logistic model.*

Table 2-4. Parameter estimates (b, c, d, and e) and standard errors of the four-parameter log-logistic model, root mean square error, and modeling efficiency for popcorn (Zea mays var. everta) yield with and without atrazine/S-metolachlor applied PRE (2,470 g ai ha^{-1}) in a field experiment conducted at the University of Nebraska–Lincoln South Central Agricultural Laboratory near Clay Center, NE in 2017 and 2018.[a]

Year	PRE treatment	*b* (±se)	*c* (±se)	*d* (±se)	*e* (±se)	RMSE	ME
			kg ha^{-1}	kg ha^{-1}	GDD		
2017	no herbicide	3.6 (0.7)	44.2 (379.3)	7497.3 (349.9)	297.4 (22.2)	753.6	0.97
	atrazine/ *S*-metolachlor	5.1 (2.8)	988.4 (1434.9)	6813.3 (278.1)	697.1 (101.5)	879.3	0.95
2018	no herbicide	2.4 (0.6)	0 (0)	6458.5 (388.6)	683.3 (67.6)	967.3	0.85
	atrazine/ *S*-metolachlor	19.4 (57.2)	4594.3 (312.3)	6509.1 (139.8)	769.0 (30.1)	575.7	0.94

[a] Abbreviations: *b*, slope; *c*, lower limit; *d*, upper limit; *e*, ED_{50}; RMSE, root mean square error; ME, modeling efficiency.

Popcorn Yield Components. There was an impact of weed removal timing on popcorn yield components including plants m^{-1} row, ears $plant^{-1}$, seeds ear^{-1}, and 100-seed weight (Figure 2-2; Table 2-5). Atrazine/*S*-metolachlor applied PRE reduced the impact of weed interference on yield components with the exception of 100-seed weights in 2018. In general, the impact of weed interference on yield components was greater in 2017 than 2018 for both main plots.

Weed-free control plots averaged 7 popcorn plants m^{-1} row. In 2017, non-treated control plots where atrazine/*S*-metolachlor was applied PRE, the average popcorn density was 5 plants m^{-1} row compared to 2 plants m^{-1} row when no herbicide was applied (Figure 2-2A). In 2018 where atrazine/*S*-metolachlor was applied PRE, the average was 7 plants m^{-1} row compared to 4 plants m^{-1} row when no PRE was applied (Figure 2-2B). Stand loss can be attributed to both plant death due to high weed interference and accidental removal during pre- and post-weed removal as weeds were removed by hoe and hand weeding as needed. Smaller plants were observed as duration of interference increased and when no PRE herbicide was applied (data not shown); this decreased standability. As previously discussed, popcorn is generally shorter, and has weaker and thinner stalks that make it more prone to lodging compared to field corn (Ziegler 2001). Adigun et al. (2014) reported similar stand reductions due to late season weed competition and mechanical weed removal by hoe in cowpea [*Vigna unguiculata* (L.) Walp.]. Evans et al. (2003a) reported no decrease in field corn density with increasing duration of weed interference, which was not a significant yield component factor determining yield reduction.

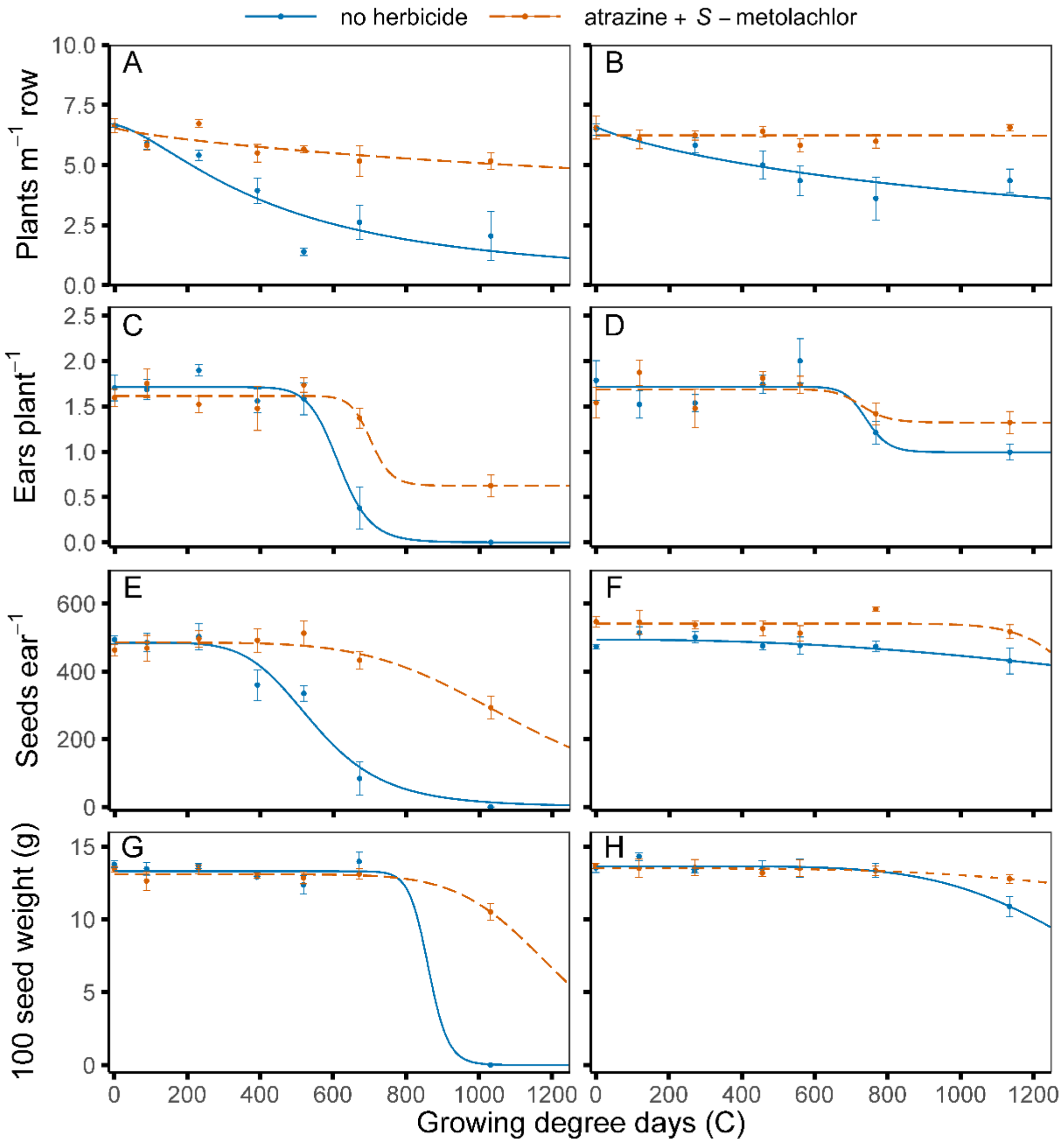

*Figure 2-2. Popcorn (*Zea mays *var.* everta*) plant density (plants m^{-1} of row) at harvest in (A) 2017 and (B) 2018, ears m^{-1} row in (C) 2017 and (D) 2018, seeds ear^{-1} in (E) 2017 and (F) 2018, and 100 seed weight (g) in (G) 2017 and (H) 2018 in response to increasing duration of weed interference as represented by growing degree days (GDD after emergence, C) in popcorn with and without atrazine/*S*-metolachlor applied PRE (2,470 g ai ha^{-1}) in a field experiment conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE. Regression lines represent the fit of a four-parameter log-logistic model.*

Weed-free plots averaged 1.65 ears plant^{-1}. In 2017, non-treated control plots where atrazine/*S*-metolachlor was applied PRE resulted in 0.6 ears plant^{-1} compared to 0 ears plant^{-1} when no herbicide was applied (Figure 2-2C). This means that where atrazine/*S*-metolachlor was applied PRE, 40% of the plants were barren; however, without PRE herbicide all plants were barren in non-treated plots. In 2018 where atrazine/*S*-metolachlor was applied PRE, the average was 1.3 ears plant^{-1} compared to 1 ear plant^{-1} when no herbicide was applied (Figure 2-2D). This indicated that season-long weed interference in 2017 reduced the number of ears plant^{-1} by 100 and 64% with no PRE herbicide applied and with atrazine/*S*-metolachlor applied PRE, respectively, and 39 and 21% in 2018, with no PRE herbicide applied and with atrazine/*S*-metolachlor applied PRE. Reduction in ears plant^{-1} were not observed until the R1 weed removal timing. Similarly, Evans et al. (2003a) reported a reduction in ears plant^{-1} with increasing duration of weed interference which accounted for less than 10% of the subsequent grain yield reduction in field corn.

*Table 2-5. Parameter estimates (*b, c, d, *and* e*) and standard errors of the four-parameter log-logistic model, root mean square error, and modeling efficiency for popcorn (*Zea mays *var. everta) yield components with and without atrazine/*S*-metolachlor applied PRE (2,470 g ai ha*$^{-1}$*) in a field experiment conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE in 2017 and 2018.*[a]

Yield component	Year	PRE treatment	*b*	*c*	*d*	*e*
						GDD
Plants m^{-1} row	2017	no herbicide	1.5 (0.4)	0 (0)	6.7 (0.5)	436.2 (74.8)
		atrazine/*S*-metolachlor	0.8 (0.5)	0 (0)	6.6 (0.4)	4,746.9 (5,458.6)
	2018	no herbicide	0.9 (0.4)	0 (0)	6.6 (0.5)	1,547.4 (621.5)
		atrazine/*S*-metolachlor	2.4 (0.2)	0 (0)	6.2 (0.2)	74,276.0 (1,677,700.0)
Ears plant^{-1}	2017	no herbicide	14.3 (5.0)	0 (0)	1.7 (0.1)	615.7 (22.8)
		atrazine/*S*-metolachlor	25.0 (69.0)	0.6 (0.1)	1.6 (0.1)	702.8 (91.0)
	2018	no herbicide	24.4 (36.4)	1.0 (0.2)	1.7 (0.1)	740.7 (53.2)
		atrazine/*S*-metolachlor	20.0 (46.0)	1.3 (0.1)	1.7 (0.1)	729.1 (116.2)
Seeds ear^{-1}	2017	no herbicide	5.6 (1.6)	0 (0)	484.9 (22.3)	548.5 (26.9)
		atrazine/*S*-metolachlor	5.1 (2.0)	0 (0)	485.1 (14.4)	1,116.8 (69.9)
	2018	no herbicide	2.3 (1.9)	0 (0)	493.7 (14.4)	2,598.2 (1,821.8)
		atrazine/*S*-metolachlor	14.8 (31.6)	0 (0)	541.2 (0)	2,598.2 (1,821.8)
100 seed weight	2017	no herbicide	31.9 (29.1)	0 (0)	13.3 (0.2)	861.7 (160.4)
		atrazine/*S*-metolachlor	9.0 (13.8)	0 (0)	13.1 (0.2)	1,205.3 (284.7)
	2018	no herbicide	5.9 (4.4)	0 (0)	13.6 (0.2)	2,462.2 (6782.8)
		atrazine/*S*-metolachlor	3.7 (13.5)	0 (0)	13.5 (0.4)	2,426.2 (6,782.8)

[a] Abbreviations: *b*, slope; *c*, lower limit; *d*, upper limit; *e*, ED_{50}; GDD, growing degree days

Seeds ear^{-1} was reduced for no herbicide and herbicide applied treatments in 2017 and for no herbicide treatments in 2018. Seeds ear^{-1} averaged 478 and 510 in 2017 and 2018 in weed-free plots, respectively. In 2017 non-treated control plots where atrazine/*S*-metolachlor was applied PRE, the average was 293 seeds ear^{-1} compared to 0 seeds ear^{-1} when no PRE herbicide was applied (Figure 2-2E). In 2018 where atrazine/*S*-metolachlor was applied PRE, the average was 517 seeds ear^{-1} compared to 431 seeds ear^{-1} when no herbicide was applied (Figure 2-2F). The earliest removal timing to observe a reduction in seeds ear^{-1} was at V9 in 2017 with no herbicide applied. Maddonni and Otegui (2004) suggested kernel number may be affected as early as the V7 field corn growth stage with interplant competition. Cox et al. (2006) reported a significant impact on seeds ear^{-1} in field corn when weeds were removed at V5/V6 growth stage or later. Evans et al (2003a) reported that the response of field corn seeds ear^{-1} in response to increasing duration of weed interference mirrored the response of final grain yield.

Weed interference reduced 100-seed weight for both herbicide and no herbicide applied main plots in 2017 and in plots that did not receive an herbicide in 2018. Weed-free 100-seed weights averaged 13.6 g. In the 2017 non-treated control plots where atrazine/*S*-metolachlor was applied PRE, the average 100-seed weight was 10.5 g compared to 0 g when no PRE herbicide was applied (Figure 2-2G). Although the 100-seed weight when no PRE herbicide was applied was reported as 0 g, it should be noted that this was due to all plants within the subsample being barren. No 100-seed weight reduction was observed in the R1 removal time for either main plot. In 2018, non-treated control plots where atrazine/*S*-metolachlor was applied PRE, the average 100-seed weight was 12.8 g compared to 10.8 g when no herbicide was applied (Figure 2-2H).

Similarly to 2017, no 100-seed weight reduction was observed at the R1 weed removal timing. Atrazine/*S*-metolachlor applied PRE limited reduction in 100-seed weight from season-long weed interference in 2017 and prevented reduction in 100-seed weight from season-long interference in 2018. Cox et al. (2006) did not observe reduction in field corn seed weight with increasing duration of weed interference compared to the V3/V4 weed removal timing, except when weeds were allowed to compete with corn season-long (27% reduction); however it was not significantly less than the weed-free control (17% reduction). Evans et al. (2003a) reported that field corn seed weight was less variable than seeds ear^{-1} and accounted for only a minor portion of the observed yield loss. Seed weight reduction has been attributed to a reduced crop growth rate 2 to 6 wk after R1 (Maddonni et al. 1998). Cathcart and Swanton (2004) reported a field corn seed weight reduction of 8 to 12% when green foxtail (50 to 300 plants m^{-2}) was allowed to compete season long.

Results suggest that weed interference had an impact on yield components and that atrazine/*S*-metolachlor applied PRE reduced the impact of weed interference on popcorn yield components and protected certain yield components such as seeds ear^{-1} and 100 seed weight. Assuming plants ha^{-1} was fixed, seeds ear^{-1} has been reported to have the greatest impact on corn yield than any other yield component (Andrade et al. 1999; Evans et al. 2003a; Otegui 1997; Tollenaar 1977).

Popcorn Yield Loss. Popcorn YL increased as weed removal timing was delayed (Figure 2-3; Table 2-6). Greater YL was observed in plots without herbicide compared to plots with atrazine/*S*-metolachlor applied PRE. Yield losses varied among years; therefore, data were presented separately. Without a PRE herbicide, yield of the non-treated control plots were reduced 95 and 84% in 2017 and 2018, respectively. Yield loss curves fit the data well with RMSE ranging from 7.2 to 14.2 and ME from 0.86 to 0.97. Tursun et al. (2016) reported 50 to 79% popcorn YL from season-long weed interference in Turkey.

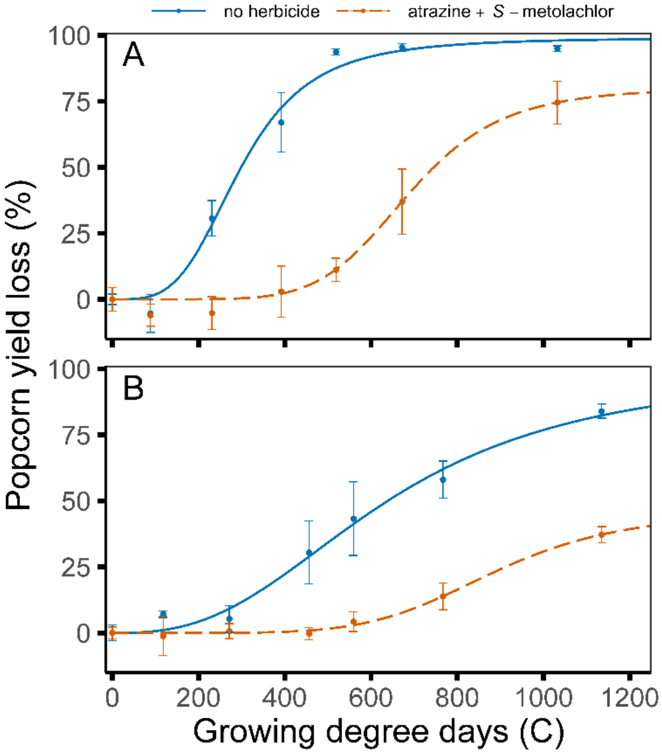

Figure 2-3. Popcorn (Zea mays var. everta) yield loss (%) response to increasing duration of weed interference as represented by growing degree days (GDD after emergence, C) in popcorn with and without atrazine/S-metolachlor applied PRE (2,470 g ai ha–1) in (A) 2017 and (B) 2018 in a field experiment conducted at the University of Nebraska–Lincoln South Central Agricultural Laboratory near Clay Center, NE. Regression lines represent the fit of a four-parameter log-logistic model.

*Table 2-6. Parameter estimates (*b, c, d, *and* e*) and standard errors of the four-parameter log-logistic model, root mean square error, and modeling efficiency used to determine the critical time for weed removal for popcorn (*Zea mays *var. everta) with and without atrazine/*S*-metolachlor applied PRE (2,470 g ai ha^{-1}) in a field experiment conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE in 2017 and 2018.*[a]

Year	PRE treatment	*b*	*c*	d	e	RMSE	ME
				% YL	GDD		
2017	no herbicide	−3.6 (0.7)	0 (0)	99.1 (5.0)	297.3 (20.0)	10.5	0.97
	atrazine/ *S*-metolachlor	−6.4 (3.2)	0 (0)	80.2 (13.9)	688.5 (68.5)	13.5	0.95
2018	no herbicide	−2.7 (0.6)	0 (0)	100 (0)	638.6 (44.5)	14.2	0.86
	atrazine/ *S*-metolachlor	−5.8 (5.1)	0 (0)	45.9 (26.6)	884.4 (257.8)	7.2	0.96

[a] Abbreviations: *b*, slope; *c*, lower limit; *d*, upper limit; *e*, ED_{50}; GDD, growing degree days; RMSE, root mean squared error; ME, modeling efficiency; YL, yield loss

Critical Time for Weed Removal. The CTWR based on 5% popcorn YL varied between years; therefore, data were analyzed separately (Table 2-7; Figure 2-3). The CTWR without PRE herbicide ranged from 133 to 213 GDD in 2017 and 2018. This corresponds to the V4 to V5 growth stages or 16 to 21 d after popcorn emergence. When atrazine/*S*-metolachlor was applied PRE, the CTWR ranged from 450 to 617 GDD in 2017 and 2018, corresponding to the V10 to V15 popcorn growth stages or 41 to 53 d after emergence. The difference in CTWR between years can be attributed to the difference in relative time of weed emergence compared to the crop, differences in weed composition, and competitiveness of velvetleaf in 2017. The CTWR for popcorn production without PRE herbicide in Turkey was reported to be VE (Tursun et al. 2016). The CTWR in sweet corn was reported to be V4 for planting during the first week of May and tasseling (VT) for planting during the third week of June in Illinois (Williams 2006). Differences in CTWR has been reported in other crops such as field corn (Hall et al. 1992; Evans et al. 2003a, 2003b), soybean (Gustafson et al. 2006a, 2006b; Knezevic et al. 2003; Van Acker et al. 1993), and sunflower (Knezevic et al. 2013). Evans et al. (2002a) reported the CTWR for field corn ranged between V4 and V7 between years and across locations in Nebraska. One major factor that can affect the CTWR is the field weed composition and relative time of weed emergence; with low weed density and late weed emergence further delaying the CTWR (Evans et al. 2003a; Norsworthy and Oliceira 2004).

Leaf Area. Popcorn VAI measurements taken at the R1 popcorn growth stage described popcorn YL well with RMSE of 16.1 and ME of 0.83 (Figure 2-4). Greater YL was observed in 2017 with VAI ranging between 0 and 5 at the R1 growth stage compared with relatively less YL in 2018 and VAI ranging between 1 and 7. Cox et al. (2006) reported field corn LAI reduction at R1 growth stage when weeds were allowed to compete without PRE herbicide and then removed at V5 to V6, V7 to V8, and season-long to be 35, 47, and 50%, respectively; however, no LAI reduction was observed when weeds were allowed to compete until V3 to V4. Hall et al. (1992) reported that weed interference increased the rate of senescence of lower corn leaves.

Management Implications. The results of this study suggest that popcorn producers in Nebraska should not allow weeds to interfere in their fields for more than 133 to 213 GDD, equivalent to V4 (16 DAE, days after emergence) to V5 (21 DAE) popcorn growth stage. When atrazine/*S*-metolachlor is utilized as a PRE herbicide, the delay of CTWR was an additional 25 to 32 d compared to when no PRE herbicide was applied, equivalent to 450 to 617 GDD or V10 (41 DAE) to V15 (53 DAE) days after popcorn emergence. Atrazine/*S*-metolachlor partially protected popcorn yield by delaying weed emergence and reducing weed density. Selection of a PRE herbicide based on known weed composition of the field may increase PRE herbicide efficacy and further delay the critical time for weed removal.

*Table 2-7. The critical time for weed removal in popcorn (*Zea mays *var. everta) with and without atrazine/*S-*metolchlor applied PRE (2,470 g ai ha^{-1}) expressed in growing degree days (GDD$_c$), crop growth stage, and days after crop emergence in a field experiment conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE in 2017 and 2018.*[a]

Year	PRE treatment	GDD$_c$	Growth stage	DAE
2017	no herbicide	133	V4	16
	atrazine/*S*-metolachlor	450	V10	41
2018	no herbicide	213	V5	21
	atrazine/*S*-metolachlor	617	V15	53

[a] Abbreviations: DAE; days after crop emergence; GDD, growing degree days

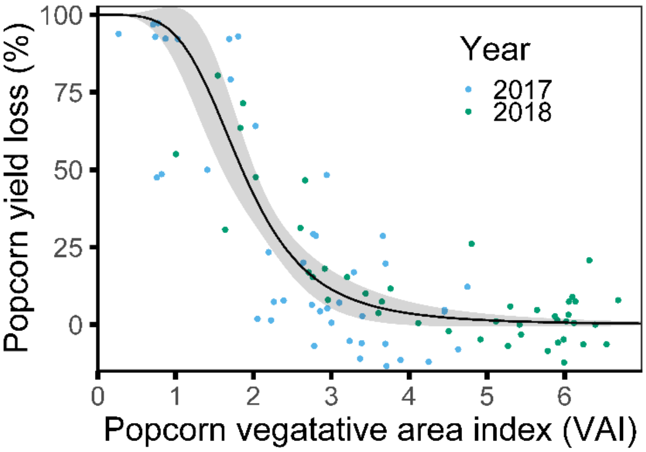

*Figure 2-4. Popcorn (*Zea mays *var.* everta*) yield loss (%) related to decreasing popcorn vegetative area index (VAI) at the R1 popcorn growth stage in a field experiment conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE in 2017 and 2018. Grey ribbon represents 95% confidence interval of the line. Regression lines represent the fit of a four-parameter log-logistic model (RMSE=16.1; ME=0.83). Model parameter values are 4.1 [slope], 1.9 [ED$_{50}$], 0.0 [lower limit], and 100.0 [upper limit].*

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CHAPTER 3 WEED CONTROL AND RESPONSE OF YELLOW AND WHITE POPCORN TO HERBICIDES

3.1 ABSTRACT

Popcorn is an important field crop to many Midwestern United States producers. While there is considerable research on field corn and sweet corn sensitivity to herbicides, there is a lack of information on popcorn sensitivity to herbicides. Field experiments were conducted in 2017 and 2018 to evaluate herbicides labeled for yellow popcorn in commercially available popcorn hybrids for weed control and crop response in Nebraska. The experiments were arranged in a split-plot design. The main plot treatments consisted of two white and six yellow popcorn hybrids. Ten sub-plot treatments consisted of non-treated control, weed-free control, and four pre-emergence (PRE) followed by (fb) post-emergence (POST) herbicide treatments applied at labeled rates (1X) and double the labeled rates (2X). Across hybrids, PRE herbicide treatments resulted in 4-8% injury. Across all PRE herbicide treatments, a yellow hybrid, R265, displayed the greatest average plant injury (11%). At labeled rates, broadleaf weed control in both years, and foxtail control in 2017, ranged from 95 to 99% with all treatments; however, foxtail control was limited (72-86%) for most treatments in 2018. Weed biomass reduction in all herbicide treatments ranged from 90 to 98% and 68 to 97% control in 2017 and 2018, respectively. Yield losses ranged from 0 to 7% in herbicide treatments, with a 42% yield loss in the untreated control. Although slight hybrid differences in herbicide sensitivity were detected, the differences were not linked to popcorn color. Data and information reported in this research are the first that determined popcorn sensitivity to herbicides.

3.2 INTRODUCTION

Popcorn (*Zea mays* L. var. everta) is an important field crop to many producers in the Midwestern United States. Popcorn is grown on nearly 90,000 ha of land annually in the United States (USDA NASS, 2018). Illinois, Indiana, Iowa, Kansas, Kentucky, Michigan, Nebraska, and Ohio are eight major contributing states for popcorn production in the United States (USDA NASS, 2018). Nebraska is the leading producer of popcorn in the United States, producing 160 million kg on over 28,000 ha of the total 356 million kg popcorn produced overall (45% of the national production) in 2012 (USDA NASS, 2018). The next three top popcorn producing states combined (Indiana, Illinois, and Ohio) produced 42% of the nation's harvest in 2012 (USDA NASS, 2018). Popcorn is usually produced under contracts, which specify the hybrids and area to be planted (D'Croz-Mason and Waldren, 1978; Ziegler, 2001). The majority of popcorn is produced under conservation tillage systems (Pike et al., 2002). Popcorn emerges slower, produces narrower and more upright leaves, and tends to have shorter and thinner stalks, as compared to field corn (*Zea mays* L. var. indentata) (Ziegler, 2001). For these reasons, growers depend on herbicides for weed control in popcorn production (Pike et al., 2002). However, popcorn is generally more susceptible than field corn to yield loss due to weed competition and herbicide injury. Additionally, white popcorn is generally more sensitive to herbicides than yellow popcorn (Loux et al., 2017).

A pest management strategic plan was created by a group of popcorn growers, agronomists, university scientists, and agricultural specialists to communicate current pest management practices in popcorn production and to identify the challenges associated with pest management (Pike et al., 2002). This strategic plan was based on the information gathered in the crop profile for popcorn (Bertalmio et al., 2003). The top regulatory priority outlined in the plan was to identify and expand the number of herbicides with distinct sites of action for weed control and crop safety. Pike et al. (2002) suggested that the market opportunity gained by herbicide manufacturers if popcorn was included in the registration package to the United States Environmental Protection Agency (USEPA) was not enough to warrant field testing and subsequent inclusion of popcorn onto the herbicide label. Many herbicides labeled for field corn are not labeled for popcorn, such as premixes of isoxaflutole plus thiencarbazone (Corvus; Bayer CropScience, 2016), and tembotrione plus thiencarbazone (Capreno; Bayer CropScience, 2012; Table 3-1). A premix of acetochlor plus clopyralid plus flumetsulam (Surestart II; Corteva Agriscience, 2014) is labeled for field corn but not for popcorn. Additionally, certain pre-mixtures such as acetochlor plus clopyralid plus mesotrione (Resicore; Corteva Agriscience, 2017) and atrazine plus bicyclopyrone plus mesotrione plus *S*-metolachlor (Acuron; Syngenta Crop Protection, 2017) are labeled for PRE and POST application for field corn, but are not labeled for POST application for popcorn. Lack of inclusion could be due to sensitivity of popcorn or lack of research and interest by the herbicide industry to register these herbicides in a minor crop. For example, mesotrione (Callisto; Syngenta Crop Protection, 2001) was first labeled for field corn in 2001, however yellow popcorn was added to the label in 2004 (Syngenta Crop Protection, 2004).

Table 3-1. Common and chemical names of herbicides.

Common name	Chemical name
acetochlor	2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide
atrazine	6-chloro-N2-ethyl-N4-(propan-2-yl)-1,3,5-triazine-2,4-diamine
bentazon	3-(1-methylethyl)-(1H)-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide
bicyclopyrone	rac-(1R,5S)-4-hydroxy-3-{2-[(2-methoxyethoxy)methyl]-6-(trifluoromethyl)(pyridine-3-carbonyl)}bicyclo[3.2.1]oct-3-en-2-one
carfentrazone	X,2-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoic acid
clopyralid	3,6-dichloropyridine-2-carboxylic acid
dicamba	3,6-dichloro-2-methoxybenzoic acid
diflufenzopyr	2-[(1E)-1-{2-[(3,5-difluorophenyl)carbamoyl]hydrazinylidene}ethyl]pyridine-3-carboxylic acid
dimethenamid-P	2-chloro-N-(2,4-dimethylthiophen-3-yl)-N-[(2S)-1-methoxypropan-2-yl]acetamide
flumetsulam	N-(2,6-difluorophenyl)-5-methyl[1,2,4]triazolo[1,5-a]pyrimidine-2-sulfonamide
fluthiacet	[(2-chloro-4-fluoro-5-{[(1Ξ)-3-oxo-5,6,7,8-tetrahydro-1H,3H-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene]amino}phenyl)sulfanyl]acetic acid
foramsulfuron	2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-(formylamino)-N,N-dimethylbenzamide
halosulfuron	3-chloro-5-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-1-methyl-1H-pyrazole-4-carboxylic acid
isoxaflutole	(5-cyclopropyl-1,2-oxazol-4-yl)[2-(methanesulfonyl)-4-(trifluoromethyl)phenyl]methanone)
mesotrione	2-[4-(methanesulfonyl)-2-nitrobenzoyl]cyclohexane-1,3-dione
nicosulfuron	[2-[[(4,6-dimethoxypyrimidin-2-yl)aminocarbonyl]aminisulfonyl]-N,N-dimethyl-3-pyridinecarboxamide]
primisulfuron	2-[[[[[4,6-bis(difluoromethoxy)-2-pyrimidinyl]amino]carbonyl]amino]sulfonyl]benzoic acid
pyroxasulfone	3-{[5-(difluoromethoxy)-1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl](methanesulfonyl)}-5,5-dimethyl-4,5-dihydro-1,2-oxazole
saflufenacil	2-chloro-4-fluoro-5-[3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydropyrimidin-1(2H)-yl]-N-[methyl(propan-2-yl)sulfamoyl]benzamide
S-metolachlor	mixture of 80–100% 2-chloro-N-(2-ethyl-6-methylphenyl)-N-[(2S)-1-methoxypropan-2-yl] acetamide and 20–0% 2-chloro-N-(2-ethyl-6-methylphenyl)-N-[(2R)-1-methoxypropan-2-yl] acetamide
tembotrione	2-{2-chloro-4-(methanesulfonyl)-3-[(2,2,2-trifluoroethoxy)methyl]benzoyl}cyclohexane-1,3-dione
thiencarbazone	4-{[3-methoxy-4-methyl-5-oxo-4,5-dihydro(1H-1,2,4-triazole-1-carbonyl)]sulfamoyl}-5-methylthiophene-3-carboxylic acid
topramezone	[3-(4,5-dihydro-1,2-oxazol-3-yl)-4-(methanesulfonyl)-2-methylphenyl](5-hydroxy-1-methyl-1H-pyrazol-4-yl)methanone

The top research priority outlined in the Popcorn Pest Management Strategic Plan for the North Central Region is to determine hybrid sensitivity to herbicides (Pike et al., 2002). Some herbicides that are labeled for field corn, sweet corn (*Zea mays* L. var. saccharata), and yellow popcorn specifically exclude white popcorn from the label, including atrazine plus mesotrione plus *S*-metolachlor (Lumax; Syngenta Crop

Protection, 2009) and atrazine plus bicyclopyrone plus mesotrione plus *S*-metolachlor (Acuron; Syngenta Crop Protection, 2017). A different formulation of a premix of atrazine plus mesotrione plus *S*-metolachlor (Lexar; Syngenta Crop Protection, 2012) than previously mentioned (Lumax) is labeled for field corn, seed corn, sweet corn, and even in grain sorghum [*Sorghum bicolor* (L.) Moench ssp. Bicolor], but is not labeled for white popcorn. Herbicide labels often have statements indicating the selectivity of the herbicide on popcorn is unknown and to test in a small area or contact supplier or University specialist to verify sensitivity. Research has been conducted to determine the sensitivity of sweet corn to a number of herbicides labeled for field corn (Bollman et al., 2008; Meyer et al., 2010; Nordby et al., 2008; Williams and Pataky, 2010; Williams et al., 2008); however, scientific literature does not exist regarding weed control and response of commercially-grown yellow and white popcorn hybrids to herbicides. The objectives of this research were to evaluate weed control and crop growth and yield response in Nebraska commercially available yellow and white popcorn hybrids treated with PRE- and POST-emergence herbicides labeled in yellow popcorn.

3.3 MATERIALS AND METHODS

Site Description. Field experiments were conducted at the University of Nebraska-Lincoln, South Central Agricultural Laboratory near Clay Center, NE (40.5752, –98.1428, 552 m elevation above mean sea level) in 2017 and 2018. The soil type was Hastings silt loam (montmorillonitic, mesic, Pachic Argiustolls; 17% sand, 58% silt, and 25% clay) with a pH of 6.5, and 2.5-3% organic matter. In early spring, the site was disked with a tandem disk at a depth of 10 cm and fertilized with 202 kg ha^{-1} of nitrogen in the form of anhydrous ammonia (82-0-0) applied with an anhydrous ammonia coulter on 96 cm spacing. Starter fertilizer ammonium polyphosphate (APP; 10-34-0) was applied in-furrow at 6 kg ha^{-1} during planting. The predominant broadleaf weed species were velvetleaf (*Abutilon theophrasti* Medik.), common lambsquarters (*Chenopodium album* L.), common waterhemp [*Amaranthus tuberculatus* (Moq.) J. D. Sauer], and Palmer amaranth (*Amaranthus palmeri* S. Watson). Grass weed species consisted of green foxtail [*Setaria viridis* (L.) P. Beauv.] and yellow foxtail [*Setaria pumila* (Poir.) Roem. & Schult.], which have been grouped and referred to as "foxtails".

Treatments and Experimental Design. The treatments were arranged in a split-plot design with three replications. The main plot treatments consisted of eight commercially available hybrids (Table 3-2). Ten sub-plot treatments consisted of a non-treated control, weed-free control, and four PRE followed by (fb) POST herbicide treatments (Table 3-3). The herbicide treatments were applied at the labeled PRE and POST rates (1X) and double the labeled PRE and POST rates (2X). PRE herbicide treatments consisted of one of five commercially available premix combinations and represented ten different herbicides (Table 3-3). POST herbicide treatments consisted of one of five commercially available premix combinations with an additional five chemicals represented (Table 3-3).

Table 3-2. Commercially available white and yellow popcorn hybrids tested in field experiments conducted at the University of Nebraska-Lincoln, South Central Agricultural Laboratory near Clay Center, NE, in 2017 and 2018.

Hybrid	Kernel type	Supplier
M2101	yellow	Conagra Brands, LLC, Chicago, IL 60654
VYP315	yellow	Conagra Brands, LLC, Chicago, IL 60654
VYP220	yellow	Conagra Brands, LLC, Chicago, IL 60654
VWP111	white	Conagra Brands, LLC, Chicago, IL 60654
N1H820	yellow	Zangger Popcorn Hybrids, North Loup, NE 68859
R265	yellow	Crookham Company, Caldwell, ID 83606
SH3707W	white	Schlessman Seed Company, Milan, OH 44846
AP2507	yellow	Agricultural Alumni Seed Improvement Association, Inc, Romney, Indiana 47981

Table 3-3. Herbicide treatments tested in a commercial popcorn hybrid field experiment at the University of Nebraska-Lincoln South Central Agricultural Laboratory near Clay Center, NE, in 2017 and 2018.

Herbicide treatment	Timing†	Rate g ai ha^{-1}	Relative rate	Trade name	Adjuvant§	Label stipulations¶
non-treated control		0	NA			
weed-free control						
S-metolachlor/atrazine	PRE	2,470	1x	Bicep II Magnum		None
S-metolachlor/atrazine	POST	3,240	1x	Bicep II Magnum		None
pyroxasulfone/fluthiacet	PRE	188	1x	Anthem MAXX		None
dicamba/tembotrione	POST	597	1x	Diflexx DUO	COC	Contact seed provider or test in small area first.
pyroxasulfone/fluthiacet	PRE	376	2x	Anthem MAXX		
dicamba/tembotrione	POST	1,194	2x	Diflexx DUO	COC	
acetochlor/atrazine	PRE	4,200	1x	Degree Xtra		None
mesotrione/fluthiacet	POST	110	1x	Solstice	NIS	Yellow popcorn only; No UAN or AMS, use NIS; contact seed company, field man, or university specialist.
acetochlor/atrazine	PRE	8,400	2x	Degree Xtra		
mesotrione/fluthiacet	POST	220	2x	Solstice	NIS	
clopyralid/acetochlor/mesotrione	PRE	2,300	1x	Resicore		Yellow popcorn only; PRE only
topramezone/dimethenamid-P	POST	940	1x	Armezon PRO	MSO	Refer to seed company recommendations; 951 g ai ha^{-1} maximum
clopyralid/acetochlor/mesotrione	PRE	4,600	2x	Resicore		
topramezone/dimethenamid-P	POST	1,880	2x	Armezon PRO	MSO	
saflufenacil/dimethamid-P	PRE	730	1x	Verdict		verify with supplier
diflufenzopyr/dicamba	POST	392	1x	Status	NIS+AMS	verify with supplier
saflufenacil/dimethamid-P	PRE	1,460	2x	Verdict		
diflufenzopyr/dicamba	POST	784	2x	Status	NIS+AMS	

† Abbreviations: AMS, ammonium sulfate (DSM Chemicals North America Inc., Augusta, GA); COC, crop oil concentrate (Agridex, Helena Chemical Co., Collierville, TN); PRE, pre-emergence; POST, post-emergence; MSO, methylated seed oil (Southern Ag Inc., Suwanee, GA); NIS, nonionic surfactant (Induce, Helena Chemical Co., Collierville, TN)

§ AMS at 5% (v/v), COC or MSO at 1% (v/v), and NIS at 0.25% (v/v) were mixed with herbicides.

¶ Summery of language used in product labels. Product labels can be found at https://cdms.net.

Plot dimensions were 9 m long by 3 m wide. On May 8, 2017 and April 30, 2018 popcorn hybrids (Table 3-2) were planted with a row spacing of 76 cm at a depth of 4 cm and a planting density of 89,000 seeds ha^{-1}. PRE herbicides were applied on May 9, 2017 and May 2, 2018 and POST herbicides were applied on June 19, 2017 and June 12, 2018 using a handheld CO_2-pressurized backpack sprayer equipped with four AIXR 110015 flat-fan nozzles (TeeJet® Technologies, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60187) spaced 60 cm apart. The sprayer was calibrated to deliver 140 L ha^{-1} at 276 kPa at a constant speed of 4.8 km h^{-1}. Weed-free control plots were kept weed-free using PRE and POST herbicide treatments of *S*-metolachlor plus atrazine as well as by hoeing as needed (Table 3-3).

Data Collection. Air temperature and rainfall data throughout the growing season were obtained from the High Plains Regional Climate Center automated weather station, which was located only about 350 m from the experimental field. Weed control was assessed visually on a scale of 0 to 100%, with 0% representing no injury and 100% representing plant death, at 28 days after PRE (DA PRE) and 21 days after POST (DA POST) herbicide application. Weed density was assessed from two randomly placed 0.5 m^2 quadrats in the middle two popcorn rows from each plot at 21 DA PRE (May 30, 2017; May 28, 2018) and 21 DA POST (July 10, 2017; July 1, 2018). Popcorn injury was assessed on a scale of 0 to 100%, with 0% representing no injury and 100% representing plant death, at 21 DA PRE and 21 DA POST. Popcorn stand counts were measured 21 DA PRE in 2 meters of the middle two rows. Popcorn plant height was measured from 6 plants per plot from the soil surface to the top leaf collar at 35 DA PRE and 21 DA POST. Popcorn lodging (%) was assessed 60 DA POST from the entire length of the middle two rows. Aboveground weed biomass was assessed from two randomly placed 0.5 m^2 quadrats in the middle two rows from each plot at 45 DA POST. Surviving weeds were cut near the soil surface, placed in paper bags, dried in an oven at 50 C for 10 d, and dry biomass weight was recorded. Popcorn was harvested from the middle two rows with a plot combine and the yields were adjusted to 14% grain moisture content. Percent stand reduction, percent biomass reduction, percent height reduction, and percent yield loss were calculated using the equation (Wortman, 2014):

$$Y = [(C\text{-}B)/C] \times 100 \qquad [1]$$

where C represents the popcorn stand from the non-treated control, weed biomass from the non-treated control, popcorn height in the weed-free control, or yield in the weed-free control in the corresponding replication block and B represents the popcorn stand, weed biomass, popcorn height, or yield of the treated plot.

Statistical Analysis. Statistical analyses were subjected to ANOVA in R version 3.5.1 utilizing the base packages (R Core Team, 2019) and the Agricolae: Statistical Procedures for Agricultural Research Package (Mendiburu, 2017). ANOVA was performed using the *sp.plot* (split plot) function where hybrid was treated as the main plot and herbicide treatment was considered as the subplot effect. Replications nested within years were considered random effects in the model. ANOVA assumptions of normality and homogeneity of variance were tested (Kniss and Streibig, 2018). Improvement in normality was not gained through transformation of data; therefore, data were analyzed without transformation. If the random effect of year was significant, data was analyzed with years separated. Treatment means were separated at $P \leq 0.05$ using Fisher's protected least significant difference test.

3.4 RESULTS

Average air temperatures were similar both years (Figure 3-1). Total precipitation (rainfall plus supplemental irrigation) totaled about 60 cm for each year (Figure 3-1). Irrigation amounts were 22 cm in 2017 and 14.2 cm in 2018 (Figure 3-1; Table 3-4). The GDD totals were 1032 and 1135 for 2017 and 2018, respectively.

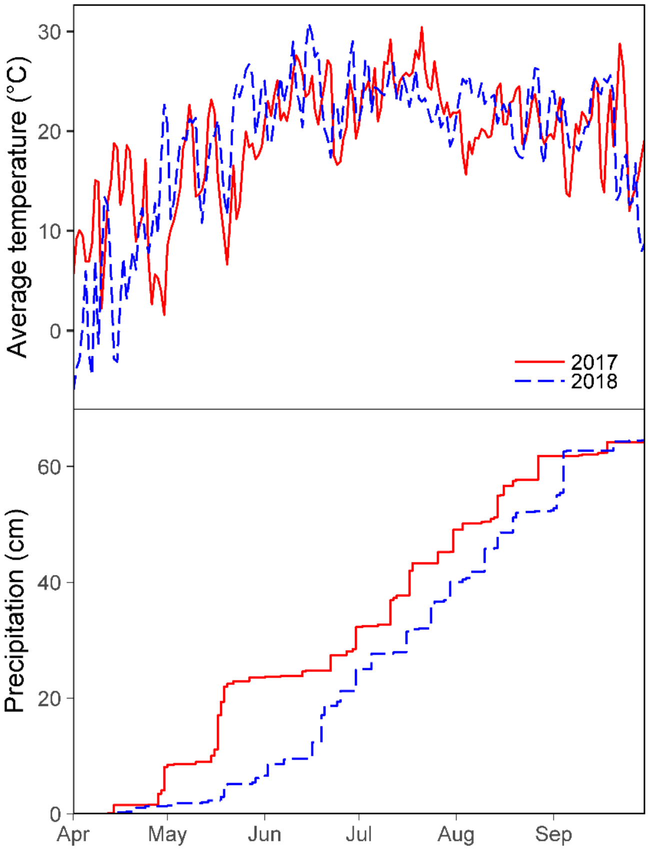

Figure 3-1. Average daily air temperature, total precipitation (rainfall + irrigation) during 2017 and 2018 growing seasons at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE

Table 3-4. Irrigation amounts in field experiments conducted at the University of Nebraska-Lincoln, South Central Agricultural Laboratory near Clay Center, NE, in 2017 and 2018.

2017		2018	
	cm		cm
June 22	2.7	June 16	2.8
June 30	3.8	July 16	3.6
July 11	4.2	July 24	3.9
July 17	3.7	August 10	3.9
July 31	3.8		
August 14	3.8		

Popcorn Herbicide Sensitivity. There were no interactions between popcorn hybrids and herbicide treatments with popcorn injury. Averaged among popcorn hybrids, PRE herbicides resulted in 4 to 8% popcorn injury 21 DA PRE (Table 3-5). Although statistically similar in several treatments, labeled herbicide rates (1X rates) resulted in 4 to 6% injury as compared with 6 to 8% injury at 2X rates. Popcorn hybrid injury ranged from 3% to 11% with the two white hybrids resulting in 3 to 6% injury from PRE herbicide treatments. Popcorn hybrid response to PRE herbicides varied among hybrids with the most sensitive hybrid, R265 – a yellow popcorn, resulting in 11% injury among the treatments (Table 3-5). Stand reductions were not observed compared to the non-treated control 21 DA PRE (data not shown). There was an interaction between popcorn hybrids and herbicide treatments with popcorn height 35 DA PRE only in 2017. Popcorn height reduction compared with the weed-free control was reduced by saflufenacil/dimethamid-P at 2X rate in VYP220 (28%), VWP111 (14%), AP2507 (13%), and R265 (13%) and from pyroxasulfone/fluthiacet at 2X rate in N1H820 (17%) and VYP220 (16%)(Table 3-6).

Popcorn injury 21 DA POST was only observed in 2018 and no interaction between popcorn hybrids and herbicide treatments occurred (Table 3-5). Saflufenacil/dimethamid-

P fb diflufenzopyr/dicamba at 2X rate resulted in 5% crop injury 21 DA POST in 2018. No interaction occurred for popcorn lodging between popcorn hybrids and herbicide treatments. No interaction between popcorn hybrids and herbicide treatments occurred for popcorn height reduction 21 DA POST. Height reduction among herbicide treatments was minimal (1 to 5%) as compared to the weed-free control; however, the non-treated control resulted in a 15% reduction in height compared with the weed-free control (data not shown). Lodging 60 DA POST was similar to non-treated and weed-free controls and was not influenced by hybrid or herbicide treatment (data not shown). No interaction between popcorn hybrids and herbicide treatments occurred for popcorn yield loss with losses due to herbicide treatments resulting in only 1-7% compared to the weed-free control (Table 3-5). Yield losses were not greater at 2X rates compared to labeled rates (Table 3-5).

Weed Control. At labeled rates, clopyralid/acetochlor/mesotrione provided greatest control of velvetleaf (98%) 28 DA PRE (Table 3-7). Saflufenacil/dimethamid-P resulted in 93% control of velvetleaf and pyroxasulfone/fluthiacet and acetochlor/atrazine resulted in 87 and 86% control of velvetleaf 28 DA PRE, respectively. All PRE herbicides at labeled rates (1X) provided 95 to 98% control of common lambsquarters 28 DA PRE, except pyroxasulfone/fluthiacet (87%). Common waterhemp and Palmer amaranth were controlled 94 to 99% and 98 to 99% with all herbicides at labeled rates 28 DA PRE, respectively. Foxtails in 2017 were controlled 95 and 98% with labeled rates of pyroxasulfone/fluthiacet and clopyralid/acetochlor/mesotrione, respectively, whereas, acetochlor/atrazine and saflufenacil/dimethamid-P resulted in 90 to 91% control. Control of foxtails in 2018 ranged from 87 to 90% 28 DA PRE with all herbicides, except pyroxasulfone/fluthiacet (78%).

Broadleaf weed control ranged from 95 to 99% from all herbicide treatments at labeled rates 21 DA POST (Table 3-7). Foxtail control ranged from 91 to 99% and 72 to 96% in 2017 and 2018, respectively. The greatest foxtail control 21 DA POST was achieved with acetochlor/atrazine fb mesotrione/fluthiacet (95%), clopyralid/acetochlor/mesotrione fb topramezone/dimethenamid-P resulting in 99% in 2017, and 96% control in 2018.

Table 3-5. Popcorn 2017 and 2018 PRE injury, 2018 POST injury, and 2017 and 2018 yield loss from herbicide treatments and 2017 and 2018 PRE injury by hybrid in field experiments conducted at the University of Nebraska-Lincoln, South Central Agricultural Laboratory near Clay Center, NE, in 2017 and 2018.

Herbicide treatment†	Relative rate†	Injury 21 DA PRE‡	2018 injury 21 DA POST‡ §	Yield loss‡
			%	
non-treated control	---	0 d	0 c	42 a
pyroxasulfone/fluthiacet fb	1x	4 c	1 bc	4 b
dicamba/tembotrione	2x	6 abc	2 b	1 b
acetochlor/atrazine fb	1x	6 bc	1 bc	2 b
mesotrione/fluthiacet	2x	7 ab	1 bc	1 b
clopyralid/acetochlor/mesotrione fb	1x	6 abc	0 c	7 b
topramezone/dimethenamid-P	2x	6 ab	1 bc	4 b
saflufenacil/dimethamid-P fb	1x	5 bc	2 b	3 b
diflufenzopyr/dicamba	2x	8 a	5 a	1 b
weed-free control	1x	5 bc	1 bc	0 b
Hybrid				
VWP111 – white		3 e	ns	ns
VYP315 – yellow		8 b	ns	ns
VYP220 – yellow		4 de	ns	ns
M2101 – yellow		4 cde	ns	ns
SH3707W – white		6 bc	ns	ns
AP2507 – yellow		5 bcd	ns	ns
N1H820 – yellow		3 e	ns	ns
R265 – yellow		11 a	ns	ns

† Abbreviations: DA PRE, days after PRE-emergence application; DA POST, days after POST-emergence application; fb, followed by; x, labeled rate reported in Table 3

‡ Means presented within the same column with no common letter(s) are significantly different according to Fisher's Protected LSD where α = 0.05

§ Popcorn POST injury for 2018 only. There was no observable POST injury in 2017.

Table 3-6. Popcorn height reduction from herbicide treatments 35 DA PRE in field experiments conducted at the University of Nebraska-Lincoln, South Central Agricultural Laboratory near Clay Center, NE, in 2017.

Herbicide treatment†	Relative rate†	Height reduction							
		VWP111 white	VYP315 yellow	VYP220 yellow	M2101 yellow	SH3707W white	AP2507 yellow	N1H820 yellow	R265 yellow
		%							
non-treated control	---	6 abc	12 a	4 c	11 a	4 a	0 b	6 ab	13 a
pyroxasulfone/	1x	9 abc	3 ab	0 c	5 a	5 a	0 b	8 ab	1 b
fluthiacet	2x	10 abc	8 ab	16 b	8 a	5 a	5 ab	17 a	3 b
acetochlor/	1x	5 bc	3 ab	2 c	7 a	3 a	0 b	5 ab	4 ab
atrazine	2x	6 abc	4 ab	3 c	12 a	4 a	3 b	3 ab	7 ab
clopyralid/acetochlor/	1x	6 abc	1 b	4 c	3 a	3 a	0 b	8 ab	0 b
mesotrione	2x	9 abc	9 ab	6 c	9 a	4 a	0 b	9 ab	1 b
saflufenacil/	1x	7 abc	1 b	7 bc	5 a	2 a	4 b	4 ab	1 b
dimethamid-P	2x	14 a	10 ab	28 a	6 a	3 a	13 a	5 ab	13 a
weed-free control	1x	0 c	0 b	0 c	0 a	0 a	0 b	0 b	0 b

† Abbreviations: DA PRE, days after PRE-emergence application; x, labeled rate reported in Table 3

‡ Means presented within the same column with no common letter(s) are significantly different according to Fisher's Protected LSD where α = 0.05

Table 3-7. Weed control in popcorn from herbicide treatments 28 DA PRE and 21 DA POST by species in field experiments conducted at the University of Nebraska-Lincoln, South Central Agricultural Laboratory near Clay Center, NE, in 2017 and 2018.

		Weed species											
		velvetleaf‡		common lambsquarters‡		common waterhemp‡		2018 Palmer amaranth‡§		2017 grasses‡		2018 grasses‡	
Herbicide treatment†	Relative rate†	28 DA PRE	21 DA POST	28 DA PRE	21 DA POST	28 DA PRE	21 DA POST	28 DA PRE	21 DA POST	28 DA PRE	21 DA POST	28 DA PRE	21 DA POST
		------ % ------											
non-treated control	---	0 d	0 c	0 d	0 c	0 c	0 c	0 c	0 c	0 e	0 d	0 e	0 d
pyroxasulfone/fluthiacet fb	1x	87 c	98 a	87 c	98 ab	95 ab	99 a	98 ab	99 a	95 a-d	93 bc	78 d	72 c
dicamba/tembotrione	2x	92 b	98 a	90 bc	98 ab	99 ab	99 a	99 a	98 ab	98 abc	97 ab	86 bc	86 b
acetochlor/atrazine fb	1x	86 c	95 b	95 ab	97 b	94 b	97 b	99 a	97 b	90 cd	95 abc	90 bc	86 b
mesotrione/fluthiacet	2x	93 b	98 a	96 a	99 a	97 ab	99 a	94 b	99 a	99 ab	98 a	85 cd	83 b
clopyralid/acetochlor/	1x	98 a	99 a	99 a	99 a	99 a	99 a	99 a	99 a	98 ab	99 a	87 bc	96 a
mesotrione fb topramezone/dimethenamid-P	2x	98 a	99 a	99 a	99 a	99 a	99 a	99 a	99 a	99 a	99 a	92 abc	97 a
saflufenacil/dimethamid-P fb	1x	93 b	99 a	96 ab	99 a	95 ab	98 ab	99 a	99 a	91 bcd	91 c	90 bc	85 b
diflufenzopyr/dicamba	2x	96 ab	99 a	97 a	99 a	96 ab	99 a	99 a	99 a	90 d	98 ab	93 ab	87 b
weed-free control	1x	99 a	99 a	99 a	99 a	99 a	99 a	99 a	99 a	99 a	99 a	99 a	99 a

† Abbreviations: DA PRE, days after PRE-emergence application; DA POST, days after POST-emergence application; fb, followed by; x, rate reported as g ai ha^{-1} in Table 3

‡ Means presented within the same column with no common letter(s) are significantly different according to Fisher's Protected LSD where α = 0.05

§ Popcorn POST injury for 2018 only. There was no observable POST injury in 2017.

Table 3-8. Weed density, weed biomass, and weed biomass reduction in popcorn from herbicide treatments in field experiments conducted at the University of Nebraska-Lincoln, South Central Agricultural Laboratory near Clay Center, NE, in 2017 and 2018.

Herbicide treatment†	Relative rate†	weed density‡				weed biomass‡		biomass reduction‡	
		2017	2018	2017	2018	2017	2018	2017	2018
		21 DA PRE		21 DA POST		45 DA POST		45 DA POST	
		----------- plants m^{-2} -----------				----- g m^{-2} -----		----- % -----	
non-treated control	---	172 a	259 a	83 a	171 a	1534 a	1250 a	0 d	0 e
pyroxasulfone/fluthiacet fb dicamba/tembotrione	1x	13 b	229 ab	9 b	67 b	151 b	407 b	90 c	68d
	2x	3 b	188 abc	4 bcd	46 bcde	33 c	370 bc	98 ab	70 d
acetochlor/atrazine fb mesotrione/fluthiacet	1x	1 b	196 abc	3 cd	47 bcd	29 c	367 bc	98 ab	71 d
	2x	1 b	169 bcd	3 cd	37 cde	19 c	217 d	99 a	84 b
clopyralid/acetochlor/mesotrione fb topramezone/dimethenamid-P	1x	0 b	123 cde	1 cd	42 cde	43 c	36 e	97 ab	97a
	2x	0 b	105 de	1 d	27 de	0 c	13 e	100 a	99a
saflufenacil/dimethamid-P fb diflufenzopyr/dicamba	1x	10 b	103 de	7 bc	49 bc	85 bc	320 bcd	95 b	74 cd
	2x	0 b	55 ef	1 cd	25 e	0 c	239 cd	100 a	80 bc
weed-free control	1x	0 b	0 f	0 d	0 f	0 c	0 e	100 a	100 a

† Abbreviations: DA PRE, days after PRE-emergence application; DA POST, days after POST-emergence application; fb, followed by; x, rate reported as g ai ha^{-1} in Table 3

‡ Means presented within the same column with no common letter(s) are significantly different according to Fisher's Protected LSD where $\alpha = 0.05$

Weed density 21 DA PRE was reduced from 172 plants m^{-2} to 0-13 plants m^{-2} when PRE herbicides were applied at labeled rates in 2017 (Table 3-8). In 2018, saflufenacil/dimethamid-P and clopyralid/acetochlor/mesotrione at labeled rates resulted in the lowest weed densities 21 DA PRE (103 to 123 plants m^{-2}) compared with the non-treated control (259 plants m^{-2}). Foxtail density 21 DA PRE in the non-treated control in 2017 was 71 plants m^{-2} and was reduced to 0-1 m^{-2} by herbicide treatments. In 2018, foxtail density was 158 plants m^{-2} in the non-treated control and was reduced to 88 plants m^{-2} by labeled rates of saflufenacil/dimethamid-P (data not shown). Weed density 21 DA POST was reduced from 83 plants m^{-2} to 1-9 plants m^{-2} from all herbicide treatments at labeled rates in 2017. In 2018, herbicide treatments at labeled rates reduced weed density from 171 plants m^{-2} to 42-67 plants m^{-2}. Weed densities at labeled rates were similar to 2X rates at 21 DA PRE and 21 DA POST.

Weed biomass 45 DA POST in the non-treated control averaged 1534 and 1250 g m^{-2} in 2017 and 2018, respectively (Table 3-8). Weed biomass reductions from 95 to 98% were achieved from all herbicide treatments in 2017, except pyroxasulfone/fluthiacet fb dicamba/tembotrione (90%). In 2018, 97% biomass reduction was achieved with clopyralid/acetochlor/mesotrione fb topramezone/dimethenamid-P. All other herbicide treatments at labeled rates in 2018 resulted in weed biomass reductions from 68 to 74%. Biomass reduction was similar in 1X rate herbicide treatments to 2X rates.

Popcorn Yield Loss. Averaged among popcorn hybrids, yield loss ranged from 1 to 7% and did not differ among herbicide treatments and weed-free control (Table 3-5). The non-treated control resulted in 42% yield loss compared with the weed-free control. Yield loss was similar among 1X and 2X rates. Yield loss did not vary by hybrid.

3.5 DISCUSSION

The two white popcorn hybrids tested, VWP111 and SH3707W, did not result in more herbicide injury than the six yellow popcorn hybrids tested in this research. These findings are inconsistent with the assumption that white popcorn hybrids are inherently more sensitive to herbicides than yellow hybrids (Loux et al., 2017), however the few hybrids tested are not enough to make a generalized conclusion about the effect of hybrid color. Height reduction due to PRE herbicides was observed only in 2017 at 2X rates and was dependent on hybrid. Clopyralid/acetochlor/mesotrione (Resicore) is not labeled for white popcorn (Corteva Agriscience, 2017). However, this herbicide did not result in a high level of injury even at the 2X rate, suggesting that there may not be a strong reason or justification for keeping white popcorn off of the label; however more white popcorn hybrid screening may be warranted prior to labeling herbicides for use in white popcorn. Mesotrione/fluthiacet (Solstice) is also only labeled for yellow popcorn; however, when following label instructions for yellow popcorn [Not to add urea ammonium nitrate (UAN) or ammonium sulfate (AMS) and to use nonionic surfactant (NIS)] (FMC Corporation, 2013), minimal injury occurred, regardless of the application rate or hybrid color. HPPD-inhibiting herbicide tolerance in sweet corn hybrids has been reported to be hybrid dependent (Bollman et al., 2008; O’Sullivan et al., 2002). Further investigation into sensitivity of sweet corn to HPPD-inhibiting herbicides concluded that hybrid sensitivity was linked to a mutation of the P450 allele and that hybrids that are homozygous for the non-mutated allele are rarely injured at labeled rates (Williams and

Pataky, 2008; 2010). This allele in sweet corn confers tolerance to other P450-metabolized herbicides such as bentazon, carfentrazone, dicamba/diflufenzopyr, foramsulfuron, halosulfuron, and primisulfuron (Nordby et al., 2008; Williams et al., 2008). Although greater injury was observed from saflufenacil/dimethamid-P fb diflufenzopyr/dicamba at a 2X rate, popcorn yield loss was not influenced.

Broadleaf weed control was achieved with all herbicide treatments; however, grass weed control was poor for all treatments, except clopyralid/acetochlor/mesotrione fb topramezone/dimethenamid-P. The high foxtail density and the subsequent lack of control provided by most herbicide treatments was a major contributor to increased total weed density and biomass in 2018 compared with 2017. The efficacy of topramezone on grass weeds is an advantage for popcorn production fields with a history of high grass weed densities as it has shown to be effective on a number of grass weed species (Grossmann and Ehrhardt, 2007). Additionally, growers have been using nicosulfuron (Accent Q; Corteva Agriscience; 2009) and nicosulfuron/mesotrine (Revulin Q; Corteva Agriscience, 2015) herbicides for grass weed control in yellow popcorn production. Sarangi and Jhala (2018) reported 95, 91, and 82% control of velvetleaf, Palmer amaranth, and foxtails, respectively, 28 DAT with saflufenacil/dimethenamid; and reported velvetleaf, Palmer amaranth, and foxtail densities 42 DAT of 6, 17, and 16 plants m^{-2}, respectively, which is consistent with the control achieved in this study. In a dose response study with 10 cm tall common waterhemp, mesotrione/fluthiacet resulted in >90% control and biomass reduction 21 DAT, similar to the results obtain in this study (Ganie et al. 2015). Similar Palmer amaranth control (95-100%) with acetochlor/atrazine has been reported in the literature (Janak and Grichar, 2016). Chahal et al. (2017)

reported 90% Palmer amaranth control and 82% biomass reduction 28 DA POST from saflufenacil/dimethamid-P fb diflufenzopyr/dicamba. Hauver et al. (2017) reported 93% control of Palmer amaranth with clopyralid/acetochlor/mesotrione. Parks et al. (1995) reported 96% common lambsquarters control 56 DAT with rates of dicamba similar to the ones used in this study.

Recommendations and Practical Implications. Weed control in popcorn is important and challenging due to limited herbicide options compared with field corn. The research was designed to determine the response of commonly grown yellow and white popcorn hybrids in Nebraska to PRE and POST herbicides. Selected yellow and white popcorn hybrids were not sensitive to herbicides tested in this research with low observed injury and minimal yield loss, even at higher than labeled rates. Although a few hybrid differences to herbicide tolerance were detected, the differences did not appear to be linked to hybrid kernel color and did not translate into detectable yield losses. The tested herbicide treatments provided adequate control of broadleaf weeds and, if labeled, can be recommended to popcorn growers. Additional measures for grass weed control may be necessary, depending on field history and herbicide treatment. The tested herbicide treatments combine PRE fb POST herbicide application and herbicides with multiple sites of action, which are keys to delay the evolution of herbicide-resistant weeds (Norsworthy et al., 2012). Not all herbicide treatments are labeled in white popcorn and producers should refer to label instructions. Results from this research are the first to determine popcorn sensitivity to herbicides and can be of immediate use in practical applications to popcorn producers, crop consultants, popcorn companies, and herbicide manufacturers and can contribute to enhance popcorn production efficiency.

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CHAPTER 4 CONTROL OF VELVETLEAF (*ABUTILON THEOPHRASTI*) WITH POST-EMERGENCE HERBICIDES AT TWO GROWTH STAGES IN NEBRASKA POPCORN

4.1 ABSTRACT

Velvetleaf is an economically important weed in popcorn production fields in Nebraska. Many PRE herbicides commonly applied in popcorn have limited residual activity or can partially control velvetleaf. POST herbicides are limited in popcorn compared to field corn, necessitating the evaluation of POST herbicides for control of velvetleaf. The objectives of this study were to (1) evaluate the efficacy and crop safety of labeled POST herbicides for controlling velvetleaf that survived S-metolachlor/atrazine applied PRE and (2) determine effect of velvetleaf height on POST herbicide efficacy, popcorn injury, and yield. Field experiments were conducted in 2018 and 2019 near Clay Center, Nebraska. The experiments were arranged in a split-plot design with four replications. The main plot treatments were velvetleaf heights (12 to 15 cm and 24 to 30 cm) and sub-plot treatments included a no-POST herbicide control, and eleven POST herbicide programs. Fluthiacet-methyl, fluthiacet-methyl/mesotrione, carfentrazone-ethyl, dicamba, and dicamba/diflufenzopyr provided > 96% velvetleaf control 28 DAT, reduced velvetleaf density to < 7 plants m^{-2}, achieved 99 to 100% biomass reduction, and no popcorn yield reduction in both years. Herbicide programs tested in this study provided > 98% control of velvetleaf 28 DAT in 2019. Most POST herbicide programs in this study provided > 90% control of 12 to 15 and 24 to 30 cm velvetleaf and no differences between velvetleaf heights in density, biomass reduction, or popcorn yield were observed, except topramezone and nicosulfuron/mesotrione 28 d after treatment (DAT) in 2018. Based on contrast analysis, herbicide programs with fluthiacet-methyl (98%) or

dicamba (95-96%) provided similar control 28 DAT compared with 87-90% control without them regardless of velvetleaf height in 2018. It is concluded that POST herbicides are available for control of 12 to 30 cm tall velvetleaf in popcorn production fields.

4.2 INTRODUCTION

Popcorn is grown on nearly 90,000 ha in the United States every year (USDA NASS 2019). States that produce over 500 ha of popcorn annually include Illinois, Indiana, Iowa, Kansas, Kentucky, Michigan, Nebraska, and Ohio (USDA NASS 2018). Global popcorn sales have increased by an average of 4 million kg each year from 1970 to 2017 with sales of over 540 million kg in 2017 (Popcorn Board 2019). Nebraska produces the greatest amount of popcorn (167 million kg) grown on about 25,900 ha, which attributed to 34% of the United States' total production in 2017 (USDA NASS 2018). Popcorn production is normally contracted by the popcorn processor and the farmer (D'Croz-Mason and Waldren 1978; Ziegler 2001). Popcorn management varies from field corn in several ways with shorter and thinner stalks, producing narrower and more upright leaves, and emerging slower than field corn (Ziegler 2001). Due to these characteristics, popcorn is less competitive with weeds compared with field corn (Ziegler 2001).

The majority of popcorn production in the United States is under conservation tillage systems and herbicides are the primary method of weed control (Pike et al. 2002). Weed control is a challenge for popcorn producers due to limited herbicide options compared to field corn. For example, herbicide premixes such as isoxaflutole/thiencarbazone (Corvus; Anonymous 2016a), tembotrione/thiencarbazone (Capreno; Anonymous 2012), rimsulfuron/mesotrione (Instigate; Anonymous 2016b), and acetochlor/flumetsulam/clopyralid (Tripleflex II; Anonymous 2014c; Surestart II; Anonymous 2014b) are labeled in field corn but not in popcorn. Commercially available popcorn hybrids are non-genetically-modified so commonly used POST herbicides in herbicide-resistant field corn, such as glyphosate and/or glufosinate, cannot be used for weed control in popcorn (Fernandez-Cornejo et al. 2014; Ziegler 2001).

Velvetleaf is a large seeded annual broadleaf weed (Bazzaz et al. 1989) native to China where it was cultivated as a fiber crop (Sattin et al. 1992). It was introduced to North America in the 17th century for fiber production (Defelice et al. 1988, Spenser 1984). Velvetleaf is now a major agricultural weed in corn, cotton (*Gossypium hirsutum* L.), soybean (*Glycine max* L. Merr.), and sorghum (*Sorghum bicolor* L. Moench) production fields in North America (Spenser 1984). A statewide survey conducted in 2015 reported velvetleaf as the fourth most difficult to control weed in Nebraska (Sarangi and Jhala 2018a). Widespread occurrence and seed bank persistence of velvetleaf is partially attributed to its longevity and long term success (Warwick and Black 1988). For example, Toole and Brown (1946) reported that velvetleaf buried for 39 years in Virginian soil had 43% seed viability. A similar study in Nebraska reported 25 and 35% viability in eastern and western Nebraska, respectively, after 17 years of velvetleaf seed burial (Burnside et al. 1996). Its growth potential and canopy architecture enables velvetleaf to compete for light with most agronomic crops (Bazzaz et al. 1989).

Velvetleaf interference in field corn has been primarily attributed to light competition (Lindquist et al. 1998). Velvetleaf competition in field corn has been reported to result in substantial yield losses. Campbell and Hartwig (1982) reported 70% yield reduction in field corn after 6 weeks of competition. Lindquist et al. (1996) reported yield loss from velvetleaf ranged from 0 to 80% depending on year in Nebraska. Terra et al. (2007) also reported variable field corn yield loss due to velvetleaf competition ranging from 0 to 72% at 20 velvetleaf plants m^{-1} row. Liphadzi and Dille (2006) reported maximum field corn yield losses from velvetleaf competition ranged from 41 to 100%. Werner et al. (2004) reported 37% field corn yield loss from 21 velvetleaf plants m^{-2} in Pennsylvania. Similarly, Scholes et al. (1995) reported 37% yield loss from 24 velvetleaf plants m^{-2} in South Dakota. Soil water level affects competition between velvetleaf with field corn (Vaughn et al. 2007; Vaughn et al. 2016). Increased field corn populations and velvetleaf that emerge after field corn emergence have resulted in less velvetleaf seed production (Teasdale et al. 1998). Field corn yield loss due to velvetleaf interference has been reported to be greater on higher levels of nitrogen fertilizer (Barker et al. 2006; Bonifas et al. 2005).

Herbicides applied PRE such as atrazine/fluthiacet-methyl/pyroxasulfone (1,260 g ai ha^{-1}) and acetochlor/clopyralid/flumetsulam (1,190 g ai ha^{-1}) have reported 78-90 and 74-79% velvetleaf control, respectively at 28 d after treatment in field corn in Nebraska (Sarangi and Jhala 2018b). Liphadzi and Dille (2006) showed that the competiveness of surviving velvetleaf in field corn was reduced due to isoxaflutole and flumetsulam applied PRE. Similarly, velvetleaf that survived dicamba, halosulfuron-methyl, or flumiclorac applied POST were less competitive with field corn than velvetleaf in plots not treated with a POST herbicide (Terra et al. 2007). Although velvetleaf plants that survive PRE or POST herbicide are likely to be less competitive, seed production from survivors is a concern because they contribute to soil seedbank (Liphadzi and Dille 2006; Murphy and Lindquist 2002; Terra et al. 2007).

Weed height at the time of herbicide application can influence herbicide efficacy (Wiles et al. 1992; Wilkerson et al. 1991). King and Oliver (1992) reported reduced herbicide efficacy as time after weed emergence increased for a number of weed species. Herbicide application to weeds at the proper weed height is a tactic used to delay the evolution of herbicide resistance (Norsworthy et al. 2012). Fluthiacet-methyl can be applied from 4.8 to 7.2 g ai ha^{-1} to velvetleaf until they are up to 91 cm tall (Anonymous 2011). The recommended height for broadleaf summer annual weeds is 3 to 8 cm when applying dicamba at rates ranging from 210 to 1,120 g ai ha^{-1} (Diflexx; Anonymous 2018a). Scientific literature is not existing on effect of velvetleaf height on labeled POST herbicide efficacy in popcorn.

Atrazine and *S*-metolachlor are commonly used herbicides applied in a premix or tank-mixture in popcorn because of their crop safety in yellow and white popcorn (Barnes et al. 2019b). For instance, it was estimated that 99% and 11% of popcorn fields were treated PRE and/or POST with atrazine and *S*-metolachlor, respectively in 1999 in the United States (Bertalmio et al. 2003). Sarangi and Jhala (2018a) reported in a statewide survey that *S*-metolachlor/atrazine was the third most common PRE herbicide applied in field corn in eastern Nebraska. A premix of *S*-metolachlor and atrazine is a commonly used, labeled PRE herbicide in popcorn production but partially controls velvetleaf (Anonymous 2014a; Taylor-Lovell and Wax 2001). For example, Barnes et al. (2019a) reported 16 velvetleaf plants m^{-2} in 2017 and 18 m^{-2} in 2018 when 2,470 g ai ha^{-1} *S*-metolachlor/atrazine was applied PRE compared to 21 and 16 velvetleaf m^{-2} in 2017 and 2018, respectively when no PRE herbicide was applied. Additionally, due to rain and other unexpected events, often it is not possible for growers to apply PRE herbicide. For example, in 2019 growing season in Nebraska and several other popcorn producing states, spring was extremely wet. Several growers were able to plant popcorn but were not able to apply PRE herbicide; therefore, they had to rely on POST herbicides for weed control. Herbicide-resistant popcorn has not been developed, so non-selective herbicides that can be used in glyphosate/glufosinate-resistant field corn cannot be used in popcorn. Additionally, relatively new pre-mixture herbicides such as atrazine/bicyclopyrone/*S*-metolachlor/mesotrione (Acuron; Anonymous 2017a) and acetochlor/clopyralid/mesotrione (Resicore; Anonymous 2017b) are labeled to apply PRE in popcorn but not POST. Under this situation, often times popcorn growers have to rely on POST herbicides for weed control.

Scientific literature is not available for control of velvetleaf in popcorn with POST herbicides. The objectives of this research were (1) to evaluate the efficacy and crop safety of labeled POST herbicides for controlling velvetleaf that survived *S*-metolachlor/atrazine applied PRE in Nebraska popcorn and (2) to determine effect of velvetleaf height on POST herbicide efficacy, popcorn injury, and yield. We hypothesized that POST herbicides are available for control of velvetleaf and their efficacy may be reduced when applied to velvetleaf at 24 to 30 cm compared with 12 to 15 cm.

4.3 MATERIALS AND METHODS

Site Description. Field experiments were conducted at the University of Nebraska-Lincoln, South Central Agricultural Laboratory near Clay Center, NE (40.5752, –98.1428, 552 m elevation above mean sea level) in 2018 and 2019. The soil type was Hastings silt loam (montmorillonitic, mesic, Pachic Argiustolls; 17% sand, 58% silt, and

25% clay) with a pH of 6.5 and 3.0% organic matter. In early spring, the site was disked with a tandem disk at a depth of 10 cm and fertilized with 202 kg ha^{-1} of nitrogen in the form of anhydrous ammonia (82-0-0) applied with an anhydrous ammonia coulter on 96 cm spacing. Starter fertilizer ammonium polyphosphate (APP; 10-34-0) was applied in-furrow at 6 kg ha^{-1} during planting.

Treatments and Experimental Design. The treatments were arranged in a split-plot design with four replications. The main plot treatments consisted of two velvetleaf heights (12 to 15 cm and 24 to 30 cm tall) and eleven sub-plot POST herbicide programs (Table 1). A no-POST herbicide control was included for comparison. Plot dimensions were 9 m long by 3 m wide. A yellow popcorn hybrid (VYP 321, Conagra Brands, LLC, Chicago, IL 60654) was planted on April 30, 2018 and May 1, 2019 with a row spacing of 76 cm at a depth of 4 cm and a planting density of 89,000 seeds ha^{-1}. *S*-metolachlor/atrazine (Bicep II Magnum, Syngenta Crop Protection, Greensboro, NC 27419) was applied at 2,470 g ai ha^{-1} on May 2, 2018 and May 2, 2019 using a tractor sprayer to entire research site to achieve early season control of small seeded weeds (Geier et al. 2009; Grichar et al. 2003; Steele et al. 2005). This PRE herbicide resulted in high survival of velvetleaf and low survival of other weed species in the experiment (Anonymous 2014a; Taylor-Lovell and Wax 2001). Except for *S*-metolachlor/atrazine applied PRE, POST herbicides were applied with a CO_2 pressurized backpack sprayer and a boom equipped with five TTI 110015 flat-fan nozzles for treatments included dicamba (TeeJet, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189) or five AIXR 110015 flat-fan nozzles spaced 51 cm apart for other herbicide treatments. POST herbicides were applied to 12 to 15 cm tall velvetleaf on June 8 and June 10 in 2018 and 2019, respectively and 24 to 30 cm tall velvetleaf on June 22 and June 17 in 2018 and 2019, respectively. Popcorn growth stages when velvetleaf reached 12 to 15 and 24 to 30 cm were V6 and V9, respectively in 2018; and V5 and V8 in 2019.

Data Collection. Velvetleaf control was assessed visually on a scale of 0 to 100%, with 0% representing no control and 100% representing complete control at 14 and 28 d after treatment (DAT). Popcorn injury was assessed on a scale of 0 to 100%, with 0% representing no injury and 100% representing plant death at 14 and 28 DAT. Velvetleaf densities were assessed from two randomly placed 0.5 m2 quadrats in each plot at 14 and 28 DAT. Velvetleaf aboveground biomass was assessed from two randomly placed 0.5 m^2 quadrat in each plot at 45 d after POST herbicides were applied. Surviving velvetleaf plants were cut near the soil surface, dried in paper bags at 65 C for 10 d, and dry weight was recorded. Percent biomass reduction compared with the no-POST herbicide control was calculated using the equation (Wortman 2014):

$$\% \text{ Biomass reduction} = [(C-B)/C] \times 100 \qquad [1]$$

where C represents the velvetleaf biomass from the no-POST herbicide control plot in the corresponding replication block and B represents the biomass of the treatment plots. At popcorn harvest, five velvetleaf plants (if present) from each plot were collected and capsules were counted. Popcorn was harvested from the middle two rows with a plot combine and the yields were adjusted to 14% grain moisture content.

Statistical Analysis. Data were subjected to ANOVA in R version 3.5.1 utilizing the base packages (R Core Team, 2019) and the Agricolae: Statistical Procedures for Agricultural Research Package (Mendiburu 2017). ANOVA was performed using the sp.plot (split plot) function where velvetleaf height (12 to 15 cm or 24 to 30 cm) was treated as the main plot and POST herbicides were considered as the subplot effect. Replications nested within years were considered random effects in the model. ANOVA assumptions of normality and homogeneity of variance were tested (Kniss and Streibig 2018). Improvement in normality were gained for density and biomass data with a logit transformation. Back-transformed data are presented in tables for interpretation. If the random effect of year was significant, data were analyzed with years separated. Treatment means were separated at $P \leq 0.05$ using Fisher's protected least significant difference (LSD) test. Orthogonal contrast analysis was conducted to compare velvetleaf control between herbicide programs that include fluthiacet-methyl, dicamba, and programs that do not include fluthiacet-methyl or dicamba.

Table 4-1. Herbicide programs for POST control of velvetleaf in popcorn in field experiments conducted at the University of Nebraska, South Central Agricultural Laboratory near Clay Center, NE in 2018 and 2019.

Herbicide program	Rate g ai ha^{-1}	Trade name	Manufacturer	Adjuvant[b]
No-POST herbicide[a]	---			
Carfentrazone-ethyl	17.5	Aim EC	FMC	NIS 0.25% v/v
Fluthiacet-methyl	7.2	Cadet	FMC	NIS 0.25% v/v + AMS 2.86 kg ai ha^{-1}
Topramezone	24.5	Impact	Amvac	MSO 1.5% v/v
Tembotrione	98	Laudis	Bayer	MSO 1% v/v
Halosulfuron-methyl	52.5	Permit	Gowan	0.5% v/v
Dicamba	560	DiFlexx	Bayer	NIS 0.25% v/v + AMS 2.86 kg ai ha^{-1}
Dicamba/diflufenzopyr	392	Status	BASF	NIS 0.25% v/v + AMS 2.86 kg ai ha^{-1}
Dicamba/tembotrione	597	DiFlexx DUO	Bayer	COC 1% v/v
Fluthiacet-methyl/mesotrione	2.8	Solstice	FMC	NIS 0.25% v/v
Nicosulfuron/mesotrione	118	Revulin Q	Corteva	NIS 0.25% v/v
Dicamba/ halosulfuron-methyl	190	Yukon	Gowan	NIS 0.25% v/v

[a] The experimental site was treated with 2,470 g ai ha^{-1} of *S*-metolachlor/atrazine applied PRE including the no-POST herbicide control plots.

[b] Abbreviations: AMS, ammonium sulfate; COC, crop oil concentrate; NIS, non-ionic surfactant.

4.4 RESULTS AND DISCUSSION

Average daily temperatures and precipitation in 2018 and 2019 growing season were similar to the 30-yr average for the experimental site (Figure 4-1). Year was significant for all measured variables, except velvetleaf seed capsules; therefore, data were analyzed separately.

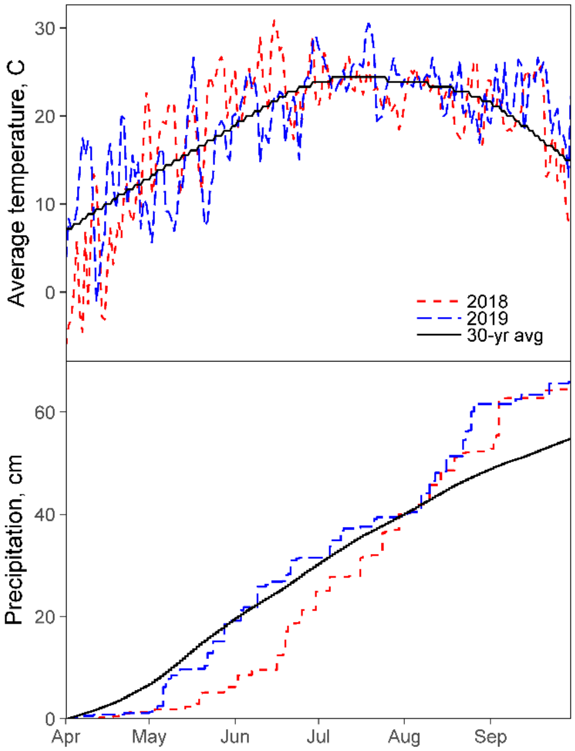

Figure 4-1. Average daily air temperature, total precipitation during 2018 and 2019 growing seasons at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE.

Velvetleaf Control. Velvetleaf control 14 DAT varied across years and an interaction between velvetleaf height and herbicide program occurred in both years of the study; however, at 28 DAT in 2019, velvetleaf height did not affect herbicide efficacy (Table 2). Most POST herbicide programs controlled velvetleaf ≥ 95% at 14 and 28 DAT regardless of velvetleaf height at application. Cafentrazone, fluthiacet-methyl, dicamba, dicamba/diflufenzophyr, and fluthiacet-methyl/mesotrione resulted in ≥ 95% control 14

DAT regardless of velvetleaf height at the time of application in 2018 and 2019. Similarly, Barnes et al. (2019b) reported velvetleaf that survived a PRE herbicide were controlled 99% with dicamba/diflufenzopyr, 98% with dicamba/tembotrione, and 95% with fluthiacet-methyl/mesotrione 21 DAT in popcorn. Sarangi and Jhala (2018c) reported velvetleaf that survived flumioxazin/pyroxasulfone applied PRE was controlled 98% 14 DAT with 5 g ai ha–1 fluthiacet-methyl applied when velvetleaf was 12 cm tall. Bussan et al. (2001) reported 0 to 7% survival of 5 cm velvetleaf treated with dicamba at 560 g ai ha–1 plus 28% nitrogen at 1.25% v/v.

Topramezone provided 91% control of 12 to 15 cm velvetleaf but only 64% control of 24 to 30 cm velvetleaf at 14 DAT in 2018. Topramezone is labeled for velvetleaf control that are < 20 cm tall (Anonymous 2019); therefore, relatively less control of 24 to 30 cm velvetleaf was expected. In contrast, topramezone resulted in ≥ 98% control in 2019 regardless of velvetleaf height. The lower level of velvetleaf control with topramezone in 2018 might be due to lower than average precipitation and higher than average maximum daily temperatures in June 2018 compared with June 2019 (Figure 4-2). When weeds are under stress, herbicide efficacy reduces. For example, Godar et al. (2015) reported greater control, shorter plants, and greater mortality in Palmer amaranth (Amaranthus palmeri S. Watson) treated with mesotrione at low temperatures (25/15 C day/night) compared to high temperatures (40/30 C day/night) with 85% control obtained with 14.9 and 80.8 g ai ha^{-1} in low and high temperatures, respectively. Tembotrione resulted in 80-81% control of 12 to 30 cm tall velvetleaf 14 DAT in 2018. Control with tembotrione of 12 to 15 cm tall velvetleaf was 99% compared to 81% control of 24 to 30 cm velvetleaf in 2019. In 2018, halosulfuron-methyl resulted in 84 and 90% control of 12 to 15 and 24 to 30 cm velvetleaf 14 DAT, respectively. In 2019, halosulfuron provided 99 and 95% control of 12 to 15 and 24 to 30 cm tall velvetleaf 14 DAT, respectively.

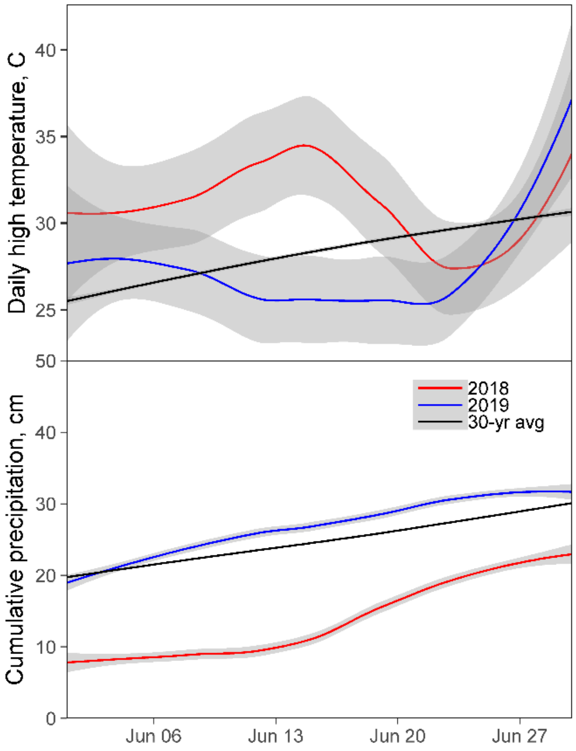

Figure 4-2. Maximum daily air temperature and total cumulative precipitation during June 2018 and 2019 compared to the 30-year average at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE.

Control of velvetleaf with dicamba/tembotrione was 88 to 94% in 2018 and 99% in 2019. Similarly, control of velvetleaf at 14 DAT with nicosulfuron/mesotrione (99%) and dicamba/halosulfuron-methyl in 2019 was 96-99% than control with nicosulfuron/mesotrione (84-92%) and dicamba/halosulfuron-methyl (86-90%) in 2018. Orthogonal contrasts indicated that POST herbicide programs with fluthiacet-methyl applied to 12 to 15 cm tall velvetleaf resulted in 99% control 14 DAT compared with herbicide programs without fluthiacet-methyl or dicamba in 2018 (89%) (Table 4-2). POST herbicide programs with dicamba applied to 12 to 15 cm velvetleaf resulted in 93% control 14 DAT compared with herbicide programs without fluthiacet-methyl or dicamba (89%) in 2018. Similarly, herbicide programs with fluthiacet-methyl resulted in 98% control compared to 92% control with dicamba based programs and 83% control without fluthiacet-methyl or dicamba for 24 to 30 cm tall velvetleaf 14 DAT in 2018. In 2019, herbicide programs with or without fluthiacet-methyl or dicamba resulted in 98 to 99% control of 12 to 15 cm tall velvetleaf 14 DAT. With 24 to 30 cm velvetleaf, herbicide programs that included fluthiacet-methyl (99%) or dicamba (98%) achieved slightly better control than without either (94%) 14 DAT in 2019. New dicamba products labeled for use in dicamba-resistant soybean do not allow for ammonium sulfate because it increases dicamba volatility (Zollinger et al. 2016). However, ammonium sulfate is commonly used as a water conditioner and the ammonium increases herbicide absorption and translocation (Zollinger et al. 2016). Tank-mixing ammonium sulfate with dicamba has been shown to increase dicamba efficacy in redroot pigweed (*Amaranthus retroflexus* L.) and common lambsquarters (*Chenopodium album* L.) (Roskamp et al. 2013). Nebraska Extension recommends that ammonium sulfate not be added to dicamba to reduce off target injury (Klein et al. 2018). Ammonium sulfate was added in this study to dicamba and dicamba/diflufenzopyr as recommended in the product labels; therefore, if recommendations to exclude ammonium sulfate from dicamba applications is followed, relatively less control of velvetleaf is expected or warrants an investigation.

Velvetleaf control at 28 DAT varied across years. Velvetleaf (12 to 15 cm tall) control ranging from 80 to 89% was achieved with dicamba/halosulfuron-methyl, halosulfuron-methyl, and tembotrione 28 DAT in 2018. More than 95% control of 12 to 15 cm tall velvetleaf was achieved with the rest of herbicide treatments 28 DAT in 2018. Carfentrazone-ethyl applied at 35 g ai ha^{-1} has been reported to provide 98% control of velvetleaf 30 DAT, but it was applied when velvetleaf was not more than 10 cm tall (Durgan et al. 1997). Sarangi and Jhala (2018c) reported 95% control of velvetleaf 28 DAT with 5 g ai ha^{-1} fluthiacet-methyl applied to 12 cm velvetleaf that survived flumioxazin/pyroxasulfone applied PRE.

Dicamba/halosulfuron-methyl, halosulfuron-methyl alone, and nicosulfuron/mesotrione provided 90 to 92% control of 24 to 30 cm tall velvetleaf 28 DAT in 2018. Schuster et al. (2008) reported 91 to 93% control of 5 to 8 cm velvetleaf with 105-140 g ai ha^{-1} nicosulfuron/mesotrione 21 DAT. Tembotrione and topramezone achieved 85 and 69% control of 24 to 30 cm tall velvetleaf 28 DAT, respectively in 2018. Bollman et al. (2008) reported 95 to 98% control of 5 to 10 cm velvetleaf 35 DAT with 12 g ai ha^{-1} topramezone and 96 to 100% control with tembotrione at 92 g ai ha^{-1} in plots that were treated with *S*-metolachlor applied PRE. All other herbicide programs achieved > 95% control of 24 to 30 cm velvetleaf 28 DAT in 2018. Velvetleaf control was not affected by plant height in 2019 (Table 2). All herbicide programs achieved 99% control of 12 to 15 and 24 to 30 cm velvetleaf 28 DAT in 2019. Herbicide programs with fluthiacet-methyl or dicamba did not differ from other herbicide programs 28 DAT in 2019. These herbicides are not necessarily labeled for control of velvetleaf at this size. For example, dicamba is labeled to provide effective control of 3 to 8 cm tall velvetleaf, fluthiacet-methyl/mesotrione for velvetleaf < 13 cm, and tembotrione for velvetleaf < 15 cm. In contrast, few herbicides evaluated in this research are labeled for velvetleaf height within the tested height range including topramezone (up to 20 cm), dicamba/halosulfuron-methyl (up to 23 cm), nicosulfuron/mesotrione (up to 25 cm), halosulfuron-methyl (up to 30 cm), carfentrazone-ethyl (up to 61 cm), and fluthiacet-methyl (up to 91 cm). Fluthiacet-methyl/mesotrione and nicosulfuron/mesotrione are labeled only in yellow popcorn and should not be applied in white popcorn. Contrast analysis of 12 to 15 cm velvetleaf control 28 DAT indicated that herbicide programs with fluthiacet-methyl (98%) and dicamba (95%) were similar to each other but provided greater control than herbicide programs without fluthicet-methyl or dicamba (90%) in 2018. Similarly, control of 24 to 30 cm velvetleaf 28 DAT with herbicide programs containing fluthiacet-methyl (98%) or dicamba (96%) provided greater control than herbicide program without fluthiacet-methyl or dicamba (87%) in 2018. Herbicide programs with fluthiacet-methyl, dicamba, or neither of them resulted in 99% control of 12 to 30 cm velvetleaf 28 DAT in 2019.

Velvetleaf Density. Velvetleaf density at 28 DAT varied by year. Velvetleaf height at POST herbicide application did not influence velvetleaf density in either year of the study (Table 3). Velvetleaf density in no-POST herbicide plots was 83 plants m^{-2} in 2018 compared with 113 plants m^{-2} in 2019. Velvetleaf density in herbicide programs ranged from 2 to 58 plants m^{-2} in 2018. Velvetleaf density following application of carfentrazone-ethyl, fluthiacet-methyl, dicamba, dicamba/diflufenzopyr, dicamba/tembotrione, and fluthiacet-methyl/mesotrione was $\leq$ 9 plants m^{-2} in 2018. The greatest velvetleaf densities of 52, 53, and 58 plants m^{-2} resulted from topramezone, halosulfuron-methyl, and tembotrione, respectively in 2018. As expected based on control ratings 28 DAT, velvetleaf density ranged from 0 to 1 plants m^{-2} for all herbicide programs in 2019. Orthogonal contrasts indicated that herbicide programs with fluthiacet-methyl reduced velvetleaf density (2 plants m^{-2}) greater than herbicide programs without fluthiacet-methyl or dicamba (37 plants m^{-2}) in 2018 (Table 3). Similarly, herbicide programs that included dicamba resulted in less velvetleaf density (12 plants m^{-2}) than herbicide programs without fluthiacet-methyl or dicamba. When herbicide programs with fluthiacet-methyl and dicamba are compared, treatments with fluthiacet-methyl resulted in 2 plants m^{-2} compared to 12 plants m^{-2} with dicamba in 2018. Sarangi and Jhala (2018c) reported 3 velvetleaf plants m^{-2} at 28 DAT with 5 g ai ha^{-1} fluthiacet-methyl applied to 12 cm velvetleaf that survived flumioxazin/pyroxasulfone applied PRE compared to 16 plants m^{-2} when only the PRE herbicide was applied.

Biomass Reduction. Velvetleaf biomass reduction varied by year. Velvetleaf plant height at the time of POST herbicide application did not affect velvetleaf biomass reduction. In 2018, 78 to 100% biomass reduction was observed across POST herbicide programs (Table 3). Carfentrazone-ethyl, fluthiacet-methyl, dicamba, dicanba/diflufenzophyr, dicamba/tembotrione, and fluthiacet-methyl/mesotrione reduced velvetleaf biomass 99 to 100%. The least biomass reduction across herbicide programs was 78% from topramezone, halosulfuron-methyl with 83% reduction, and tembotrione with 84% biomass reduction. In 2019, all herbicide programs resulted in at least 98% biomass reduction. Zhang et al. (2013) reported 90% velvetleaf biomass reduction with topramezone at 15.84 g ai ha^{-1} plus 0.3% methylated seed oil (MSO). Hart (1997) reported halosulfuron-methyl at 9 g ai ha^{-1} plus dicamba at 140 g ai ha^{-1} plus crop oil concentrate (COC) or MSO 1% v/v resulted in 87% velvetleaf biomass reduction.

Table 4-2. Comparison of POST herbicide programs for control of 12 to 15 and 24 to 30 cm tall velvetleaf in popcorn at 14 and 28 d after treatment (DAT) in field experiments conducted at the University of Nebraska, South Central Agricultural Laboratory near Clay Center, NE in 2018 and 2019.

		Velvetleaf control 14 DAT				Velvetleaf control 28 DAT		
		2018		2019		2018		2019
		Velvetleaf height[b]						
Herbicide program[a]	Rate	12 to 15 cm	24 to 30 cm	12 to 15 cm	24 to 30 cm	12 to 15 cm	24 to 30 cm	12 to 15 and 24 to 30 cm
	g ai ha^{-1}	%						
No-POST herbicide	---	0 e	0 g	0 b	0 c	0 e	0 f	0 b
Carfentrazone-ethyl	17.5	98 a	98 ab	99 a	99 a	98 a	98 ab	99 a
Fluthiacet-methyl	7.2	99 a	99 a	99 a	99 a	98 a	99 a	99 a
Topramezone	24.5	91 abc	64 f	99 a	98 a	94 ab	69 e	99 a
Tembotrione	98	81 d	80 e	99 a	81 b	80 d	86 d	99 a
Halosulfuron-methyl	52.5	84 cd	90 bcd	99 a	95 a	85 cd	91 bcd	99 a
Dicamba	560	96 a	95 abc	97 a	97 a	98 a	98 ab	99 a
Dicamba/diflufenzopyr	392	98 a	97 ab	99 a	99 a	97 a	99 a	99 a
Dicamba/tembotrione	597	94 ab	88 cde	99 a	99 a	97 a	95 abc	99 a
Fluthiacet-methyl/mesotrione	2.8	99 a	98 ab	99 a	99 a	99 a	98 ab	99 a
Nicosulfuron/mesotrione	118	92 abc	84 de	99 a	99 a	96 a	90 cd	99 a
Dicamba/halosulfuron-methyl	190	86 bcd	90 bcd	99 a	96 a	89 bc	92 bcd	99 a
P-Value		<0.001		<0.001		<0.001		<0.001
Orthogonal contrasts[c]								
Fluthiacet-methyl herbicides vs. non-dicamba herbicides		99 vs. 89***	98 vs. 83***	99 vs 99 NS	99 vs. 94***	98 vs. 90***	98 vs. 87***	99 vs. 99 NS
Dicamba herbicides vs. non-fluthiacet-methyl herbicides		93 vs. 89***	92 vs. 83***	98 vs. 99 NS	98 vs. 94**	95 vs. 90***	96 vs. 87***	99 vs. 99 NS
Fluthiacet-methyl herbicides vs. dicamba herbicides		99 vs. 93***	98 vs. 92***	99 vs. 98 NS	99 vs. 98 NS	98 vs. 95 NS	98 vs. 96 NS	99 vs. 99 NS

[a] The experimental site was treated with 2,470 g ai ha^{-1} of *S*-metolachlor/atrazine as a preemergence including the no-POST control.

[b] Means presented within the same column with no common letter(s) are significantly different according to Fisher's Protected LSD where α = 0.05.

[c] Significance levels: NS, non-significant; *P < 0.05; **P < 0.01; * * *P < 0.001.

Table 4-3. Comparison of velvetleaf density 28 DAT, velvetleaf biomass 45 days after 24 to 30 cm application, and 2019 velvetleaf capsule yield in herbicide programs for control of 12 to 15 and 24 to 30 cm tall velvetleaf in popcorn in field experiments conducted at the University of Nebraska, South Central Agricultural Laboratory near Clay Center, NE in 2018 and 2019.

		Density 28 DAT[b]		Biomass reduction[b]		Capsules[b,c]
Herbicide program[a]	Rate	2018	2019	2018	2019	2018/2019
	g ai ha^{-1}	-------- m^{-2} --------		------------- % -------------		capsules plant^{-1}
No-POST herbicide	---	83 a	113 a	0	0	9 a
Carfentrazone-ethyl	17.5	4 ef	0 b	100 ab	100	0 b
Fluthiacet-methyl	7.2	2 fg	1 b	100 a	100	0 b
Topramezone	24.5	52 ab	1 b	78 e	99	2 ab
Tembotrione	98	58 ab	0 b	84 de	100	0 b
Halosulfuron-methyl	52.5	53 ab	1 b	83 de	99	0 b
Dicamba	560	5 def	0 b	100 a	100	0 b
Dicamba/diflufenzopyr	392	6 cde	1 b	99 a	99	1 ab
Dicamba/tembotrione	597	9 cde	0 b	97 bc	100	0 b
Fluthiacet-methyl/ mesotrione	2.8	2 fg	0 b	100 a	100	0 b
Nicosulfuron/ mesotrione	118	17 cd	1 b	99 ab	98	3 ab
Dicamba/ halosulfuron-methyl	190	30 bc	0 b	94 cd	100	0 b
P-Value		<0.001	<0.001	<0.001	NS	<0.001
Orthogonal contrasts[c]						
Fluthiacet-methyl herbicides vs. non-dicamba herbicides		2 vs. 37 ***	0 vs. 0 NS	100 vs. 89 ***	100 vs. 99 NS	0 vs. 1 NS
Dicamba herbicides vs. non-fluthiacet-methyl herbicides		12 vs. 37 **	0 vs. 0 NS	98 vs 89 **	100 vs. 99 NS	0 vs. 1 NS
Fluthiacet-methyl herbicides vs. dicamba herbicides		2 vs. 12 *	0 vs. 0 NS	100 vs. 98 NS	100 vs. 100 NS	0 vs. 0 NS

[a]The experimental site was treated with 2,470 g ai ha^{-1} of *S*-metolachlor/atrazine applied PRE including the no-POST herbicide treatment.

[b]Means presented within the same column with no common letter(s) are significantly different according to Fisher's Protected LSD where $\alpha = 0.05$.

[c]There was no significant difference between number of velvetleaf seed capsules; therefore, data of both years were combines.

[d]Significance levels: NS, non-significant; *P < 0.05; **P < 0.01; * * *P < 0.001.

Velvetleaf Seed Capsules. The no-POST herbicide plots resulted in an average of 9 capsules plant^{-1} (Table 3). Lindquist et al. (1995) report that each velvetleaf capsule contains about 40 seeds. Dicamba/diflufenzopyr, topramezone, and nicosulfuron/mesotrione resulted in 1, 2, and 3 capsules plant^{-1}, respectively without difference among other treatments. The amount of seed production is most likely reduced with all herbicide programs considering the capsule production and velvetleaf density 28

DAT observed in this study. Schmenk and Kells (1998) reported 50% less seed production in velvetleaf that escaped atrazine than non-treated plants. Murphy and Lindquist (2002) reported that velvetleaf that survived halosulfuron, dicamba, or flumiclorac applied POST produced the same number of capsules plant^{-1} as the no-POST herbicide; however, velvetleaf density was reduced resulting in significantly less seed production. Terra et al. (2007) reported velvetleaf treated with dicamba, halosulfuron, or flumiclorac produced fewer capsules and seeds than non-treated velvetleaf. Bussan et al. (2001) reported velvetleaf seed production is correlated with velvetleaf biomass in corn and soybean production systems.

Popcorn Injury and Yield. Popcorn injury was not observed in any of herbicide programs tested in this study during both years. Yield varied by year; however, there was no effect of herbicide program or velvetleaf height on yield (Table 4). Popcorn yields in 2018 ranged from 4,691 kg ha^{-1} with halosulfuron-methyl to 5,597 kg ha^{-1} with fluthiacet-methyl/mesotrione. The no-POST herbicide plots yielded 5,198 kg ha^{-1} in 2018. Popcorn yield in 2019 was poor due to significant rain events in May that resulted in poor crop stand and hail and wind damage in August that resulted in lodging. In 2019, the no-POST herbicide treatment resulted in 803 kg ha^{-1}. It has to be noted that no-POST herbicide plots in this study also received atrazine/S-metolachlor applied PRE that provided partial control of velvetleaf. Additionally, the majority of velvetleaf in no-POST herbicide plots emerged after popcorn emerged; therefore, were not very competitive. A lack of yield loss due to surviving velvetleaf plants is not unexpected because it has been reported in the literature. For example, Liphadzi and Dille (2006) reported that velvetleaf that escaped flumetsulam applied POST (3 plants m^{-2}) caused only 3% field corn yield loss compared to 38% yield loss with the same density allowed to compete without herbicide program. Weaver (1991) reported no soybean yield loss with velvetleaf densities up to 11 plants m^{-2} that survived metribuzin applied preplant incorporated. Schmenk and Kells (1998) reported no field corn yield loss from velvetleaf that survived atrazine or pendimethalin applied PRE. Terra et al. (2007) reported that velvetleaf surviving dicamba, halosulfuron-methyl, or flumiclorac were less competitive with field corn than plants in no-POST herbicide plots. Reduced competitiveness in other weeds surviving an herbicide has been reported such as Palmer amaranth in field corn treated with isoxaflutole or flumetsulam (Liphadzi and Dille 2006) and common cocklebur (*Xanthium strumarium* L.) and sicklepod (*Senna obtusifolia* L. H.S. Irwin & Barneby) in soybean treated with alachlor and metribuzin.

Table 4-4. Comparison of popcorn yield in POST herbicide programs for control of 12 to 15 and 24 to 30 cm tall velvetleaf in field experiments conducted at the University of Nebraska, South Central Agricultural Laboratory near Clay Center, NE in 2018 and 2019.

		Yield	
Herbicide program[a]	Rate	2018[b]	2019[b]
	g ai ha^{-1}	------- kg ha^{-1} -------	
No-POST herbicide		5,198	803
Carfentrazone-ethyl	17.5	5,492	782
Fluthiacet-methyl	7.2	5,439	942
Topramezone	24.5	5,207	1,382
Tembotrione	98	5,264	908
Halosulfuron-methyl	52.5	4,440	925
Dicamba	560	4,818	902
Dicamba/diflufenzopyr	392	4,747	1,256
Dicamba/tembotrione	597	4,853	1,308
Fluthiacet-methyl/mesotrione	2.8	5,383	1,222
Nicosulfuron/mesotrione	118	5,300	1,473
Dicamba/halosulfuron-methyl	190	4,725	988
P-Value[b]		NS	NS

[a] The experimental site was treated with 2,470 g ai ha^{-1} of *S*-metolachlor/atrazine applied PRE including the no-POST herbicide treatment.

[b] Significance levels: NS, non-significant; *P < 0.05; **P < 0.01; * * *P < 0.001.

Practical Implications. Weed management in no-till popcorn is chiefly dependent on herbicides. Selecting POST herbicide is challenging in popcorn than field corn due to exclusionary herbicide labels. There are few control options for weeds that escape PRE herbicides and reach height above most label recommendations because popcorn has limited labeled herbicides. Velvetleaf was effectively controlled by a number of POST herbicides tested in this study at heights ranging from 12 to 30 cm. Fluthiacet-methyl and carfentrazone-ethyl provided 98 to 99% control and are labeled for up to 91 and up to 62 cm tall velvetleaf, respectively. With the addition of ammonium sulfate, dicamba and dicamba/diflufenzopyr also provided ≥ 95% velvetleaf control. Results of this study conclude that effective POST herbicides are available for control of 12 to 30 cm velvetleaf in popcorn production. Popcorn yield reduction was not observed with any herbicide program, including in the no-POST herbicide control. This is not unexpected based on information known about the reduced competitiveness of weeds that survive herbicide as previously discussed. The reduction in velvetleaf seed production observed with near complete control provided by some of the most effective herbicides in 2019 is an important weed management principle especially considering the longevity of velvetleaf in the seedbank. As of 2019, only atrazine resistant velvetleaf has been reported (Heap 2019); however, multiple effective sites of action herbicide programs should be used to delay the evolution of herbicide-resistant velvetleaf.

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CHAPTER 5 DOSE RESPONSE OF WHITE AND YELLOW POPCORN HYBRIDS TO GLYPHOSATE, 2,4-D CHOLINE/GLYPHOSATE, OR DICAMBA AT TWO GROWTH STAGES

5.1 ABSTRACT

Commercial popcorn production fields are in proximity to field corn and soybean production fields. The objective of this research was to determine the effect of glyphosate, 2,4-D choline/glyphosate, or dicamba on the injury, above ground biomass reduction, height reduction, and yield loss of commercially available white and yellow popcorn hybrids at two growth stages. Field experiments were conducted near Clay Center, Nebraska in 2017 and 2018. Treatments included non-treated control and four rates of glyphosate, 2,4-D choline/glyphosate, or dicamba applied POST at V5 or V8 popcorn growth stages. A three-parameter log-logistic model was fitted to each herbicide. Glyphosate and 2,4-D choline/glyphosate applied at V5 growth stage had greater popcorn injury, biomass reduction, height reduction, and yield loss than the V8 growth stage. The white popcorn hybrid had greater injury and biomass reduction from 2,4-D choline/glyphosate and greater biomass reduction from glyphosate at the V5 growth stage compared with the yellow popcorn hybrid. At the V8 application, 2,4-D choline/glyphosate resulted in greater injury in the white hybrid; however no hybrid differences in glyphosate sensitivity were observed at the V8 growth stage. Glyphosate and 2,4-D choline/glyphosate at 0.25X rates resulted in complete plant death in both hybrids, whereas the highest dicamba dose (2X) caused 11% injury and no biomass reduction, plant height reduction, or yield loss. These results demonstrate the sensitivity of popcorn to glyphosate, 2,4-D choline/glyphosate, or dicamba and can be of immediate

educational use and practical implementation for popcorn producers, herbicide applicators, and agronomists.

5.2 INTRODUCTION

Herbicide labels often exclude white popcorn as it is thought to be more sensitive to herbicides than yellow popcorn (Loux et al., 2017). Exclusionary labels include premixes of atrazine plus mesotrione plus *S*-metolachlor (Syngenta Crop Protection, 2009; Syngenta Crop Protection, 2012), acetochlor plus mesotrine plus clopyralid (Corteva Agriscience, 2017b), and atrazine plus bicyclopyrone plus mesotrione plus *S*-metolachlor (Syngenta Crop Protection, 2017). Despite the general perception that white popcorn hybrids are less tolerant to herbicides, evidence has not been published. Transgenic, herbicide-resistant hybrids of popcorn are not commercially available; therefore, popcorn is not resistant to commonly used POST herbicides such as glyphosate or glufosinate in much of the field corn grown in the United States (Fernandez-Cornejo et al., 2014; Ziegler, 2001).

A statewide survey reported glyphosate as the most used POST herbicide in glyphosate-resistant corn and soybean in Nebraska (Sarangi and Jhala, 2018a). According to Kniss (2017), there were 0.9 glyphosate area treatments in field corn in 2014 and 1.4 glyphosate area treatments in soybean in 2015 in the United States. This accounts for 26 and 43% of all herbicide area treatments in corn and soybean, respectively. Group 4 site of action herbicides (synthetic auxins), have an average of 0.36 area treatments per year in corn from 1990 to 2014 (Kniss, 2017). Dicamba use has increased in recent years with rapid evolution of glyphosate-resistant weed biotypes and the commercialization of dicamba-resistant soybean. In Nebraska, about 19% of the 2.3 million ha of soybean planted in 2017 were dicamba-resistant of which an estimated 80% were treated with dicamba (Werle et al., 2018). Bayer Crop Science estimated 16.2 million ha planted to dicamba-resistant soybean in 2018 or around half of the soybean production area in the United States (Werle et al., 2018). The adoption of dicamba-resistant soybean increased to about 50% of total soybean planted in Nebraska for 2018 (Jhala, 2018). In 2018, 94% of the United States and 96% of Nebraska soybean planted had an herbicide-resistant trait (USDA NASS, 2019). The possible herbicide-resistant traits in soybean include glyphosate, glyphosate/dicamba, or glufosinate. Herbicide-resistant field corn represents 80% of the field corn in the United States and 84% of Nebraska field corn in 2018 (USDA NASS, 2019). Corn resistant to 2,4-D choline/glyphosate/glufosinate was commercialized in 2018; and soybean resistant to these herbicides will be available commercially in the near future. That outcome will inevitably increase the use of 2,4-D choline particularly for control of glyphosate-resistant common waterhemp [*Amaranthus tuberculatus* (Moq.) J. D. Sauer] and Palmer amaranth (*Amaranthus palmeri* S. Watson) in Nebraska.

Given the aforementioned production dynamics and current and future potential challenges with popcorn production, a pest management strategic plan was generated following a workshop by key players of the popcorn industry, including popcorn growers, agronomists, university faculty and agricultural professionals, and popcorn breeders to communicate pest, pesticides, and pest management practices available in popcorn and to identify the challenges associated with pest management in popcorn (Pike et al., 2002). This strategic plan was based on the information gathered in the crop profile for popcorn (Bertalmio et al., 2003). The pest management strategic plan for popcorn prioritized regularity, research, and education priorities for enhancing popcorn production and production efficiency (Pike et al., 2002). The strategic plan outlined the importance of educating custom applicators about the lack of herbicide-resistant traits in popcorn compared to field corn and the necessity of checking herbicide labels for special instructions or reduced rates in regard to popcorn when herbicides are labeled for both crops (Pike et al., 2002). Popcorn is grown in proximity primarily to corn, soybean, and wheat (*Triticum aestivum* L.) in the Midwest. One regulatory priority in the strategic plan is the critical issue of hybrid sensitivity to new herbicides as they are being registered for use in corn/soybean with the potential of drift or carryover injury to popcorn (Pike et al., 2002). Drift issues present significant challenges for both popcorn and other crop production in terms of herbicide applications, especially in regions with high wind speeds during the growing seasons such as Nebraska and other Midwestern states. For example, in south central Nebraska, the monthly average growing season wind speeds at 2 m height ranges from 2.6 to 4.5 m sec^{-1} from March through October, respectively, which are much greater than most other states in the United States (Irmak et al., 2006), further exacerbating the herbicide drift problem.

Conventional field corn response to glyphosate has been documented in several studies (Brown et al., 2009; Buehring et al., 2007; Ellis et al., 2002; Ellis et al., 2003; Reddy et al., 2010). Additionally, Banks and Shroeder (2002) reported the response of sweet corn to simulated drift rates of glyphosate. Growth stage has been reported to influence herbicide efficacy in field corn in simulated drift studies (Ellis et al., 2003; Reddy et al., 2010). Popcorn production fields in Nebraska are commonly grown in rotation with soybean and field corn and thus are in close proximity to corn and soybean production fields. Herbicides applied POST in corn/soybean such as glyphosate, 2,4-D choline, or dicamba may result in off target movement and popcorn injury. Misapplication, drift, and tank contamination risk of glyphosate, 2,4-D choline/glyphosate, or dicamba to popcorn fields has not been assessed. The objective of this research was to determine the effect of simulated drift rates of glyphosate, 2,4-D choline/glyphosate, or dicamba on the injury, above ground biomass, height reduction, and yield loss of yellow and white popcorn hybrids at two growth stages.

5.3 MATERIALS AND METHODS

Site Description. Field experiments were conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE (40.5752˚N, 98.1428˚W and 552 m above mean sea level) in 2017 and 2018. The soil texture at the experimental site was Hastings silt loam (montmorillonitic, mesic, Pachic Argiustolls; with particle size distribution of 17% sand, 58% silt, and 25% clay) with a pH of 6.5, and 2.5-3% organic matter. The experimental site was disked before planting with a tandem disk at a depth of 10 cm and fertilized with 202 kg ha^{-1} of nitrogen in the form of anhydrous ammonia (82-0-0) and was irrigated using a linear-move irrigation system.

Experimental Design and Treatments. The study was arranged in a split plot design with main plots consisted of a 2 × 2 × 3 factorial comprising of two hybrids, two growth stages, and three herbicides. Popcorn hybrids consisted of a commonly grown white (VWP111; Conagra Brands 222 W. Merchandise Mart Plaza Chicago, IL 60654) and yellow (VYP315; Conagra Brands) popcorn hybrids. Split plots included non-treated control and four rates of glyphosate (0.25X, 0.125X, 0.063X, and 0.031X), 2,4-D choline/glyphosate (0.25X, 0.125X, 0.063X, and 0.031X), or dicamba (2X, 1X, 0.5X, and

0.25X) applied POST at V5 (EPOST) or V8 (LPOST) popcorn growth stages. The glyphosate (479.3 g ae L^{-1}) labeled rate (1X) is 1,680 g ae ha^{-1} (Durango, Corteva, 9330 Zionsville Rd, Indianapolis, IN 46268). The 2,4-D choline/glyphosate (191.7 g ae L^{-1} of 2,4-D choline [48.5%]; 203.7 g ae L^{-1} of glyphosate [51.5%]) labeled rate is 2,200 g ae ha^{-1} (Enlist DUO, Corteva, 9330 Zionsville Rd, Indianapolis, IN 46268). The dicamba (350 g ae L^{-1}) labeled rate is 560 g ae ha^{-1} (XtendiMax, Bayer CropScience, Research Triangle Park, NC 27709). The rates of glyphosate and 2,4-D choline/glyphosate are similar to the glyphosate rates tested in the literature on sweet and field corn (Banks and Schroeder, 2002; Buehring et al., 2007). Dicamba is labeled in popcorn; therefore, a maximum of 2X of the labeled rate was selected as a worst-case scenario. Plot dimensions were 9 m long by 3 m wide. On April 27, 2017 and April 26, 2018 popcorn hybrids were planted in rows spaced 76 cm apart at 4 cm depth with a planting density of 89,000 seeds ha^{-1}. Ammonium polyphosphate (APP; 10-34-0) was applied in-furrow as starter fertilizer at 6 kg ha^{-1} during planting. Atrazine/*S*-metolachlor (Bicep II Magnum, Syngenta Crop Protection, Greensboro, NC 27419) was applied PRE at 2,470 g ai ha^{-1} for early season weed control on April 27, 2017 and May 2, 2018. POST herbicide treatments were applied when popcorn reached V5 or V8 growth stages, June 14 and 29 in 2017 and May 31 and June 18 in 2018, using a handheld CO_2-pressurized backpack sprayer equipped with four AIXR 110015 flat-fan nozzles (TeeJet® Technologies, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60187) spaced 51 cm apart and calibrated to deliver 140 L ha^{-1} at 276 kPa at a constant speed of 4.8 km h^{-1}.

Data Collection. Air temperature and rainfall data were obtained from the nearest High Plains Regional Climate Center automated weather station that was located 350 m from the experimental field. Popcorn injury was assessed on a scale of 0 to 100%, with 0% representing no injury and 100% representing plant death at 21 d after early POST (DAEPOST) and 21 d after late POST (DALPOST). Popcorn plant height was measured from 6 plants plot^{-1} at 21 DAEPOST and 21 DALPOST by measuring from the soil surface to the arch of the tallest collared leaf of each plant. Aboveground popcorn biomass was collected from 4 sequential plants in the middle two rows from each plot at 70 DALPOST. Plants were cut near the soil surface, put in paper bags, and dried in an oven at 50 C for 10 d to constant weight, and dry biomass weight was recorded. Percent biomass reduction and percent height reduction compared with the non-treated control was calculated using the equation (Wortman, 2014):

$$Y = [(C–B)/C] \times 100 \qquad [1]$$

where, *Y* represents the percent biomass reduction or height reduction compared to the non-treated control plot in the corresponding replication block, *C* represents the biomass or height from the non-treated control plot and *B* represents the biomass or height of the treatment plot. Popcorn was harvested with a plot combine from the middle two rows to avoid edge effect and the yields were adjusted to 14% grain moisture content. Relative yield loss was calculated as:

$$\mathrm{YL} = 100 \times (1–\mathrm{P}/\mathrm{C}) \qquad [2]$$

where, YL is the yield loss relative to the non-treated control, P is the plot yield, and C is the yield of the non-treated control.

Statistical Analysis. R (R Core Team, 2019) base packages and the *drc: Analysis of Dose-Response Curves* package (Ritz et al., 2015) were utilized for data analysis. Injury, biomass reduction, and relative yield loss data were analyzed using the three-parameter log-logistic model (Knezevic et al., 2007):

$$Y = D/\{1 + \exp[B(\log X - \log E)]\} \qquad [3]$$

where, Y is popcorn injury, biomass reduction, height reduction, or yield loss, D is the upper limit (maximum effect; not allowed to exceed 100%), E is the ED_{50} (herbicide rate where 50% response between lower and upper limit occurs; inflection point), and B is the slope of the line at the inflection point. Models fits with separate curves for fixed effects (treatments) or random effects (years) were subjected to F-tests separated at alpha <0.05 to test for significance of effects and compared to the overall model not distinguishing between years or treatments (single curve). Data were pooled over years if the random effect of year was not significant. When the treatments effect was significant, each treatment (hybrids and growth stage combinations) was fitted to the model separately. Model parameters, B, D, and E, and the ED_5 (herbicide rate causing 5% response) were statistically compared between hybrids and growth stages using T-tests separated at alpha <0.05 utilizing the *compParm* function in R. A Pearson's correlation analysis was conducted between popcorn yield loss and popcorn injury, biomass reduction, and height reduction (Deeds et al., 2006).

Model Goodness of Fit. Root mean squared error (RMSE) and modeling efficiency (ME) were calculated to evaluate goodness of fit for popcorn injury, biomass reduction, height reduction, and yield loss models. The RMSE was calculated with equation (Barnes et al., 2018; Roman et al., 2000; Sarangi and Jhala, 2018b):

$$\mathrm{RMSE} = [1/n \sum_{i=1}^{n} (Pi - Oi)^2]^{1/2} \qquad [4]$$

where, Pi and Oi are the predicted and observed values, respectively, and n is the total number of comparisons. The smaller the RMSE, the closer the model predicted values to the observed values. The ME was calculated using following equation (Barnes et al., 2017; Mayer and Butler, 1993):

$$\mathrm{ME} = 1 - [\textstyle\sum_{\mathrm{i}=1}^{\mathrm{n}}(Oi - Pi)^2 / \sum_{\mathrm{i}=1}^{n}(Oi - \bar{O}i)^2] \qquad [5]$$

where, $\bar{O}i$ is the mean observed value and all other parameters are the same as equation 4. ME differs from coefficient of determination (R^2) only in not having a lower limit. ME values closest to 1 indicate the most accurate (perfect) predictions (Sarangi et al., 2015).

5.4 RESULTS AND DISCUSSION

The average daily air temperature and total water supply (rainfall + irrigation) to the experimental field during 2017 and 2018 growing seasons at the research site were similar (Figure 5-1). The fit of the three-parameter log-logistic model was significantly different for every response variable when curves were fitted to each year was not significantly different for any response variable compared to a single curve with years combined for glyphosate, 2,4-D choline/glyphosate, or dicamba; therefore, data were pooled across years (Table 5-1). When curves were fitted to each treatment (growth stage and hybrid combinations) the fits differed from that of a single curve, with treatments combined, for glyphosate and 2,4-D choline/glyphosate but not for dicamba visual estimates of crop injury (Table 5-1). Dicamba estimates of visual injury were pooled across treatments and a single curve was fitted.

Table 5-1. P-values of F-test (alpha < 0.05) between three-parameter log-logistic model and three-parameter log-logistic model with treatment or year effect in field experiments conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE in 2017 and 2018.

Herbicide	Response	Treatment	Year
Glyphosate	injury	<0.001	0.137
	biomass reduction	<0.001	0.244
	height reduction	<0.001	0.710
	yield loss	<0.001	0.288
2,4-D choline/ glyphosate	injury	<0.001	0.772
	biomass reduction	<0.001	0.201
	height reduction	<0.001	0.570
	yield loss	0.004	0.105
Dicamba	injury	1	0.614
	biomass reduction	NA	NA
	height reduction	NA	NA
	yield loss	NA	NA

Growth Stage. Popcorn injury response to glyphosate was greatest at the V5 growth stage (Figure 5-2A). The ED_{50} values at the V5 growth stage averaged 23 g ae ha^{-1} compared with 73 g ae ha^{-1} at the V8 growth stage (Table 5-2). The same trend was observed in the ED_5 values with glyphosate applied at the V5 growth stage averaging 5 g ae ha^{-1} compared with 46 g ae ha^{-1} at the V8 growth stage. A greater slope resulted from the V5 than for the V8 growth stage application. The upper limit reached or exceeded 100% injury for both growth stages and was not allowed to exceed 100%. With conventional field corn, Reddy et al. (2010) reported 45 and 55% injury 21 DAT when glyphosate at 105 g ae ha^{-1} was applied at V2-V4 and V6-V8 growth stages, respectively. Results of dose response in this study predicted 105 g ae ha^{-1} of glyphosate to result in 71-78% and 56-60% injury when applied at the V5 and V8 popcorn growth stages, respectively. This suggests that popcorn in this research is more sensitive to glyphosate than conventional field corn reported by Reddy et al. (2010). Ellis et al. (2003) reported

140 g ae ha^{-1} of glyphosate resulted in 64-78% injury 7 DAT when applied at the V6 conventional field corn growth stage and 0-40% injury at 7 DAT when applied at the V9 growth stage. Glyphosate at 140 g ae ha^{-1} resulted in popcorn injury of 75-82% and 73-76% when applied at the V5 and V8 popcorn growth stage, respectively; lower than that reported in the literature for conventional field corn. In general, glyphosate application at the V5 growth stage resulted in greater biomass reduction than the V8 growth stage, which may indicate greater sensitivity of popcorn to herbicide at V5 stage (Figure 5-2C). The ED_{50} and ED_5 were lower for the V5 growth stage than the V8 growth stage (Table 5-3). The slope and upper limit were similar between both growth stages. Glyphosate applied at the V5 growth stage resulted in greater height reductions compared with the V8. For example, ED_{50} for glyphosate applied at V5 stage averaged41 g ae ha^{-1} for the two hybrids compared with 82 g ae ha^{-1} at the V8. Similarly, a greater amount of glyphosate was needed to reduce popcorn height by 5% (ED_5) at the V8 growth stage (Figure 5-2E). The maximum popcorn height reduction (upper limit) following glyphosate application was greater for the V5 application (91%) than the V8 application (75%) for the white hybrid (Table 5-4). This was not the case for the yellow hybrid. The slopes were similar between hybrids and growth stages. Ellis et al. (2003) reported 10-87% height reduction from 140 g ae ha^{-1} of glyphosate application at 7 DAT at the V6 conventional corn growth stage and 4-32% reduction at 7 DAT at the V9 growth stage. Glyphosate applied at V5 resulted in greater yield loss than the V8 (Figure 5-2G). For instance, the ED_{50} averaged 16 g ae ha^{-1} for the two hybrids at the V5 and 64 g ae ha^{-1} at the V8 growth stage (Table 5-5). The ED_5 followed a similar trend as the ED_{50} shifting from 1 g ae ha^{-1} at the V5 to 13 g ae ha^{-1} at the V8 growth stage. The slope and upper

limit did not vary between growth stages. Ellis et al. (2003) reported 78 and 33% yield loss with glyphosate at 140 g ae ha^{-1} applied at the V6 and V9 growth stages, respectively, in conventional corn.

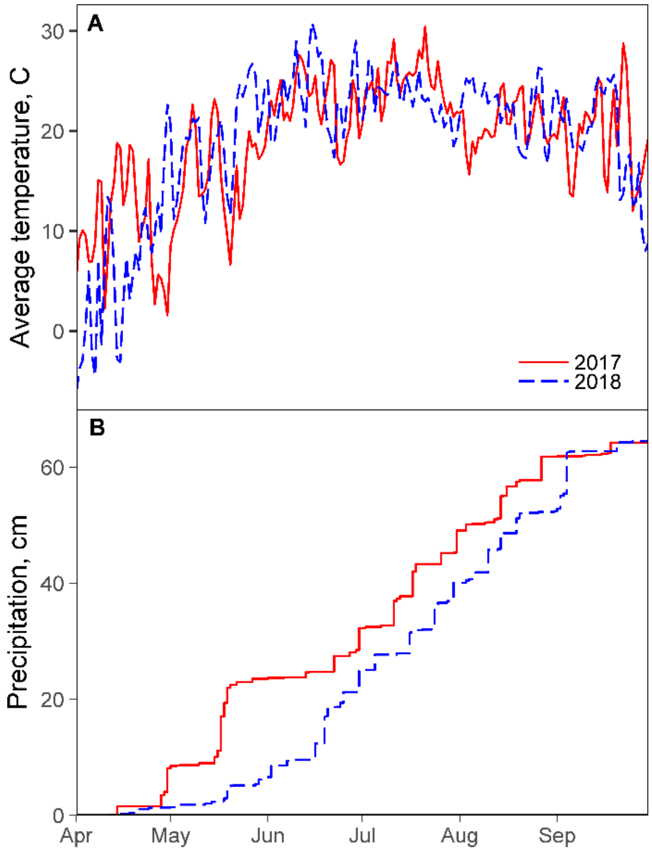

Figure 5-1. (A) Average daily air temperature and (B) total water supply (rainfall + irrigation) during 2017 and 2018 growing seasons at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, Nebraska.

Similar to glyphosate alone, 2,4-D choline/glyphosate applied at the V5 stage resulted in more injury than when applied at the V8 growth stage (Figure 5-2B). For example, the white popcorn hybrid at the V5 growth stage resulted in ED_{50} of 35 g ae ha^{-1} compared with 77 g ae ha^{-1} at the V8 (Table 5-2). Similarly, the yellow popcorn hybrid at the V5 growth stage resulted in ED_{50} of 72 g ae ha^{-1} compared with 98 g ae ha^{-1} at the

V8 stage. The ED_5 was similar for the V5 and V8 growth stages in the yellow popcorn hybrid; however, the ED_5 in the white popcorn hybrid was 6 and 19 g ae ha^{-1}, respectively. The slope between the V5 and V8 application growth stages within hybrids were not significantly different. 2,4-D choline/glyphosate applied at the V5 growth stage resulted in similar biomass reduction compared to the V8 growth stage in the yellow hybrid (Figure 5-2D). However, with the white hybrid, greater biomass reduction occurred following the V5 application compared to the V8 application with ED50 of 47 g ae ha^{-1} compared with 114 g ae ha^{-1}, respectively (Table 5-3). Popcorn height reduction and yield loss due to 2,4-D choline/glyphosate was similar between hybrids (Figures 5-2F, 5-2H; Tables 5-4, 5-5).

Table 5-2. Parameter estimates (b, d, and e) and ED_5 with standard errors of the three-parameter log-logistic model, root mean squared error, and modeling efficiency used to determine the dose-response of two popcorn [Zea mays (L.) var. everta] hybrids at two growth stages to glyphosate, 2,4-D choline/glyphosate, and dicamba based on visual observations of injury in field experiments conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE in 2017 and 2018. †

Herbicide	Stage	Hybrid ‡	Slope §	Upper limit	ED_{50} §	ED_5 §	RMSE	ME
				% injury	g ae ha^{-1}		% injury	
Glyphosate	V5	VWP111	-2.1 (1.1) a	100 (0)	21 (11) a	5 (7) a	1.6	1.0
		VYP315	-1.7 (0.4) a	100 (0)	24 (6) a	4 (3) a	3.8	0.99
	V8	VWP111	-6.2 (0.5) b	100 (0)	71 (2) b	44 (2) b	7.3	0.97
		VYP315	-6.1 (0.5) b	100 (0)	75 (2) b	47 (2) b	7.4	0.97
2,4-D choline/ glyphosate	V5	VWP111	-1.7 (0.5) a	100 (0)	35 (9) a	6 (4) a	2.9	0.99
		VYP315	-2.6 (0.4) a	100 (0)	72 (3) b	23 (4) bc	7.7	0.96
	V8	VWP111	-2.1 (0.3) a	100 (0)	77 (4) b	19 (4) b	7.5	0.96
		VYP315	-2.5 (0.3) a	100 (0)	98 (4) c	31 (4) c	9.9	0.94
Dicamba	pooled	pooled	-1.5 (0.4)	100 (0)	4403 (1665)	643 (81)	5.4	0.33

† Abbreviations: ED_{50}, herbicide rate where 50% response between lower and upper limit occurs and inflection point; ED_5, herbicide rate where 5% response occurs; ME, modeling efficiency; RMSE, root mean squared error

‡ Hybrid VWP111, white kernel color, Conagra brand; hybrid VYP315, yellow kernel color, Conagra brand

§ Means presented within the same column for each herbicide with no common letter(s) are significantly different according to pairwise t-tests where $\alpha = 0.05$

Table 5-3. Parameter estimates (b, d, and e) and ED_5 with standard errors of the three-parameter log-logistic model, root mean squared error, and modeling efficiency used to determine the dose-response of two popcorn [Zea mays (L.) var. everta] hybrids at two growth stages to glyphosate and 2,4-D choline/glyphosate based on biomass reduction in field experiments conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE in 2017 and 2018. †

Herbicide	Stage	Hybrid ‡	Slope §	Upper limit	ED_{50} §	ED_5 §	RMSE	ME
				% BMr	g ae ha^{-1}		% BMr	
Glyphosate	V5	VWP111	-2.1 (0.8) a	100 (0)	51 (7) a	10 (7) a	9.4	0.94
		VYP315	-1.8 (0.4) a	100 (0)	62 (6) b	12 (5) ab	15.5	0.84
	V8	VWP111	-2.8 (0.5) a	100 (0)	87 (6) c	30 (6) bc	12.3	0.91
		VYP315	-2.6 (0.5) a	100 (0)	88 (6) c	29 (6) c	14.4	0.87
2,4-D choline/ glyphosate	V5	VWP111	-1.9 (0.8) a	100 (0)	47 (12) a	10 (9) a	12.5	0.90
		VYP315	-2.5 (0.5) a	100 (0)	109 (10) b	34 (9) b	16.1	0.85
	V8	VWP111	-2.5 (0.5) a	100 (0)	114 (10) b	35 (9) b	19.3	0.80
		VYP315	-2.7 (0.6) a	100 (0)	131 (12) b	45 (12) b	14.8	0.88

† Abbreviations: BMr, biomass reduction; ED_{50}, herbicide rate where 50% response between lower and upper limit occurs and inflection point; ED_5, herbicide rate where 5% response occurs; ME, modeling efficiency; RMSE, root mean squared error

‡ Hybrid VWP111, white kernel color, Conagra brand; hybrid VYP315, yellow kernel color, Conagra brand

§ Means presented within the same column for each herbicide with no common letter(s) are significantly different according to pairwise t-tests where $\alpha = 0.05$

Table 5-4. Parameter estimates (b, d, and e) and ED_5 with standard errors of the three-parameter log-logistic model, root mean squared error, and modeling efficiency used to determine the dose-response of two popcorn [Zea mays (L.) var. everta] hybrids at two growth stages to glyphosate and 2,4-D choline/glyphosate based on height reduction in field experiments conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE in 2017 and 2018. †

Herbicide	Stage	Hybrid ‡	Slope §	Upper limit §	ED_{50} §	ED_5 §	RMSE	ME
				% HR	g ae ha^{-1}		% HR	
Glyphosate	V5	VWP111	-5.8 (11.6) a	90.9 (3.6) b	47 (11) a	29 (35) ab	9.4	0.93
		VYP315	-2.1 (1.3) a	92.0 (5.7) b	38 (11) a	10 (10) a	9.4	0.93
	V8	VWP111	-4.7 (1.1) a	75.1 (3.9) a	80 (6) b	45 (7) ab	14.7	0.83
		VYP315	-4.8 (1.3) a	79.1 (4.2) ab	84 (6) b	48 (8) b	12.0	0.89
2,4-D choline/ glyphosate	V5	VWP111	-3.2 (1.4) a	89.1 (4.8) b	69 (6) a	29 (11) a	10.3	0.92
		VYP315	-2.1 (0.6) a	94.2 (6.9) b	89 (11) ab	23 (9) a	8.8	0.94
	V8	VWP111	-2.1 (1.1) a	67.0 (7.4) a	83 (15) ab	25 (14) a	18.5	0.64
		VYP315	-2.3 (1.0) a	73.5 (8.3) ab	105 (19) b	33 (14) a	15.1	0.77

† Abbreviations: ED_{50}, herbicide rate where 50% response between lower and upper limit occurs and inflection point; ED_5, herbicide rate where 5% response occurs; HR, height reduction; ME, modeling efficiency; RMSE, root mean squared error

‡ Hybrid VWP111, white kernel color, Conagra brand; hybrid VYP315, yellow kernel color, Conagra brand

§ Means presented within the same column for each herbicide with no common letter(s) are significantly different according to pairwise t-tests where $\alpha = 0.05$

Table 5-5. Parameter estimates (b, d, and e) and ED_5 with standard errors of the three-parameter log-logistic model, root mean squared error, and modeling efficiency used to determine the dose-response of two popcorn [Zea mays (L.) var. everta] hybrids at two growth stages to glyphosate and 2,4-D choline/glyphosate based on yield loss in field experiments conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE in 2017 and 2018. †

Herbicide	Stage	Hybrid ‡	Slope §	Upper limit	ED_{50} §	ED_5 §	RMSE	ME
				% YL	g ae ha^{-1}		% YL	
Glyphosate	V5	VWP111	-1.2 (1.4) a	100 (0)	11 (23) a	1 (5) a	6.5	0.97
		VYP315	-1.1 (0.5) a	100 (0)	21 (12) ab	1 (3) ab	6.6	0.97
	V8	VWP111	-1.8 (0.4) a	100 (0)	63 (8) bc	12 (5) bc	19.8	0.78
		VYP315	-2.0 (0.4) a	100 (0)	64 (7) c	14 (5) c	18.5	0.80
2,4-D choline/ glyphosate	V5	VWP111	-2.3 (1.2) a	100 (0)	57 (11) a	16 (13) a	14.2	0.87
		VYP315	-2.3 (0.6) a	100 (0)	79 (9) ab	21 (8) a	19.9	0.79
	V8	VWP111	-2.1 (0.5) a	100 (0)	86 (10) bc	22 (9) a	16.7	0.83
		VYP315	-2.4 (0.5) a	100 (0)	110 (11) c	33 (10) a	16.3	0.84

† Abbreviations: ED_{50}, herbicide rate where 50% response between lower and upper limit occurs and inflection point; ED_5, herbicide rate where 5% response occurs; RMSE, root mean squared error; ME, modeling efficiency

‡ Hybrid VWP111, white kernel color, Conagra brand; hybrid VYP315, yellow kernel color, Conagra brand

§ Means presented within the same column for each herbicide with no common letter(s) are significantly different according to pairwise t-tests where $\alpha = 0.05$

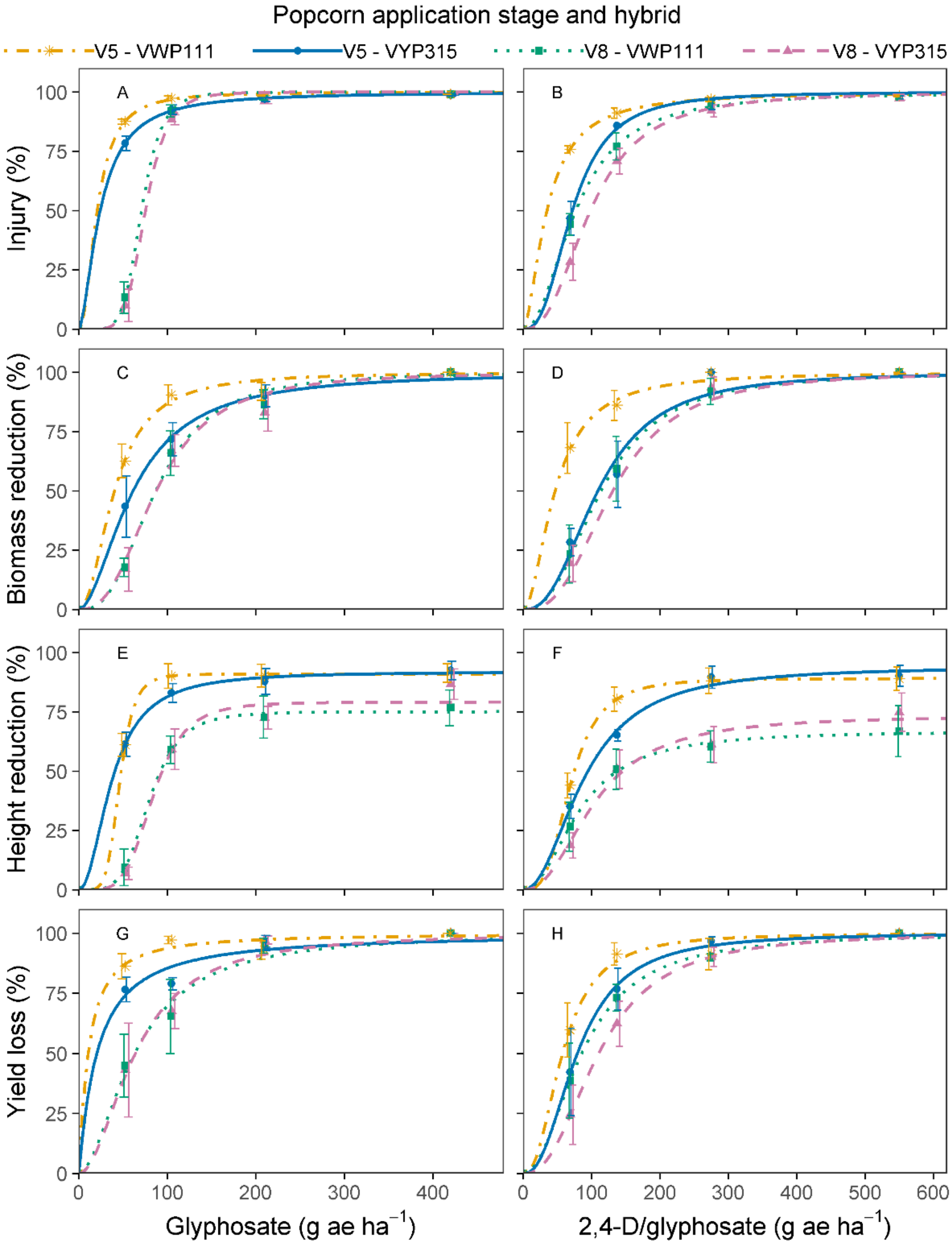

*Figure 5-2. Dose-response of two popcorn [*Zea mays *(L.) var. everta] hybrids (VWP111, white kernel color; and VYP315, yellow kernel color; Conagra Brands) at two growth stages (V5, 5-leaf stage; V8, 8-leaf stage) to glyphosate and 2,4-D choline/glyphosate based on (A/B) visual assessment of injury, (C/D) biomass reduction, (E/F) height reduction, and (G/H) yield loss in field experiments conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, Nebraska in 2017 and 2018.*

Dicamba injury ranged from 1% (±0.5) at 0.25X rate (140 g ae ha^{-1}) to 11% (±2.3) at the 2X rate (1,120 g ae ha^{-1}). The injury observed from the 2X rate of dicamba consisted of brace root malformation and mid-season lodging followed by goose-necking. There was no significant difference between the four curves (hybrids or growth stage for dicamba injury); therefore, data were combined, and a single curve was obtained (Tables 5-1,5-2; Figure 5-3). The labeled rate of dicamba is 560 g ae ha^{-1}, while the ED_5 value for dicamba injury was 643 g ae ha^{-1} (±81). There was no popcorn biomass reduction, height reduction, or yield loss from dicamba applications regardless of rate or growth stage. Late application of dicamba in field corn has been shown to result in injury consisting of fused brace roots, stalk bending and brittleness, or missing kernels similar to symptoms with late application of 2,4-D (Gunsolus and Curran, 1999). Dicamba (XtendiMax) used in this study is labeled in popcorn; however, it can be applied up to V5 growth stage and the label recommends verification with supplier for popcorn sensitivity (Bayer Crop Science, 2018). Although information regarding popcorn's sensitivity to dicamba is not available in the published literature, sweet corn sensitivity to dicamba has been researched and has been shown to be linked to a mutation on the P450 allele (Nordby et al., 2008). Similar to injury and biomass reductions, the V5 application resulted in greater yield loss with ED_{50} of 57 g ae ha^{-1} at the V5 application versus 86 g ae ha^{-1} at the V8 application for the white hybrid and 79 versus 110 g ae ha^{-1} for the yellow hybrid. The ED_5, slope, and upper limit did not vary between growth stages.

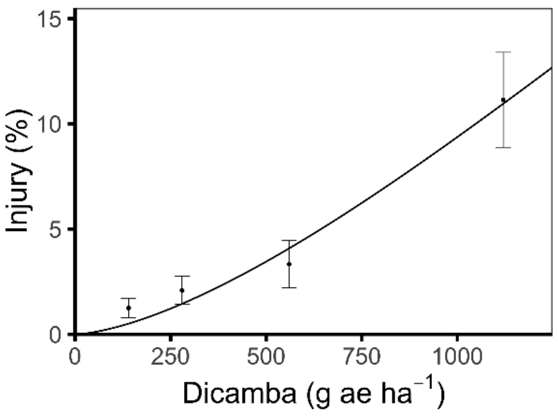

*Figure 5-3. Dose-response of two popcorn [*Zea mays *(L.) var. everta] hybrids (VWP111, white kernel color; and VYP315, yellow kernel color; Conagra Brands) at two growth stages (V5, 5-leaf stage; V8, 8-leaf stage) to dicamba based on visual assessment of injury in field experiments conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, Nebraska in 2017 and 2018.*

Hybrid. The white and yellow popcorn hybrids had similar injury response to glyphosate at all model parameters (Figure 5-2A). The ED_{50} values at the V5 growth stage for white and yellow hybrid averaged 23 g ae ha^{-1} compared with 73 g ae ha^{-1} at the V8 growth stage (Table 5-2). The same trend was observed in the ED_5 values with injury averaging 5 and 46 g ae ha^{-1}, at the V5 and V8 growth stages, respectively. The upper limit reached or exceeded 100% injury for both hybrids and was not allowed to exceed 100%. With field corn, Brown et al. (2009) reported 3-16% conventional field corn injury at 14 DAT from 100 g ae ha^{-1} of glyphosate applied at V4-V5 growth stage. In sweet corn, glyphosate applied at 92 g ae ha^{-1} resulted in 1 to 38% crop injury depending on carrier volume (12 to 281 L ha^{-1}) at 14 DAT (Banks and Schroeder 2002). Similarly, Ellis et al. (2002) reported 33-51% conventional field corn injury at 14 DAT from 140 g ae ha^{-1} of glyphosate applied at the V6 growth stage. Glyphosate at 160 g ae ha^{-1} resulted in 43% injury at 14 DAT when applied to V6-V8 conventional field corn growth stage (Buehring et al., 2007). Biomass reduction was greater in white popcorn hybrid than yellow hybrid when glyphosate was applied at the V5 growth stage; however, at the V8 growth stage, biomass reduction was similar between two hybrids (Figure 5-2C). At the V5 growth stage, the ED_{50} was 51 and 62 g ae ha^{-1} for white and yellow popcorn hybrids, respectively (Table 5-3). The ED_5, slope, and upper limit were similar for both hybrids within growth stage. Brown et al. (2009) reported 19% conventional field corn biomass reduction at 42 DAT from glyphosate at 100 g ae ha^{-1} applied at V4-V5 growth stage, substantially less than observed from popcorn in this research. Similarly, Banks and Schroeder (2002) reported sweet corn fresh weight reduction varying from 0 to 40% at 14 DAT with glyphosate applied at 92 g ae ha^{-1}. Popcorn height reduction following glyphosate application was not different between the two hybrids (Figure 5-2E; Table 5-4). Brown et al. (2009) reported 19% height reduction at 28 DAT from 100 g ae ha^{-1} of glyphosate applied at V4-V5 stage. Similarly, Ellis et al. (2002) reported 28-45% height reduction at 14 DAT from 140 g ae ha^{-1} of glyphosate. Generally, less reduction in conventional field corn height has been reported in the literature than those observed at similar glyphosate rate in popcorn in this research. This might be due to the fact that popcorn is more sensitive to herbicides and tends to have shorter and thinner stalks compared to field corn. Popcorn yield loss from glyphosate did not vary between hybrids (Figure 5-2G; Table 5-5). Brown et al. (2009) reported 9-31% conventional field corn yield loss from 100 g ae ha^{-1} of glyphosate applied at V4-V5 stage. Similarly, Ellis et al. (2002) reported 41-62% yield reduction from 140 g ae ha^{-1} of glyphosate applied at V6 field corn growth stage. Banks and Schroeder (2002) reported 0 to 51% loss in marketable sweet corn ears with glyphosate applied at 92 g ae ha^{-1} when 20-25 cm tall.

Thus, by comparing results of this research with scientific literature on conventional corn and sweet corn, it is evident that popcorn is more sensitive to glyphosate; therefore, care should be taken to avoid drift or tank contamination of glyphosate in popcorn fields to avoid yield loss.

A premix of 2,4-D choline/glyphosate applied to the white popcorn hybrid resulted in greater injury than the yellow hybrid at both growth stages (Figure 5-2B). The ED_{50} values for the white and yellow hybrids at the V5 growth stage was 35 and 72 g ae ha^{-1}, respectively (Table 5-2). The ED_{50} at the V8 growth stage was 77 and 98 g ae ha^{-1} for the same respective hybrids. The same trend occurred for the ED_5 values. No differences in model slope or upper limit (100%) occurred between hybrids within growth stages. In glyphosate-resistant field corn, 2,4-D choline/glyphosate applied at 1,720 g ae ha^{-1} resulted in 0-63% injury at 7 DAT, with the predominate injury symptom being fused brace roots and 63% injury resulting from a location where the corn was at the V4 growth stage at the time of application (Ford et al., 2014). The highest rate of 2,4-D choline/glyphosate applied in this study was 550 g ae ha^{-1} (0.25 X) which primarily resulted in glyphosate injury symptoms in both popcorn hybrids. Similar to biomass reduction from glyphosate, 2,4-D choline/glyphosate resulted in greater biomass reduction in the white popcorn hybrid at the V5 growth stage and no difference between two hybrids at the V8 growth stage (Figure 5-2D). At the V5 stage, the ED_5 were 10 and 34 g ae ha^{-1}, and the ED_{50} were 47 and 109 g ae ha^{-1} for the white and yellow hybrids, respectively (Table 5-3). The slope and upper limit were similar for both hybrids at both the V5 and V8 growth stages. Similar to glyphosate, 2,4-D choline/glyphosate resulted in no differences in height reduction between hybrids (Figure 5-2F; Table 5-4). Popcorn yield loss from applications of 2,4-D choline/glyphosate followed similar trends to that of glyphosate as the ED_5 and ED_{50} did not differ between hybrids (Figure 5-2H; Table 5-5).

Correlation Analysis and Model Fits. Correlation analysis suggests that popcorn injury ratings and biomass reduction were strongly correlated (Table 5-6). Correlation with yield loss ranged from 80 to 99% for glyphosate and 2,4-D choline/glyphosate injury ratings at 21 days after treatment (DAT) and 80 to 96% for biomass reduction at 70 DAT. Deeds et al. (2006) reported similar correlation between yield loss and glyphosate injury ratings in wheat. Popcorn height reduction was correlated less strongly with yield loss than injury or biomass reduction, ranging from 70 to 97%. The correlation between dicamba injury and yield loss was weak (10%) because no yield losses were detected. The three-parameter log-logistic model had an adequate fit for all glyphosate and 2,4-D choline/glyphosate treatments with low RMSE and high ME values; however, was a poor fit to dicamba injury because only slight injury occurred (Tables 5-2, 5-3, 5-4, and 5-5).

*Table 5-6. Correlation coefficients between popcorn yield loss and popcorn injury ratings, biomass reduction, and height reduction of two popcorn [*Zea mays *(L.) var. everta] hybrids at two growth stages to glyphosate and 2,4-D choline/glyphosate based on yield loss in field experiments conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE in 2017 and 2018.*

Herbicide	Stage	Hybrid †	Injury	Biomass reduction	Height reduction
Glyphosate	V5	VWP111	0.99	0.96	0.96
		VYP315	0.98	0.94	0.97
	V8	VWP111	0.80	0.85	0.70
		VYP315	0.81	0.80	0.78
2,4-D choline/ glyphosate	V5	VWP111	0.92	0.93	0.94
		VYP315	0.89	0.88	0.91
	V8	VWP111	0.92	0.86	0.83
		VYP315	0.93	0.85	0.83
Dicamba	combined	combined	0.10	NA	NA

† Hybrid VWP111, white kernel color, Conagra brands; hybrid VYP315, yellow kernel color, Conagra brands

Practical Implications. Unintentional application, spray drift, or improper tank clean out are challenges for popcorn producers operating in landscapes dominated by herbicide-resistant crops (USDA NASS, 2019) such as glyphosate-resistant corn and soybean in Nebraska. In this research, a constant carrier volume was used, 140 L ha^{-1}, which provided estimates of the minimum or low end of injury, biomass reduction, height reduction, and yield loss when considering drift rates. A proportional carrier volume to the herbicide rate results in greater herbicide effect (Banks and Shroeder, 2002; Smith et al., 2017). Although the white and yellow popcorn hybrids tested in this research resulted in ≤11% injury and 0% yield loss with dicamba applied at 1,120 g ae ha^{-1} (2X), they resulted in nearly 100% yield loss with glyphosate as low as 105 g ae ha^{-1} (0.063X) and 2,4-D choline/glyphosate as low as 275 g ae ha^{-1} (0.125X). Dicamba is labeled for use in popcorn up to V5 growth stage; thus, potential drift of dicamba should not pose a risk to popcorn production. As of now, 2,4-D choline is not labeled for POST application in popcorn (Corteva Agriscience, 2017a). As glyphosate-resistant corn and soybean production fields in the United States receive on average 0.9 and 2.4 glyphosate area treatments per year (Kniss, 2017), there are plenty of opportunities for application mistakes involving glyphosate. An increase in 2,4-D choline and 2,4-D choline/glyphosate application can be expected with the 2018 commercialization of 2,4-D choline/glyphosate/glufosinate-resistant corn (Enlist E_3; Dow AgroSciences LLC) and Enlist E3 soybean commercialized in 2019. Field corn and popcorn are nearly indistinguishable from each other during vegetative stages. This means that communication with applicators and agronomists must be maintained to avoid application mistakes to reduce off-target injury.

Although not evaluated in this research, popcorn is not resistant to glufosinate. The use of glufosinate is expected to increase with the integration of glufosinate-resistance into stacked herbicide-resistant traits in corn and soybean; which will inevitably increase the risk of popcorn injury by glufosinate. The differences observed between white and yellow popcorn herbicide sensitivity in this study are inconclusive. With some measurements, the white popcorn hybrid (VWP111) was more sensitive to the tested herbicides than the yellow hybrid (VYP315) and with other measurements, there were no differences. In an experiment evaluating the response of two white and six yellow commercially available popcorn hybrids to several PRE and POST herbicides, Barnes et al. (2019) did not observe differences in injury between yellow and white popcorn hybrids. Although there is a perception that white popcorn is more sensitive to herbicides than yellow popcorn, there is only a single gene, *Y1*, responsible for yellow/white kernel color in maize (Buckner et al., 1996; Ford, 2000). This is the first research to characterize white and yellow popcorn hybrid response to glyphosate, 2,4-D choline/glyphosate, and dicamba in the published literature. This research will play an important role in educating applicators and agronomists about sensitivity of white and yellow hybrids to glyphosate, 2,4-D choline/glyphosate, and dicamba.

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CHAPTER 6 RISK ASSESSMENT OF POLLEN-MEDIATED GENE FLOW FROM *GA1-M* DENT CORN TO DENT-STERILE *GA1-S* POPCORN

6.1 ABSTRACT

The United States' popcorn industry is at risk of genetic contamination because it utilizes the *gametophyte factor 1* gene (*Ga1*) as a barrier against pollen-mediated gene flow (PMGF) from dent corn. Popcorn with the *Ga1-s* allele accepts pollen from only *Ga1-s* corn, allowing for dent corn and popcorn to be nearby without isolation. Germplasm is being introduced to the United States to increase dent corn diversity that unknowingly contains the *ga1-m* allele, which can overcome *Ga1-s* selectivity and pollinate popcorn. The risk to the popcorn industry has been under-assessed. Experiments were conducted to model the frequency of PMGF from *Ga1-m* dent corn to *Ga1-s* popcorn under field conditions and to evaluate the role of wind speed and direction using a concentric donor-receptor design. PMGF to white popcorn was detected using a dent corn pollen donor with yellow kernel color (dominate) and confirmed molecularly. Popcorn kernels were harvested from cardinal and ordinal directions and distances from 1 to 70 m, and more than 7 million kernels were screened to detect the PMGF. Information-theoretic criteria was used to select the best-fit model of PMGF out of 564 double exponential decay models. The greatest PMGF (1.6- 4.1%) was detected at 1 m and declined with distance. PMGF was detected at 70 m, the maximum distance tested. Wind and meteorological parameters did not improve the fit of the model despite PMGF varying by direction. This is the first assessment of PMGF from dent corn to popcorn and results are alarming for the popcorn industry.

6.2 INTRODUCTION

Popcorn (*Zea mays* L. var. everta) is a popular healthy snack food in the United States and is increasing popularity worldwide (1, 2). Popcorn is a recommended snack to meet daily ChooseMyPlate.gov whole grain consumption guidelines (3, 4). This is because popcorn contains high fiber, no cholesterol, and low fat and several vitamins, minerals, and polyphenols (5, 6). Popcorn is produced on nearly 90,000 ha annually in the United States (USDA 2019). Popcorn is commonly grown in rotation with soybean [Glycine max (L.) Merr]/dent corn (*Zea mays* L. var. indentata) and produced under contracts with the private company/manufacturer (7, 8). Producers tend to financially benefit from the contract production of popcorn on their farms primarily in recent years where corn, soybean, and wheat (*Triticum aestivum* L.) price is low (9, 10). The four largest popcorn-producing states in the United States are Nebraska, Indiana, Ohio, and Illinois. Additionally, aforementioned states are also among the eight largest dent corn-producing states (11). Therefore, popcorn and dent corn are grown in proximity with usually similar planting and flowering time. While 92% of dent corn planted in the United States is genetically-modified (GM), there is no GM popcorn commercially available in the market place (12).

Popcorn relies on *gametophyte factor 1* (*ga1*) for protection against pollen mediated gene flow from dent corn (8, 13). The *ga1* system is utilized elsewhere such as in organic production systems (14). The *ga1* system is the only utilized genetic system for preventing gene flow from dent/sweet corn to popcorn (15). The *ga1* locus has the *ga1*, *Ga1-s*, and *Ga1-m* alleles (15, 16). The *ga1* allele is the most prevalent because it is carried by almost all commercially available dent corn in the United States and has no barrier which allows for it to be pollinated by all three alleles (15, 17).The *Ga1-s* allele is utilized in popcorn breeding programs and commercial cultivation as it is nonreciprocal cross-sterile which prevents pollination from *ga1* dent corn (15, 18, 19). The *Ga1-m* allele is cross neutral which can pollinate and accept pollen from any *gametophyte factor 1* allele including *Ga1-s* (15, 16). *Ga1-m* is not prevalent in the United States but is being unintentionally introduced from Mexican and Central American germplasm to increase the genetic base of dent corn (14, 15). Ten inbred lines have been released from North Carolina State University that unknowingly contained *Ga1-m* (14, 15). A 2008 study concluded that 55% of commercial dent corn hybrids planted in Mexican tropical, subtropical, and highlands were *Ga1-m* homozygous (20). Popcorn relies on *Ga1-s* to maintain genetic purity; however, the introduction of *Ga1-m* puts the popcorn production system at risk from dent corn cross-pollination and genetic impurity that can affect popcorn export market (14, 15).

The major factors that contribute to corn genetic contamination are accidental seed impurity, sowing equipment and practices, cross-fertilization, volunteer plants, products mixing during harvest, transport, and storage processes (21). Pollen-mediated gene flow (PMGF) is the largest potential biological source of on farm mixing of genetic material in corn (22). PMGF from genetically-modified crops to non-genetically-modified crops and organic crops (23) or wild relatives (24) is a concern. PMGF in corn depends on number of factors including but not limited to pollen viability, synchronization of flowering, and the relative concentrations of pollen in the donor and receptor fields (25–27). Additional factors such as wind direction and intensity, rainfall, and distance between the pollen source and pollen receptor are also important (26, 28). An individual dent corn tassel produces 2 to 5 million pollen grains (29, 30). Additionally, pollen shed can last for five to six days and silks are receptive for about 5 days (31, 32). The second ear on a plant silks later than the first, increasing the timeframe that a field will have silks receptive to pollen (33). Corn pollen is approximately 100 microns compared to 17 to 58 microns in most other wind pollinated plants (34). Despite pollen of dent corn being relatively large and heavy, gene flow in corn has been shown at larger distances with favorable meteorological conditions (25, 28, 35, 36). Isolation distances of 200 m and 300 m are typically recommended to maintain 99% and 99.5% purity standards in dent corn (36–38). Complete isolation of dent corn fields planted at the same time require 1,600 m (39).

Popcorn has a larger tassel that produces more pollen than dent corn (8). Popcorn is prolific and generally produces two ears per plant but has smaller ear shoots than dent corn (8). Isolation distance in popcorn is 200 m to avoid cross contamination with other popcorn or *Ga1-s* specialty corn (8). Due to presence of dent-sterility in popcorn, a physical isolation from dent corn is not required to prevent cross-pollination (8). However, dent corn with the *Ga1-m* allele can cross-pollinate with popcorn and genetic contamination of popcorn breeding or production programs from *Ga1-m* dent corn would have significant impacts. Scientific information is not available in the literature about PMGF from dent corn to popcorn. The objectives of this research were to model the frequency of PMGF from a homozygous *Ga1-m* dent corn to homozygous *Ga1-s* popcorn under field conditions and to evaluate the role of wild speed and direction. We hypothesized that PMGF from *Ga1-m* dent corn to *Ga1-s* popcorn is possible and the frequency of gene flow will reduce with increasing distance from the pollen source.

6.3 MATERIALS AND METHODS

Plant Materials and Field Experiments. The dent-sterile popcorn hybrid VWP111 (Conagra Brands, Chicago, IL), a white kernelled popcorn hybrid, was selected as a female parent in this study. The *ga1-m* homozygous dent corn NC390.NC394 (North Carolina State University, Raleigh, NC), a yellow kernelled F2 inbred, was selected as a male parent. Field experiments were conducted in 2017 and 2018 at the University of Nebraska-Lincoln's Eastern Nebraska Research and Extension Center (ENREC) near Mead, Nebraska (41.1704°N, 96.4615°W). The soil at the research site was a Yutan silty clay loam (fine-silty, mixed, superactive, mesic Mollic Hapludalfs) with a pH of 6.8 and 2.7% soil organic carbon. The experiments were under center pivot irrigation and were irrigated as needed. The experimental site was disked with a tandem disk to a depth of 10 cm and fertilized with 202 kg ha^{-1} of nitrogen in the form of anhydrous ammonia (82-0-0) in early spring. To achieve weed control, herbicide pre-mixture atrazine/*S*-metolachlor (Bicep II Magnum; Syngenta Crop Protection, Greensboro, NC 27419) was applied at 2,470 g ai ha^{-1} pre-emergence after planting but before crop emergence and post-emergence at 2,470 g ai ha^{-1} when corn was at three-leaf stage during both years.

A concentric donor-receptor design (i.e., Nelder wheel) was used for field experiments where the pollen donors were surrounded by pollen receptors (23). The experiments were 120 m by 120 m with a central square of 20 m by 20 m for the pollen donor-block and the entire outside square for the pollen receptor-block. The dent corn pollen donor (NC390.NC394) and popcorn pollen receptor (VWP111, white popcorn hybrid) were planted on May 16 in 2017. In 2018, the dent corn pollen donor was planted 11 days before the popcorn pollen receptor on April 27 and May 8, respectively. The receptor-block was divided into eight directional blocks (cardinal: N, S, E, and W; and ordinal: NE, NW, SE, and SW) and flags placed at 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, and 50 m in all eight directions and additionally at 60 and 70 m for ordinal directions.

Flowering and Seed Harvest. The pollen donor and receptor blocks were monitored for beginning and ending anthesis and silking dates and flowering synchrony was calculated as the percentage of days where donor block pollen shed and receptor block silking overlapped (40). At maturity, sixty ears from each direction and distance combination were hand harvested. Total kernels per treatment were determined by the average kernel number per ear of a six-ear subsample per treatment and multiplied by the total number of ears harvested in that treatment. Yellow kernels per treatment were counted for the entire sample. The percentage of gene flow in each treatment was calculated by dividing the number of yellow kernels by the total kernels examined (26, 51).

Meteorological Data. Meteorological data were recorded every 30 minutes (26) by installing a weather station (METER Group, Inc. USA, Pullman, WA) at the experimental site which recorded half hour averages of air temperature, relative humidity, wind speed, and wind direction. The weather station also recorded total precipitation and maximum wind gust speed for each half an hour. Data during days with flower synchrony were used for modeling PMGF.

Statistical Analysis. Gene flow frequency follows a binomial distribution with two possible outcomes in this study: yellow kernels: gene flow occurred, and white kernels: no gene flow occurred. The probability of gene flow is a function of the covariate, distance from pollen donor (43, 45). Distance from the pollen donor can take on any real value; however, the probability of gene flow ranges between 0 and 1. Therefore, a Logit function, or log-odds, must be used to transform the probability of gene flow data to remove the range and floor restrictions (52):

$$\text{Logit, } \eta_{\text{I}} = \text{logit}(\text{p}_{\text{i}}) = \ln\left(\frac{p_i}{1+p_i}\right) \qquad [1]$$

Data are back transformed for presentation.

A set of 564 possible models was constructed to explain the frequency of gene flow using an exponential decay function with distance from the pollen donor, direction of the pollen receptor relative to the pollen donor, average wind speed, wind frequency, wind direction, and/or maximum wind gust (43, 45). Additionally, models were evaluated with and without thresholds of air temperature, relative humidity, and the dual threshold of air temperature and relative humidity (RH). Models with air temperature thresholds considered gene flow only when temperatures were less than 35 C. Temperatures above 35 C during anthesis have been shown to decrease pollen viability (53–55). The maximum relative humidity was set at 75% because above 75% RH water forms a film on the pollen causing it to clump (56, 57). Models were evaluated considering both the entire 24 hr data during flower synchrony and a 6 hr between 0900 and 1500 Central Standard Time (CST). The 6 hr time frame is when pollen shed typically occurs in corn (26).

Model Comparison and Evaluation. Model comparison and selection was performed using the Corrected Akaike's Information Criterion (AICc):

$$\text{AICc} = -2LL + 2K(n/[n-K-1]) \qquad [2]$$

where K is the number of model parameters, LL is the maximum log likelihood, and n is the sample size (58). AICw:

$$\text{AICw}_i = \exp\left[\left(-\frac{1}{2\Delta i}\right) / \sum_{r=1}^{n} \exp\left(-\frac{1}{2\Delta r}\right)\right] \qquad [3]$$

where Δi is the difference between the model with the lowest AIC and the ith model, r represents the total number of models being compared, and Δr is the difference between the model with the lowest AIC and the rth model. The model with the lowest AICc and the highest AICw is considered the best explanation of the data within the model set (58). The best model was evaluated for goodness of fit using Pearson's chi-squared test (43, 45).

Model Goodness of Fit. Model goodness of fit was estimated using Pearson's chi-squared statistic:

$$\chi^2_{(\text{n-k-1})} = \sum_i \frac{n_i(\gamma_i - \hat{\mu}_i)^2}{\hat{\mu}_i(n_i - \hat{\mu}_i)} \qquad [4]$$

where the sum of squared difference between the observed values (γ_i) and the fitted values for the i^{th} group of observations ($\hat{\mu}_i$) is divided by the variance of γ_i that is $\mu_i(n_i - \hat{\mu}_i)/n_i$ (with μ_i estimated using $\hat{\mu}_i$), and n_i is the sample size for the i^{th} group. The degrees of freedom for the chi-squared test are $n-k-1$, where n refers to the total number of groups and k refers to the number of parameters (43, 45).

Molecular Assay for Gene Flow Confirmation. Two yellow corn kernels from each direction in each year at the farthest distance (50 to 70 m) and six kernels of the pollen donor and pollen receptor were planted in the greenhouse with 15 hr supplemental light and day temperature 27-29 C and night temperature 19-21 C for three weeks. DNA was extracted from approximately 150 mg of tissue using Quick DNA Plant/Seed Miniprep Kit (Zymo Research, Irvine, CA). The *Ga1-s* and *Ga1-m* coding sequence was amplified using forward and reverse primers (Table 6-1). PCR was performed with One Taq 2X Master Mix with standard buffer (New England Biolabs Inc., Ipswich, MA) using PCR settings of 30 s at 94 C followed by 30 s at 94 C, 60 s at 55 C, 30 s at 68 C repeated 29 times, followed by 5 min at 68 C. Sanger sequencing of a cloned PCR product was performed to confirm that the PCR amplified the correct segment of the *Ga1* gene. A restriction enzyme digest was performed using Bs1I (New England Biolabs) for 8 min at 55 C. Bs1I does not cut *Ga1-m* but does cut *Ga1-s* (Table 6-1). The products were analyzed via gel electrophoresis.

Table 6-1. Ga1-s *and* Ga1-m *gene sequences and primer sequences used for cloning of* Ga1-s *and* Ga1-m *from yellow kernelled popcorn by dent corn hybrids listed in the 5' to 3' direction. Hybrids were from a field experiment conducted at the University of Nebraska–Lincoln Eastern Nebraska Research and Education Center in 2017 and 2018.*

Genetic material	5' to 3' Sequence
Ga1-s *	CACCGTGGACTTTGTGTTTGGCAATGCCCAGGCCATGTTCCAGAGCTGCG CGCTGCTGGTGCGCCGC**CCACCGAAAGG**CAAGCACAATGTGCTGACGGC CCAGGGCTGCAACAACGCAAGCCGCGAGTCCGGCTTCTCGTTCCACATGT GCACCGTGGAAGCCGCGCCGGGCGTGGACCTCGACGGCGTGGAGACCTA CCTCGGCCGCCCCTACAGGAACTTCTCCCACGTCGCCTTCATCAAGTCGTA TCTCAGTCGCGTGGTCA
Ga1-m †	CACCGTGGACTTTGTGTTTGGCAATGCCCAGGCCATGTTCCAGAGCTGCG CGCTGCTGGTGCGCCGCCCACCGAgaAAGGCAAGCACAATGTGCTGACGG CCCAGGGCTGCAACAACGCAAGCCGCGAGTCCGGCTTCTCGTTCCACATG TGCACCGTGGAAGCCGCGCCGGGCGTGGACCTCGACGGCGTGGAGACCT ACCTCGGCCGCCCCTACAGGAACTTCTCCCACGTCGCCTTCATCAAGTCGT ATCTCAGTCGCGTGGTCA
Forward primer	GCACCGTGGACTTTGTGTTTG
Reverse primer	CTGACCACGCGACTGAGATAC

* Bold and red text annotate restriction enzyme cut site on *Ga1-s*

† Lowercase and red text annotate two base pair insertion that differentiates *Gal-m* from *Ga1-s*

6.3 RESULTS

Flowering Synchrony. In 2017, silking of the primary ear in the pollen receptor, *Ga1-s* popcorn, occurred 10 days before tasseling of the pollen donor, *Ga1-m* dent corn. The secondary popcorn ear silks were receptive to dent corn pollen for 2 days before silking ceased which accounted for 25 to 30% flowering synchrony in 2017. In 2018, overlap of *Ga1-m* dent corn pollen shed and *Ga1-s* popcorn silking occurred 7 days ensuring 100% flowering synchrony.

Meteorological Data. Average daily temperatures and accumulated precipitation during the growing season followed the trend of the 30-year average for the experimental site (Figure 6-1). During flowering synchrony, the average relative humidity (RH) and average air temperature (T) were 81% and 28 C in 2017, respectively, and 82% and 24 C in 2018, respectively (Table 6-2). When data during typical pollen shed hours (0900 to 1500 CST) were considered, the average RH was less (77-78%) and the average temperature was greater (Table 6-2).

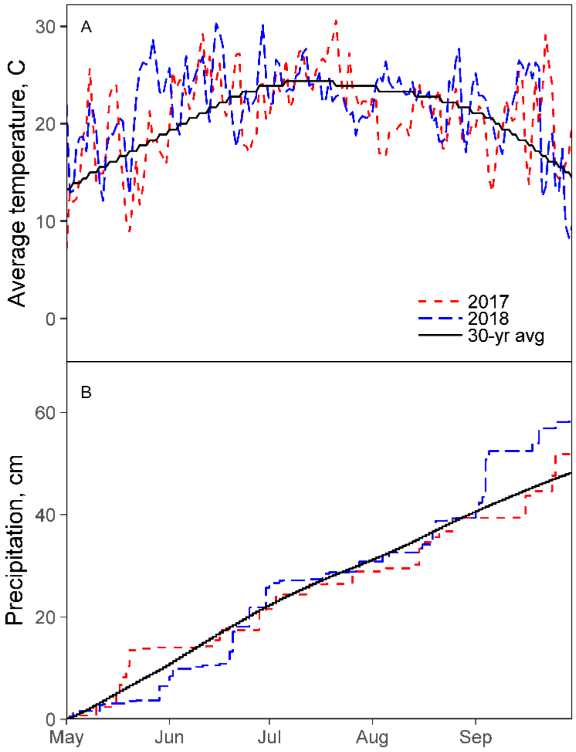

Figure 6-1. (A) Average daily air temperature and (B) precipitation during 2017 and 2018 growing seasons and 30-yr average at the University of Nebraska–Lincoln, Eastern Nebraska Research and Education Center near Mead, Nebraska

Table 6-2. Average relative humidity and average air temperature observed during dent corn and popcorn pollination synchrony in a field experiment conducted at the University of Nebraska–Lincoln Eastern Nebraska Research and Education Center in 2017 and 2018.

	0900-1500 CST		0000-2400 CST	
Date	Relative humidity	Average air temperature	Relative humidity	Average air temperature
	%	C	%	C
July 21, 2017	71	28	76	29
July 22, 2017	85	31	85	27
2017 average	78	30	81	28
July 15, 2018	69	30	77	25
July 16, 2018	77	27	80	26
July 17, 2018	91	21	89	22
July 18, 2018	80	26	84	23
July 19, 2018	78	28	82	25
July 20, 2018	71	26	80	23
July 21, 2018	73	27	80	23
2018 average	77	26	82	24

Pearson correlation coefficients (*r*) showed a low degree of correlation ($r = 0 \leq 0.29$) between frequency of gene flow and wind parameters; average wind speed, wind frequency, and wind run in most cases (Table 6-3). This suggests that wind parameters contributed slightly to the frequency of PMGF in this study in any given direction. Correlation between frequency of gene flow and wind speed increased to a moderate degree ($r = 0.29 \leq 0.49$) up to 20 m from the pollen source when only data between 0900 and 1500 CST was used. This suggests that the average wind speed during typical pollen shed hours contributes more towards frequency of gene flow than 24-hr average wind speed. The average wind speed at tassel height was 0.5 m s^{-1} and 0.7 m s^{-1} during flowering synchrony in 2017 and 2018, respectively (Figure 6-2). Wind was predominately towards the North in 2017 and the North and Southwest in 2018. The air at tassel height was calm 46 and 21% of the time in 2017 and 2018, respectively.

Table 6-3. Pearson's correlation coefficients between frequency of gene flow and wind parameters during pollination from a field experiment conducted at the University of Nebraska–Lincoln Eastern Nebraska Research and Education Center in 2017 and 2018.

Wind parameter	Time frame*	Total gene flow	Distance from pollen source 1	2	5	10	15	20	50
						m			
Wind speed	24hr	0.11	0.26	0.28	0.30	0.41	0.41	0.29	0.12
Wind frequency		0.10	0.13	-0.27	-0.19	0.32	0.24	0.09	0.30
Wind run		0.08	0.27	0.22	-0.15	0.38	0.24	0.15	0.24
Wind speed	0900-1500 CST	0.16	0.41	0.45	0.37	0.64	0.63	0.45	0.17
Wind frequency		0.07	0.22	0.16	-0.20	0.28	0.19	-0.07	0.34
Wind run		0.08	0.28	0.18	-0.16	0.42	0.23	0.04	0.31

* Data for wind parameters during flowering synchrony were tested during two-time frames; complete 24-hr data and 6-hr data collected between 0900 and 1500 CST. 6-hr data represents typical pollen shed timeframe in dent corn.

Frequency of PMGF. A total of 5,980 popcorn ears were harvested totaling over 3.5 million kernels screened in 2017. Similarly, in 2018, 6,240 popcorn ears were harvested resulting in over 3.6 million kernels screened (Table 6-4). Frequency of gene flow was highest at the closest distance (1 m) and declined quickly as distance from the pollen source increased (Figures 6-3 and 6-4). Regardless of direction from pollen source, frequency of gene flow at 1 m was 0.01615 and 0.04113 in 2017 and 2018, respectively. At the furthest distance (50 m) that all directions were sampled, the average frequency of gene flow was 0.00012 (0.012%) and 0.00058 (0.058%) in 2017 and 2018, respectively.

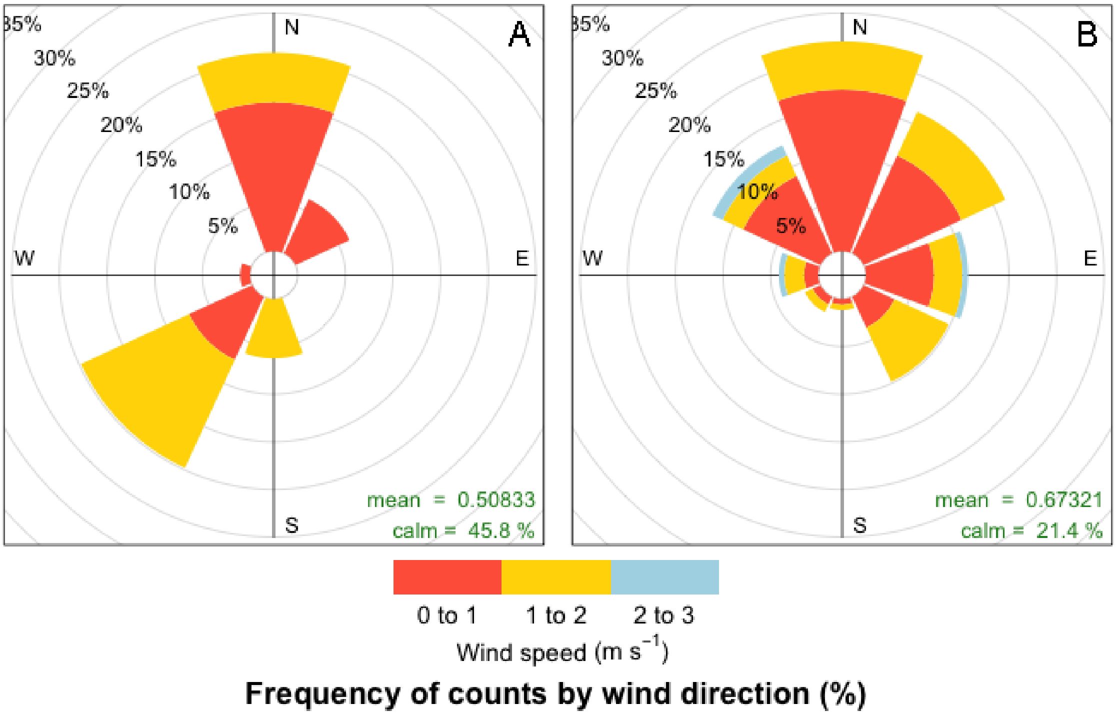

Figure 6-2. Windrose plots of the wind speed (m s^{-1}) and wind frequency (%) at tassel height during pollination synchrony in (A) 2017 and (B) 2018 at the University of Nebraska–Lincoln, Eastern Nebraska Research and Education Center near Mead, Nebraska. Wind speed was low (0-3 m^{-2}) with frequent periods of calm wind in both years.

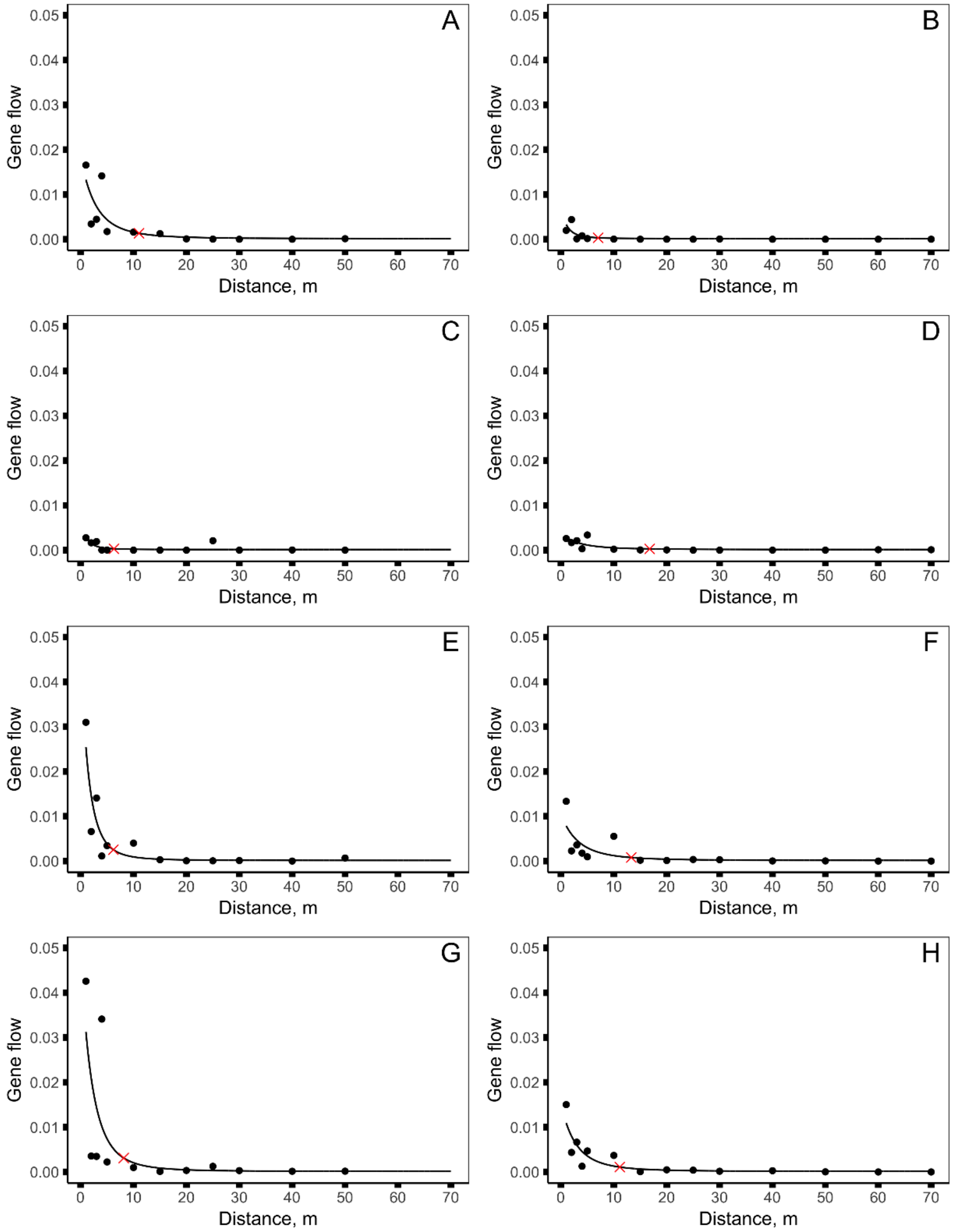

Figure 6-3. Pollen-mediated gene flow from (PMGF) Ga1-m *dent corn to* Ga1-s *popcorn as affected by distance from the pollen source in 2017. Gene flow in 2017 in eight directions were plotted against distance: (A) north, (B) northeast, (C) east, (D) southwest, (E) south, (F) southwest, (G) west, and (H) northwest. The line represents the best fit double exponential decay model where the first instance varied with year and the second instance varied with direction and year. The red × represents the point of 90% reduction in gene flow.*

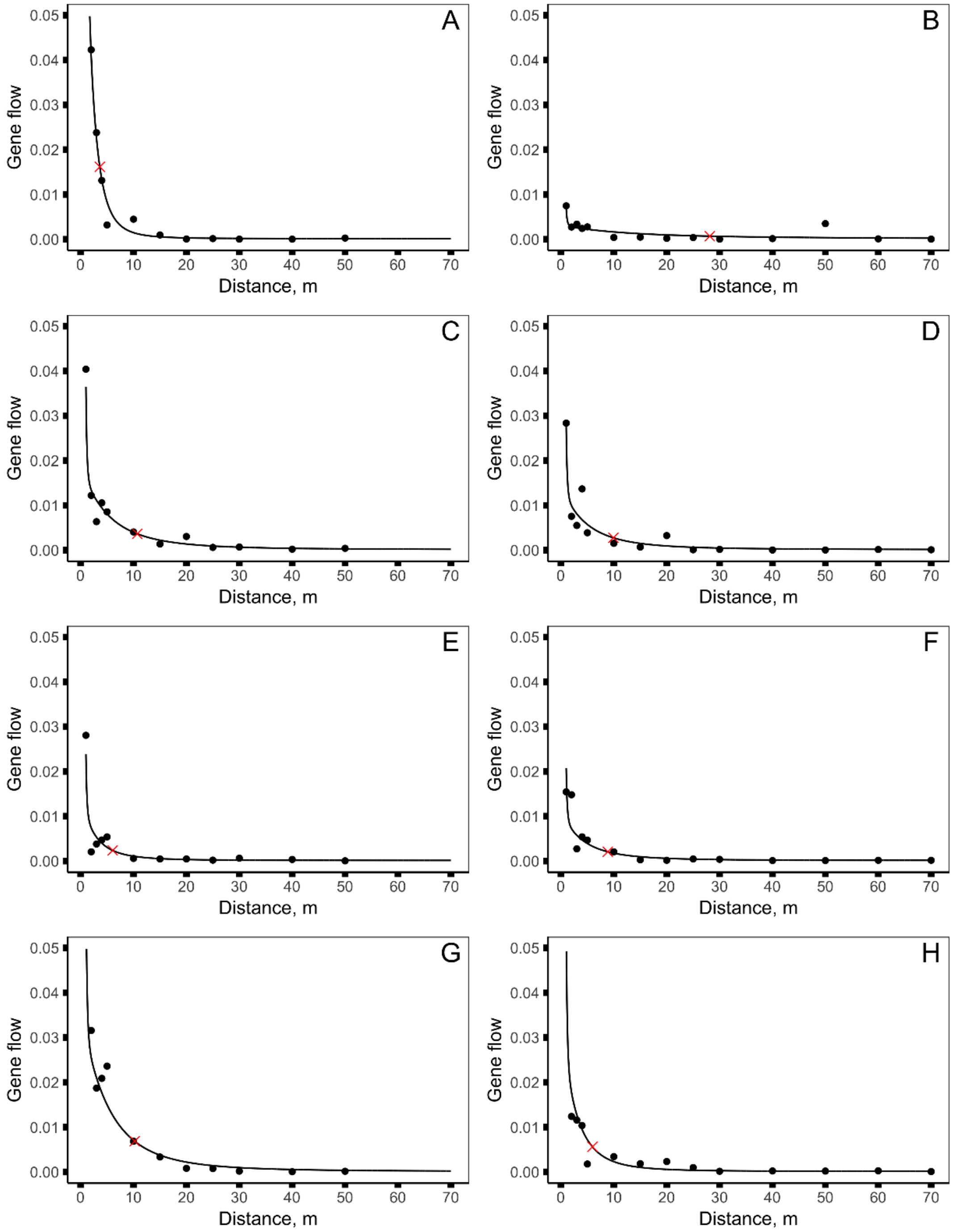

Figure 6-4. Pollen-mediated gene flow from Ga1-m *dent corn to* Ga1-s *popcorn as affected by distance from the pollen source in 2018. Gene flow in 2018 in eight directions were plotted against distance: (A) north, (B) northeast, (C) east, (D) southwest, (E) south, (F) southwest, (G) west, and (H) northwest. The line represents the best fit double exponential decay model where the first instance varied with year and the second instance varied with direction and year. The red × represents the point of 90% reduction in gene flow.*

Table 6-4. Frequency of pollen-mediated gene flow from Ga1-m *dent corn to* Ga1-s *popcorn in a field experiment conducted at the University of Nebraska–Lincoln Eastern Nebraska Research and Education Center in 2017 and 2018.*

Year	Distance from pollen source	Kernels screened	Yellow kernels *	Frequency of gene flow †	Power (1−β) [α = 0.05]	
					$H_0 = 0.01$	$H_0 = 0.00001$
	m					
2017	1	265,322	4,284	0.01615	1	1
	2	270,593	940	0.00347	1	1
	3	273,522	1,297	0.00474	1	1
	4	272,936	1,835	0.00672	1	1
	5	263,565	552	0.00209	1	1
	10	268,251	531	0.00198	1	1
	15	264,736	63	0.00024	1	1
	20	268,251	38	0.00014	1	0.99
	25	274,108	143	0.00052	1	1
	30	283,479	34	0.00012	1	0.99
	40	271,179	16	0.00006	1	0.91
	50	262,979	32	0.00012	1	0.99
	60	127,683	3	0.00002	1	<0.80
	70	137,054	3	0.00002	1	<0.80
	Total	3,503,657	9,771	0.00279	1	1
2018	1	281,157	11,564	0.04113	1	1
	2	281,222	4,328	0.01539	1	1
	3	280,645	2,610	0.00930	0.97	1
	4	280,584	2,789	0.00994	<0.80	1
	5	281,212	1,856	0.00660	1	1
	10	283,217	810	0.00286	1	1
	15	281,739	324	0.00115	1	1
	20	280,315	356	0.00127	1	1
	25	284,091	125	0.00044	1	1
	30	278,571	78	0.00028	1	1
	40	285,714	40	0.00014	1	0.99
	50	279,310	162	0.00058	1	1
	60	143,750	23	0.00016	1	0.99
	70	144,444	13	0.00009	1	0.89
	Total	3,656,414	25,083	0.00686	1	1

* Average pollen-mediated gene flow from all directions.

† Power values were calculated from a 95% confidence interval using binomial probabilities

PMGF varied between years and by direction from the pollen source. The inclusion of direction and year in the final model provided a better estimation of PMGF than without direction and year based on the corrected Akaike information criterion (AICc). PMGF followed a double exponential decay model where the first instance varied with year and the second instance varied with direction and year (Table 6-5). Pearson's chi-squared test indicated a good fit of the model and the null hypothesis that the observed and predicted frequency of gene flow are the same; therefore, the null hypothesis cannot be rejected at $\alpha = 0.05$. The distance where 90% reduction in gene flow occurred ranged from 6.2 to 16.8 m in 2017 and from 3.6 to 28.1 m in 2018 (Figures 6-3 and 6-4). The maximum distance that 90% reduction in gene flow occurred in SE direction and NE direction in 2017 and 2018, respectively.

Hybrid Confirmation. PMGF from *Ga1-m* dent corn to *Ga1-s* popcorn was determined by kernel color. Amplification of *Ga1* followed by restriction enzyme digest confirmed that yellow kernelled progeny were hybrids from the result of PMGF (Figure 6-5). The restriction enzyme, Bs1I, cut *Ga1-s* (two bands) but not *Ga1-m* (one band). The hybrids resulted in three bands; one from *Ga1-m* and two from *Ga1-s*.

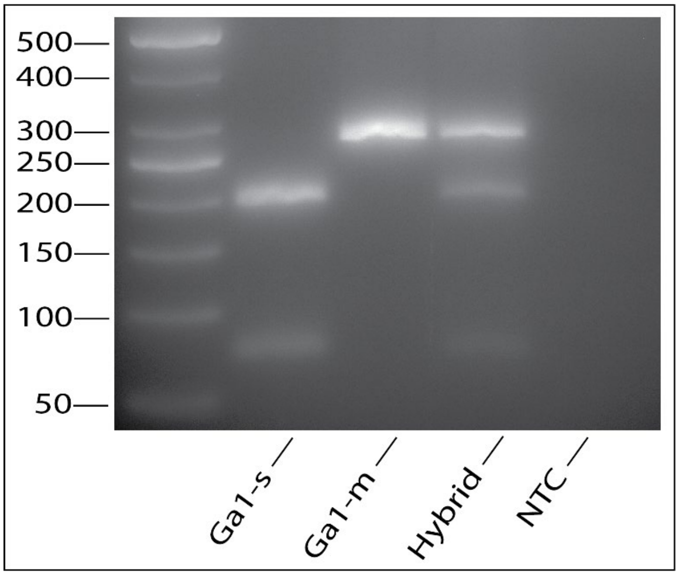

Figure 6-5. PCR amplification of the Ga1 *coding sequence was performed for the* Ga1-s *and* Ga1-m *parents and hybrid (*Ga1-s × Ga1-m*). A restriction enzyme digest was performed using Bs1I which does not cut* Ga1-m *(1 band) but does cut* Ga1-s *(2 bands). Gene flow from* Ga1-m *field corn to* Ga1-s *popcorn results in hybrids and restriction enzyme cuts half the PCR product (3 bands).*

Table 6-5. Estimation of coefficients, standard error, and test of significance for the double-exponential decay model for the prediction of pollen-mediated gene flow from Ga1-m dent corn to Ga1-s popcorn from a field experiment conducted at the University of Nebraska–Lincoln Eastern Nebraska Research and Education Center in 2017 and 2018.*

Coefficients †	Estimate	Std. Error	z value	Pr(>|z|) ‡
β_0	-8.88	0.10	-85.94	< 2E-16 ***
β_1	-1.65	3.68	-0.45	0.65
γ_1	-0.04	0.15	-0.30	0.77
β_1:Year 2	5.31	3.85	1.38	0.17
γ_1:Year 2	-3.77	1.31	-2.88	3.99E-03 **
β_2	1.36	0.17	7.83	< 2E-16 ***
γ_2	-0.28	0.08	-3.70	2.19E-04 ***
β_2:Direction N	0.19	0.06	3.26	1.13E-03 **
β_2:Direction NE	-0.03	0.08	-0.34	0.74
β_2:Direction NW	0.15	0.06	2.55	0.01 *
β_2:Direction S	0.38	0.07	5.58	0.00 ***
β_2:Direction SE	-0.21	0.10	-2.10	0.04 *
β_2:Direction SW	0.06	0.06	1.02	0.31
β_2:Direction W	0.38	0.07	5.70	1.22E-08 ***
γ_2:Direction N	0.21	0.08	2.73	0.01 **
γ_2:Direction NE	0.05	0.05	0.95	0.34
γ_2:Direction NW	0.21	0.08	2.69	0.01 **
γ_2:Direction S	0.17	0.07	2.33	0.02 *
γ_2:Direction SE	0.19	0.07	2.54	0.01 *
γ_2:Direction SW	0.21	0.08	2.72	0.01 **
γ_2:Direction W	0.20	0.08	2.67	0.01 **
β_2:Year 2	0.24	0.17	1.42	0.16
γ_2:Year 2	0.24	0.08	3.21	1.35E-03 **
β_2:Direction N:Year 2	0.17	0.06	2.84	4.51E-03 **
β_2:Direction NE:Year 2	-0.43	0.08	-5.36	8.55E-08 ***
β_2:Direction NW:Year 2	-0.03	0.06	-0.53	0.60
β_2:Direction S:Year 2	-0.43	0.07	-6.27	3.57E-10 ***
β_2:Direction SE:Year 2	0.16	0.10	1.54	0.12
β_2:Direction SW:Year 2	-0.18	0.06	-2.79	0.01 **
β_2:Direction W:Year 2	-0.26	0.07	-3.80	1.48E-04 ***
γ_2:Direction N:Year 2	-0.28	0.08	-3.64	2.77E-04 ***
γ_2:Direction NE:Year 2	-0.03	0.05	-0.65	0.52
γ_2:Direction NW:Year 2	-0.24	0.08	-3.07	2.14E-03 **
γ_2:Direction S:Year 2	-0.21	0.07	-2.93	3.42E-03 **
γ_2:Direction SE:Year 2	-0.19	0.07	-2.62	0.01 **
γ_2:Direction SW:Year 2	-0.23	0.08	-2.93	3.44E-03 **
γ_2:Direction W:Year 2	-0.20	0.08	-2.63	0.01 **

* $\text{logit}(\rho_i) = \beta_0 + \exp[\beta_1(\mathit{Year}) + \Upsilon_1(\mathit{Year}) \times \mathit{Distance}] + \exp[\beta_2(\mathit{Direction{:}Year}) + \Upsilon_2(\mathit{Direction{:}Year}) \times \mathit{Distance}]$, where ρ_i is frequency of gene flow of the i*th* observation; β_0 is the overall intercept; β_1 and β_2 are the intercepts for the first and second instances, respectively; and γ_1, and γ_2 are the decay rates.

† $\beta 2$ and $\gamma 2$ vary with the direction and the year.

‡ P-values show the test of significance at $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

6.4 DISCUSSION

Flowering synchrony was 25 to 30% in 2017 when the pollen donor and pollen receptor were planted on the same day. To increase flowering synchrony in 2018, the pollen donor was planted 11 days before the pollen receptor. This resulted in 100% flowering synchrony in 2018. The results indicate that maximum frequency of gene flow, 0.01615 (1.615%) and 0.04113 (4.113%), is observed at the closest distance of 1 m. Similar results were obtained in studies of gene flow from GM dent corn to conventional dent corn (40, 26, 25, 22). Baltazar et al. (40) reported frequency of PMGF at 1 m ranged from 0.064 (6.4%) to 0.215 (21.5%) across eight field locations. The relative size of the pollen source to the pollen receptor increases the frequency of gene flow due to a greater relative area of pollen from the pollen source (22, 26). At 3.6 to 28.1 m there was 90% reduction in PMGF depending on year and direction. PMGF was detected at the maximum distance tested (70 m in the ordinal directions).

A double exponential decay model was used to describe PMGF in this study. Beckie et al. (41) used double exponential decay model to explain PMGF in spring wheat. This approach for modeling PMGF has also been successful in weedy species such as barnyardgrass (*Echinochloa crus-gali* L.) (42), glyphosate-resistant to susceptible waterhemp [*Amaranthus tuberculatus* (Moq.) J. D. Sauer] (43), inter-specific hybridization of waterhemp and Palmer amaranth (*Amaranthus palmeri* S. Watson) (44), and glyphosate-resistant to susceptible giant ragweed (*Ambrosia trifida* L.) (45).

Gene flow was affected by direction from the pollen source. Wind speed at tassel height was correlated with frequency of gene flow but only to a low degree. Studies with higher correlation of wind parameters with frequency of gene flow in waterhemp and giant ragweed have been in bare ground areas and report higher wind speeds (43, 45); whereas, with a crop canopy present, wind speed tend to be slowed down (44, 46). Baltazar et al. (40) reported a low degree of correlation between gene flow in dent corn and wind speed (x^2 = 0.01) which indicates a low association between wind speed and gene flow frequency despite corn being a wind pollinated species. Similarly, an incomplete association between wind direction and PMGF in dent corn was observed by Della Porta et al. (26). Ivanovska et al. (47) reported a higher PMGF downwind when wind is blowing in a singular pronounced direction; however, no pattern in PMGF with wind blowing from distributed directions. In this study, wind direction did not dominate from a single direction.

Currently no isolation distance is required between popcorn and dent corn. Dent corn is used in border row as isolation in many popcorn breeding programs. With low-level PMGF at distance of at least 70 m from the pollen source (maximum distance tested in this study), popcorn breeding programs are at major risk of *Ga1-m* dent corn contamination. The most important issue for the maintenance of a cross-pollinated crop species in breeder production or seed production is genetic purity (48, 49). Contamination could go undetected for several years in a breeding program as the majority of dent corn and popcorn having yellow kernel color. Popcorn seed production is also at risk of dent corn contamination. It is unlikely that contamination would be detected, and seed could be distributed to contract growers for production. The popcorn industry could be impacted by PMGF that occurs during popcorn production if the seed is checked by country inspectors and samples return positive results of GM contamination. Europe allows up to 0.9% GM contamination in crop seeds (50). Currently there is no plan in place for the testing of current and pipe-line commercial dent corn hybrids for the presence of *Ga1-m* (14). This information would be beneficial for popcorn breeders and producers when communicating with neighbors or selecting bordering dent corn hybrids. There is known *Ga1-m* resistance in sweet corn which could potentially be backcrossed into popcorn (15).

The popcorn industry should be cautious of potential *Ga1-m* dent corn contamination. Known strategies to avoid PMGF in corn such as isolation distances, border rows harvested separately, using known *Ga1* dent corn as border rows, and staggering planting dates to avoid flowering synchronization with neighboring dent corn fields, should be utilized.

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