TANK CONTAMINANT AND RESIDUAL EFFECTS OF DICAMBA

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And hereby certify that, in their opinion, it is worthy of acceptance.

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ABSTRACT: DICAMBA RESIDUES IN SPRAY EQUIPMENT REDUCES SOYBEAN (GLYCINE MAX) GROWTH AND YIELD

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Dr. Reid J. Smeda, Thesis Supervisor

Abstract

Introduction of dicamba-tolerant soybeans (Glycine max L. Merr.) will improve management of increasing populations of herbicide-resistant weeds. However, increased use will lead to risks for damage to susceptible crops such as adjacent soybeans. Research on cleaning commercial spray equipment used to apply dicamba on tolerant soybeans followed by application of other herbicides on sensitive soybeans is needed. Soybean injury and yield following application of spray tank rinsates containing dicamba was studied in 2015 and 2016. Dicamba was applied through two commercial sprayers and spray equipment was subsequently treated with water or one of four cleaning agents (water alone, ammonia, Cleanse® or Erase®). This initial treatment was considered a first rinsate, and was followed by two rinses of water (second and third rinsate). Rinsate solutions were applied on V3 or R1 soybeans. Visible damage was observed at 7 days after treatment (DAT), especially for first rinsates (6 to 16% for V3 treated plants and up to 22% for R1 plants). At 14 DAT, plant height was reduced up to 48 and 33% on V3 and R1 treated soybeans, respectively. Stunting on R1 treated soybeans persisted until the end of the season. First rinsates reduced yields up to 11 and 46% for V3 and R1 soybeans respectively, compared to the untreated control. For third rinsates, yields for R1 plants were reduced up to 6%, with no effect on V3 plants. Cleaning agents can reduce residues of dicamba in contaminated equipment, but dilution of residues by a triple rinse procedure is most important.
Chapter 1: Literature Review

Introduction

Dicamba (2-methoxy-3,6-dichlorobenzoic acid) is a selective benzoic acid herbicide that was first registered for use in the United States in 1967. Targeted use includes postemergence (POST) control of broadleaf weeds in lawns, corn, barley, oats, wheat and other grass crops (USDA-Crops Research Division 1967). Additionally, non-crop areas treated include industrial, roadside, and pasture areas (Al-Khatib and Peterson 1999, Egan and Mortensen 2012, EPA 2006).

Dicamba belongs to a group of herbicides referred to as plant growth regulators (PGRs) (Steckel et al. 2005). PGRs mimic the plant hormone auxin and were introduced for use after World War II (Grossmann 2010). Dicamba was introduced commercially in 1967 (EPA 2006). The Weed Science Society of America (WSSA) places benzoic acids and PGRs in Group 4 with other synthetic auxins (WSSA 2007). Today, there are over 10 distinct herbicides in use that act as synthetic auxins (Baumann et al. 2008).

Dicamba effects sensitive plants by mimicking a natural plant compound. Within plants, hormones are chemical messengers that facilitate communication between cells (Taiz and Zeiger 2002). Hormones control plant development and are generally referred to as plant growth regulators. Auxin was the first plant growth regulator to be discovered (Bonner 1933), and is referred to as indole-3-acetic acid (IAA). Auxin is the most abundant hormone in plants (Taiz and Zeiger 2002) and effective at very low (parts per million [ppm]) concentrations (Weidenhamer et al. 1989).
Auxin influences nearly every aspect of plant growth and development, and mimicking this process was considered a prime target for development of herbicides (Marth and Mitchell 1944). Synthetic auxins such as 2,4-D and MCPA were tested in the early 1940s and were shown to act in the same way as IAA in plants. However, unlike the low concentrations of IAA that are quickly inactivated through conjugation and degradation, synthetic auxins are introduced at higher doses and are much more stable in the plant (Grossmann 2010).

Expression of herbicidal activity by synthetic auxins is dose dependent and occurs in three distinct phases (Gleason et al. 2011). The first phase occurs within hours after application and results in symptoms such as leaf cupping and stem as well as petiole epinasty (Lingenfelter and Curran 2013), which is the result of deregulated growth and accumulation of abscisic acid (ABA) (Grossmann 2010). After absorption, the herbicide binds to auxin receptors which leads to acidification of the cell wall. Acidification makes cell walls more plastidic and this is seen as twisting of stem and petioles. Ethylene production is also boosted following acidification of extracellular space, and this increases ABA synthesis. Elevated ABA levels stop plant growth by closing the stomata, preventing carbon dioxide conversion in the plant. In the second phase, growth ceases as carbon fixation is stopped and transpiration is reduced. Hydrogen peroxide levels also increase and this compound contributes to membrane lipid peroxidation and cell death (Gleason et al. 2011). The third phase is characterized by foliar senescence, wilting and necrosis, which results from the loss of membrane integrity (Grossmann 2010). Disruption of xylem and phloem integrity interferes with water and nutrient transport in the plant, further contributing to plant death. At low concentrations, auxinic herbicides
increase RNA levels by promoting RNA polymerase activity, and subsequently, greater protein biosynthesis (WSSA 2007). Uncontrolled cell division and growth disrupts the function of the xylem and phloem. Visibly, callous growth occurs along cracks that form on the petiole and stem (Grossmann 2010). At high concentrations, cell division and growth are inhibited in the meristematic regions where phloem transport accumulates the herbicide (WSSA 2007).

Auxins such as dicamba exhibit non-selective on broadleaf plants with little activity on grasses. Grasses and other monocots rapidly metabolize dicamba into 5-hydroxy-2-methoxy-3,6-dichlorobenzoic acid (5-hydroxy), a non-harmful metabolite, before extensive injury occurs in the plant (Broadhurst et al. 1966). In grasses, up to 50% of dicamba can be metabolized in 1 day while only 10% of dicamba was detoxified 20 days after treatment in broadleaves (Chang and Vanden Born 1971).

For many years, dicamba has been used for broadleaf weed control in grass crops such as corn (Wax et al. 1969). In addition to corn, dicamba is also labeled for weed control in other monocot crops such as small grains, proso millet, sorghum, and sugarcane. For sensitive crops, dicamba and other PGR’s are strictly applied prior to planting (burndown). In non-crop areas such as fallow crop ground, grass grown for seed, hay, pasture, rangeland, sod farms and turf, higher rates are used to control broadleaf perennial weeds (Anonymous 2010a).

In cropping systems such as soybean, dicamba is combined with other pre-plant herbicides to eliminate early season weed competition (Kelley et al. 2005). However, dicamba and other PGR herbicides have plant-back restrictions for soybeans to limit early season injury, usually around 14 days (Anonymous 2010a, 2010b). Weather conditions
can sometimes delay applications, resulting in injury to emerging soybeans of up to 38% at 35 days after planting when dicamba is applied only seven days before planting (Thompson et al. 2007).

The introduction of glyphosate-tolerant crops in the 1990s provided growers with a tool to non-selectively manage weeds prior to crop planting as well as in-season. As adoption increased, development of new herbicide modes of action (MOA) declined (Green 2007). The use of other herbicides for soybeans, cotton, canola and corn declined, including dicamba (Duke 2012) as growers shifted traditional weed control costs to “technology fees” added to the price of the seed (Gianessi 2008). In soybeans, the number of different MOAs used (applied to at least 10% of soybean hectares) declined from seven to one (glyphosate) between 1995 and 2002, while applications of PRE herbicides such as pendimethalin and trifluralin dropped from over 20% of soybean hectares to less than 10% in the same time period (Young 2006). During this similar period, the cost to introduce a new active ingredient increased from $152 million in 1995 to $286 million from 2010-2014; research, development and registration costs all increased substantially (Phillips McDougall 2016). The low cost and effectiveness of glyphosate increased pressure on industry to develop new herbicides that were comparable economically. As a result, the number of chemical screenings required to commercialize one new herbicide increased from 1000 in 1950 to over 500,000 in 2007 (Green 2007).

As reliance on glyphosate increased, the occurrence of GR weeds began to grow as well (Duke and Powles 2009). In 1996, the year GR crops were introduced to the market, only one species (Annual ryegrass [Lolium rigidum Gaudin]) had evolved
resistance to glyphosate (Heap 2016). By 2016, over 250 individual cases of resistance had been reported across 37 species (Heap 2016), including problematic soybean weeds such as horseweed (*Conyza canadensis* (L.) Cronq.), common waterhemp (*Amaranthus rudis* Sauer), Palmer amaranth (*Amaranthus palmeri* S. Wats.), giant ragweed (*Ambrosia trifida* L.) and common ragweed (*Ambrosia artemisiifolia* L.) (Behrens et al. 2007). Poor control of these weeds resulted in substantial yield losses. In Kansas, Palmer amaranth and common waterhemp at 8 plants m\(^{-1}\) of row density reduced yields 78 and 56%, respectively (Bensch et al. 2003) while giant ragweed at 2 plants per 9 m of soybean row reduced soybean yield up to 50% (Baysinger and Sims 1991).

With fewer new herbicides coming to the market and an increase in the development of GR weeds, the agricultural industry has considered new approaches to controlling troublesome weeds in soybeans (Behrens et al. 2007). Dicamba controls many of the GR weeds found in soybeans, and to date there are few weeds with demonstrated resistance (Heap 2016) despite its long-term use (Green and Owen 2011). Species such as horseweed, common waterhemp, Palmer amaranth, giant ragweed and common ragweed are effectively controlled by dicamba (Behrens et al. 2007). Through the efforts of the University of Nebraska, BASF and Monsanto, dicamba-resistant soybeans and cotton were recently developed (Johnson et al. 2012). Availability of dicamba allows growers to control GR broadleaf weeds while also adding an additional mode of action (MOA) for weed control in soybeans.

**Dicamba and Soybeans**

Development of dicamba-tolerant (DT) soybeans (Anonymous 2016a) and recent approval of the genetically modified crop as well as label approval in the U.S. will
significantly increase dicamba usage (Mortensen et al. 2012). Although available long-term in corn, only 2% of corn acres were sprayed with dicamba in 2005 (USDA et al. 2006). Adoption of DT soybeans and DT cotton is largely in response to widespread infestations of GR weeds. Heap (2016) estimated that across the U.S., GR weeds had infested over six million ha by 2012. Recent soybean seed sales in Missouri indicate up to 30% of the states two million hectares will be dicamba-tolerant (Jason Weirich, personal communication). Many growers may also feel compelled to plant dicamba-resistant soybeans to reduce the risk of crop injury from neighboring fields planted with resistant varieties (Hurley and Frisvold 2016).

Soybean tolerance to dicamba occurs via metabolism (Chang and Vanden Born 1971). *Pseudomonas maltophilia*, a bacterium found in the soil, converts dicamba to 3,6-dichlorosalicylic acid (DCSA) via dicamba monoxygenase (DMO), resulting in a non-herbicidal metabolite (Behrens et al. 2007). DMO is a genetically engineered gene that encodes a Rieske protein which is capable of metabolizing dicamba in transgenic plants, and is not commonly found in nature. The gene from *P. maltophilia* that encodes for DMO was inserted into soybean cultivars, resulting in dicamba tolerance. Plants that express the inserted gene product and are exposed to high doses of dicamba exhibit little to no symptomology (Johnson et al. 2012). The specificity of dicamba tolerance does not impact sensitivity to other PGRs (Peterson et al. 2016).

Use of dicamba on tolerant soybeans will include burndown applications near the time of planting, but also POST applications up to initial flowering (R1). As currently labeled (Anonymous 2016b), up to 2.24 kg ae ha\(^{-1}\) is permitted per year, with rates at each application recommended at 0.56 kg ha\(^{-1}\). Applications during the growing season
will subject adjacent dicamba-sensitive soybeans to injury and is of great concern in the agriculture community (Griffin et al. 2013).

**Sources of Dicamba and Soybean Injury**

The effectiveness of dicamba is concerning, as very low concentrations can significantly injure exposed, non-tolerant plants. Damage to non-tolerant plants can occur via drift and volatilization (Boerboom 2004). Increased dicamba use will also contaminate spray equipment, requiring thorough cleaning.

Drift and volatilization occur at or soon after herbicide application, and the extent is influenced by environmental conditions. Drift is the movement of herbicides in liquid form during spray application, while volatility occurs when the chemical changes from a liquid to a gaseous state after application (Zimdahl 2013). Drift is most affected by higher wind speeds and boom height (greater distance between applicator nozzle and intended target) (Smith et al. 1982). Higher spray pressure also impacts drift potential by decreasing herbicide droplet size. Volatility occurs after an herbicide has reached its intended target, where chemical properties such as vapor pressure can result in active herbicide moving off-site as a gas. Dicamba is especially susceptible to volatility (Behrens and Lueschen 1979), with a vapor pressure of $1.25 \times 10^{-5}$ mm Hg at 25 C versus glyphosate, which is $9.8 \times 10^{-8}$ mm Hg (128-fold higher) (PubChem Compound Database 2017).

Ensuring dicamba reaches its target site is critical in reducing dicamba damage to sensitive soybeans. Between 1 and 8% of the herbicide applied from pesticide applicators drifts beyond the spray swath (Maybank et al. 1978). In turfgrass, researchers found that proper nozzle selection can greatly reduce drift. Compared to TeeJet XR8004 flat fan
nozzles (Spraying Systems Co., Wheaton, IL 60188), RA-6 Raindrop nozzles (Delavan Inc., West Des Moines, IA 50265) reduced drift by 55% at distances ranging from 90 to 210 cm downwind from application by increasing the average droplet size from 269 to 330 µm (Hatterman-Valenti et al. 1995, Hurto 1988). Using a spray-gun system where diluted herbicide solutions are applied at high volumes and low pressure, drift was reduced by 96% compared to XR8004 flat fan nozzles (Hatterman-Valenti et al. 1995).

Environmental conditions during and after herbicide application can impact the likelihood of drift or volatility of dicamba. Both higher air temperature and lower relative humidity are correlated with higher drift rates. Higher air temperature and lower humidity results in more rapid drying of droplets during application, increasing drift (Egan and Mortensen 2012). Higher humidity may also result in greater injury by facilitating uptake of off-target herbicide on susceptible plants. Volatility is also increased under higher air temperatures. Behrens and Lueschen (1979) reported that soybeans exhibited almost 40% more injury at 30 C than 15 C when exposed to dicamba vapors for six hours. Use of dicamba later in the growing season will correspond with weather conditions favoring both drift and volatility, increasing the probability of off-target movement.

A number of PGRs, including 2,4-D and dicamba are subject to volatility. In particular, the form of the active ingredient (salt) for each acid form of the herbicide influences the volatility (Zimdahl 2013). Dicamba in its active form is an acid, and is stabilized by being formulated as a salt. The particular cation used for the salt impacts the likelihood of volatility (Petersen et al. 1985). With the use of air samplers, Mueller et al.(2013) found that the dimethylamine (DMA) salt formulation of dicamba was twice as likely to be detected in the air as the diglycolamine (DGA) salt formulation. Using the
DGA salt to determine whether application timing affected volatility, Mueller et al. (2013) determined that volatility occurred most often at mid-day, regardless of application timing. These findings correspond to weather conditions favoring volatility (higher air temperatures) (Behrens and Lueschen 1979). Dicamba has moved up to 60 m downwind from application sites, with consequent soybean injury resulting up to three days after application to adjacent fields (Behrens and Lueschen 1979). Sciumbato et al. (2004) showed that volatility of 0.05% of a normal use rate of dicamba (0.56 kg ha\(^{-1}\)) damaged cotton and soybean in a greenhouse setting, while in field settings 0.1 and 0.5% volatilization rates damaged cotton and soybean, respectively. Recently developed formulations of dicamba for use on DT soybean (Engenia from BASF and Xtendimax from Monsanto) are up to 93% less volatile than DGA formulations (Gavlick et al. 2016).

Damage by dicamba to sensitive soybeans depends upon the dose as well as stage of soybean at the time of exposure. Weidenhamer et. al. (1989) sought to evaluate injury symptoms in soybeans and relate these symptoms to yield losses. Soybeans were treated at both the vegetative and reproductive growth stages with dicamba from 0.04 to 80 g ai ha\(^{-1}\). Resulting injury included a significant reduction in plant height, which was correlated to reduced yield. Injury symptoms consistent with dicamba damage at low rates include cupped leaves and swollen as well as twisted petioles and stems (Auch and Arnold 1978, Wax et al. 1969, Weidenhamer et al. 1989). Additionally, dicamba damage can lead to malformed pods, delayed maturity and reduced germination capability of harvested seed (Auch and Arnold 1978, Wax et al. 1969). Exposure of soybeans in the reproductive stage is especially detrimental. Dicamba translocates to the meristematic regions of the plant, effecting the growth of new tissues (Chang and Vanden Born 1971).
In the vegetative stage, exposure to dicamba contributes to greater branching allowing soybeans to recover from injury prior to flowering (Wax et al. 1969). However, in the early bloom stage soybeans accumulate dicamba in reproductive tissue and subsequent growth is minimal. In Louisiana, soybeans at the V3/V4 growth stage treated with 4.4 to 17.5 g ha\(^{-1}\) dicamba reduced yields 4 to 15%, while at R1 growth stage, these same rates reduced yields 10 to 36% (Griffin et al. 2013). Applications of 4.4 g ha\(^{-1}\) have been shown to reduce yields 4% following applications at V4 and 23% when applied on R1 soybeans in Illinois (Wax et al. 1969). In Kansas, yields were reduced 45% for vegetatively treated soybeans (56 g ha\(^{-1}\)) while dicamba at 5.6 g ha\(^{-1}\) reduced yields 1.6% (Al-Khatib and Peterson 1999). Andersen et al. (2004) reported yields for V3 treated soybeans were reduced 24 and 77% for 5.6 and 56 g ha\(^{-1}\) dicamba applied, respectively.

Quantifying yield loss in soybeans following exposure to dicamba can be difficult due to environmental conditions and the plasticity of soybeans. Soybean plasticity is the ability of plants to adapt to stressful situations by increasing lateral branching and expanding overall leaf area (Robinson and Conley 2007). This characteristic depends upon factors such as soil type, plant stress, weather and soybean variety. In South Dakota, 56 g ha\(^{-1}\) of the DMA salt of dicamba applied on V3 soybean reduced yield 20.1% compared to the untreated check (Auch and Arnold 1978). However, in another South Dakota study, the same rate of the DGA salt at a similar growth stage reduced yield nearly 80% (Andersen et al. 2004). The authors speculated drought conditions following dicamba application contributed to greater yield reductions. In drought-stressed years, applications as low as 1.3 g ai ha\(^{-1}\) of dicamba at mid-bloom reduced yield by 10%,
while in years with higher rainfall, rates as high as 15 g ai ha\(^{-1}\) were necessary to reduce yields by 10% (Weidenhamer et al. 1989).

**Dicamba Contaminated Spray Equipment**

In addition to off-target movement, spray equipment following application of dicamba becomes contaminated, which is also a concern (Boerboom 2004). Applicators typically use the same field spray equipment to apply herbicides to all crops, relying upon proper cleaning to reduce injury to subsequently treated crops (Johnson et al. 1997, Thompson et al. 2007). Numerous studies have focused on negative effects of low concentrations of growth regulators when applied through contaminated spray equipment and onto sensitive crops (Derksen 1989, Johnson et al. 1997, Steckel et al. 2005).

Derksen (1989) found that dicamba rates as low as 0.5% of labeled field rates reduced sunflower (*Helianthus annuus* L.) dry weight by 20% in greenhouse studies and yields up to 6% in field studies. Osborne et. al (2015) sampled tank-cleanout residue collected from commercial spray equipment in Colorado and found that after three rinses, some applicators only removed 98% of dicamba residues from the spray tank. Addition of herbicides to contaminated spray equipment can also facilitate removal of herbicides. Glyphosate, for example, removes adsorbed dicamba particles from equipment and into the spray solution, increasing the probability that dicamba can damage subsequently treated sensitive crops (Steckel et al. 2005).

Unlike glyphosate, which is highly water soluble, PGRs readily bind to porous materials in the spray tank (Haefner 2011). Dicamba adheres to many areas within the sprayer, including plastic parts, rubber hoses, the spray tank, and nozzles (Peachey 2009). Rubber hoses and plastic components are porous and especially susceptible to herbicide
buildup; water rinsing alone is unable to adequately displace dicamba and remove particles in the rinsate (Davis and Barber 2015).

Proper cleaning of spray equipment is important for all herbicides to minimize damage following applications to subsequently treated sensitive plants. This is especially true for PGRs, which are active at ppm. Guidelines for proper tank cleanout include repetitive practices that are time-consuming. For example, many extension publications recommend adding fresh water to the spray tank along with ammonia (1% v/v) or a commercial tank cleaner and allowing the mixture to stand overnight (Johnson et al. 1997, Pringnitz 1997, Steckel et al. 2005). The following day, applicators are advised to flush the system with fresh water twice to ensure the maximum herbicide residues are removed. The current dicamba labels also recommend a triple-rinse cleaning procedure, with a commercial cleaning agent or ammonia included in the first rinsate (Anonymous 2016b).

The role of the cleaning agent is to solubilize or displace herbicide particles to permit removal of the herbicide from the sprayer (Peachey 2009). Solubilizers such as ammonia increase the solubility of an herbicide in the rinsate, thereby removing the herbicide when the rinsate is flushed through the system (Johnson et al. 1997). Cleaning agents that increase solubility are composed of phosphate groups, which attach water molecules to hydrophobic molecules such as the herbicide, allowing water to remove the herbicide particles. Other cleaning agents reduce surface tension and allow the cleaning agent to penetrate cracks and displace herbicide particles into solution (Peachey 2009).

**Dicamba in the Soil**

Dicamba usage primarily results from POST applications, but residues appear to
persist in the soil following application and exhibit herbicidal affects (Burnside and Lavy 1966, Grossmann et al. 2002). Friesen (1965) found that 12 weeks after application dicamba resulted in severe injury to seedlings of newly planted Tartary buckwheat (*Fagopyrum tataricum* L.). Expression of residual activity may expand the utility of dicamba and reduce selection pressure for resistance.

The extent of dicamba persistence is affected by factors such as soil type and environmental conditions. Breakdown of dicamba in the soil occurs primarily through microbial activity (Smith and Cullimore 1975). Degradation of dicamba is enhanced with conditions favoring microbial activity, such as soil temperatures (15 to 35 C) where breakdown was highest at 30 C (Fogarty and Tuovinen 1995). Using the acid form of dicamba, Burnside and Lavy (1966) reported that under dry conditions, dicamba persisted up to three months longer in the soil at 15 C than at 35 C. Furthermore, dicamba persisted up to 1 month longer in a sandy loam soil compared to a silt loam or clay loam soil.

Rapid breakdown of dicamba was observed with moisture at 80% of field capacity, while soils with 13% of field capacity resulted in minimal breakdown of dicamba four months after application (Burnside and Lavy 1966). In Oklahoma, Altom and Stritzke (1973) reported rapid breakdown of dicamba in the first 20 days after application at three sites. Subsequently, dicamba in grassland soils continued to rapidly metabolize while residues in two forest sites exhibited slower breakdown, most likely the result of greater microbial activity in the grassland site (Altom and Stritzke 1973).

While microbes play a large role in metabolism of dicamba, tillage practices and soil organic matter levels can also impact soil fate of dicamba. Between 1992 and 1996, the National Water Quality Assessment found dicamba in 0.13% of groundwater samples.
collected throughout the United States (Kolpin et al. 2000). Watts and Hall (2000) sought to test the effects of mulch tillage on the leaching and runoff of dicamba. Using extraction columns, researchers discovered that tillage did not influence dicamba leaching, but found that conventionally tilled fields resulted in greater dicamba losses than mulch or no-tilled fields, due to soil erosion. Soils high in organic matter had ten-fold higher ED$_{50}$ values (effective dose to kill 50% of plants) than sand or sandy loam soils (Donaldson and Foy 1965), as dicamba strongly adsorbs to organic matter.

Once dicamba reaches the soil, movement is also affected by rainfall. Grover (1977) found that herbicide mobility is inversely related to the adsorptive values of the soil, as soils higher versus lower in organic matter required more water to leach dicamba through the soil profile. In a silty clay soil, Harris (1964) discovered that repeated simulated rainfall events resulted in dicamba leaching through soil columns. After planting oats at different depths in soil columns, dicamba was applied, followed by repeated applications of various rainfall increments. At depths of 20-30 cm, smaller, more frequent increments of rainfall (0.6 cm) resulted in 40% less oat biomass (fresh weight) compared to soil columns receiving 2.5 cm rainfall increments (Harris 1964). Friesen (1965) noted that dicamba is water soluble and readily moves within the soil profile with water. Using soil columns and Tartary buckwheat as a bioassay, dicamba was detected at depths just above the deepest penetration of water and caused the greatest damage at depths where moisture content was the highest in loam and sandy loam soils (Friesen 1965).
Purpose of Research

Broad adoption of DT soybean will result in a significant increase in POST applications, resulting in the need to clean spray equipment. Currently, little research apart from extension publications have described the impact of commercial spray cleaners, water or ammonia for cleaning commercial spray equipment. The proposed research will identify the impact of rinsates on soybean response (growth, yield) at two growth stages (V3, R1). The objective of this study was to compare four tank cleaning agents (water, ammonia, Cleanse® [Universal Crop Protection Alliance, LLC, Eagan, MN] and Erase® [Precision Laboratories, Waukegan, IL]) for removal of dicamba residues from commercial spray equipment with three successive rinsates.

A second study was conducted to determine the residual effects of dicamba in the soil following rainfall, which are not well described. Under greenhouse conditions, the objective of this research was to identify the soil activity of dicamba on emerging common waterhemp and morningglory (Ipomoea spp.) following simulated rainfall.
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CHAPTER 2: DICAMBA RESIDUES IN SPRAY EQUIPMENT REDUCES SOYBEAN (GLYCINE MAX) GROWTH AND YIELD

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Abstract

Introduction of dicamba-tolerant soybeans (Glycine max L. Merr.) will improve management of in-crop weeds. However, this poses a risk for contamination of spray equipment which may then be used to treat susceptible soybeans. Field studies were conducted in 2015 and 2016 at two locations in Central Missouri to assess soybean injury and yield following application with spray tank rinsates containing dicamba residues. Dicamba at 0.56 kg ae ha\(^{-1}\) was applied through a pulsating-pressure and constant-pressure sprayer. Spray equipment was subsequently treated with water or one of four cleaning agents (water alone, ammonia, Cleanse® or Erase®). This initial treatment was considered a first rinsate, and was followed by two rinses of water (second and third rinsate). A portion of rinsate solutions were applied on V3 or R1 soybeans. Visible damage was found at 7 days after treatment (DAT) for first rinsates, ranging from 6 to 16% for V3 treated plants and up to 22% for R1 plants. Overall, V3 plants recovered following rinsate applications by 28 DAT. However, R1 plants exhibited 24 to 39% injury following applications of first rinsates. At 14 DAT, plant height was reduced up to 48 and 3% for first and third rinsates, respectively on V3 treated soybeans. Stunting of R1 treated soybeans ranged from 16 to 33% for first rinsates at 14 DAT, and 29 to 45% by 28 DAT. For second and third rinsate treatments that resulted in up to 26% shorter soybeans, reductions in plant height were significant at 28 DAT. Soybean yields were
reduced up to 46% for R1 treated soybeans by first rinsates compared to the untreated control, while V3 treated plants exhibited yield losses up to 11% compared to the control. For third rinsates, yields for V3 plants increased up to 10% compared to the untreated control, while yields ranged from 6% lower to 11% higher on R1 plants following application of third rinsates. First rinsates contained between 44 and 105 ppm dicamba while typical third rinsates had less than 9 ppm dicamba. A number of cleaning agents can minimize equipment contamination damage by dicamba to soybeans, but the use of triple rinsing and exposure of soybeans before the reproductive stage are critical.

**Nomenclature:** dicamba; soybean, *Glycine max*.

**Keywords:** cleaning agent; contamination.
**Introduction**

Dicamba is a plant growth regulator (PGR) herbicide used traditionally for controlling broadleaf weeds in grass crops (USDA-Crops Research Division 1967). Dicamba is selectively active on broadleaves, as grasses and other monocots rapidly metabolize dicamba before extensive injury occurs to the plant (Broadhurst et al. 1966). Use in broadleaf crops has been strictly limited to pre-plant conditions (Anonymous 2010).

Development of dicamba-tolerant (DT) crops, including soybean, is an attractive option for growers to add a new mode of action not previously available in soybean. Effective loss of control of key broadleaf weeds because of resistance to glyphosate and other herbicides has left few postemergence (POST) compounds for use (Duke, 2012; Heap, 2016). DT soybeans will allow safe use of dicamba from emergence through R1 (Anonymous, 2016; Mortensen et al., 2012). Introduction of DT soybeans should improve control of many troublesome broadleaf weeds such as giant ragweed (*Ambrosia trifida* L.) and common waterhemp (*Amaranthus rudis* Sauer) (Behrens et al. 2007).

The effectiveness of dicamba is also a potential weakness, as inadvertent exposure of sensitive plants is problematic (Johnson et al. 2012b). Dicamba results in significant injury to sensitive soybeans at extremely low concentrations (Cenkci et al. 2010) because the herbicide mimics the hormone auxin (Grossmann 2010). Rates as low as 0.01% of the labeled rate (560 g ha$^{-1}$) have reduced soybean yields by 10% (Weidenhamer et al. 1989). The extent of dicamba damage depends on the dose of dicamba and the stage of soybean at time of exposure. Soybeans in the early-bloom stage (R1) appear to be more susceptible to yield losses than soybeans in the pre-bloom stage (V3). Auch and Arnold
(1978) reported soybean yields ranged from 2% higher to 9% lower for plants treated with 11 g ae ha\(^{-1}\) of dicamba in the V3 and R1 growth stages, respectively. Griffin et al. (2013) reported yield reductions of 4 to 15% for soybeans treated with 4.4 to 17.5 g ha\(^{-1}\) dicamba at the V3/V4 stage, but a 10 to 36% yield reduction for comparable rates at the R1 growth stage. Applications of 4.4 g ha\(^{-1}\) have been shown to reduce yields 4% in V4 applications and 23% at R1 soybeans in Illinois (Wax et al. 1969). An example of the risk for damage to dicamba-sensitive soybeans following dicamba applications to adjacent DT soybeans is widespread damage (more than 400 complaints) in southeast MO in 2016 (Bennett 2016).

Off-target damage from dicamba has been studied within the growing season, but symptoms do not always result in yield reductions. Behrens and Lueschen (1979) devised an injury rating scale to rate dicamba injury from 0 (no damage) to 100% (plant death) and found significant yield reductions associated with injury ratings above 60%. Experiments in North Carolina determined soybean injury ratings of 20% at 1 week after treatment (WAT) and 30% at 2 WAT resulted in yield reductions following treatment with 11 g ha\(^{-1}\) dicamba (Johnson et al. 2012a). Al-Khatib and Peterson (1999) reported soybean injury between 12 and 66% at 7 days after treatment (DAT) and 30 to 92% at 14 DAT with dicamba at rates of 5.6 to 187 g ha\(^{-1}\) on V3/V4 soybeans. Plant heights were reduced 12% at 60 DAT compared to the untreated control for rates of 5.6 g ha\(^{-1}\) dicamba, but only resulted in yield reductions of 2%. Andersen et al. (2004) reported soybean biomass was reduced 16, 15, 16 and 35% at 6, 12, 24 and 48 DAT, respectively for V3 soybeans treated with 5.6 g ha\(^{-1}\) dicamba. This rate resulted in yield losses of 24%.
Off-target damage in soybean can result from evaporation in gas form from treated areas (volatility) or particle movement after release from the spray equipment but before deposition in targeted areas (drift). Much research has focused on off-target movement of dicamba through vapor or particle drift. Al-Khatib and Peterson (1999) showed that simulated drift of a dicamba solution caused severe epinasty and leaf curling within three hours after treatment, and shoot and petiole epinasty within a week of treatment. Behrens and Lueschen (1979) found that dicamba volatility caused injury ratings of up to 38% for V1 soybeans placed 30 m downwind after dicamba application.

Improper cleaning of spray equipment, also referred to as tank contamination, can also result in unintended damage. Simulated tank contamination of 0.5% of labeled field rates of dicamba reduced sunflower (Helianthus annuus L.) dry weight by 20% in greenhouse studies and yields up to 6% in field studies (Derksen 1989). Osborne et al. (2015) found that even after three rinses, some commercial applicators only removed 98% of dicamba residues from the spray tank, with remaining residues sufficient to damage soybean.

Herbicide application equipment consists of an extensive network of hoses and plastic fittings connected to the spray tank. Many of these materials are porous (Haefner 2011) and not efficiently cleaned by rinsing with water (Davis and Barber, 2015; Johnson et al, 1997). PGR’s are not highly water soluble, and readily bind to porous materials in the spray tank (Haefner 2011). Therefore, use of water alone is often not efficient for removal of damaging levels of dicamba (Davis and Barber, 2015; Johnson et al., 1997).

Typical removal of dicamba from commercial spray equipment often follows a triple-rinse cleaning procedure with the use of a commercial cleaning agent or ammonia.
Cleaning agents are intended to solubilize or deactivate the herbicide within the spray tank to prevent injury to subsequently treated areas (Peachey 2009). Solubilizers such as ammonia increase the solubility of a contaminant, thereby allowing water to displace the particle into solution (Johnson et al. 1997). Deactivating cleaning agents prevent further damage by oxidizing pesticide particles and decomposing herbicide molecules into inactive compounds (Peachey 2009).

Commercial application equipment must be sufficiently clean to preclude damage to sensitive soybeans following dicamba applications on DT soybeans. However, effective cleaning agents and the required number of rinsates to remove damaging levels of dicamba from commercial sprayers is not well described. Therefore, the objective of this research was to utilize commercial sprayers to compare water with three chemical cleaners and multiple rinsates for minimizing dicamba damage to subsequently-treated sensitive soybeans.

**Materials and Methods**

Field trials were established in 2015 and 2016 at two locations in central Missouri: the Bradford Research and Extension Center (Bradford) near Columbia (38.89°N, 92.19°W), and the Kendall Kircher farm near Boonville (Boonville) (38.99°N, 92.67°W). The soil type at Bradford was a Mexico silt loam (fine, smectitic, mesic Vertic Epiqualfs) with 2.1% organic matter and a pH of 6.2, while the soil type at Boonville was a Lowmo silt loam (coarse-silty, mixed, superactive, mesic Fluventic Hapludolls) with 1.4% organic matter and a pH of 6.0.
At each location, a glyphosate-resistant (MorSoy 39X14) variety of soybeans was planted (76 cm rows) into conventionally tilled conditions at a population of 346,000 seeds ha\(^{-1}\). Planting occurred on June 7, 2015 and June 2, 2016 at Boonville in areas with corn planted the previous year. In 2016, poor emergence led to replanting 271,700 seeds per hectare on June 13. At Bradford, soybeans were planted June 10, 2015 and May 4, 2016. In 2015, poor emergence resulted in termination of existing stand of soybeans with glufosinate (0.23 kg ai ha\(^{-1}\)) and replanting of soybeans on June 25. Plots at Bradford in 2015 followed fallow conditions in 2014. In 2016, soybeans were planted into a no-till area which contained soybeans the previous year.

All experimental areas were maintained weed-free to properly assess effects of dicamba-contaminated spray solution. At Boonville, PRE applications of 0.26 kg ai ha\(^{-1}\) sulfentrazone + 0.017 kg ai ha\(^{-1}\) chlorimuron ethyl and 1.39 kg ai ha\(^{-1}\) S-metolachlor were applied at planting. Escape weeds were removed with POST applications of 0.21 kg ai ha\(^{-1}\) clethodim, 0.22 kg ai ha\(^{-1}\) lactofen and 0.146 kg ai ha\(^{-1}\) pyroxasulfone + 0.0044 kg ai ha\(^{-1}\) fluthiacet-methyl along with 0.25% v/v crop oil concentrate on June 24, 2015 and June 29, 2016. At Bradford 0.26 kg ha\(^{-1}\) sulfentrazone + 0.034 kg ai ha\(^{-1}\) cloransulam-methyl were applied on June 11, 2015 and May 5, 2016. Glyphosate at 1.59 kg ae ha\(^{-1}\) was also applied in 2016 at the time of the PRE application. POST applications of 1.93 kg ha\(^{-1}\) glyphosate were made June 29, 2016. An infestation of Japanese beetles (Popillia japonica Newm.) resulted in an application of 0.028 kg ai ha\(^{-1}\) lambda cyhalothrin on July 14\(^{th}\), 2016 at Bradford. Applications of 0.022 kg ai ha\(^{-1}\) zeta-cypermethrin and 0.066 kg ai ha\(^{-1}\) bifenthrin were made at Boonville for Japanese beetle control in both 2015 and 2016. All pesticides were applied with a CO\(_2\) pressurized back pack sprayer and 3 m boom.
Spray conditions included a spray volume of 140 L ha\(^{-1}\) and spray pressure of 117 to 138 kPa.

Plots (3 x 7.6 m) were arranged at each location as a split plot with four replications. The main plot variable was treatment timing for soybean, V3 or R1. The sub-plot variables comprised four cleaning agents (water, ammonia, Cleanse® or Erase®) three rinsates, and two commercial field sprayers in a randomized complete block. An untreated control resulted in a total of 25 treatments at each soybean timing.

Collection of rinsate occurred using commercial field equipment (MFA Incorporated). The sprayers used were a 3330 Case IH Patriot with Aim Command (Case IH, Racine, WI) and 1084 Rogator (AgCo, Hutchinson, MN). Each sprayer was equipped with a 3,800 L stainless-steel tank and 30.5 m booms. To prevent contamination of the entire boom, only the center section of each sprayer was exposed to dicamba, and all rinsate was collected from nozzles in the center section. Rinsate was collected a total of four times. Collections occurred before soybeans reached the V3 or R1 growth stage in each year, and each rinsate was applied to soybeans at both locations.

To test the effectiveness of various cleaning agents at removing dicamba from contaminated equipment, four separate cleaning agents were used; fresh water, ammonia (LA’s Totally Awesome clear ammonia, Buena Park, CA), Cleanse® (Universal Crop Protection Alliance, Eagan, MN) and Erase® (Precision Laboratories, Waukegan, IL). Ammonia acts a solubilizing cleaning agent while Cleanse and Erase are considered deactivating cleaning agents. All cleaning agents were added during the first rinsate only.

Rinsates were collected following a specific order for each cleaning agent, with timing of collection shortly before soybeans reached the targeted size. Initially, dicamba
(Clarity® herbicide, BASF Corporation, Research Triangle Park, NC 27709) was added to the spray tank of each respective sprayer at a rate of 0.56 kg ae ha\(^{-1}\) with 190 L of fresh water, circulated through the spray system for five minutes and sprayed through the nozzle tips. Following application, the tank was drained and fresh water as well as one of the cleaning agents (ammonia, 1% v/v; Cleanse, 0.25% v/v; Erase, 0.5% v/v; and water alone) was added to the tank with a total liquid volume of 190 L. Spray solution was allowed to circulate in the spray tank for five minutes and 37 L of solution was then sprayed through the boom. During this process, 3.8 L was collected from multiple nozzles and considered the first rinsate. Following collection, the tank was drained and 190 L of fresh water was added. Water was circulated for five minutes and 37 L was sprayed as described above; 3.8 L was again collected and considered the second rinsate. For a third time, the solution was drained and refilled with 190 L of fresh water. Following circulation for five minutes, 37 L was sprayed and 3.8 L of rinsate was collected (third rinsate). After the third rinsate, dicamba in water was added to the spray tank and the entire process was repeated until all cleaning agents and associated rinsates were collected.

Following rinsate collection, solutions were stored in a cooler until use on soybeans. Rinsate was collected for application to each growth stage in a particular year at both locations (V3 and R1). Applications were sprayed at 4.8 km h\(^{-1}\) with a CO\(_2\) pressurized backpack sprayer equipped with XR8002 TeeJet (TeeJet® Spraying Systems, Wheaton, IL) flat fan nozzle tips calibrated to deliver 140 L ha\(^{-1}\). To prevent contamination of spray equipment, individual booms were built for each cleaning agent, and rinsates were sprayed in the following order: third, second and first rinsate.
Data collection began 1 day after treatment (DAT) and concluded when soybeans were harvested. Visual injury ratings were taken at 1, 3, 7, 14, and 28 DAT and were based on a 0 to 100% scale, with 0 indicating no visible injury and 100 representing plant death. Plant height was estimated at 14 and 28 DAT, as well as prior to harvest. Height was considered from ground level to the top of the apical meristem from five random plants in the center two rows of each plot. Yield was determined by harvesting the two center rows of each plot with an 8 XP Massey Ferguson Multi Plot Research Plot Combine (Kincaid Equipment Manufacturing, Haven, KS) on October 15, 2015 and October 24, 2016 at Boonville and October 21, 2015 and October 21, 2016 at Bradford. Yields were corrected to 13% moisture content and expressed as kilograms per hectare. Seed samples from each harvested plot were collected to determine the effect of dicamba on seed size. Two samples of 100 seeds were counted and weighed from each plot to determine if spray solutions impacted seed size.

In addition to assessing response on soybeans, each rinsate was analyzed to determine the quantity of dicamba in the solution. A sample of each rinsate was collected and frozen following collection. Samples were thawed and 10 mL of each sample was packaged in dry ice and shipped to Dr. Thomas Mueller the University of Tennessee (Knoxville, TN 37996). Rinsate samples were received via overnight shipping and stored at -20 C until analysis. Samples were thawed and allowed to come to room temperature, and then each was diluted with one mL aliquot of each sample added to 19 mL of methanol, for a net 5% dilution. These diluted samples were filtered through a 0.45 µM filter to remove all particulates. Dicamba concentrations were determined using an LCMS system including a 1100 liquid chromatography system coupled in line with a
single quad 6120 mass spectrometer, both from Agilent Technologies (Agilent.com). An external standard was added at known concentrations of 10, 100, and 1000 mg Kg\(^{-1}\) to generate a standard curve. Detector response was linear within this range, and a conservative lower limit of detection was 10 mg Kg\(^{-1}\). Chromatographic separation was accomplished using a C18 column (3 µM), 150 mm* 4.6 mm analytical column (phenomenex.com) and a linear mobile phase consisting of acetonitrile:water at 70:30 v:v (both with 0.1% formic acid). Mass spectrophotometric operating conditions included drying gas flow = 12.0 L, nebulizer pressure of 35 psig, drying gas temperature of 250 C, vaporizer temperature of 200 C, capillary voltage of 2500, corona current - 0, charging voltage = 1200, fragmentor operated at 50, and single ion monitoring in negative mode at 219.00 was used. The retention time in the system was 4.1 minutes. An analysis of samples was conducted from both 2015 and 2016 rinsates.

Data were subjected to ANOVA using PROC GLIMMIX in SAS 9.4 (SAS Institute Incorporated, Cary, NC). Years and location were considered random effects, whereas cleaning agents, growth stage, sprayer, replication and rinsates were considered fixed effects in the model. A significant main effect for location and location by year interaction resulted in separate analyses for each year and location. The main effect for sprayers and interactions between other variables and sprayers were not significant. Therefore, data were combined over sprayers. Means were separated using Fisher’s Protected LSD at P=0.05.

**Results and Discussion**

Initial plant injury was observed as soon as 1 DAT (data not shown). By 7 DAT, first rinsates resulted in visual injury ranging from 6.6 to 16.1% for V3 plants and from
9.6 to 21.5% for R1 plants (Table 2.1). Across locations, injury was generally greater in 2015 than 2016. At the V3 timing, first rinsates of all cleaning agents in 2015 at both locations resulted in similar injury. However, in 2016, injury was up to 5.5% higher with Cleanse and Erase compared to ammonia and water, suggesting more effective removal of dicamba residues. At the R1 timing, soybean injury 7 DAT was overall lower for water compared to the other cleaning agents; injury was up to 26.5% lower compared to first rinsates of Erase in 2016 at Bradford (Table 2.1).

Soybean injury at 7 DAT was significantly lower for the second and third rinsate of all cleaning agents compared to the first rinsate for both V3 and R1 plants (Table 2.1). Similar to the results for the first rinsate, Cleanse and Erase resulted in up to 4% greater injury in 2016 compared to ammonia and water for V3 soybean. With all cleaning agents, third rinsates caused less than 3% damage to V3 soybeans. Third rinsates on R1 soybeans sustained 5% injury or less; the exception was Erase in 2016 at each location. Cleanse and Erase resulted in greater injury to R1 plants in 2016 at both locations when compared to ammonia and water for both second and third rinsates.

Soybean injury persisted beyond 7 DAT, and for many treatments increased at the 28 DAT evaluation (Table 2.2). Injury to both V3 and R1 soybeans increased significantly for first rinsates compared to 7 DAT first rinsates. Injury with the first rinsate ranged from 17.6 to 37.6% for V3 soybeans and 29.1 to 39.3% for R1 soybeans. Similar to 7 DAT evaluations, Cleanse and Erase resulted in up to 11% greater visual injury compared to water and ammonia with the first rinsate in 2016 to V3 plants at 28 DAT. All plants exposed to second rinsates sustained visual injury between 5.6 and 23.4%, with the exception of V3 plants at Boonville in 2015, where injury rating was 3.3
to 4.5%. Over all site years, soybeans exposed to second rinsates following water and ammonia as the cleaning agent resulted in up to 53 and 62.9% less injury than plants treated following Cleanse and Erase for V3 and R1 soybeans, respectively. Second rinsate treatments with water as the cleaning agent resulted in the least damage among cleaning agents for all site years but V3 plants at Boonville in 2015. Third rinsate damage ranged from 0.6 to 6.1% for V3 plants and 0.6 to 17% for R1 plants. Third rinsates following use of Cleanse and Erase overall resulted in greater injury to R1 soybeans compared to water and ammonia. At Bradford in 2016, Cleanse and Erase had significantly greater damage at 28 DAT than water and ammonia for all three rinsates for R1 soybeans.

Besides typical visual symptoms associated with dicamba activity, plant height is also a mechanism to estimate soybean response. At 14 DAT, reductions in soybean plant height were readily evident (Figures 2.1 and 2.2). For all first rinsates, plant heights were reduced up to 48 and 32.9% for V3 and R1 plants, respectively, compared to the untreated control. Similar to visual injury, first rinsates using Cleanse and Erase resulted in no impact on V3 plants in 2015 in 2016 when compared to water and ammonia (Figure 2.1A), but reductions of up to 15.1% for V3 plants in 2016 were noted (Figure 2.2A). These results support the findings of Griffin et al. (2013), where V3 plants exhibited greater stunting than R1 plants, which was attributed to V3 plants more actively growing vegetatively. All plants exposed to third rinsate treatments had heights from 8.5% shorter to 7.3% greater than the untreated control at 14 DAT (Figures 2.1 and 2.2). R1 plants following Erase at three of four site years and use of Cleanse at Boonville in 2016 were up to 10.2% shorter than the untreated control.
By 28 DAT, dicamba impacts on plant height for both V3 and R1 treated plants revealed plant recovery or more intensive injury (Figures 2.3 and 2.4). First rinsates for V3 plants were 23.2 to 45.8% shorter than the untreated control height. In 2015, no V3 plants treated with second or third rinsates were shorter than the untreated control, although second rinsates following Cleanse and Erase were up to 6.4% shorter than water and ammonia second rinsates. First rinsates applied to R1 plants resulted in 29.4 to 45.1% reductions in height compared to the untreated control. Plant heights for R1 plants treated with third rinsates ranged from 18.3% shorter to 4.1% taller than the untreated control. In 2016, second rinsates of Cleanse and Erase resulted in significantly shorter R1 plants (up to 19.5%) than water and ammonia. Third rinsates following Cleanse and Erase were up to 18.3% shorter than the untreated control at both locations in 2016, as were all R1 plants following third rinsates at Boonville in 2015; the exception was the third rinsate following water as the cleaning agent.

Development of injury symptoms or reductions in soybean height is helpful to assess initial affects, but the most important determinant of dicamba injury is the impact on yield. Overall, yield losses from dicamba injury were greater for R1 treated soybeans, as yields were 12.4% lower than in V3 treated plants (Table 2.3). Despite injury of up to 38% at 28 DAT, first rinsates on V3 plants only resulted in yield reductions of up to 11.4% compared to the untreated control; some treatments yielded 3.5% more than the untreated control. For first rinsates containing Cleanse, yields were numerically overall lower compared to the other cleaning agents in both 2015 and 2016. Despite sustaining greater injury and stunting throughout the study, soybean plants recovered for Cleanse and Erase first rinsate treatments, and yields were statistically similar to ammonia and
water for V3 treated soybeans in 2016. All first rinsates on R1 plants resulted in yields significantly lower than the untreated control; yield losses were between 12 and 46.4% for all cleaning agents. For R1 first rinsates at Bradford in 2016, Erase and Cleanse yielded 25.6 and 29.8% less than water as the cleaning agent, respectively.

With the majority of dicamba particles being removed in the first rinsate, second and third rinsates only resulted in slight yield reductions. Comparing rinsates within each cleaning agent separately, soybeans exposed to second rinsates yielded up to 15 and 45% more than first rinsates on V3 and R1 soybeans, respectively (Table 2.3). For V3 plants, yields for plants treated with second rinsates were only reduced up to 2.9% for V3 treated soybeans, and up to 11.7% for R1 soybeans compared to the untreated control.

Comparing the cleaning agents within second rinsates, treatments following water as a cleaning agent resulted in the greatest yield in all years except for soybeans at Bradford in 2016, when yields were 1.8% less than the second rinsate of Erase. Yields for third rinsate treatments ranged from 7.8% lower to 11% greater than the untreated check and were not significantly different. Yields of third rinsate treated soybeans were not consistently higher within any specific cleaning agent. Yields were similar across years for the Boonville location but were significantly greater for Bradford in 2016 due to an earlier planting date.

Initial injury to soybean was less related to the extent of injury and height reduction, and more aligned with the growth stage of soybean when injury occurred. Soybeans treated in both the V3 and R1 growth stage with first rinsates sustained visual injury up to 26.6% at 28 DAT. The V3 plants recovered and only sustained average yield losses of 2.9%, while the R1 plants yielded up to 37.7% less than the untreated control.
At 7 DAT visual injury ratings of 3.9% for second rinsates on R1 plants reduced yields by 5.7%, while V3 yields increased by up to 5.3%. The third rinsate of Erase at R1 Boonville soybeans in 2016 were 18.3% shorter than the untreated check at 28 DAT, but yielded within 0.6% of the untreated check. Observations of greater injury and plant stunting early in the growing season generally resulted in lower yields for R1 soybeans but had little effect on V3 soybeans. The inability of R1 plants to initiate new meristems from lateral shoots likely limited the recovery of plants.

Differences in the response of soybean plants to dicamba were related to the concentration of herbicide in the rinsate solution (Table 2.4). The majority of dicamba particles were removed from the sprayer in the first rinsate, ranging from 79.8 to 93.3% of the total dicamba removed from the combined three rinses. The labeled rate of dicamba contained an average of 2,465 parts per million (ppm) in 2015 and 1,846 ppm in 2016. Cleaning agents with two water rinsates reduced the dicamba concentration up to 97.7%, but this was not enough to preclude soybean injury and yield. In 2015, first rinsates containing water removed more dicamba than the other cleaning agents, while in 2016, water was the poorest cleaning agent. However, as soybean yields indicated, a single rinsate is not sufficient to prevent yield losses, especially for R1 treated plants. In 2015 and 2016, second rinsates following Cleanse and Erase for V3 plants and second as well as third rinsates for R1 plants removed up to 19-fold more dicamba than water alone.

These data suggest that over time, commercial cleaning agents may be more effective in removing or neutralizing dicamba during the cleaning process. Yield results indicate that for V3 plants, soybeans exposed to up to 13 ppm of dicamba can recover and not sustain losses when compared to the untreated control (Table 2.3). However, in R1 soybeans,
similar levels of dicamba can reduce yields up to 7.2%, with dicamba levels of 5 ppm or less required to have little impact on yield.

Proper tank cleanout is essential to reducing risk for sensitive soybeans treated with pesticide equipment that is also used to apply dicamba. Previous research suggests that soybeans in the reproductive growth stage are more susceptible to yield loss from dicamba exposure than soybeans in the vegetative growth stage (Auch and Arnold 1978, Griffin et al. 2013, Wax et al. 1969). Griffin et al. (2013) showed that dicamba rates of 1.4 and 11.2 g ha\(^{-1}\) reduced yields 1 and 10%, respectively on V3 treated soybeans and 3 and 24%, respectively on R1 treated soybeans. This research supports those claims as first rinsates reduced yields 12 to 46% for R1 treated soybeans but only up to 11% for V3 treated soybeans. Visual injury and stunting were also greater in R1 soybeans.

There is limited research on effectively removing dicamba from commercial applicators, as it is difficult to determine if all dicamba particles have been removed during the clean-out process. Extension publications suggest using a triple-rinse procedure with the use of a commercial cleaning agent or ammonia is effective for cleaning out commercial applicators. This research suggests that three rinses are necessary to effectively clean the pesticide applicator and that cleaning agents may aid in dicamba removal. While yields were similar between second and third rinsates, damage was observed at both 7 and 28 DAT for third rinsates, indicating that two rinsates do not remove all dicamba particles. Indeed, dicamba concentrations up to 8.51 ppm were detected in third rinsate solutions. Although water caused similar or significantly less injury than the other cleaning agents at 28 DAT, this was likely due to water removing less dicamba from the sprayer and having less dicamba in the rinsate (Table 2.4).
Therefore, with water-only rinsates, dicamba particles may remain adhered to application equipment and levels build-up over time to a point where they could potentially result in economic damage to a sensitive crop. This research did not consider the use of herbicides tank-mixed with the cleaning agents and subsequent water rinsates. It is expected that glyphosate may also be used in the spray equipment. Glyphosate has been shown to act as a cleaning agent and remove herbicides adhered to spray equipment (Haefner 2011). Removing essentially all dicamba particles is vital to eliminating off-target movement of dicamba through tank contamination, especially for soybeans treated in the reproductive stage. Our research suggests that at least two rinses with fresh water following the use of a cleaning agent are necessary to effectively minimize the impact of dicamba on sensitive soybeans.

Acknowledgement

The authors would like to thank MFA Incorporated for the use of their equipment throughout this experiment, as well as their time, supplies and land. In addition, the authors thank Dr. Tom Mueller and his group at the University of Tennessee for analysis of dicamba rinsate samples.
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Washington D.C. 1-72 p


Table 2.1. Visual injury ratings (0=no injury, 100=plant death) for soybeans at 7 DAT at two locations in central Missouri. Means with the same letter within timing, year and location are not significantly different using Fisher’s Protected LSD at P=0.05.

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Table 2.2. Visual injury ratings (0=no injury, 100=plant death) for soybeans at 28 DAT at two locations in central Missouri. Means with the same letter within timing, year and location are not significantly different using Fisher’s Protected LSD at P=0.05.

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with the same letter within timing, year and location are not significantly different using Fisher’s Protected LSD at P=0.05.
Table 2.3. Soybean yields in kg/ha at two locations in central Missouri. Means with the same letter within timing, year and location are not significantly different using Fisher’s Protected LSD at P=0.05.

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Table 2.4. Mean dicamba concentrations in parts per million (ppm) for rinsates collected from two commercial sprayers. Measured concentrations of dicamba at the labeled rate of 0.56 kg/ha were 2464.96 and 1846.17 ppm in 2015 and 2016, respectively. Means with the same letter within timing and year are not significantly different using Fisher’s Protected LSD at P=0.05.

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Figure 2.1. Plant heights as a percent of the untreated control at 14 DAT for Bradford (A) and Boonville (B) in 2015. Means followed by the same letter within application timing are not significantly different using Fisher’s Protected LSD at P=0.05. Abbreviations: Am, ammonia; Cl, Cleanse®; Er, Erase®.
Figure 2.2. Plant heights as a percent of the untreated control at 14 DAT for Bradford (A) and Boonville (B) in 2016. Means followed by the same letter within application timing are not significantly different using Fisher’s Protected LSD at $P=0.05$. Abbreviations: Am, ammonia; Cl, Cleanse®; Er, Erase®.
Figure 2.3. Plant heights as a percent of the untreated control at 28 DAT for Bradford (A) and Boonville (B) in 2015. Means followed by the same letter within application timing are not significantly different using Fisher’s Protected LSD at P=0.05. Abbreviations: Am, ammonia; Cl, Cleanse®; Er, Erase®.
Figure 2.4. Plant heights as a percent of the untreated control at 28 DAT for Bradford (A) and Boonville (B) in 2016. Means followed by the same letter within application timing are not significantly different using Fisher’s Protected LSD at P=0.05. Abbreviations: Am, ammonia; Cl, Cleanse®; Er, Erase®.
CHAPTER 3: DICAMBA EXHIBITS SOIL RESIDUAL ACTIVITY ON MORNINGGLORY (IPOMOEA SPP.) AND COMMON WATERHEMP (AMARANTHUS RUDIS)

Andy J. Luke and Reid J. Smeda

Abstract

Dicamba is a postemergence herbicide, but field observations suggest residual activity may also be exhibited. In a greenhouse setting, the influence of varying rates of dicamba and subsequent activating rainfall on common waterhemp (Amaranthus rudis), and morningglory (Ipomoea spp.) emergence was measured. Polypropylene containers were filled with field soil and PVC rings were fitted into the soil. Weed species were seeded individually in rings and containers were treated with dicamba ranging from 0.14 to 1.12 kg ae ha\(^{-1}\) or 0.072 kg ae ha\(^{-1}\) flumioxazin; simulated rainfall ranging from 0 to 1 cm was then applied to each container. Optimum emergence of waterhemp and morningglory was reached in control areas by 9 days after treatment (DAT). At 9 DAT, treatments with flumioxazin that received rainfall of 0.51 or 1 cm showed no waterhemp emergence while morningglory emergence was up to 96% less than the untreated control after 1 cm of rainfall. Dicamba reduced waterhemp emergence from 40 to 63% as rates increased from 0.14 to 1.12 kg ha\(^{-1}\) following 1 cm of rainfall at 9 DAT. By 15 (DAT), morningglory emergence was similar to the control for treatments receiving no rainfall. For treatments receiving 1 cm rainfall, morningglory emergence was reduced from 19 to 80% for dicamba rates ranging from 0.14 to 1.12 kg ha\(^{-1}\) at 15 DAT. By 21 DAT, waterhemp emergence was reduced between 92 and 100% following 1.12 kg ha\(^{-1}\) dicamba. Across all dicamba only treatments, waterhemp emergence was reduced 81%
versus 62% at high (1 cm) compared to low (0.13 cm) rainfall amounts, respectively, suggesting dicamba activity was promoted by rainfall. For seedlings that did emerge, biomass of morningglory was reduced up to 73% while waterhemp plants attained up to 99% less biomass than the untreated control. Under controlled conditions, dicamba exhibits soil activity for at least 21 days, but should be viewed as a supplement and not a substitute for use of a traditional soil residual herbicide.

**Nomenclature:** dicamba; flumioxazin; common waterhemp, *Amaranthus rudis* Sauer, AMATA; morningglory, *Ipomoea* spp.

**Keywords:** Preemergence; rainfall; soil-applied.
Introduction

The herbicide dicamba was introduced for commercial use in 1967 (EPA 2006) and for over 50 years has targeted emerged broadleaf weeds. Dicamba effects sensitive plants by mimicking the plant hormone auxin. Auxins influence nearly every aspect of plant growth and development, and unlike the low concentrations of auxin that are quickly inactivated through conjugation and degradation, synthetic auxins are introduced at higher doses and are much more stable in the plant (Grossmann 2010). Dicamba also results in significant injury on sensitive broadleaf plants at extremely low concentrations (Cenkci et al. 2010).

Despite use of dicamba on emerged plants, a number of researchers have demonstrated soil activity of dicamba. Indirectly, restrictions are in place to delay the planting of sensitive broadleaf crops (Anonymous 2016) following burndown applications. Weather conditions can sometimes delay applications, resulting in injury to emerging soybeans of up to 38% at 35 days after planting when dicamba is applied only seven days before planting (Thompson et al. 2007) Sheets et al. (1968) reported up to a 4% reduction in snap bean (*Phaseolus vulgaris* L.) biomass when 0.5 ppm dicamba acid was applied to soil at 16 weeks before planting. Applications of 2 or 8 ppm dicamba acid resulted in significant reductions in biomass of snap bean planted up to eight weeks after application. Soil applications of 0.28 kg ae ha\(^{-1}\) dicamba resulted in up to 98% control of common lambsquarters (*Chenopodium album* L.) and greater than 90% control of horseweed (*Conyza canadensis* L. Cronq.) (Johnson et al. 2010) in field experiments at three weeks after application. However, control was less than 60% for velvetleaf (*Abutilon theophrasti* Medik), Palmer amaranth (*Amaranthus palmeri* S. Wats.), common
waterhemp (*Amaranthus rudis* Sauer), giant ragweed (*Ambrosia trifida* L.) and morning glory (*Ipomoea* spp.) throughout the U.S. (Johnson et al. 2010). Burnside et al. (1971) in NE showed no yield impact to field beans (*Phaseolus vulgaris* L.) planted two years after application of 22 kg ha$^{-1}$ dicamba.

Persistence of dicamba is affected by the level of microbial activity in the soil. Smith and Cullimore (1975) discovered that no degradation of dicamba occurred in steam-sterilized soils. Studies in OK reported rapid breakdown of dicamba in the first 20 days after application in grassland and forest soils (Altom and Stritzke 1973). Thereafter, dicamba was rapidly metabolized in the treated grassland soil, while the rate of degradation in the two forest sites was slower; likely a result of greater microbial activity in the grassland site. After conducting further studies to estimate the length of dicamba persistence, Altom and Stritzke (1973) determined the half-life for dicamba to be 17 to 32 days, with the half-life related to the extent of vegetative cover and microbial activity of each soil. Voos and Groffman (1997) reported dicamba degraded more rapidly in soils with higher microbial activity. No degradation occurred 80 days after treatment (DAT) in soils from an aquifer setting (sand and gravel) while no dicamba was detected in a wetland soil with high organic matter and an associated high microbial population. With environmental conditions favoring microbial activity, Burnside and Lavy (1966) also reported rapid breakdown of dicamba. Soil persistence was up to three months less in soils at 35 C compared to 15 C. Optimal breakdown of dicamba occurred at soil temperatures of 30 C (Fogarty and Tuovinen 1995).

Soil activity of dicamba is also impacted by rainfall. Harris (1964) found that dicamba showed considerable movement both downward and upward in a silty clay soil.
following rainfall and subsurface irrigation, respectively. Smaller and more frequent rainfall events versus larger, single events reduced fresh oat biomass up to 40% at a depth of 20 to 30 cm in soil columns. Friesen (1965) used Tartary buckwheat (Fagopyrum tataricum L.) in soil columns to demonstrate that plant damage occurred up to six inches below the surface following dicamba application. Movement of dicamba results because the acid form is quite soluble in water (Senseman 2007). Dicamba strongly adsorbs to organic matter, as Donaldson and Foy (1965) showed ten-fold higher ED$_{50}$ values on high organic matter versus sand or sandy loam soils. Grover (1977) found that herbicide mobility is inversely related to the adsorptive values of the soil, as soils higher versus lower in organic matter required more water to leach dicamba through the soil profile.

Registration of dicamba-tolerant soybeans will lead to significant increases in dicamba use in-season for weed management. A key question is whether the persistence of dicamba in soybean production areas can result in meaningful suppression of broadleaf weed emergence or seedling growth. Demonstrated residual activity of dicamba may extend control of broadleaf weeds in soybeans, reducing the need for subsequent postemergence herbicides. However, little research has looked at the duration of control and effects of rainfall on dicamba persistence. Therefore, the objectives of this research were to determine the influence of rainfall on emergence and seedling growth of common waterhemp (Amaranthus rudis Sauer) and morningglory (Ipomoea spp. L.) following dicamba application under greenhouse conditions.

**Materials and Methods**

In the spring of 2016, trials were initiated in a greenhouse at the University of Missouri-Columbia. Field soil (silt loam: 15% sand, 60% silt, 25% clay) was added to 40
by 67 cm plastic containers (Sterilite Corporation Townsend, MA) to a total depth of 15 cm. Soil tests indicated a pH of 7.4 with 1.4 to 1.8% organic matter. In addition, high pressure sodium lamps supplemented sunlight and allowed for a 14 h day, 10 h night. Light levels at the soil surface averaged 300 to 500 µE•s⁻¹•m⁻².

Two PVC rings (20 cm diameter) were placed on the surface of the soil in each container (Figure 3.1) and the soil was moistened with water. Seeds of morningglory (60 seeds per ring) and common waterhemp (200 seeds in trial 1, 250 seeds in trial 2) were added to individual PVC rings. Morningglory seeds were covered with 1 cm of soil to improve germination. For waterhemp, acid washed quartz sand was lightly sprinkled on the soil surface to facilitate soil contact.

Within 24 hours of establishing weed species, chemical treatments were applied (Table 3.1). Treatments consisted of various rates of dicamba as well as a commonly used residual herbicide (flumioxazin). Applications were made with a CO₂ pressurized backpack sprayer equipped with XR8002 TeeJet (TeeJet® Spraying Systems, Wheaton, IL) flat fan nozzle tips calibrated to deliver 140 L ha⁻¹. All soil surfaces in the container were treated and then returned to the greenhouse.

Immediately after herbicide application, simulated rainfall was added to each individual ring. Plastic buckets (25 cm diameter) with 90 to 110 holes (1.6 mm diameter) drilled in the bottom were placed on the rings to allow water to slowly drip onto the soil, simulating a gentle rainfall. Buckets were slowly rotated as water was added to prevent simulated rainfall from dripping repeatedly on the same area. Rainfall amounts of 0, 0.13, 0.51 and 1.02 cm were added to designated PVC rings, with both rings in the same container receiving the same amount of rainfall. Following rainfall, no water was added.
inside the rings for the duration of the study. To ensure continued germination, soil outside of the rings received water throughout the experiment. One liter of water was added to the container as needed to maintain a soil moisture content of approximately 90% of field capacity throughout the experiment.

Emerged waterhemp and morningglory seedlings were recorded beginning at 3 days after treatment (DAT) and repeated every three days until trial termination at 36 DAT. A seedling was considered emerged when the hypocotyl with open cotyledons was observed. To preclude interference of emergence when the soils surface in PVC rings was shaded, emerged seedlings were harvested at soil level at 15 DAT for morningglory and 21 DAT for waterhemp. Emerged seedlings were again harvested at soil level at 33 DAT for morningglory and 36 DAT for waterhemp. Following harvest, seedlings were dried at 50 C for two days and biomass recorded. In addition, visual injury of seedlings in each PVC ring were recorded every 6 DAT. Injury levels ranged from 0-100, with zero indicating no injury and 100 representing complete plant death.

Experimental design was a split plot with five replications and the trial was repeated. The main plot variable was rainfall and the sub-plot variable was herbicide treatment and species. Factors included rainfall and chemical treatment as fixed effects and time and replication as random effects. Data were subjected to an ANOVA using Proc Glimmix in SAS 9.4 (SAS Institute Incorporated, Cary, NC). Dry weights and percent control data couldn’t satisfy the assumptions of ANOVA; data were ranked as outlined by Conover and Iman (1981) and subjected to Proc Glimmix. Emergence data were transformed using a Poisson distribution. Untransformed data are presented for
simplicity, while the ANOVA was based on transformed data. Differences among means were separated using Fisher’s Protected LSD at P=0.05.

Results and Discussion

Waterhemp and morningglory emergence data represent the average of five replications combined over both experiments. Waterhemp emergence data at 9 and 21 DAT were selected for display of results (Figure 3.2). At 9 DAT, emergence of waterhemp reached >90% of maximum; emergence at 21 DAT reflected any change (new emergence or death of existing seedlings) in emergence for waterhemp. Fewer than 3 waterhemp seedlings emerged between 22 and 36 DAT (data not shown). Mean emergence in the untreated controls at 9 DAT was 4.2, 4, 20.8 and 33 common waterhemp plants per ring at 0, 0.13, 0.51 and 1.02 cm of rainfall, respectively (data not shown). This suggests initial rainfall was important for waterhemp emergence. By 21 DAT, emergence in untreated control treatments was relatively unchanged, averaging 4.8, 4.4, 19.8 and 32.4 plants at 0, 0.13, 0.51 and 1.02 cm, respectively.

All treatments at both 9 and 21 DAT reduced waterhemp emergence compared to the untreated control (Figure 3.2A). Flumioxazin, traditionally a residual herbicide with activity on waterhemp, reduced waterhemp emergence from 81 to 100% at 9 DAT; emergence did not occur with 0.51 or 1.02 cm of rainfall. For all rates of dicamba, waterhemp emergence at 21 versus 9 DAT declined between 19.2 and 100%. This reflects injury of emerging waterhemp seedlings, many of which died between 9 and 21 DAT. Higher rates of dicamba resulted in overall greater reductions in waterhemp emergence. Low rates of dicamba (0.14, 0.28 and 0.42 kg ha\(^{-1}\)) reduced emergence 49 to 80% after rainfall of 0, 0.51 or 1.02 cm, while 0.13 cm of rainfall only reduced
emergence 10 to 37% at the same herbicide rates. At higher rates of dicamba (0.56, 0.7 and 0.84 kg ha\(^{-1}\)), emergence was reduced 63 to 92% across all rainfall amounts. Emergence following 1.12 kg ha\(^{-1}\) dicamba was reduced 24 to 90% by 9 DAT, but between 92 and 100% at 21 DAT (Figure 3.2B). For the highest rates of dicamba (0.84 and 1.12 kg ha\(^{-1}\)) waterhemp emergence was comparable to the flumioxazin alone or flumioxazin + dicamba treatment.

Emergence of waterhemp alone was not a clear indicator of the impact of dicamba. Biomass of emerged waterhemp was reduced up to 99% across all dicamba treatments compared to the untreated control (Figure 3.3). Waterhemp treated with 0.072 kg ha\(^{-1}\) flumioxazin and no rainfall exhibited a statistically similar biomass to the untreated control. However, the addition of 0.56 kg ha\(^{-1}\) dicamba resulted in 92 and 95% reductions in biomass compared to flumioxazin alone and the untreated control, respectively, at 0 cm rainfall. Dicamba reduced biomass 72 to 99% per plant across all rainfall amounts, with a rate of 0.56 kg ha\(^{-1}\) reducing biomass 90 to 95% compared to the untreated control. Although dicamba at 0.14 to 0.42 kg ha\(^{-1}\) resulted in inconsistent reductions in waterhemp biomass, results were consistent at rates greater than or equal to 0.56 kg ha\(^{-1}\), and were not impacted by rainfall. From the biomass data, little rain was needed to effect a response to dicamba, and rainfall up to 1.02 cm did not negatively influence the effectiveness of dicamba.

Unlike waterhemp emergence, morningglory emergence was less impacted by the rate of dicamba than the amount of rainfall (Figure 3.4). At 9 DAT, morningglory emergence for the no rainfall treatments was equal to or exceeded emergence of the untreated control, independent of dicamba rate (Figure 3.4A). This likely reflected the
positioning of seed at a depth of 1 cm and minimal contact with dicamba during emergence. At lower rates of dicamba (0.14 to 0.56 kg ha$^{-1}$), increasing rainfall numerically lowered morningglory emergence, but results were only significant with 1.02 cm rainfall. Above 0.56 kg ha$^{-1}$ dicamba, morningglory emergence was lower compared to the untreated control, and was reduced step-wise with increasing rainfall. At 1.02 cm rainfall, dicamba at rates greater than 0.56 kg ha$^{-1}$ reduced emergence up to 84%. By 15 DAT, additional seedlings of morningglory were observed in most treatments lacking rainfall (Figure 3.4B). This resulted in greater separation among no rainfall versus rainfall across all dicamba treatments. Flumioxazin alone had little impact on morningglory emergence at 9 or 15 DAT unless followed by 1.02 cm rainfall (94.1% reduction). The addition of 0.56 kg ha$^{-1}$ dicamba to flumioxazin reduced emergence 33 to 100% compared to each herbicide alone; higher rainfall improved suppression of emergence. Unlike waterhemp, emergence of morningglory was not reduced between 9 and 15 DAT, indicating little if any seedling death from dicamba injury. Additional morningglory emergence occurred between 15 and 33 DAT, with between 1 and 12 seedlings emerging in each plot (data not shown). However, herbicide treatment had little impact on morningglory emergence or plant biomass.

Per plant biomass of morningglory was relatively unaffected by dicamba rate (Figure 3.5). In the absence of herbicide, morningglory biomass was 69 to 122 mg at 15 DAT. At 0 and 0.13 cm rainfall, biomass per plant was minimally impacted by herbicide treatment. Total biomass was reduced 20.1 to 57.1% and 25 to 72.8% after receiving 0.51 and 1.02 cm of rainfall, respectively. The addition of dicamba to flumioxazin reduced
biomass up to 55% compared to flumioxazin alone, demonstrating a benefit of using a combination of these two herbicides.

There is limited research regarding the influence of dicamba as a preemergence herbicide. Myers and Harvey (1993) showed that the addition of 0.56 kg ha\(^{-1}\) dicamba to 2.2 kg ha\(^{-1}\) metolachlor increased visible control of common lambsquarters from 43% to up to 97% at 43 to 44 DAT. Birschbach et al. (1993) reported identical rates of dicamba and metolachlor increased smooth pigweed (Amaranthus hybridus L.) control from 16% with metolachlor alone to 81% versus metolachlor + dicamba. The population of emerged seedlings was reduced by 87.7% when dicamba was applied with metolachlor. This research supports these findings, as the addition of 0.56 kg ha\(^{-1}\) dicamba to 0.072 kg ha\(^{-1}\) flumioxazin reduced waterhemp emergence up to 33% at 21 DAT and morningglory emergence up to 81% at 15 DAT.

Increasing the application rate of dicamba can lead to greater persistence in the soil, causing greater injury to subsequently emerging seedlings. Sheets et al. (1968) reported that the biomass of snap beans planted 16 weeks after dicamba applications of 0.5 ppmw on a sandy loam soil were reduced by 2%, while applications of 2 ppmw reduced biomass 67%. In a clay soil, 0.5 and 2 ppmw dicamba reduced snap bean biomass 1 and 87%, respectively, when planted 16 weeks after application. In Nebraska, applications of 22.4 kg ha\(^{-1}\) dicamba limited soybean yields by up to 74% three years after application while applications of 11.2 kg ha\(^{-1}\) only resulted in reductions of 15% (Burnside et al. 1971). This research showed that, increasing the rate of dicamba from 0.14 to 1.12 kg ha\(^{-1}\) reduced morningglory biomass between 5 and 78% at 15 DAT and reduced waterhemp biomass from 9 to 79% at 21 DAT across all rainfall amounts.
Rainfall can also significantly impact the movement and persistence of dicamba. For morningglory seeds planted 1 cm below the soil surface, rainfall was necessary to expose seeds to dicamba, with biomass reduced up to 65% at 15 DAT when compared to the same treatment receiving no rainfall. The rate of dicamba had a greater influence on waterhemp activity than rainfall. Significant injury to waterhemp resulted at low rates of dicamba (0.14 kg ha\(^{-1}\)) and were not impacted by rainfall. In this study, rates of dicamba greater than 0.84 kg ha\(^{-1}\) resulted in similar impacts to waterhemp emergence as 0.072 kg ha\(^{-1}\) flumioxazin.

Dicamba alone does impact emerging seedlings when applied directly to soil. For a large seeded broadleaf (morningglory), rainfall was necessary to move dicamba through the soil profile where absorption or contact by morningglory was possible. Surprisingly, rainfall did not negatively impact activity on waterhemp emergence. When tank mixed with traditional PRE herbicides such as flumioxazin, dicamba improved control of broadleaf weeds, especially in the absence of rainfall when the PRE herbicide was not activated. Therefore, dicamba should not take the place of conventional herbicides, but should be included in PRE herbicide applications to not only increase herbicide activity, but to provide early season broadleaf weed control in the absence of an activating rainfall.
Literature Cited


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Table 3.1. Herbicide treatments for determining residual activity of morningglory (*Ipomoea* spp.) and common waterhemp (*Amaranthus rudis*). Herbicides were applied February 17 and April 13, 2017 for the initial and repeated experiment.

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<tr>
<td>Dicamba</td>
<td>0.14</td>
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<tr>
<td>Dicamba</td>
<td>0.28</td>
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<tr>
<td>Dicamba</td>
<td>0.42</td>
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<td>0.56</td>
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<td>Dicamba</td>
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</tr>
<tr>
<td>Dicamba</td>
<td>0.84</td>
</tr>
<tr>
<td>Dicamba</td>
<td>1.12</td>
</tr>
<tr>
<td>Flumioxazin</td>
<td>0.072</td>
</tr>
<tr>
<td>Dicamba + Flumioxazin</td>
<td>0.56 + 0.072</td>
</tr>
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</table>
Figure 3.1. Representative arrangement of PVC rings (20.3 x 7.6 cm) in 40 x 67 cm plot area. Prior to application of herbicides and rainfall, seeds of morningglory were added to the soil and incorporated up to 1 cm; common waterhemp seeds were added to the surface and lightly covered with quartz sand.
Figure 3.2. Emergence of waterhemp plants calculated from 10 observations at 9 (A) and 21 (B) DAT as a percentage of the emerged seedlings in the untreated control. Untreated control emergence at 9 DAT averaged 4.2, 4, 20.8 and 33 for 0, 0.13, 0.51 and 1.02 cm of rainfall, respectively. Untreated control emergence at 21 DAT averaged 4.8, 4.4, 19.8 and 32.4 for 0, 0.13, 0.51 and 1.02 cm of rainfall, respectively. Means followed by the same
letter within each rainfall amount are not significantly different using Fisher’s Protected LSD at $P=0.05$. Abbreviations: Dic, dicamba; Flu, flumioxazin.
Figure 3.3. Mean biomass per waterhemp plant at 21 DAT (average of 10 observations). A page break is shown between 11 and 20 mg to separate biomass between treatments. Means followed by the same letter within each rainfall amount are not significantly different using Fisher’s Protected LSD at P=0.05. Abbreviations: Dic, dicamba; Flu, flumioxazin.
Figure 3.4. Emergence of morningglory plants calculated from 10 observations at 9 (A) and 15 (B) DAT as a percentage of the emerged seedlings in the untreated control. Untreated control emergence at 9 DAT averaged 1.4, 3, 1.8, and 3.4 for 0, 0.13, 0.51 and 1.02 cm of rainfall, respectively. Untreated control emergence at 15 DAT averaged 1.4, 3.6, 2, and 4.2 for 0, 0.13, 0.51 and 1.02 cm of rainfall, respectively. Means followed by
the same letter within each rainfall amount are not significantly different using Fisher’s Protected LSD at P=0.05. Abbreviations: Dic, dicamba; flu, Flumioxazin.
Figure 3.5. Average biomass of emerged morningglory plants from 10 observations harvested 15 DAT. Means followed by the same letter within each rainfall amount are not significantly different using Fisher’s Protected LSD at P=0.05. Abbreviations: Dic, dicamba; Flu, flumioxazin.