SUSCEPTIBLE CROP RESPONSE TO SOIL RESIDUES AND FOLIAR EXPOSURE OF DICAMBA

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

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In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Plant Sciences

March 2016

Fargo, North Dakota

North Dakota State University
Graduate School

Title

Susceptible crop response to soil residues and foliar exposure of dicamba

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Dry bean, soybean, sugarbeet, and sunflower were tested for tolerance to dicamba residue in soil. Visible injury was seen on dry bean and soybean depending on location, but yield differences were not present. Increasing rates of glyphosate and dicamba caused injury to dry edible bean; however, final bean weight only differed from the nontreated when 18 g ha^{-1} dicamba was included. Dicamba applied at 1.8 g ha^{-1} caused a consistent delay in physiological maturity. Dicamba concentration in plants did not predict yield loss. Across the four market classes in the field, dicamba caused yield loss, but glyphosate did not. Effects of the herbicide on yield was the same in each market class.

ACKNOWLEDGEMENTS

This thesis is the combined effort of many people that have helped me in the past two years. First, I would like to thank my major advisor, Dr. Rich Zollinger, for all of his enthusiasm, patience, and guidance. I would also like to acknowledge the time that Dr. Kirk Howatt has put into my writing. Dr. Ted Helms has been equally patient to help me understand the statistics needed to complete this thesis and discuss alternative experiment designs. Dr. Abbey Wick has included me in many Soil Health Extension tours and meetings which has peaked my interest in interdisciplinary research incorporating understanding of soil and weeds.

I would like to acknowledge the funding and support of Monsanto and Northarvest Bean Growers. A special thanks to Steve Valenti who was able to work with me, help us establish a second location for the soil residue study, and have great conversations. Also, all of the faculty and staff of the Plant Sciences Department have welcomed me to North Dakota and always been willing to help when I ask.

The most gracious of my thanks go to my friends and family that have been supporting me the past two years in the best and worst of times. Thank you to all of the graduate students that have peer reviewed my paper, provided a wonderful learning community, and made my North Dakota my temporary home. Thank you to my family whose tradition in agriculture has led me to scientific research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

USDA..United States Department of Agriculture

NASS ....National Agriculture Statistics Service

POST...postemergence

PRE ....preemergence

ae ....acid equivalent

pK_a ....acid dissociation constant

K_{ow} ....octanol-water partition coefficient

pH...potential hydrogenii (potential hydrogen)

CEC..cation exchange capacity

OM ....organic matter

DAE ....days after emergence

EPOST ....early postemergence

MPOST ....mid- postemergence

RCBD...randomized complete block design

NDAWN ....North Dakota Agricultural Weather Network

DAT ....days after treatment

glyt ....glyphosate

dica...dicamba

LIST OF APPENDIX TABLES

CHAPTER I. LITERATURE REVIEW

Soybean Production in North Dakota

Soybean (*Glycine max L.*) production is rising in the United States, with approximately 33 million hectares produced in 2013 and an estimated value of $41.8 billion(USDA NASS 2013b and 2014). Area planted to soybean in North Dakota was estimated at 2 million ha in 2014 with a value of $1.7 billion (Young et al. 2014). Widespread adoption of soybean cultivars resistant to glyphosate (N-phosphonomethyl glycine), an enolpyruvyl-shikimake-3-phosphate synthase inhibitor, has resulted in heavy reliance on postemergence weed control and limited modes of action used in most soybean fields (Young 2006). According to a 2012 USDA survey, herbicides were used on 98% of soybean hectares in the US; approximately 90% of the soybean hectares that used herbicides primarily used glyphosate (NASS 2013a). Repeated use of a single mode of action will select for naturally resistant weed biotypes, which has been evident in places where glyphosate has been continuously used (Johnson et al. 2009). Three glyphosate-resistant weeds have been confirmed in North Dakota: common ragweed (*Ambrosia artemisiifolia L.*) in 2007, tall waterhemp (*Amaranthus tuberculatus Moq.*) in 2010, and kochia (*Kochia scoparia L.*) in 2012 (Heap 2014).

Glyphosate Resistant Weeds and Management

Currently, soybean growers have limited options to control glyphosate-resistant weeds in soybean postemergence because effective herbicides to control many of these weeds would also injure soybean. Dicamba-resistant (3,6-dichloro-2-methoxybenzoic acid) soybean will provide additional options for resistant-weed management (USDA 2015). Dicamba can control resilient weeds, such as common purslane (*Portulaca oleracea*) (Stacewicz-Sapuncakis et al. 1973). This chemical is also effective in controlling glyphosate-resistant and suspected -resistant weeds:

waterhemp, common ragweed, horseweed (*Conyza canadensis*), and kochia (Zollinger 2014). Although dicamba has been used for decades, reported weed resistance to dicamba and other growth regulator herbicides is relatively low compared to other modes of action (Mithila et al. 2011). Due to a combination of factors, including dicamba's low risk of weed resistance, high effectiveness on other herbicide-resistant weeds, and familiarity among producers, dicamba-resistant soybean presents a new opportunity to control herbicide-resistant weeds (Behrens et al. 2007).

Dicamba

Patented in 1958 by S.B. Richter and first registered for commercial use in 1967, dicamba is a benzoic acid plant growth regulator herbicide (Senseman et al. 2007). Dicamba is primarily used in postemergence (POST) applications to control broadleaf weeds in corn, small grains, and grasses; however, dicamba can also be used preemergence (PRE) in corn at a rate of 0.56 kg ae ha^{-1} and for fall and early spring applications prior to soybean and sorghum.

Dicamba is a weak acid herbicide with a pK_a value of 1.87 and a K_{ow} of 0.29; therefore, dicamba would be in conjugate base form in most soils and likely partition with water rather than lipid portions. Burnside and Lavy (1966) demonstrated dicamba behaved as an anion in electrophoresis work, which explains why dicamba is not strongly bound to clay and travels readily with soil water: gravitationally or by capillary action. Dicamba acid has a solubility of 4500 mg L^{-1}, but often is formulated into a salt for commercial use. Some of these salt formulations are dimethylamine, sodium, diglycolamine, isopropylamine, and potassium salts of dicamba (Senseman et al. 2007). Each of these formulations are more soluble in water than the acid form, which is important for mixing and plant absorption. The most soluble formulation, dimethylamine salt, can have a solubility of 720,000 mg L^{-1} (Senseman et al. 2007).

Dimethylamine salt was the first formulation available for commercial use and was marketed as Banvel® in 1967 by the Vesicol Chemical Company (Grassi 2012). While dimethylamine salt is the most soluble formulation, the high volatility of this formulation increased risk of off-site movement to susceptible crops. Thus, less volatile formulations were created. A low-volatility formulation, diglycolamine salt, is marketed as the herbicide Clarity® that has been used since 1992 (Anonymous 2010).

Foliar Injury

Non-target crops can express herbicide-induced foliar injury due to volatilization, particle drift, or sprayer contamination. Vapor drift is defined in the Herbicide Handbook as the movement of pesticides as vapor from the area of application after the spray droplets have impinged on the target (Senseman et al. 2007). Particle drift is the lateral movement of a spray droplet by wind at the time of application. Spray tank contamination mimics drift symptoms, but is due to insufficient clean-out procedures (Steckel et al. 2006). These examples of off-site movement and misapplication are visualized when susceptible plants show symptoms of herbicide phytotoxicity. Characteristic syptoms of dicamba and most other growth regulator herbicides are epinasty, leaf cupping, strapped viens, and calloused leaves or stems (Grossmann 2010). Off-target application due to volatilization, particle drift, and sprayer contamination can result in similar foliar injury to susceptible species.

Dicamba is an herbicide that targets broadleaf weeds because of the difference in metabolism between most monocots and dicots. A study comparing herbicide movement within cereals and broadleaf weeds observed slower translocation in cereals, which are generally tolerant, and faster translocation in broadleaf plants (Chang and Born 1971a). Cereal plants can metabolize dicamba into non-phytotoxic metabolite by two processes, while broadleaves were only found to have one slow metabolism process.

New, low-volatile formulations of dicamba have become available, but there is still concern for off-target movement and misapplication of dicamba causing injury to susceptible crops. Injury can take several weeks to appear with low rates of dicamba (Altom and Stritzke 1973; Burnside and Lavy 1966; Hahn et al. 1969; Skipper et al. 1996). Once absorbed, dicamba is quickly translocated through the xylem and the phloem to accumulate in the meristematic tissue of the plant (Stacewicz-Sapuncakis et al. 1973). Furthermore, dicamba continued to translocate to the newest tissue as the plant matured (Chang and Born 1971a, 1971b). The speed of dicamba translocation was demonstrated by Chang and Born (1971b). They observed that dicamba moved upward and downward from the treated leaf within an hour of application and that dicamba reached maximum absorption within 24 hours. However, this might differ depending on plant vigor; actively growing plants will translocate herbicide quicker than stressed plants.

Dicamba translocation in the plant has been shown to correlate with the plant stage at the time of spraying. In purple nutsedge (*Cyperus rotundus L.*), as much as 40% of dicamba applied at the vegetative stage was translocated out of the treated leaf, but only 15% was translocated when applied during flowering (Magalhaes et al. 1968). Generally, the movement of dicamba to actively growing tissue was reduced if dicamba was applied after flowering (Magalhaes et al. 1968; Stacewicz-Sapuncakis et al. 1973).

Soybean and cotton (*Gossypium hirsutum L.*) have both been studied intensively to relate dicamba injury to yield loss, but other non-target crops have limited information. Current formulations have caused phytotoxic effects on soybean with as low as 0.01% of the labeled rate in corn; this could be less than that which is left in an improperly cleaned spray tank (Strachan et al. 2010). Injury caused by dicamba exposure to pre-flower soybean has not correlated to reduction in soybean yield (Wax et al. 1969). Simulated drift to soybean in South Dakota was most detrimental to yield when exposure occurred at flowering, but dicamba caused less germination of harvested seeds when applied during pod fill (Auch and Arnold 1978).

Low-volatile formulations are not necessarily safer for susceptible crops, nor can a new formulation eliminate misapplication to non-target crops. New formulations, in combination with good management practices, are meant to prevent contact of the herbicide with non-target species. Yield reduction caused by poor weed control typically is more costly than crop injury caused by dicamba; however, the potential for injury to susceptible crops due to increased dicamba usage could introduce a widespread problem of economic concern (Strachen 2010).

Soil Persistence

Label recommendations advise waiting 45 days per 560 g ha^{-1} for susceptible crop safety west of the Mississippi River (Anonymous 2010). This restriction is broad considering that the specified area encompasses environments from hot and arid to cool and wet. These environmental conditions are among factors that affect degradation and dissipation of dicamba in the soil through leaching and microbial breakdown (Senseman et al. 2007).

Herbicide movement in the soil is typically calculated as a function of soil properties, such as soil texture, available water, pH, and properties of the herbicide like solubility and charge (Grover 1977, Baes et al. 1983). In soil columns, dicamba moved with soil water (Harris 1967, Friesen 1965, Donaldson et al. 1965). Dicamba also had limited adsorption to clays, which have a negative charge. This supports that dicamba moves as an anion in soils, further confirmed by electrophoresis work by Burnside et al. (1966). Some soil textures, with high porosity, could result in more leaching of dicamba, since it is highly soluble and soils with a high bulk density could physically inhibit leaching. Equations such as those described by Grover (1977) and Baes et al. (1983) can help predict losses by leaching; however, water does not move constantly downward. Soils with high bulk density have increased capillary action, or water moving up through the soil profile due to surface evaporation and plant uptake. Dicamba moved with capillary water into the root zone while still at rates toxic to plants, supporting that surface evaporation and subsequent upward capillary movement will influence dicamba movement in soil (Harris et al. 1964). Canadian prairies have potential for long soil residual and upward movement into the planting zone during dry conditions due to the evaporation from soil surfaces that pulls soil water back into the root zone (Friesen 1965). Leaching will initially delay crop injury from dicamba soil residues; however, dry conditions could reintroduce dicamba into the root zone by capillary action.

Dicamba is also known to be degraded microbially. Ideal temperatures for microbial breakdown have been described (Burnside et al. 1966; Hahn et al. 1969; Smith et al. 1975; Fogarty et al. 1994). A slightly acidic pH seems to be ideal (Corbin et al. 1967; Fogarty et al. 1994). Texture with relation to amount of available carbon for microbial population also seems to have a role (Upchurch et al. 1962; Donaldson et al. 1964; Hahn et al. 1968). Moreover, subsoil, even in ideal temperature and moisture, has limited organic matter and oxygen compared to topsoil (Hahn 1968). At current labeled rates, dicamba is not typically persistent when these conditions are met (Burnside et al 1966). However, the northern plains experience low rainfall and early frosts which might cause prolonged dicamba residue at higher rates. Studies in Canada indicated low risk of carryover from fall application at labeled rates, but rates of dicamba above 0.6 kg ha^{-1} applied in October and applications less than 15 days before planting usually resulted in unwanted injury to legumes (Moyer et al. 1992). Fall applications tested in northern Minnesota and the Red River Valley region at rates above 1.1 kg ha^{-1} injured soybean the following spring, especially in drier winters (Magnusson and Wyse 1989). Thompson et al. (2007) observed yield reductions only in rates greater than 0.28 kg ha^{-1} dicamba applied at the time of planting. However, the risk of phytotoxic amounts of dicamba residues can vary by region or year.

The fate of dicamba is also affected by many factors that influence leaching and microbial breakdown, many of which are interconnected. Typically, soil microbiology is most diverse in the top 15 cm of a soil because oxygen becomes limited at lower depths (Skipper et al. 1996). Microbial health is also dependent on the soil management and texture. In silty loam, 80% of dicamba dissipated in 8 days at 15 C, but in clay and sandy loam, the temperature had to be over 20 C and took over 14 days (Smith et al 1975). In order for effective microbial breakdown into nontoxic components, dicamba must stay in the upper 15 cm. Leaching will provide crop safety but is somewhat reversible while microbial breakdown is a complete detoxification.

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CHAPTER II. SUSCEPTIBLE CROP RESPONSE TO SOIL RESIDUES OF DICAMBA

Abstract

Dicamba (3,6-dichloro-2-methoxybenzoic acid) is a common growth regulator herbicide used to control broadleaf weeds. The introduction of dicamba-resistant soybean (*Glycine max L.*) and increase of dicamba use rates creates an elevated risk of injury to susceptible crops in rotations. Dicamba was applied in the summer prior and immediately before planting to evaluate injury and impact yield of four susceptible, high value crops: dry bean (*Phaseolus vulgaris L.*), soybean, sugar beet (*Beta vulgaris L.*), and sunflower (*Helianthus annuus L.*). Dry bean and soybean had visible injury depending on location but no yield differences could be detected in any crop. Dry bean, soybean, sugar beet, and sunflower yields were not impacted by amounts of dicamba in the soil less than 2809 g ha^{-1} present at the time of planting, which is more than double the suggested rate for dicamba-resistant soybean. More research is needed to ensure the safety of crops across the region and in different environments.

Nomenclature: dicamba, 3,6-dichloro-2-methoxybenzoic acid, diglycolamine salt; glyphosate, *N*-(phosphonomethyl)glycine; dry bean, *Phaseolus vulgaris L.*; soybean, *Glycine max L.*; sugar beet *Beta vulgaris L.*; and sunflower *Helianthus annuus L.*

Key words: soil residual herbicide, auxin-type herbicides, bioassay

Introduction

Dicamba-resistant soybean will provide additional options for resistant-weed management, especially for glyphosate-resistant weeds (USDA 2015). Dicamba has been used for broadleaf weed control within the crop season and preemergence. The maximum rate for fall and early spring burndown is 0.56 kg ae ha^{-1} prior to soybean varieties without the dicamba-resistant trait.

Dicamba residue in the soil has been phytotoxic to crops planted the year after an application (Schweizer et al. 1971; Magnusson et al. 1987; Moyer et al. 1992). Dicamba dissipation depends on environmental conditions (Friesen et al. 1965, Skipper 1996, Magnusson et al. 1987) and soil properties (Upchurch et al. 1962; Burnside et al. 1966; Hahn et al. 1969). Dicamba concentrations in the soil are reduced by microbial breakdown and leaching; therefore, the herbicide is not typically phytotoxic to crops grown in warm climates with adequate rainfall and well drained soils (Burnside et al. 1966).

Equations such as those described by Grover (1977) and Baes et al. (1983) can help predict losses by leaching; however, water does not move constantly downward. Soils with high bulk density have increased capillary action, or water moving up through the soil profile due to surface evaporation and plant uptake. Dicamba moved with capillary water into the root zone while still at rates toxic to plants, supporting that surface evaporation and subsequent upward capillary movement will influence dicamba movement in soil (Harris et al. 1964).

The fate of dicamba is also affected by many factors that influence leaching and microbial breakdown, many of which are interconnected. Typically, soil microbiology is most diverse in the top 15 cm of a soil because oxygen becomes limited at lower depths (Skipper et al. 1996). Microbial health is also dependent on the soil management and texture. In silty loam, 80% of dicamba dissipated in 8 days at 15 C, but in clay and sandy loam, the temperature had to be over 20 C and took over 14 days (Smith et al 1975). In order for effective microbial breakdown into nontoxic components, dicamba must stay in the upper 15 cm. Leaching will provide crop safety but is somewhat reversible while microbial breakdown is a complete detoxification.

Label recommendations advise waiting 45 days per 560 g dicamba ha^{-1} for susceptible crop safety west of the Mississippi River (Anonymous 2010). This restriction is broad considering that the specified area encompasses environments from hot and arid to cool and wet. Therefore, our objectives were to test the safety of rotational crops that could follow new dicamba-resistant soybean in the North Dakota Red River Valley.

Materials and Methods

Proposed dicamba use rates in dicamba-resistant soybean allow up to 1120 g ha^{-1} to be applied PRE and up to 1120 g ha^{-1} across multiple timings during the season (Anonymous 2015). Two experiments tested crop response to potential dicamba residue. Separate field trials were established for 'Ensign' navy bean, 'AG0832' soybean, 'SV36272RR' sugarbeet, and 'P63ME70' sunflower to evaluate crop response to both sets of treatments at two locations: Hillsboro (47º19'46.9"N 97 º 05'28.1"W) and Erie, ND (47 º 04'05.1"N 97 º 24'37.8"W). Soil type at Hillsboro was a Gardena coarse-silty, mixed, superactive, frigid Pachic Hapludolls (14% sand, 71% silt, 15% clay) and at Erie was a Barnes fine-loamy, mixed, superactive, frigid, Calcic Haludolls- Svea fine-loamy, mixed, superactive, frigid Pachic Hapludolls (40% sand, 38% silt, 22% clay) (USDA-NRCS 2014; USDA-NRCS 2016). Each site had a pH of 7, CEC of 19, and OM content of 4 to 5% (USDA-NRCS 2014). The field sites were fertilized according to extension publication guidelines for each crop based on soil test analysis (Franzen 2013). All crops were planted in rows spaced 0.8 m apart on May 22, 2015. Experiments were maintained weed-free by labeled chemical control options that would not cause injury to the crop.

Methods common to both experiments

All herbicide treatments were applied with a CO_2-pressurized backpack sprayer and hand boom system at 80 L ha^{-1} and 276 kPa using TT11002 nozzles. Plant populations of 6 m of row in each plot were recorded both 1 day after emergence (DAE) and the day of harvest. Each crop was evaluated for visible injury 7, 14, 21, and 28 DAE. Yield was estimated by harvesting 6 m from each of two treated rows in dry bean, soybean, and sunflower and harvesting 6 m from one treated row of sugarbeet. Sugarbeet was also analyzed for sugar content.

Experiment 1

Dicamba treatments were applied in 2014 to bare ground field sites and kept weed free throughout the season. Field studies were established in a randomized complete block design (RCBD) with 4 replications at Hillsboro and 3 replications at Erie. At Hillsboro, herbicide treatments were applied to the center 3 m the length of plots 4.5 m by 9 m long. At Erie, herbicide was applied to the center 1.5 m width of plots that were 3 m wide by 9 m long. Applications in 2014 were timed to mimic approximate spray application timing in soybean: PRE (May 24), EPOST (June 13), and MPOST (July 11). PRE rates ranged from 1120 to 2240 g dicamba ha^{-1}, EPOST from 560 to 1120 g ha^{-1}, and MPOST from 560 to 4480 g ha^{-1} in various combinations. All treatments were compared to a nontreated control that had no dicamba applied, and clopyralid at 158 g ae ha^{-1} applied MPOST served as a standard for persistent herbicide residue. Experiment was not tilled in the fall or spring following dicamba treatments and plots were planted in 2015.

Experiment 2

Each crop was a separate experiment arranged in an RCBD with four replicates at each location. The center 1.5 m was treated in plots 3 m wide by 9 m long the day of planting (May 22, 2015). Plots were treated with increasing rates of dicamba at 0,140, 351, 702, 1054, 1405, 1756, 2107, 2458, and 2809 g ha^{-1}.

Statistical Analysis

Analysis of variance across environments was conducted using SAS 9.3 (SAS Version 9.3, Institute, Inc., Cary, NC) for each data parameter, with treatments and location as fixed variables. Data were combined over locations if variances were considered similar (means square error values within a factor of 10). Means were separated using Fisher's protected LSD at the 5% level of significance.

Results and Discussion

Sugarbeet and sunflower populations were not affected by treatment at emergence or harvest in either study (Tables A1 and A2). At the dicamba rates tested, injury was not observed in sugarbeet or sunflower at either location, in either study (Table A1 and A2). Sunflower yield was only harvested at Erie because high winds resulted in approximately 75% plant loss at Hillsboro. Sunflower yield at Erie was similar across all treatments in Experiment 1 (Table 2.1) or Experiment 2 (Table 2.2). Also, sugarbeet extractable sugar was not affected by treatment in either experiment (Table 2.1 and Table 2.2).

The maximum dicamba rate applied, 2809 g ha^{-1} was at planting more than double of labeled rates that would be applied during the previous season; however, no yield impact was observed. In a previous study conducted by Walker et al. (1992), sunflower yield was not reduced when dicamba was applied the day before but was reduced when dicamba was applied immediately after planting. However, dicamba rates in their study of 560 g ha^{-1} or less were lower than our study. In another study, sunflower was observed to be injured and yield reduced from fall applied dicamba at 1100 g ha^{-1} in Morris, MN ≈ 243 km SE from Erie, ND (Magnusson et al. 1987). The site at Morris did have a higher pH than our sites (7.7 compared to 7) and pH higher than 6.5 has been reported to slow dicamba degradation (Fogarty et al 1994). Organic matter levels in our study at Hillsboro and Erie and Magnusson et al. (1987) study at Morris were higher than 4%, which should encourage microbial breakdown. Other factors such as temperature and rainfall were also similar between the two sites, so it is unclear why injury and yield loss occurred at Morris and not in our studies (Figure 2.1). Magnusson et al. (1987) data did indicate injury and impact on yield was different at other MN sites depending on year and incidents of surface movement of dicamba into other plots resulted in one year of data in some locations.

Studies in Colorado documented over 90% sugarbeet death in all plots treated with fall applied dicamba at 2200 g ha^{-1} or greater (Schweizer et al. 1971). Two winters after the initial application, the highest rate of dicamba at 6700 g ha^{-1} caused yield reduction. The soil type in Colorado had a high pH of 8.4 and low organic matter of 1.9%. Possible reasons for injury in Colorado compared to North Dakota might be the high pH, low organic matter, and the dry conditions. Rainfall was not mentioned apart from the irrigation needed to grow sugarbeets, which may have attributed to the long persistence of dicamba. Based on the literature and observations of this study, dicamba persistence in sunflower and sugarbeet fields still depends on many factors.

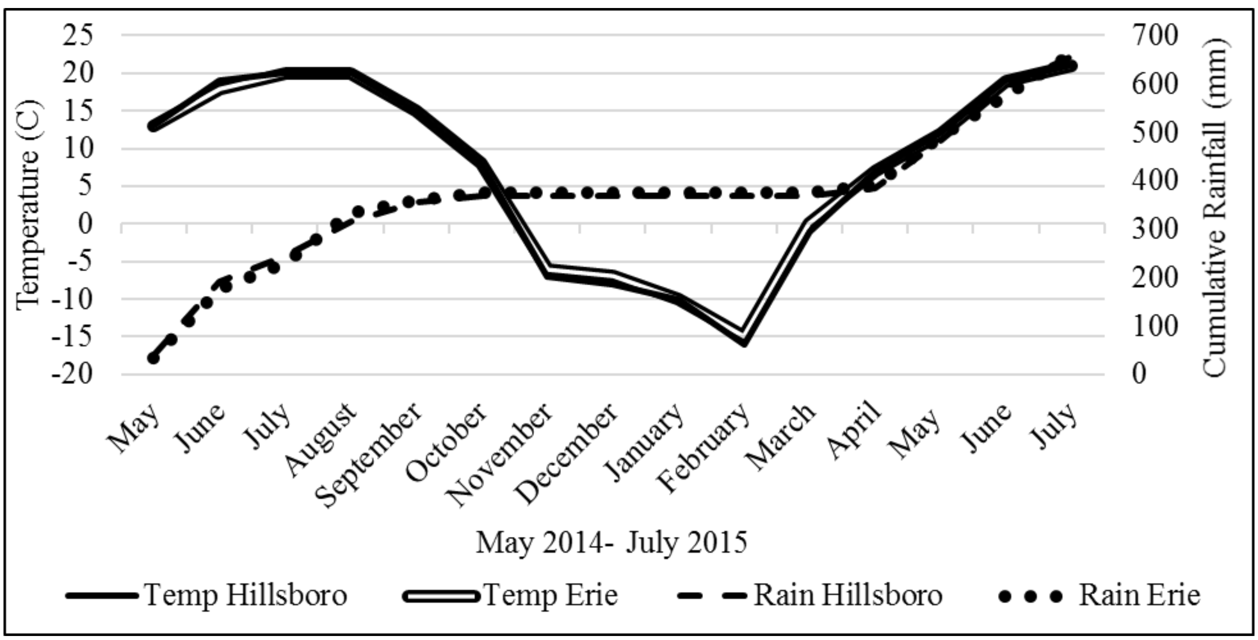

Figure 2.1. Monthly average temperature and cumulative rainfall after the first dicamba application in May 2014 to the end of injury in July 2015 at both sites. Hillsboro and Erie, ND 2014 and 2015. Data acquired from (NDAWN 2016).

Table 2.1. Estimated grain yield of dry bean, soybean, and sunflower and extractable sugar of sugarbeet planted the year after various rates and timings of dicamba were applied to bare soil and compared to clopyralid as a standard. Hillsboro and Erie, ND 2015.

Treatment[a]	Dry Bean	Soybean	Sugarbeet	Sunflower [b]
g ha^{-1}		kg ha^{-1}		
Untreated	-[c]	2930	4820	1660
Dica 1120, 0, 0	2230	2790	4390	2310
Dica 1120, 560, 0	-[c]	2650	4740	1620
Dica 1120, 560, 560	2410	2890	4710	1860
Dica 2240, 0, 0	2380	2820	5130	1940
Dica 2240, 1120, 0	2340	2690	4900	1830
Dica 2240, 1120, 1120	2200	2830	4780	1850
Dica 0, 0, 560	2350	2840	4750	1960
Dica 0, 0, 1120	2330	2730	4400	2260
Dica 0, 0, 2240	2330	2980	4970	1720
Dica 0, 0, 4480	2040	2860	4800	2100
Dica 0, 560, 560	2320	2830	4430	1780
Dica 0, 1120, 1120	2380	2680	5110	1970
Clop 0, 158, 0	2270	2850	5580	1740
LSD	NS	NS	NS	NS

[a]Rates of herbicide applied at each timing: PRE (May 24), EPOST (June 13), and MPOST (July 11) in 2014. Abbreviations: Dica, dicamba; Clop, clopyralid.
[b]Sunflower yield is only the mean of Erie due to crop loss at Hillsboro.
[c]Omitted due to application error.

Soybean plant population did not differ by treatment in either study (Tables A1 and A2). Injury to soybean was not observed in Experiment 1 at either location. Soybean yield in Experiment 1 was unaffected by treatment (Table 2.1). Thompson et al. (2007) observed little to no injury in soybean when dicamba was applied more than 21d before planting, consistent with lack of injury in Experiment 1. Our results were also similar to Magnusson et al. (1987) results at Waseca and Lamberton, MN, where they found no yield decrease or significant injury until dicamba rates of 4500 g ha^{-1} were applied the fall before soybean planting. However, in the same study, injury to soybean increased in sites farther north, such as Morris, MN, which recorded soybean injury and yield reduction when dicamba was applied at 2200 g ha^{-1} in the fall before soybean. As previously discussed, Morris, MN and our sites at Hillsboro and Erie, ND have similar pH, organic matter, temperature, and rainfall, so it was unclear why injury and yield loss occurred at Morris and not in our studies.

Table 2.2. Estimated grain yield of dry bean, soybean, and sunflower and extractable sugar of sugarbeet planted immediately after application of increasing rates of dicamba were applied to bare soil. Hillsboro and Erie, ND 2015.

Treatment	Dry edible bean	Soybean	Sugarbeet[a]	Sunflower[b]
g ae ha^{-1}		kg ha^{-1}		
Untreated	2300	2880	2360	2110
140	2320	2880	2130	1900
351	1990	2790	2170	1930
702	2130	2890	2250	2010
1054	2060	2860	2120	1890
1405	1860	2840	2300	2050
1756	2330	2820	2270	2030
2107	2310	2880	2080	1860
2458	2290	2790	2190	1950
2809	2510	2870	2240	2000
LSD	NS	NS	NS	NS

[a]Sunflower yield is only the mean of Erie due to crop loss at Hillsboro.

In Experiment 2, injury to soybean was observed at Erie (Table 2.3). Generally, injury increased with rate, with as much as 80% injury at 7 DAE in plants treated with dicamba at 2809 g ha^{-1}. Injury was less than 25% in plots treated with 1120 g ha^{-1} or less 7 DAE. Dicamba applied at 1120 g ha^{-1} would be the maximum amount of dicamba that can be applied to dicamba-resistant soybean in the previous year, and this study applied 1120g ha^{-1} at the time of planting. By 28 DAE maximum injury was 23% and no injury was observed 42 DAE. Despite early injury in soybean at Erie, soybean yield did not differ by treatment. Additionally, location and treatment did not interact for yield response (Table 2.2). Thompson et al. (2007) reported that yield decreased when 280 g ha^{-1} dicamba was applied at planting. In our study, rates of 10x the label rate tested by Thompson et al. (2007) did not result in yield loss. Injury to soybean in the study by Thompson et al. (2007) was variable by year, as was yield. Injury to soybean was higher in 2005 and yields were lower, which was attributed to environmental differences, such as moisture.

Table 2.3. Estimated yield and visual injury of soybean planted immediately after application of increasing rates of dicamba were applied to bare soil. Erie, ND 2015.

	Injury				Yield
Treatment	7	14	21	28	Grain weight
g ha^{-1}	%				kg ha^{-1}
0	0	0	0	0	2860
140	16	1	14	4	2870
351	3	0	0	0	2720
702	3	0	11	0	2820
1054	18	6	16	5	2830
1405	28	15	19	9	2720
1756	29	13	15	14	2830
2107	55	24	34	18	2920
2458	64	21	28	24	2760
2809	76	34	31	21	2900
LSD	27	11	NS	10	NS

None of the treatments affected dry bean population at emergence or harvest within either study (Table A1 and A2). Injury was observed at both locations in Experiment 2 but only at Erie in Experiment 1. Dry bean in Experiment 1 were injured by all treatments at Erie 7 DAE but there was no injury at Hillsboro. Injury at Erie persisted in dry bean treated with 2240 and 4480 g ha^{-1} dicamba applied MPOST the previous year (Table 2.4). In Experiment 2, at both locations, injury was significant by treatment at 7, 14, and 21 DAE with injury as high as 70% 7 DAE in plants treated with dicamba at 2809 g ha^{-1}. Injury was less than 20% with dicamba at 1120 g ha^{-1} or less (Table 2.5). Despite injury early in the season, yield was not influenced by dicamba treatments in either study (Table 2.4 and 2.5).

Table 2.4 Estimated yield and visual injury of dry edible bean planted the year after various rates and timings of dicamba were applied to bare soil, compared to clopyralid as a standard. Erie, ND 2015.

	Injury				Yield
Treatment	7	14	21	28	Grain weight
g ha^{-1}		%			kg ha^{-1}
Untreated	-[b]	-	-	-	-
Dica 1120, 0, 0	10	2	0	2	1780
Dica 1120, 560, 0	-	-	-	-	-
Dica 1120, 560, 560	3	0	3	2	2410
Dica 2240, 0, 0	17	1	0	0	2230
Dica 2240, 1120, 0	10	3	0	2	1920
Dica 2240, 1120, 1120	12	7	2	0	2160
Dica 0, 0, 560	22	0	3	3	2210
Dica 0, 0, 1120	12	2	0	2	2180
Dica 0, 0, 2240	19	12	13	7	1760
Dica 0, 0, 4480	31	32	30	45	2100
Dica 0, 560, 560	8	3	0	3	2560
Dica 0, 1120, 1120	20	3	3	3	2230
Clop 0, 157.5, 0	13	0	0	0	1940
LSD	NS	15.4	16	16	NS

[a]Rates of herbicide applied at each timing: PRE (May 24), EPOST (June 13), and MPOST (July 11) in 2014. Abbreviations: Dica, dicamba; Clop, clopyralid.
[b]Omitted due to application error.

Based on these data, dicamba injury depended on environmental conditions. In these environments, yield was not influenced by increasing dicamba rates, regardless of injury early in the season. This research supports other studies that suggest high rates of dicamba from the previous year may cause injury, but this injury does not necessarily indicate yield loss (Walker et al. 1992; Thompson et al. 2007). More research is required to evaluate the safety of crops across the region and in different environments.

Table 2.5 Estimated yield and visual injury of dry edible bean planted immediately after application of increasing rates of dicamba were applied to bare soil. Hillsboro and Erie, ND 2015.

	Injury				Yield
Treatment	7	14	21	28	Grain Weight
g ha^{-1}	%				kg ha^{-1}
0	0	0	0	0	2300
140	4	8	4	7	2320
351	9	10	7	9	1990
702	10	10	3	5	2130
1054	13	9	4	7	2060
1405	28	24	9	15	1860
1756	34	16	3	6	2330
2107	33	18	4	3	2310
2458	50	23	9	5	22901
2809	54	31	16	14	2510
LSD	10	16	7	NS	NS

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CHAPTER III. RELATING DICAMBA INJURY AND RESIDUE TO YIELD REDUCTION IN DRY BEAN

Abstract

The purpose of this study was to determine if visible injury caused by dicamba (3,6-dichloro-2-methoxybenzoic acid) and glyphosate (*N*-(phosphonomethyl) glycine) drift on dry bean or µg kg^{-1} herbicide concentration in leaf tissue could predict dry bean yield. Dicamba was applied at 0.18, 1.8, and 18 g ha^{-1}, glyphosate at 0.37, 3.7, 37 g ha^{-1}, and combined herbicides at the low, medium, and high rate, respectively. Additionally, four dry bean (*Phaseolus vulgaris L.*) market classes were compared at 18 g ha^{-1} dicamba, 37 g ha^{-1} glyphosate, and the combination in field and greenhouse. Injury was observed for all herbicide treatments, but final bean weight only differed from the nontreated when treated with 18 g ha^{-1} dicamba alone or in combination with 37 g ha^{-1} glyphosate. Dicamba at 1.8 g ha^{-1} caused a consistent delay in physiological maturity that could require a desiccation application prior to harvest. Dicamba residue found in the dry bean plants or visible injury was not useful to create a yield model for North Dakota due to low predictive value. Across the four market classes in the field, dicamba and dicamba plus glyphosate caused yield loss, but glyphosate alone did not. Yield also differed by market class but herbicide residue had the same influence on all market classes. Greenhouse experiments had biological differences similar to the field experiments.

Nomenclature: dicamba, 3,6-dichloro-2-methoxybenzoic acid, diglycolamine salt; glyphosate, *N*-(phosphonomethyl)glycine; dry bean, *Phaseolus vulgaris L.*

Key words: herbicide drift, dry bean injury, auxin-type herbicides.

Introduction

The registration of dicamba-resistant soybean varieties will provide additional options for resistant-weed management (USDA 2015); however, the increased risk of off-target movement from dicamba is a cause for concern for injury to susceptible crops in the area. Dicamba is noted for off-target movement and damage to susceptible plant species (Behrens et al. 1979; Johnson et al. 2012; Robinson et al. 2013). Susceptible crops can express herbicide-induced foliar injury, due to volatilization, particle drift, and sprayer contamination.

New, low-volatile formulations of dicamba have been developed, but there is still concern for off-target movement and misapplication of dicamba, which may cause injury to susceptible crops. Injury can take several weeks to appear with low rates of dicamba (Altom et al. 1973; Burnside et al. 1966; Hahn et al. 1969; Skipper et al. 1996). Once absorbed, dicamba is quickly translocated through the xylem and the phloem to accumulate in the meristematic tissue of the plant (Stacewicz-Sapuncakis et al. 1973) and continues to translocate to newest tissue as the plant matures (Chang et al.1971).

Translocation can differ by species, but most research has focused on soybean rather than other non-target crops. In soybean, injury caused by pre-flower exposure to dicamba has not correlated to yield reduction (Wax et al. 1969). However, simulated drift was most detrimental to yield when exposure occurred at flowering. Dicamba reduced germination of harvested seeds when applied during pod fill (Auch et al. 1978, Wax et al. 1969).

Current dicamba formulations have caused phytotoxic effects on soybean with as low as 0.01% of the labeled rate in corn (*Zea mays L.*); this could be less than that left in an improperly cleaned spray tank (Strachan et al. 2010). Andersen (2004) found residue in soybean leaf tissue up to 24 days after treatment (DAT) was correlated to yield loss.

Research on the foliar effects of dicamba to dry edible bean have not been studied. The potential for injury to susceptible crops, such as dry bean, due to increased dicamba usage could introduce a widespread problem of economic concern (Strachen 2010). Therefore, this research was conducted to address concerns of growers. The effect of dicamba drift to growth and yield of dry edible bean was evaluated.

Materials and Methods

Proposed dicamba use rates in dicamba-resistant soybean allow up to 1120 g ae ha^{-1} to be applied up to three times during the season. Application of dicamba could occur from emergence through flowering. While susceptibility of dry bean at various growth stages was not found, flowering was the most susceptible growth stage in soybean (Auch et al. 1978). Therefore, exposure during flowering was the target for these experiments. Two experiments were designed to evaluate the potential for dicamba drift exposure to injure dry edible bean by examining rate and a selection of dry bean varieties representing four market classes: red kidney, navy, black, and pinto. Field trials were established at Hillsboro and Thompson, ND. Soil type at Hillsboro was a Gardena coarse-silty, mixed, superactive, frigid Pachic Hapludolls and at Thompson, Bearden fine-silty, mixed, superactive, frigid Aeric Calciaquolls- Perella fine-silty, mixed, superactive, frigid Typic Endoaquolls (USDA-NRCS 2014; USDA NRCS 2016). The field sites were fertilized according to extension publication guidelines for dry edible bean based on soil test analysis (Franzen 2013). Experiments were kept weed free with labeled herbicides that would not injure the crop and weeding by hand when necessary.

Experiment 1: Rates of dicamba and glyphosate drift on dry edible bean

Field studies were conducted near Thompson, ND during the 2014 (47 º 45’24.7”N 97 º 06’34.1”W) and 2015 (47 º 45’07.7”N 97 º 05’23.6”W) growing seasons to evaluate the effect of foliar exposure of dry edible bean to low rates of dicamba. The experiment was set up in randomized complete block design (RCBD) with plots 3 m wide by 12 m long. 'Ensign' navy bean was planted in rows spaced 0.5 m apart in May of both years. Treatments were sprayed at flowering with a CO_2-backpack sprayer and hand boom system at 160 L ha^{-1} and 276 kPa, using TT11002 nozzles. Treatments included a nontreated control and rates of dicamba (0.18, 1.8, 18 g ha^{-1}), glyphosate (0.37, 3.7, 37 g ha^{-1}), and dicamba plus glyphosate (0.18 + 0.37, 1.8 + 3.7, 18 + 37 g ha^{-1}) to observe the additive properties of the two herbicides on dry bean.

Plant tissue of the newest growth was collected from plants evenly spaced within the plot. Destructive sampling was taken at 10 and 20 days after treatment (DAT) from two sprayed rows that would not be included in the yield estimate. Tissue samples were analyzed for dicamba and glyphosate residues, which required 80 g of plant material, 40 g for each test. Due to the small size of dry bean plants, new growth from at least 15 to 30 plants was required. Evenly spaced plants were pulled throughout each plot for representative samples. As many as two newest trifoliates per node and including any pods present were harvested for the sample. Plant tissue samples were collected in labeled paper bags and immediately stored in a cooler. Samples were delivered the day after sampling to South Dakota Agriculture Laboratories (1006 32nd Ave #105, Brookings, SD 57006) for analysis of glyphosate and dicamba residue. At maturity, two undisturbed, treated rows were harvested, threshed, and seeds were weighed to estimate yield.

Experiment 2: Dicamba drift among four dry bean market classes

Field: Field experiments were established at two sites in 2015: Thompson (47◦45'07.7"N 97◦05'23.6"W) and Hillsboro, ND (47◦19'46.9"N 97◦05'28.1"W). Both sites were planted June 11, 2015. Plots were arranged in a split block arrangement with three replications at each location. The vertical plot was assigned a herbicide treatment and the horizontal plots were assigned one of four dry bean market classes: 'Redhawk' red kidney, 'Ensign' navy bean, 'Eclipse' black bean, and 'Lariat' pinto bean. Seeding direction was perpendicular to herbicide application. Rows were spaced 0.8 m wide. Whole plots (herbicide treatment) were 3 m wide by 12 m long arranged in an RCBD.

Plants were sprayed at flowering. All four market classes were close to the same growth stage within each location and were sprayed on the same day (34 days after planting at Hillsboro and 36 days after planting at Thompson). Treatments were sprayed with a CO_2-backpack sprayer and hand boom system at 160 L $ha^{-1}$and 276 kPa, using TT11002 nozzle tips. Treatments included the highest rate of each herbicide and the combination used in Experiment 1: 37 g ha^{-1} glyphosate, 18 g ha^{-1} dicamba, glyphosate plus dicamba 37 plus 18 g ha^{-1}, and a nontreated control. Plants were evaluated for visible symptoms at 10 and 20 DAT, plants per 1 m^2 were recorded at maturity, and 1 m^2 was harvested from each subplot.

Greenhouse: A greenhouse study was conducted in the fall of 2015 to determine dicamba tolerance among different dry bean market classes. Black, navy, pinto, and red kidney were planted in pots 10 by 10 by 15 cm deep. Pots were filled to 12 cm with Sunshine mix (Sun Gro Horticulture Distribution Inc. 770 Silber Street, Agawam, MA 01001) and seeds were planted 2 cm deep. Plants were watered to maintain adequate moisture and fertilized with 10 g of 14-14-14 Osmocote (Scotts Co., Marysville, Ohio) in each pot. Lamps in the greenhouse were set to 16 h day length and temperature was maintained at ≈ 24 C.

Beans were sprayed at flowering, but each variety had a different growth pattern: kidney was sprayed 25 and 24 days after emergence (DAE), black and navy at 28 and 28 DAE, and pinto at 28 and 31 DAE, respective to first and second run. Treatments included the highest rate of each herbicide and the combination used in Experiment 1: 37 g ha^{-1} glyphosate, 18 g ha^{-1}

dicamba, and the combination. Treatments were applied by a one nozzle spray booth using TT11001 nozzle at 276 kPa to obtain 79.6 L ha^{-1}. This experiment was conducted with a completely random experimental design in two runs planted 10 days apart. Each run consisted of four replicates. Pots were rotated two times a week to reduce the effect of microenvironments. Data were collected on visible symptoms and final dry weights. Visual ratings were evaluated at 10 and 20 DAT. After the last visual rating, plants were cut at the soil surface and weighed for total mass, pod mass, and seed mass.

Statistical Analysis

Data were subjected to analysis through SAS 9.3 (SAS Version 9.3 Institute, Inc., Carry, NC). Data were combined over runs if variances were considered similar (means square error values within a factor of 10). Experimental run and location were considered random and herbicide treatment was a fixed effect. Means were separated by Fischer's protected LSD at the 5% level of significance.

Results and Discussion

Experiment 1

Injury rating, residue analysis, and yield were combinable over years; however, glyphosate and dicamba residue analysis at 10 DAT could not be combined across locations. The difference in herbicide residue content at 10 DAT could be due to the difference in rainfall in the days after application in 2014 compared to 2015 (Figure 3.1). Robinson et al. (2013) and Weidenhamer et al. (1989) reported enhanced injury symptoms and increased persistence linked to drought conditions. Their results are consistent with our results from 2014, where little to no rainfall occurred between spray application and last sampling date (Figure 3.1).

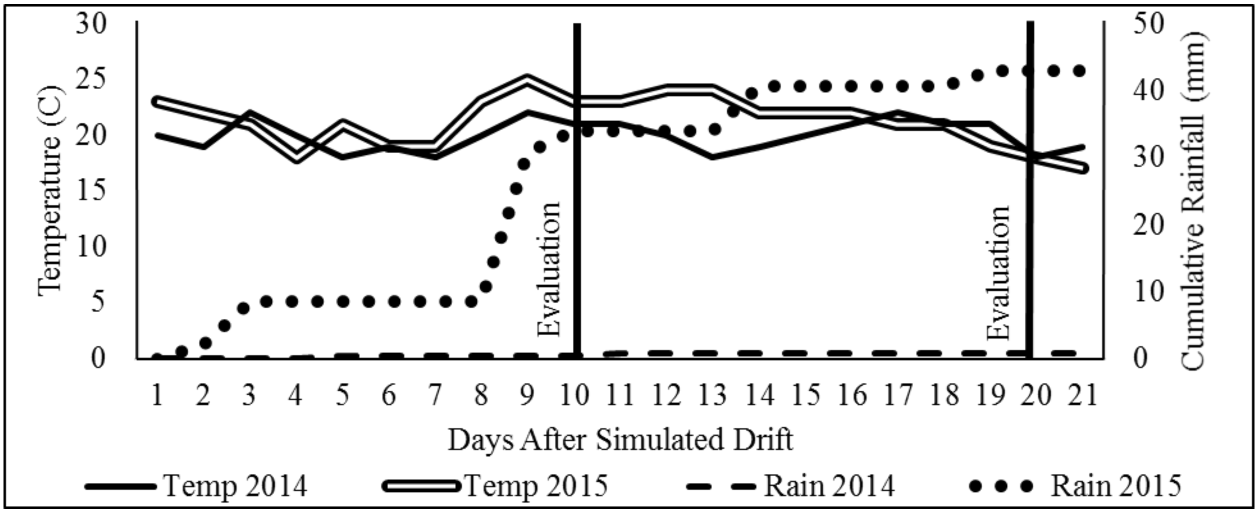

Figure 3.1. Daily temperature and rainfall in the first 21 days after simulated drift application Thompson, ND 2014 and 2015. Data acquired from (NDAWN 2016).

Dicamba at 18 g ha^{-1} and in combination with glyphosate at 37 g ha^{-1} caused injury greater than 50% (Table 3.1). At 10 DAT, dry edible bean was only slightly injured (6%) with dicamba at 0.18 g ha^{-1} compared to the nontreated control. Dicamba applied at 1.8 g ha^{-1} caused injury between 20 and 30%, but also increased number of flowers along the stem. Dry edible bean flowers were aborted when dicamba was applied at 18 g ha^{-1}. Predicting yield using visible injury after a drift occurrence can be difficult. Injury and yield were correlated using the nontreated control and the increasing rates of dicamba alone, glyphosate alone, or the combination. Correlations of injury to yield were significant ($p \leq 0.05$) for all sets of herbicides in 2014. Correlation coefficients for increasing rates of glyphosate were 0.59 and 0.60, but these yields were all similar to the nontreated control (Table 3.2). Furthermore, while dicamba and glyphosate plus dicamba treatment correlation of injury to yield had coefficients that ranged from 0.82 to 0.86 in 2014, the correlation of injury to yield was only significant at the 20 DAT evaluation in 2015 and ranged from 0.72 to 0.78. Johnson et al. (2012) reported variable injury from dicamba and 2,4-D correlated to yield loss, that resulted in low correlation coefficients (below absolute value of 0.6). They concluded no consistent trend. Using this benchmark, injury with dicamba or glyphosate have not been a consistent indicator of yield loss, but injury with glyphosate plus dicamba may be a more consistent indicator of yield loss (coefficients greater than 0.8 in 2014 and combined across years).

Table 3.1. Visual injury at 10- and 20- and residue analysis from leaf tissue 20- days after simulated drift on dry edible bean, and affect of simulated drift on final grain yield of dry edible bean combined over years. Thompson, ND 2014 and 2015.

				Residue Analysis		
		Injury		Glyphosate	Dicamba	Yield
Herbicide[a]	Rate	10	20	20	20	
	g ha^{-1}	———%———		——µg kg^{-1}——		kg ha^{-1}
Nontreated		0	0	65	2	1490
Glyphosate	0.37	1	11	72	3	1540
	3.70	1	6	82	6	1560
	37.00	8	13	78	11	1400
Dicamba	0.18	6	14	82	4	1590
	1.80	21	23	62	6	1580
	18.00	38	53	70	403	386
Glyt + Dica	0.37 + 0.18	3	10	74	9	1530
	3.70 + 1.80	27	25	75	16	1650
	37.00 + 18.00	47	63	87	561	273
LSD		6	7	NS	344	702
CV		24.4	21.9	29.8	258.5	23

[a]Abbreviations: glyt, glyphosate; dica, dicamba

Other research has published detailed injury rating scales describing symptoms rating from 1 to 100% (Sciumbato et a. 2004, Andersen et al. 2004, Robinson et al. 2013) or describe specific leaf morphology rather than assign a rating number (Weidenhamer et al. 1989). However, we observed consistent effect on reproductive structures that indicated yield loss. This was also the focus of Robinson et al. (2013) which measured several yield components of soybean affected by increasing rates of dicamba. While they detected 10% yield loss in soybean at 0.169 g ha^{-1}dicamba, we could not detect yield loss in dry bean until above 1.8 g ha^{-1} dicamba (Table 3.1). However, the plant stresses that linked dicamba injury to seed yield loss were the same. Termination of the apical meristem branching (Figure 3.2) did not result in yield reduction but did delay maturity (Figure 3.3), which could reduce yield in the event of an early frost. Robinson et al. (2013) observed flower abortion in soybean that resulted in yield loss. In our studies, flower abortion also resulted in yield loss. Delayed physiological maturity required desiccation to harvest all plots at the same timing. Yield was only reduced in plants treated with 18 g ha^{-1} dicamba (Table 3.1). Dicamba applied at 18 g ha^{-1} caused yield loss of more than half the nontreated control yield. While injury was seen in most plots at 20 DAT, yield did not differ from the nontreated control, except at the highest rate of dicamba.

Table 3.2. Simple correlation coefficients between yield and visual injury at 10 and 20 days after simulated drift. Thompson, ND 2014 and 2015.

	2014		2015		Combined	
Source	10	20	10	20	10	20
Glyphosate alone[a]	-0.59*[d]	-0.60*	-0.45	-0.49	-0.29*	-0.04
Dicamba alone[b]	-0.83*	-0.82*	-0.45	-0.78*	-0.62*	-0.73*
Glyphosate + Dicamba[c]	-0.86*	-0.83*	-0.32	-0.72*	-0.86*	-0.83*

[a]Glyphosate applied at 0, 0.37, 3.7, 37 g ae ha^{-1}.
[b]Dicamba applied at 0, 0.17, 1.7, 17 g ae ha^{-1}.
[c] Glyphosate + Dicamba applied at 0 + 0, 0.37+0.18, 3.7+1.8, 37+18 g ae ha^{-1}.
[d]Denotes correlation is significant ($p \leq 0.05$).

Figure 3.2. Dry edible bean injury 20 days after simulated drift treatment of dicamba or glyphosate + dicamba. Plants treated with dicamba at 0.18 g ae ha-1 showed few symptoms (a), while Dicamba 1.8 g ae ha-1 had more foliar damage and flower proliferation (b). Dicamba at 18 g ae ha-1 alone and in combination with glyphosate at 37 g ae ha-1 killed the apical meristem resulting in stunting and flower abortion (c and d).

There were significant differences in both years among treatments for residue analysis at 20 DAT. There were no significant differences among treatments for glyphosate residue analysis. Glyphosate could be found in high quantities (over 1000 µg kg^{-1} in some treatments in 2015) in tissue with little to no injury (data not shown). Dicamba quantities of half that concentration corresponded to yield loss. However, yield was only reduced when plots were treated with 18 g ha^{-1} dicamba alone and in combination with 37 g ha^{-1} glyphosate. Dicamba residue found in tissue from plants sprayed with 0.18 or 1.8 g ha^{-1} was similar. Dicamba concentration in plants treated between 1.8 g ha^{-1} and 18 g ha^{-1} dicamba resulted in levels of herbicide beyond the capabilities of dry edible bean to metabolize, allowing accumulation and causing permanent injury.

Residue analysis from tissue sampled at 10 and 20 DAT showed strong correlation between dicamba concentration and yield in 2014 (Table 3.3). When plants were treated with glyphosate plus dicamba, the recovery of either herbicide in residue analysis at either 10 or 20 DAT was correlated to yield loss. Glyphosate alone did not cause yield loss, yet there were large amounts of glyphosate detected in leaves; therefore, correlation was not confirmed (Tables 3.1 and 3.3). Dicamba alone was correlated to yield loss at 10 DAT but not at 20 DAT; however, in 2015, only the correlation between recovered dicamba at 20 DAT and yield was significant. Contrary to 2014, residues of dicamba and glyphosate in treatments of glyphosate plus dicamba did not correlate to yield. Combined over years, the correlation between dicamba residue and yield loss was significant, but below the 0.6 threshold identified by Johnson et al. (2012). Other studies have attempted to correlate herbicide residue analysis to yield reduction but with varied results. Auch et al. (1978) observed quick dissipation of dicamba from leaves, but sampled the whole plant, which diluted concentration of dicamba. Dicamba translocation to meristematic tissue has been confirmed, which indicated that sampling meristematic tissue would give precise information (Chang et al. 1971; Stacewicz et al. 1973). Sirons et al. (1982) found linear relationships between amount of dicamba recovered and yield reduction, but residue levels quickly dissipated in the plant, especially in top growth. Auch et al. (1978), Sirons et al. (1982), and Andersen et al. (2004) also reported the importance of early sampling; however, our data suggest 20 DAT was an adequate time to sample in the wet year of 2015.

Table 3.3. Simple correlation coefficients between yield and residue analysis at 10 and 20 days after simulated drift. Thompson, ND 2014 and 2015.

		2014		2015		Combined	
Analysis	Source	10	20	10	20	10	20
Glyphosate	Glyphosate alone[a]	-0.50	-0.02	-0.01	-0.25	-0.21	-0.25
	Glyphosate + Dicamba[b]	-0.88*[d]	-0.59*	-0.15	-0.21	-0.21	-0.47*
Dicamba	Dicamba alone[c]	-0.89*	-0.44	-0.45	-0.72*	-0.61*	-0.46*
	Glyphosate + Dicamba[b]	-0.93*	-0.63*	-0.44	-0.44	-0.69*	-0.50*

[a] Glyphosate applied at 0, 0.37, 3.7, 37 g ae ha^{-1}.
[b] Glyphosate + Dicamba applied at 0 + 0, 0.37+0.18, 3.7+1.8, 37+18 g ae ha^{-1}.
[c] Dicamba applied at 0, 0.17, 1.7, 17 g ae ha^{-1}.
[d] Denotes correlation is significant ($p \leq 0.05$).

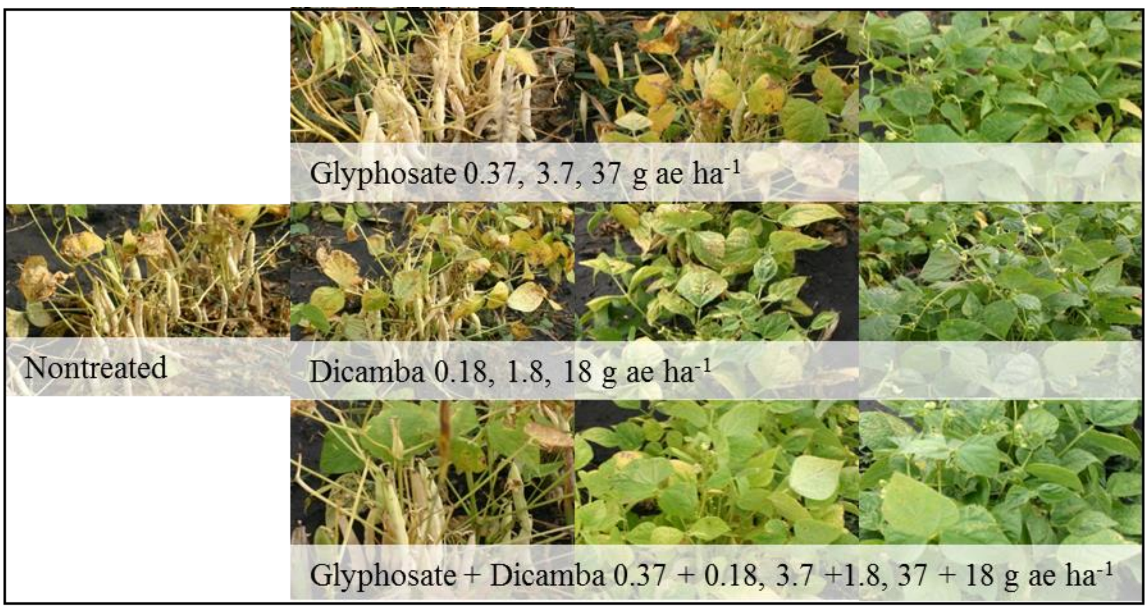

Figure 3.3. Visual differences in delay of physiological maturity 47 DAT. Plants applied with glyphosate at 0.37 and 3.7 g ha-1 and dicamba at 0.18 g ha-1, matured similar to the nontreated. Treatments applied with 1.8 g ae ha-1 dicamba and 37 g ha-1 glyphosate were filling pods, and treatements with 18 g ha-1 dicamba were beginning to flower. Pictures taken September 9, 2014.

Experiment 2

Field: In Experiment 2, visible injury was consistent with observations in Experiment 1. We observed minimal chlorosis in glyphosate-treated plants, but extensive symptoms in dicamba treated plants, including leaf strapping, cupping, epinasty, stunting, killed apical meristem, and aborted flowers. Visible injury 10 DAT could be separated by treatment effect (Table 3.4). However, there was no interaction between herbicide and dry bean class. Dicamba at 18 g ha^{-1} alone or in combination with glyphosate at 37 g ha^{-1} caused similar injury when averaged over all market classes. Glyphosate at 37 g ha^{-1} was similar to the nontreated.

Table 3.4. Injury to four dry edible bean market classes 10 days after glyphosate and dicamba simulated drift on dry edible bean and final yield combined over locations. Thompson and Hillsboro, ND 2015.

	Rate	Injury	Yield
Treatment	g ha $^{-1}$	%	kg ha $^{-1}$
Nontreated		0	2690
Glyphosate	37	2	2600
Dicamba	18	39	1830
Glyphosate + dicamba	37 + 18	39	2070
LSD		10	770
Market Class			
Red kidney		23	2150
Navy		30	2180
Black		32	1970
Pinto		23	3000
LSD		NS	680
CV		37	23

At 20 DAT, the interaction between herbicide and dry bean class was significant (Table 3.5). Within each market class, injury due to dicamba alone was similar to injury caused by glyphosate plus dicamba. This was also seen in Experiment 1. Navy bean and black bean were similar. Dicamba alone injured navy more than pinto or kidney. In combination, glyphosate plus dicamba injured kidney bean less than any other bean type.

Maturity of each bean variety differed in response to the herbicide treatment. All beans bloomed at approximately 35 days after planting, but each class was at a different stage in maturity 35 DAT. Red kidney bean was a stouter plant which had a short bloom to pod interval, even under stress. Navy and black beans aborted flowers within days of the herbicide treatment, which delayed podset. The pinto bean variety had an indeterminate growth, which allowed more vegetative growth during pod development. The increasing biomass may explain tolerance observed in different varieties.

Table 3.5. Injury to four dry edible bean market classes 20 days after glyphosate and dicamba simulated drift combined over locations. Thompson and Hillsboro, ND 2015.

		Market class			
Treatment[a]	Rate	Red kidney	Navy	Black	Pinto
	g ha $^{-1}$	——% injury——			
Nontreated		0	0	0	0
Glyphosate	37	0	2	5	6
Dicamba	18	30	56	48	38
Glyt+dica	37 + 18	28	49	44	44
LSD		——11——			
CV= 24.9					

[a]Abbreviations: glyt, glyphosate; dica, dicamba

Plant population was recorded but did not differ by treatment or class (data not shown). Final grain yield was affected by treatment and by market class (Table 3.4). Dicamba at 18 g ha^{-1} alone and in combination with glyphosate at 37 g ha^{-1} resulted in yield loss compared to the nontreated across all bean classes. Glyphosate alone at 37 g ha^{-1} produced similar yield to the nontreated. Pinto bean yields, averaged over treatments, were higher than any other bean class.

Greenhouse: Differences were not observed among treatments. However, we have reason to believe there are biological differences in growth habit. By terminating the greenhouse plants at 20 DAT, injury to bean plants were more apparent than visible injury ratings in the field. Pod weight was measured, which demonstrates the magnitude of delay in the treated plants; on average, dicamba-treated plants had 70% of the pod mass of untreated and glyphosate treated plants (Table 3.7). On average, navy beans had highest number of pods, but dicamba treated plants had less time for pod fill because of the immediate abortion of flowers after treatment (Table 3.8). Navy and black beans both had reduced pod weight in the dicamba treated plants and, consequently, many of those pods had not filled. In red kidney bean, pod weight was increased with the glyphosate treatments; however, those pods were often empty.

Table 3.6. Average plant weight, pod weight, and pod number per dry edible bean plant treated with dicamba and glyphosate simulated drift, averaged across market classes.

Treatment[a]	rate	Plant weight	Pod weight	Pod number
	g ha^{-1}		g	
Nontreated		52.1	22.6	8.7
Glyphosate	37	52.3	23.2	8.9
Dicamba	18	48.7	16.4	4.6
Glyt+dica	37 + 18	45.8	16.2	2.7
LSD		NS	NS	NS
CV		22.7	29.1	42.3

[a]Abbreviations: glyt, glyphosate; dica, dicamba

Table 3.7. Average plant weight, pod weight, and pod number per dry edible bean plant in each market class, averaged across herbicide treatments.

Market Class	Plant weight	Pod weight	Pod number
		g	
Red Kidney	47.5	10.4	4.1
Navy	50.1	33.5	6.9
Black	52.5	19.9	8.9
Pinto	48.8	14.7	5.0
LSD	NS	NS	NS
CV	22.7	29.1	42.3

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APPENDIX

Table A1. Population estimates from counts taken of four susceptible crops planted the year after various rates and timings of dicamba applied to bare soil and compared to clopyralid as a standard.

	Dry Bean		Soybean		Sugar Beet		Sunflower	
Treatment[a]	Emerge	Harvest	Emerge	Harvest	Emerge	Harvest	Emerge	Harvest
g ha^{-1}	plants ha^{-1}							
Untreated	-[b]	-	219876	289130	76087	80124	39130	41460
Dica 1120, 0, 0	118324	186957	209628	292237	75465	77020	49067	45032
Dica 1120, 560, 0	-	-	237267	273291	73291	76087	44100	40993
Dica 1120, 560, 560	133230	189130	237578	284783	69254	78883	44411	43945
Dica 2240, 0, 0	101552	172361	242857	275154	79193	77639	46274	38820
Dica 2240, 1120, 0	110870	191926	238820	309628	81057	88509	40063	38820
Dica 2240, 1120, 1120	108696	187267	220187	296274	82920	89752	43789	40838
Dica 0, 0, 560	116459	182920	239752	287889	63354	76398	43167	44410
Dica 0, 0, 1120	112733	172980	217702	289130	69565	77950	47826	38975
Dica 0, 0, 2240	106522	181987	248137	315528	71428	71739	45030	36801
Dica 0, 0, 4480	109937	170807	222672	287267	71428	85093	43167	43167
Dica 0, 560, 560	105589	185404	213354	286024	72672	80435	46585	39130
Dica 0, 1120, 1120	104348	179502	227639	291615	74535	77328	49689	41304
Clop 0, 157.5, 0	107765	171740	226398	278261	78261	81367	46585	40684
LSD	NS	NS	NS	34900	NS	NS	NS	NS

[a]Rates of herbicide applied at each timing: PRE (May 24), EPOST (June 13), and MPOST (July 11) in 2014. Abbreviations: Dica, dicamba; Clop, clopyralid.
[b]Omitted due to application error

Table A2. Population estimates from counts taken of four susceptible crops planted immediately after application of increasing rates of dicamba applied to bare soil.

	Crop							
	Dry Bean		Soybean		Sugar Beet		Sunflower	
Treatment	Emerge	Harvest	Emerge	Harvest	Emerge	Harvest	Emerge	Harvest
g ha^{-1}	plants ha^{-1}							
0	104000	193000	222000	288000	51100	56800	41000	35100
140	73000	190000	241000	283000	52400	59500	38900	37400
351	89300	176000	241000	287000	45900	50800	45900	31500
702	79900	190000	216000	284000	52700	51630	41000	34100
1054	88100	183000	228000	272000	52200	56800	43500	30800
1405	107000	188000	206000	278000	50800	56000	45700	34500
1756	74000	191000	243000	272000	54300	58400	44800	34600
2107	98500	193000	223000	280000	49200	57600	40500	32900
2458	91300	185000	229000	275000	57600	58200	42400	31000
2809	69900	182000	232000	275000	49700	50500	44300	33000
LSD	NS	NS	NS	NS	NS	NS	NS	NS

Table A3. Injury, yield, and residue analysis from leaf tissue at 10 and 20 days after simulated drift on dry edible bean. Thompson, ND 2014.

		Injury		Glyphosate		Dicamba		Yield
		10	20	10	20	10	20	
		%		mg kg $^{-1}$				kg ha $^{-1}$
Nontreated		0	0	42	46	5	4	966
Glyphosate	0.37	2	20	64	55	8	6	721
	3.70	0	12	68	72	0	11	989
	37.00	15	25	210	67	14	21	683
Dicamba	0.18	10	27	102	69	14	7	745
	1.80	13	37	46	35	34	7	754
	18.00	35	55	34	37	533	530	156
Glyt+dica	0.37 + 0.18	6	20	35	49	2	18	889
	3.70 + 1.80	23	43	34	55	34	17	795
	37.00+ 18.00	52	73	197	78	523	810	100
LSD		7	9	60	NS	35	NS	202

Table A4. Injury, yield, and residue analysis from leaf tissue at 10 and 20 days after simulated drift on dry edible bean. Thompson, ND 2015.

		Injury		Glyphosate		Dicamba		Yield
		10	20	10	20	10	20	
		%		mg kg $^{-1}$				kg ha $^{-1}$
Nontreated		0	0	2100	84	2.7	0.23	1040
Glyphosate	0.37	0	3	5700	88	8.7	0.73	1620
	3.70	3	1	4700	92	2.4	0.20	1130
	37.00	1	0	790	89	7.7	0.00	1430
Dicamba	0.18	2	2	4400	94	14.0	0.70	1670
	1.80	29	9	2900	88	37.0	4.30	1640
	18.00	40	52	3000	100	520.0	275.00	455
Glyt+dica	0.37 + 0.18	1	1	4900	99	7.1	0.67	1270
	3.70 + 1.80	31	7	2600	95	46.0	14.00	1690
	37.00+ 18.00	43	53	2000	96	270.0	311.00	340
LSD		4	5	NS	NS	NS	170.00	420