DICAMBA VOLATILIZATION AND THE EFFECT OF SYNTHETIC AUXIN HERBICIDES ON SENSITIVE SOYBEAN

by

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A Thesis

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Master of Science

Department of Botany and Plant Pathology

West Lafayette, Indiana

May 2020

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ACKNOWLEDGMENTS

First, I would like to thank my advisor, Dr. Bryan Young, for his guidance and support throughout my time at Purdue. I would also like to thank my committee of Drs. Bill Johnson, Josh Widhalm, and Bryan Young for their support and involvement with my research. This research would not have been possible without the continued support of past and present graduate students in the Young and Johnson Labs, whom I thank greatly. I would also like to thank Julie Young, who was critical in the accomplishment of this research. I also appreciate efforts of Drs. Josh Widhalm and Manoj Ghaste in accomplishing the sample analysis needed in such a timely manner. I am also appreciative of the United Soybean Board and the Indiana Crop Improvement Association for providing funding and services necessary to complete this research. Lastly, I would like to thank my family, who have provided unwavering support throughout my education.

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ABSTRACT

The broad adoption of soybean resistant to synthetic auxin herbicides has led to increased risk for off-target exposure of sensitive plants to these herbicides through both tank-contamination and drift. New dicamba and 2,4-D formulations have been commercialized in an attempt to reduce the potential for off-target movement. A great deal of research has been conducted on soybean response to auxin herbicides alone, but when other postemergence herbicides are introduced into the equation, the effects of 2,4-D and dicamba have not been sufficiently studied. Additionally, the volatilization of dicamba formulations available prior to the registration of new formulations for use in dicamba-resistant soybean has been characterized in order to determine factors that influence off-target movement. However, the volatilization of these new formulations has not been extensively investigated.

Field experiments were conducted to determine 1) the response of glyphosate-resistant soybean to dicamba and 2,4-D, and 2) the influence of a full rate of dicamba applied with tank-contamination doses of 2,4-D on the response of dicamba/glyphosate-resistant soybean. Glyphosate-resistant soybean response to 2,4-D and dicamba were similar to published literature. The ED_{10} values for injury 14 days after the V2 and R1 exposure timings were 0.03 and 0.18 g ae ha^{-1}, respectively, for dicamba and 35 and 31 g ae ha^{-1}, respectively for 2,4-D. For soybean grain yield, ED_{10} values for dicamba were 4.61 and 1.66 g ha^{-1} at the V2 and R1 timings, respectively, and 34 g ha^{-1} for 2,4-D, combined across exposure timings. Additionally, dicamba/glyphosate-resistant soybean yield response to 2,4-D (ED_{10} = 34 g ha^{-1}) was similar to glyphosate-resistant soybean (ED_{10} = 34 g ha^{-1}) and the addition of a full rate of dicamba to 2,4-D tank-contamination did not increase soybean yield response to 2,4-D (ED_{10} = 59 g ha^{-1}). Thus, no interaction of 2,4-D

plus dicamba was apparent on the dicamba/glyphosate-resistant soybean and practices to mitigate exposure to 2,4-D on these soybeans would be no different than other sensitive varieties.

An experiment was conducted to evaluate soybean response to dicamba in conjunction with other labeled postemergence herbicides that are known to cause soybean injury, such as lactofen, acetochlor, and 2,4-DB. Soybean injury at 28 days after the R1 application was influenced primarily by the timing of dicamba exposure rather than the labeled POST herbicide, with up to 14% injury from POST herbicides alone and up to 37% injury from dicamba exposure at a dose of 5.6 g ha^{-1} alone at R1. Soybean yield reduction in response to dicamba alone was greater during the 2017 growing season with a 37% reduction in yield from dicamba exposure at R1 in 2017 compared with a 17% reduction in 2018. Regardless of the difference in yield response between years, the primary factor that influenced yield was the timing of dicamba exposure. In general, glyphosate-resistant soybean response to a reduced rate of dicamba was not influenced by additional postemergence herbicides applied at either the V3 or R1 growth stage.

Controlled environment experiments were conducted to evaluate the relative volatilization of dicamba formulations applied with drift reduction agents, turbid water carrier, ions in spray solution, and a spray solution pH range. Drift reduction agents and turbid water carrier did not affect dicamba volatilization. Spray pH levels of 4 and above did not result in increased levels of volatilization, while a spray pH of 3 increased volatilization by 2.8X and 3.9X for the DGA + VG and BAPMA formulations, respectively, compared with each respective dicamba formulation applied alone at a native pH of 5.4 and 6.4, respectively. Of the ions tested, diammonium and ferrous sulfate increased dicamba volatilization by 5X and 9X for the DGA + VG formulation, respectively, and 11X for the BAPMA formulation compared with dicamba alone. Additionally, the sulfate and chloride anions present in other ions tested did not cause an increase in volatilization.

These results indicate the importance of spray application parameters and the continual attention to details such as tank-mix pH and carrier water ion content that must be practiced prior to an application of a synthetic auxin herbicide to avoid off-target movement.

CHAPTER 1. LITERATURE REVIEW

1.1 Off-Target Movement of Herbicides

The movement of herbicides to non-target areas has been studied for many years and the need for further research persists today. Sources for off-target herbicide movement include primary drift, secondary drift, and sprayer contamination. Primary drift is the physical movement of spray droplets away from the target area at the time of a chemical application (Maybank et al. 1978). Primary drift can be influenced by many factors such as wind velocity, droplet size, application speed, spray nozzle height, relative humidity, and spray carrier volume (Hill 1976). Primary drift often results in a gradient of off-target movement with higher concentrations of herbicide adjacent to the application site and decreasing concentrations as distance from the application site increases (Maybank et al. 1978).

Secondary drift occurs when herbicide is deposited into the target area, but is not retained within the boundaries of this area (Combellack 1982). In reference to herbicide off-target movement research, secondary drift is defined as the off-target movement of herbicide that occurs after a period of 15 to 30 min following the application, which is considered a reasonable amount of time to allow for spray droplets to settle (Farrell et al. 2017, Mueller et al. 2013). One cause of secondary drift is herbicide volatilization. Volatilization is the process through which a compound transitions into the gaseous phase (Bedos et al. 2002). Herbicide volatilization can be affected by many factors, such as ambient air temperature, the rate of herbicide vapor movement away from the treated surface, and the vapor pressure of the herbicide (Spencer et al. 1973).

Sprayer contamination occurs as a result of inadequate sprayer and/or mix system cleanout (Bretthauer 2006). Modern field sprayers currently used in agricultural production have complex plumbing systems that require a great deal of attention to detail during the cleanout process

(Whitford et al. 2015). Sprayer contamination can result in a variety of injury patterns ranging from small, isolated areas to entire fields. Although improper sprayer cleanout is often the cause of sprayer contamination, there are sources upstream in the pesticide mixing and loading process that can result in contamination such as mixing equipment, transport tanks, and transfer hoses (Whitford et al. 2015). Many of the potential risks associated with herbicide drift and sprayer contamination can be avoided though the use of proper application methods and adherence to a rigorous cleaning procedure.

1.2 Adoption of Auxin-resistant Soybean Technology

Auxin-resistant soybean are soybean which are genetically-engineered to be resistant to either the synthetic auxin herbicide 2,4-D or dicamba (Behrens et al. 2007, Wright et al. 2010). The introduction of auxin-resistant soybean varieties and the labeling of dicamba and 2,4-D for postemergence applications to soybean has resulted in a change in the use pattern of auxin herbicides. Prior to the adoption of auxin-resistant soybean, dicamba and 2,4-D were used primarily for selective control of broadleaf weeds in cereal grains, corn, and pastures (Mortensen et al. 2012). The growing season of 2017 was the first year when dicamba-resistant soybean was available along with dicamba herbicides labeled for postemergence use. During that year, 8 million of the 36 million ha of soybean grown in the United States were dicamba-resistant, indicating a rapid adoption of this technology by soybean producers (Lingenfelter 2017). In addition to dicamba-resistant soybean, 2,4-D-resistant soybean were made widely available for commercial planting for the first time during the 2019 growing season. This technology provides growers with another auxin herbicide option for postemergence control of broadleaf weeds in soybean. Both dicamba- and 2,4-D-resistant soybean were available for commercial planting in 2019.

Since the introduction of dicamba-resistant soybean, only two formulations of dicamba have been approved for postemergence use. These formulations are the diglycolamine (DGA) salt with VaporGrip® and the N,N-Bis-(3-aminopropyl) methylamine salt (BAPMA) (Anonymous 2019a, 2019b). The labels of these herbicides restrict application methods, such as observing nontreated border areas between application areas and sensitive crops, wind speed limits, and list environmental conditions unsuitable for application (Anonymous 2019a, 2019b). The dicamba herbicide labels for 2017 and 2018 permitted the application of dicamba to dicamba-resistant soybean up to, and including, the R1 growth stage (Anonymous 2018a, 2018b). Applications of 2,4-D in 2,4-D-resistant soybean can be performed up to, and including the R2 growth stage (Anonymous 2019c, 2019d). Since soybean planting dates vary widely throughout the soybean-producing regions of the United States, there exists a lengthy period, with a wide range of environmental conditions, when auxin herbicides may be applied (Egan et al. 2014). Soybean growers have expressed concern over the potential for off-target movement of these herbicides to sensitive crops. One especially sensitive crop that is grown in very close proximity to auxin-resistant soybean is auxin-sensitive soybean, which includes conventional, glyphosate-resistant, and glufosinate-resistant soybean. Auxin-sensitive soybean can demonstrate herbicide injury symptoms at very low doses of 2,4-D and dicamba (Robinson et al. 2013a, Weidenhamer et al. 1989).

1.3 Soybean Response to Auxin Herbicide Exposure

Symptoms of low-dose dicamba exposure in soybean are often described as leaf cupping and crinkling, while higher doses may cause death of the apical meristem (Behrens and Lueschen 1979, Zimmer et al. 2019). The dicamba doses necessary to cause 10% visual injury 14 days after treatment (DAT) on vegetative and reproductive soybean were 0.203 and 0.285 g ae ha^{-1}, respectively (Robinson et al. 2013b). Visual injury symptoms at 14 DAT up to 21% occurred following dicamba exposure at the V3 growth stage at a dose of 0.028 g ae ha^{-1} (Solomon and Bradley 2014). A recent meta-analysis of soybean response to dicamba by Kniss (2018) reported the dose necessary to cause 5% foliar injury symptoms pooled across growth stages ranged from 0.038 to 0.046 g ae ha^{-1} across various rating times. The wide range of soybean foliar injury responses to similar doses of dicamba indicates that soybean foliar injury can vary significantly depending on several factors such as environmental conditions around exposure, growth stage at the time of exposure, and soybean variety evaluated.

In addition to foliar injury symptoms, soybean height can be another indicator of exposure to dicamba. Soybean height at maturity was reduced 28 and 44% following an R1 exposure of dicamba at doses of 17.5 and 70 g ae ha^{-1}, respectively (Griffin et al. 2013). Mature soybean height when exposed to a dose of 16 g ae ha^{-1} at the mid-bloom growth stage was reduced 25% (Weidenhamer et al. 1989). The final height of soybean treated with 5.6 g ae ha^{-1} at the V3, V7, and R2 growth stage was reduced by 23, 35, and 19%, respectively (Kelley et al. 2005). There is a high correlation of soybean height with grain yield, but predicting yield loss from height reduction may be difficult, as the timing of soybean exposure and varietal differences may cause widely variable responses (Weidenhamer et al. 1989). Thus, soybean height may be a valuable tool in assessing soybean response to dicamba, but the relationship between dicamba dose and height depends on the growth stage, as soybean height may be reduced to a greater extent if exposure occurs during vegetative growth compared with reproductive growth.

From a soybean grower's perspective, the most important soybean response to dicamba exposure is the potential loss of grain yield. Soybean grain yield was reduced 0.5% when treated with 56 g ae ha^{-1} at the V1 to V2 growth stage (Auch and Arnold 1978). The same rate of dicamba caused a 45% reduction in yield when applied at the V2 to V3 growth stage (Al-Khatib and Peterson 1999). The dicamba dose necessary to cause 10% soybean grain yield reduction was 15 and 1.3 g ae ha^{-1} in 1980 and 1981, respectively (Weidenhamer et al. 1989). This 11.5-fold difference was attributed to dry conditions in 1981 that resulted in a 240 mm difference in rainfall during June through August between the two years, and demonstrates the impact that environmental conditions can have on soybean response to dicamba exposure. Kniss (2018) summarized that the average doses necessary to cause 5% yield reduction were 1.9, 5.7, and 0.89 g ae ha^{-1} at early vegetative (V1 to V3), late vegetative (V4 to V7), and reproductive (R1 to R2) growth stages, respectively. Although yield reductions of less than 5% may be economically significant to a soybean grower, a more reliable dose estimate can be determined at the 5% response level, as opposed to lower levels of yield reduction where estimates may have a higher degree of uncertainty (Kniss 2018). When exposure occurs during vegetative growth, yield loss can be difficult to predict, since favorable growing conditions may increase the ability of the soybean to mitigate yield loss and adverse growing conditions may exacerbate the effects of the dicamba (Kniss 2018). As the growing season progresses, soybean have less time to recover from dicamba exposure, thus, increasing the likelihood of reduced yield.

In addition to dicamba exposure alone, another factor that may affect soybean response to dicamba is the addition of labeled postemergence herbicides. Applications of dicamba to dicamba-resistant soybean often contain other herbicides, such as glyphosate, to broaden the spectrum of weed control. Improper tank cleanout may also result in the presence of dicamba in spray tanks when applicators switch from applying dicamba to other postemergence herbicides. Increased soybean injury occurred when glyphosate-resistant soybean at the V3 or V7 growth stage were treated with imazethapyr, imazamox, or fomesafen at a labeled field uses rate in combination with

5.6 g ae ha^{-1} of dicamba as a tank contaminant, compared with treatments where only dicamba was applied (Kelley et al. 2005). Additionally, soybean grain yield reduction was observed when imazethapyr, imazamox, or fomesafen were applied at V7 in combination with dicamba, compared with the dicamba-only treatments. These findings indicate that herbicides which are labeled for use across soybean with various herbicide-resistance traits may influence the extent of soybean response to dicamba when dicamba tank-contamination occurs.

Another potential interaction between labeled soybean herbicides and sensitive soybean exposure to dicamba is a result of the time it takes for symptoms to develop following a dicamba exposure event. When dicamba-sensitive soybean are exposed to dicamba, symptoms may not be evident immediately following exposure. During this time, labeled herbicide applications may be made to soybean that have been exposed to dicamba, but have not yet begun to show symptoms. Soybean injury was increased and grain yield was reduced from a simulated dicamba/diflufenzopyr drift event followed by a chlorimuron-ethyl application two to four days later compared with dicamba/diflufenzopyr drift alone (Brown et al. 2009). The issue of off-target dicamba movement that dicamba-sensitive soybean growers face may be exacerbated as the labeled postemergence herbicides that they choose to apply may result in additional yield reduction compared with off-target dicamba exposure alone. As such, additional research examining interactions between dicamba and other herbicides labeled for use in soybean, aside from those already investigated in the available literature, on dicamba-sensitive soybean is of interest.

Like dicamba, injury from soybean exposure to 2,4-D can also occur at very low doses compared with field use rates and responses can vary widely. Soybean injury from 2,4-D is described as leaf strapping and leaf/petiole twisting or bending (Zimmer et al. 2019). Petiole bending as a result of 2,4-D exposure can be apparent immediately following exposure (Wax et al.

1969). The 2,4-D doses necessary to cause 10% soybean injury 14 DAT were 30, 8, and 33 g ae ha^{-1} at the V2, V5, and R2 growth stages, respectively (Robinson et al. 2013a). Soybean exposure to 2,4-D at a dose of 56 g ae ha^{-1} caused 8, 22, and 19% soybean injury when exposure occurred at V3, V7, and R2, respectively (Kelley et al. 2005). Applications of 11.2, 56, or 112 g ae ha^{-1} of 2,4-D applied at the V3 growth stage resulted in 5, 25, or 35% visual crop response at 12 DAT, respectively (Andersen et al. 2004).

In regard to soybean height following exposure to 2,4-D, final soybean height was reduced 18, 25, and 21% following an application of 180 g ae ha^{-1} of 2,4-D at the V3, V7, and R2 growth stages, respectively (Kelley et al. 2005). Soybean height was not reduced following an application of 28 g ae ha^{-1} at the V3 or R2 growth stage 28 DAT (Solomon and Bradley 2014). The doses necessary to cause 5% height reduction were 97, 33, and 40 g ae ha^{-1} at the V2, V5, and R2 growth stages, respectively (Robinson et al. 2013a). Soybean grain yield from the same experiment was reduced 0, 7.2, and 31.7% for the 11.2, 56, and 112 g ae ha^{-1} rates, respectively. At both the V2 and R2 treatment timings, the 2,4-D dose necessary to cause 5 and 10% yield reduction was 116 and 202 g ae ha^{-1}, respectively (Robinson et al. 2013a). The above-listed results confirm that significantly higher doses of 2,4-D are necessary to cause similar soybean response compared with dicamba. Like dicamba, the adoption of 2,4-D-resistant soybean will increase the overall use of postemergence 2,4-D in soybean and increase the potential for off-target movement to sensitive soybean.

Since both 2,4-D- and dicamba-resistant soybean were commercially available in 2019, the potential exists for sprayers to become contaminated when switching between these herbicides. Some soybean growers have the misconception that dicamba- and 2,4-D-resistant soybean are resistant to both 2,4-D and dicamba, but this is not the case. Dicamba resistance in dicamba-resistant soybean is conferred by the expression of dicamba monooxygenase (DMO) in the chloroplast (Behrens et al. 2007). The DMO gene encodes a Reiske nonheme oxygenase that catalyzes the oxidative demethylation of dicamba to 3,6-dichlorosalicylic acid (Dumitru et al. 2009). DMO was first identified in *Pseudomonas maltophilia* DI-6 as a part of a three-component enzyme system (Wang et al. 1997). This system requires a ferredoxin which is similar to the ferredoxin found in plant chloroplasts, thus expression of the DMO gene in the chloroplasts results in a transgenic plant capable of rapidly detoxifying dicamba (Behrens et al. 2007). The mechanism for resistance in 2,4-D-resistant soybean is conferred by the constitutive expression of aryloxyalkanoate dioxygenase-12 (AAD-12). The AAD-12 enzyme is responsible for the cleavage of 2,4-D into dichlorophenol and glyoxylate, thus effectively metabolizing 2,4-D into non-herbicidal compounds (Wright et al. 2010). The expression of AAD-12 or DMO in soybean result in robust mechanisms of resistance to 2,4-D or dicamba, respectively. The substrate specificity of these enzymes results in a herbicide resistance mechanism that is unique for each. Thus, dicamba-resistant soybean technology should not impart any differential response to 2,4-D and vice versa.

1.4 Dicamba Volatilization

Herbicides can volatilize and move away from the intended target area, resulting in a herbicide concentration in the air that is high enough to cause injury to sensitive plants (Strachan et al. 2013). Potential for volatilization is determined by the chemical vapor pressure, which is a measure of the tendency of a liquid or solid to transition into a gas (Spencer et al. 1973). A higher vapor pressure indicates that a compound has a greater likelihood to volatilize. The vapor pressure for dicamba acid is 3.4×10^{-5} mm Hg compared with a relatively non-volatile herbicide such as glyphosate with a vapor pressure of 1.8×10^{-10} mm Hg (Shaner 2014). Vapor pressure is not the only determinant of herbicide volatilization, which can be affected by targeted surface properties, environmental conditions, and other characteristics of the herbicide (Spencer et al. 1973). Dicamba is a weak acid, and multiple formulated products utilize different salts to act as counter ions (conjugate bases). Dicamba acid is significantly more volatile than dicamba products that are formulated as salts (Behrens and Lueschen 1979). When dicamba is in the acid form, the molecule is non-polar, rendering it more hydrophobic than the dicamba ion. Dicamba formulations utilizing salts have been developed to mitigate the risk of off-target movement by decreasing the potential for volatilization by favoring the association of dicamba with the conjugate base in the salt formulation over forming the free acid of dicamba (MacInnes 2017). Although salt formulations of dicamba may have reduced volatilization potential compared with dicamba acid, different salt formulations have not resulted in a dicamba product that is non-volatile (Behrens and Lueschen 1979, Egan and Mortensen 2012, Sciumbato et al. 2004).

Two principle methods for quantifying herbicide volatilization have been implemented for auxin herbicides: controlled environment and field studies (Mueller 2015). Research on volatilization often begins in a controlled environment due to lower costs and faster turnaround times compared with field studies. Upon completion of controlled environment studies, results can then be used to select parameters to test in field studies. As studies move from controlled environment settings to the field, the ability to maintain precise control of the environment decreases, making the results of field studies more complicated to interpret. However, controlled environment studies cannot always accurately predict how a herbicide will behave in a field setting; therefore, field trials have value since they simulate realistic environmental conditions (Mueller 2015). Air sampling and bio-indicator plants can be used in both types of studies to provide a greater understanding of herbicide volatilization. Air sampling consists of the use of a filtration media that traps airborne herbicide molecules. The herbicide is then extracted from the media and analyzed using chromatography in order to quantify the air concentration of the herbicide that volatilized during the sampling period (Gavlick et al. 2016). Bio-indicator plants can be placed in a closed system with a herbicide-treated surface, or placed in/around a field trial at different strategic positions to determine movement direction, distance, and relative amount of volatilization (Behrens and Lueschen 1979, Sciumbato et al. 2004). The utility of bio-indicator plants in volatilization research is that sensitive plants can respond to very low rates of volatilization and are easier to use since they do not require additional equipment in order to obtain results (Mueller 2015).

1.5 Factors Affecting Dicamba Volatilization

Numerous studies have documented the potential for dicamba to volatilize (Behrens and Lueschen 1979, Burnside and Lavy 1966, Egan and Mortensen 2012, Farrell et al. 2017, Henry and Smeda 2018, Mueller et al. 2013, Mueller and Senseman 2015, Mueller and Steckel 2018, Oseland et al. 2018, Sciumbato et al. 2004, Strachan et al. 2010, 2013). The off-target movement of dicamba has resulted in a large number of complaints to state regulatory agencies by dicamba-sensitive crop growers during the 2017 and 2018 growing seasons (Bradley 2018). Many of these complaints have been attributed to applications made in violation of current label requirements, but there are still complaints for which a definitive cause has not yet been identified. When sensitive soybean are exposed to off-target dicamba movement, small yield losses could result from exposure at a dose as low as 0.56 g ae ha^{-1} (Egan et al. 2014). This dicamba dose has been documented to move off-target through volatilization and subsequent re-deposition when the dimethylamine salt of dicamba was applied to glyphosate-resistant soybean and quantified using indicator plants (Egan and Mortensen 2012).

One factor that has been identified as influencing dicamba volatilization is pH. The relative level of dicamba volatilization decreases as pH increases when applied to glass surfaces (Behrens and Lueschen 1979). However, this effect was not observed in the field when corn leaves were sprayed (Behrens and Lueschen 1979). The herbicide labels for dicamba products intended for use in dicamba-resistant soybean indicate that spray tank pH levels below 5 may increase the potential for dicamba volatilization and should be avoided (Anonymous 2019a, 2019b). In addition to spray solution pH, dicamba volatilization can be affected by the pH of soil that it is applied to. As soil pH decreased from 8.3 to 4.3, dicamba volatilization increased (Oseland et al. 2018). The aforementioned information indicates that both the pH of the treated soil surface and spray carrier solution can have an impact on dicamba volatilization.

Water is a common carrier used to apply herbicides in the United States (Devkota et al. 2016). Carrier water may be sourced from above-ground or below-ground sources. Different water sources may contain varying levels of dissolved cations such as calcium and magnesium as well as suspended soil particles. In Indiana ground water, the following hard water cations have been identified: calcium, magnesium, sodium, potassium, iron, manganese, and aluminum (IDNR 1999). The negative effect of hard water on weak acid herbicide efficacy in foliar applications has been documented by numerous studies (Buhler and Burnside 1983, Devkota et al. 2016, Zollinger et al. 2010). The efficacy of glufosinate, a weak acid herbicide, was negatively influenced by increasing water hardness levels when applied to giant ragweed (*Ambrosia trifida*) (Devkota and Johnson 2016). Calcium and magnesium found in hard water are capable of interacting with the carboxyl functional group of another weak acid herbicide, glyphosate, resulting in the formation of glyphosate salts that are less readily absorbed into plant tissue (Thelen et al. 1995). The potential for a similar interaction between hard water cations exists with dicamba. If dicamba and cations associate in solution, these complexes may not be as readily absorbed as the formulated dicamba product. If dicamba is not absorbed, it will remain on the leaf surface for a longer time, increasing the potential for volatilization.

Another water quality factor that can influence herbicide performance is the presence of organic matter or soil in the spray solution (Chahal et al. 2012). Herbicides may become adsorbed to soil particles in water and form complexes that reduce the availability of the herbicide to plants (Oschwald 1972). The soil adsorption coefficient (K_d) of dicamba can range from 0.01 to 0.40 L kg^{-1} depending on the soil type and soil water content (Ochsner et al. 2006). The average organic carbon-water partition coefficient (K_{oc}) of dicamba is 2 mL g^{-1}, indicating that dicamba is weakly adsorbed to soil (Shaner 2014). Although the sorption coefficients of dicamba are known, the interaction of soil suspended in spray carrier solution with the conjugate bases that form the counter ion in dicamba formulations is unknown. Since many negative charges are present on clay particles and organic matter found in soil, the potential exists for an interaction between the conjugate bases and these negatively charged particles that may alter the properties of herbicide solutions. If the conjugate base is bound to the soil particles and unavailable to bind to the herbicide, then the solution pH will influence the formation of the dicamba acid, which is substantially more volatile than formulated dicamba products. Although many studies have documented the effect of water quality on herbicide efficacy, information about the effect of water quality on herbicide volatilization is not readily available.

Another potential factor that may affect dicamba volatilization is the addition of a drift reduction additive (DRA) that is added to the spray solution prior to application. A DRA is a spray solution additive that is intended to reduce the potential for physical spray drift by reducing the amount of fine spray droplets with a diameter of less than 150 μm, which are more susceptible to drift (Bouse et al. 1988). A DRA is required by the label when certain tank-mix partners, especially glyphosate formulations, are included in dicamba applications made to dicamba-resistant soybean. Applicators of dicamba to dicamba-resistant soybean are required to check a supplemental online label to determine which herbicide tank-mix partners require the use of a DRA, and which DRAs are acceptable for use. There are three distinct primary functioning agents that comprise the currently approved, commercially-available drift reduction additives: hydroxypropyl guar, polyvinyl polymer, and polyacrylamide. Hydroxypropyl guar and polyvinyl polymer DRAs are available as formulated products with primarily hydroxypropyl guar or polyvinyl polymer, respectively. Polyacrylamide DRAs are available in premixes with alkyl polyglucoside surfactant and other water conditioning agents. Although these DRAs are required when applications of dicamba are made, information about their effect on dicamba volatilization is not available.

1.6 Justification

The adoption of dicamba-resistant soybean technology has resulted in a larger application area and a wider application window during the growing season for dicamba to be applied. During the first years that dicamba was labeled for use in dicamba-resistant soybean, many complaints of off-target dicamba movement were filed. The commercialization of 2,4-D-resistant soybean will result in a similar shift in the use pattern of 2,4-D. The prevalence of the off-target movement of dicamba has resulted in a reality where many soybean growers must react to dicamba-affected soybean on a yearly basis. Information about sensitive soybean response to these auxin herbicides is needed to provide soybean growers with a knowledge base that will allow them to maintain their profitability when off-target movement occurs. Additionally, the number of cases of off-target movement warrant further research into application factors that can reduce the potential for dicamba volatilization. Therefore, the objectives of this research were to determine the effect of application factors on dicamba volatilization and how soybean growers can maintain profitability when off-target movement does occur. The specific research objectives were:

1. To determine the effect of 2,4-D tank-contamination on dicamba-resistant soybean injury, growth parameters, and yield.
2. To determine the effect of dicamba exposure on soybean in conjunction with labeled postemergence herbicides regarding injury, growth parameters, and yield.
3. To determine the effect of application factors on dicamba air concentration in a controlled environment.

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CHAPTER 2. DICAMBA-RESISTANT SOYBEAN RESPONSE TO 2,4-D TANK CONTAMINATION DURING A DICAMBA APPLICATION

2.1 Abstract

The commercialization of soybean resistant to synthetic auxin herbicides has increased the likelihood of sensitive soybean exposure to these herbicides. With various herbicide-resistant soybean traits available, the spectrum of soybean herbicides has also increased, resulting in an increased importance of sprayer cleanout when switching between soybean technologies and associated herbicides that are enabled, especially the auxin herbicides. The combined effects of 2,4-D tank-contamination and a labeled dicamba application on dicamba/glyphosate-resistant soybean is unclear. Field experiments were conducted in 2016 and 2017 to determine 1) the response of glyphosate-resistant soybean to dicamba and 2,4-D, and 2) the influence of a full rate of dicamba applied with tank-contamination doses of 2,4-D on the response of dicamba/glyphosate-resistant soybean. Herbicides were applied to soybean at the V2 or R1 growth stage. Dicamba/glyphosate-resistant soybean sensitivity to 2,4-D was similar to glyphosate-resistant soybean, with ED_{10} values all within the 30 to 35 g ae ha^{-1} range for soybean injury 14 DAT, across the V2 and R1 growth stages. Yield reduction was also similar between soybean types, with an ED_{10} value of 34 g ha^{-1} of 2,4-D pooled across the V2 and R1 exposure timings for both types. Dicamba/glyphosate-resistant soybean injury was increased across the dose range when 2,4-D was applied alone, compared with 2,4-D with a full rate of dicamba and glyphosate. Aside from visual injury, the response of dicamba/glyphosate-resistant soybean to simulated 2,4-D tank-contamination in any other data parameters collected was not influenced by the presence of a full rate of dicamba. Additionally, simulated 2,4-D tank-contamination did not affect dicamba/glyphosate-resistant soybean seedling progeny grown from parent plants in this study in

commercial seed testing or greenhouse assays. These results indicate that the resistance-mechanisms in dicamba/glyphosate-resistant soybean to dicamba and glyphosate are not compromised by accidental exposure to 2,4-D in the form of tank-contamination and that no interaction was evident between 2,4-D tank-contamination rates and dicamba.

2.2 Introduction

When soybean plants are exposed to 2,4-D, the extent of vegetative malformation and yield reduction depends on the dose and growth stage at the time of exposure (Kelley et al. 2005). The commercialization of 2,4-D-resistant crops including corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), and soybean has enabled the use of 2,4-D in these cropping systems. Likewise, applications of dicamba to dicamba-resistant soybean is labeled up to the R1 growth stage (Anonymous 2019a, 2019b). These application windows allow for dicamba and 2,4-D applications to resistant crops throughout the entire duration of vegetative growth stages, at which time auxin-sensitive soybean may be growing at a similar growth stage. As a result, auxin-sensitive soybean are currently more likely to be exposed to the off-target movement from commercial applications of 2,4-D and dicamba.

Off-target herbicide movement can result from both drift and sprayer contamination (Maybank et al. 1978). Sprayer contamination is the outcome of an inadequate sprayer and/or mix system cleanout (Bretthauer 2006). Modern field sprayers currently used in agricultural production have complex plumbing systems that require a great deal of attention to detail during the cleanout process (Whitford et al. 2015). With a an increasingly diverse assortment of soybean herbicide options, the possibility for spray tank-contamination continues to rise. For example, when rinsate samples were collected from commercial applicators throughout the cleanout process following

2,4-D application, an average of 1% of the initial 2,4-D concentration remained in the spray tank after the third rinse (Osborne et al. 2015).

Injury from soybean exposure to 2,4-D is described as leaf strapping and leaf/petiole twisting or bending (Zimmer et al. 2019). In addition to soybean injury, plant height and grain yield reductions can also result from unintended exposure to 2,4-D. Exposure at 1% of the field use rate of 2,4-D resulted in visual injury of 5%, although this dose did not cause yield reduction (Andersen et al. 2004). While no yield reduction was observed at this low dose, this level of herbicide contamination can occur even when triple-rinse procedures are followed while cleaning a field sprayer. Increased doses of 2,4-D exposure that could occur as a result of drift or tank-contamination can result in economically important grain yield reductions of 5 and 10% when soybean are exposed to 116 and 202 g ha^{-1}, respectively (Robinson et al. 2013a). In addition to herbicide dose, the timing of exposure can have an effect on the yield reduction resulting from soybean exposure to 2,4-D. A meta-analysis by Egan et al. (2014) reported that the 2,4-D dose range necessary to cause 50% grain yield reduction at vegetative (emergence to flowering) or reproductive (flowering to maturity) growth stages were 651 or 461 g ha^{-1}, respectively.

Soybean injury from unintended dicamba exposure at low doses can be described as leaf cupping and crinkling, while higher dose exposure may cause death at the apical meristem (Behrens and Lueschen 1979, Zimmer et al. 2019). In addition to foliar injury symptoms, height reduction and yield reduction can occur as a result of unintended dicamba exposure. Soybean height reduction in response to dicamba exposure at a dose of 5.6 g ae ha^{-1} (1/100X of a field use rate of dicamba) resulted in height reductions of 23, 35, and 19% at the V3, V7, and R2 growth stages, respectively (Robinson et al. 2013b). Likewise, grain yield reductions following dicamba exposure can vary by growth stage at the time of exposure. Kniss (2018) reported that the doses necessary to cause 5% yield reduction were 1.9, 5.7, and 0.89 g ha^{-1} at early vegetative (V1-V3, late vegetative (V4 to V7), and reproductive (R1 to R2) growth stages, respectively.

In addition to growth stage, environmental conditions surrounding the time of exposure have been documented to contribute to overall crop response to auxin herbicides (Egan et al. 2014). Kniss (2018) reported similar results when soybean is exposed to dicamba, with growth stage at the time of exposure and environmental conditions resulting in variable soybean responses to dicamba. Thus, soybean response to auxinic herbicides under specific environmental conditions of an isolated field experiment may have limited value when a larger inference is desired.

Although soybean response to 2,4-D has been evaluated, little information is available regarding the response of dicamba-resistant soybean to 2,4-D tank-contamination in combination with other postemergence herbicides. The potential for a synergistic interaction of a labeled postemergence herbicide and tank-contamination with another herbicide has been documented. Kelley et al. (2005) reported a synergistic injury response when an application of glyphosate was made to glyphosate-resistant soybean with dicamba as a tank contaminant.

Dicamba resistance in dicamba-resistant soybean is conferred by the expression of dicamba monooxygenase (DMO) in the chloroplast (Behrens et al. 2007). This enzyme system requires the ferredoxin found in plant chloroplasts, thus expression of the DMO gene in the chloroplasts results in a transgenic plant capable of rapidly detoxifying dicamba (Behrens et al. 2007). The expression of DMO in soybean results in a robust mechanism of resistance to dicamba. The substrate specificity of this enzyme system results in a herbicide resistance mechanism that is unique to dicamba. Thus, dicamba-resistant soybean technology should not impart any differential response to 2,4-D.

With the increase in the adoption of soybean resistant to dicamba and 2,4-D, the potential for tank-contamination of these herbicides has also been increased. Labeled herbicides in combination with tank-contamination from auxin herbicides can result in increased crop response. Although other tank contaminant interactions have been evaluated on soybean, the interaction of 2,4-D tank-contamination during a labeled application of dicamba and glyphosate to dicamba-resistant soybean has not been documented. The combined activity of 2,4-D and potentially unmetabolized dicamba may have an increased influence on auxin reception which could induce a plant response beyond 2,4-D alone.

Therefore, the objectives of this research were to evaluate the effect of tank-contamination doses of 2,4-D or dicamba on glyphosate-resistant soybean injury, yield components, total grain yield, and seedling vigor and germination and to characterize the interaction of 2,4-D tank-contamination during an application of dicamba to dicamba-resistant soybean regarding injury, yield components, total grain yield, and seedling vigor and germination.

2.3 Materials and Methods

Field experiments were conducted in Arkansas, Illinois, Indiana, Mississippi, Missouri, and Wisconsin during 2016 and 2017 to evaluate the effects of auxin herbicide tank-contamination on sensitive soybean. A soybean variety resistant to glyphosate and another variety resistant to both dicamba and glyphosate were established in separate, yet adjacent, field areas and grown according to common agronomic practices at each location. The soybean variety at all sites had an indeterminate growth habit. Site-specific soil type and properties are presented in Table 2.1. Preemergence blanket applications of residual herbicides in combination with a single postemergence application of glyphosate were made to maintain weed-free conditions across all sites.

As stated previously, separate field experiments were conducted at each location for the dicamba/glyphosate-resistant soybean and glyphosate-resistant soybean for two reasons: 1) reduce the potential for full rates of dicamba to move off-target to sensitive soybean, and 2) isogenic soybean cultivars were not available for direct comparison of the individual traits anyhow. A rate titration of dicamba (Clarity® BASF Corporation, Research Triangle Park, NC) included 0, 0.056, 0.56, 5.6 and 56 h ha^{-1} or 2,4-D (Weedar® 64 Nufarm Inc. Alsip, IL) at 0, 0.56, 5.6, 56, and 560 g ha^{-1} was applied to glyphosate-resistant soybean at the V2 and R1 soybean growth stage to simulate a range of off-target movement doses during vegetative and reproductive growth. For the dicamba/glyphosate-resistant soybean trial, 2,4-D at 0, 0.56, 5.6, 56, and 560 g ha^{-1} was applied alone or in combination with dicamba at a rate of 560 g ha^{-1} to simulate 2,4-D tank contamination during a labeled application of dicamba plus glyphosate to dicamba/glyphosate-resistant soybean. Glyphosate (Roundup PowerMax® Bayer Crop Science, Research Triangle Park, NC) at a rate of 1120 g ae ha^{-1} was included in all treatments. Plot size ranged from 3 to 4 m wide by 9 to 14 m long with four soybean rows per plot. Herbicides were applied to the entire plot width with a CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} using TTI 110015 nozzles (Teejet Technologies, Springfield, IL).

Visual injury symptoms on soybean were estimated using a standard crop injury scale (0 = no injury; 100 = complete death), hereafter referred to as “injury” , and a scale developed by Behrens and Lueschen (1979) specifically for auxin herbicide injury on soybean, hereafter referred to as “BL injury”, 14 and 28 days after treatment (DAT). Soybean plant height reduction estimates and growth stage were also recorded at 14 and 28 DAT. Soybean plant population and overall height from the soil surface to the top of the main stem were recorded at physiological maturity. Five plants from each of the center two rows of each plot were collected at maturity for a total of ten plants to measure harvest index parameters. Nodes per plant, reproductive nodes per plant, pods per plant, 100-seed mass, and total seed mass were recorded from these selected plants. From these harvest index parameters and the plant population, yield components were calculated (Board and Modali 2005). Grain yield was determined by harvesting the center two rows of each plot and adjusted to 13% moisture content.

In addition to field-based assessments, soybean seed samples were collected from the Arkansas and Indiana sites in 2017 and retained for germination testing. A sub-sample of seed from each individual plot was collected and delivered to a commercial seed testing laboratory (Indiana Crop Improvement Association, Lafayette, IN) for warm, cold, and accelerated aging germination testing. In addition to formal seed analysis, greenhouse evaluations of progeny collected from field studies were conducted to evaluate seed germination and seedling growth of 25 seeds. These studies were conducted in a greenhouse at an average temperature of 27 C and natural light was supplemented with high pressure sodium lamps delivering 1,100 μmol $m^{-2}s^{-1}$ to achieve a 16-h day length. Seeds were planted in a 27.9 by 54.3 cm^{-1} plastic greenhouse flat at a depth of 1.5 cm in a 2:1 potting soil (Pro-Mix FLX, Premier Horticulture Inc., Quakertown, PA) sand medium and watered daily. Flats were fertilized weekly when the seedlings reached the unifoliate growth stage (Jack's Professional 20-20-20 General Purpose Fertilizer, JR Peters Inc Allentown, PA). Emerged seedlings were counted at 4, 7, 14, and 21 days after planting and malformed seedlings (irregular leaf margin or other malformation) were counted at 7, 14, and 21 days after planting. Greenhouse studies were completely randomized designs with four replications, where each seed sample from an experimental unit in the field represented the same in the seed lab or greenhouse for a given treatment.

2.3.1 Statistical Analysis

Each field experiment was designed as a randomized complete block with four replications. Height reduction, mature plant height, and yield were normalized to a percent of the nontreated prior to analysis. For 2,4-D and dicamba exposure in glyphosate-resistant soybean, dicamba and 2,4-D were analyzed separately. Data were subjected to ANOVA using PROC GLIMMIX in SAS 9.4 (SAS Institute, Inc., Cary, NC) with herbicide dose and exposure timing as factors. If the effect of exposure timing and the interaction of exposure timing and dose were non-significant, then the data were pooled across exposure timing for further analysis.

Additional analysis was performed using nonlinear regression to predict plant injury, height reduction, mature plant height, grain yield, and harvest index parameters following glyphosate-resistant soybean exposure to dicamba or 2,4-D. This analysis was conducted using the drc package in R (R, Version 3.5.3, www.r-project.org) using a two-parameter log-logistic model ($y = 100 / (1 + \exp(b(\log(x) - \log(e))))$) for injury, BL injury, and height reduction where y is the response variable, x is the herbicide dose, e is the herbicide dose causing 50% response, and b is a parameter describing the slope at e. A separate, three-parameter log-logistic model ($y = 0 + ((d - 0) / (1 + \exp(b(\log(x) - \log(e)))))$) was used to analyze mature height and grain yield where y is the response variable, x is the herbicide dose, d is the upper limit, e is the herbicide dose causing 50% response, and b is a parameter describing the slope at e. Harvest index parameters (Board and Modali 2005) were analyzed with a three-parameter Weibull model ($y = 0 + (d - 0) \exp(-\exp(b(\log(x) - e))$) where y is the response variable, x is the herbicide dose, b is the relative slope of the curve, d is the upper limit, and e is the inflection point. A lack-of-fit test ($\alpha = 0.05$) was used to determine that the model adequately described the data. From the dose-response models, effective dose (ED) causing 5, 10, and 20% response levels were calculated using the ED function within the drc package. These levels were selected based on previously reported levels of commercially unacceptable yield loss or crop response (Kniss 2018, Robinson et al. 2013a).

For the dicamba/glyphosate-resistant soybean experiment, similar analyses to the glyphosate-resistant soybean trial were conducted. However, the ANOVA was conducted with 2,4-D dose, presence of dicamba in the spray mixture, and exposure timing as the main factors. Depending on the significant factors and interactions, further analysis with non-linear regression was performed appropriately.

Harvest index parameters as described by Board and Modali (2005) were analyzed using ANOVA in the same manner previously described to determine 1) the effect of dicamba and 2,4-D on glyphosate-resistant soybean and 2) the response of dicamba/glyphosate-resistant soybean response to 2,4-D with and without a full rate of dicamba. Data from seed evaluations were subjected to ANOVA using similar methods to field trials.

2.4 Results and Discussion – Glyphosate-Resistant Soybean

2.4.1 Vegetative Response

The ED_{10} value for dicamba injury symptoms at the V2 exposure timing was 0.03 g ha^{-1} at 14 DAT and increased to 0.42 g ha^{-1} at 28 DAT, a 14X increase which suggest a rapid reduction in injury symptoms with time (Table 2.2). The ED_{10} value for exposure at the R1 soybean growth stage decreased from 0.18 to 0.07 g ha^{-1}, a 3X reduction between the 14 and 28 DAT rating timings, respectively (Table 2.2). These results suggest soybean recovery from dicamba is more rapid during vegetative growth compared with reproductive growth and demonstrate the variability of phytotoxic responses of soybean to dicamba exposure throughout the growing season. These results, where greater sensitivity during vegetative growth were observed at 14 DAT compared with 28 DAT and soybean response increasing from 14 DAT to 28 DAT following exposure at R1, are consistent with previous reports (Robinson et al. 2013b).

Soybean injury response to 2,4-D was more consistent across both exposure and rating timings, with ED_{10} values of 35 and 31 g ha^{-1} at the V2 and R1 exposure timings, respectively (Table 2.3). For visual injury at 28 DAT, data were pooled over growth stage and ED values were nearly double that of the 14 DAT timing (Table 2.3), demonstrating an overall dissipation of soybean response with time. The greater sensitivity at the V2 exposure timing is consistent with previous reports of 2,4-D exposure to soybean (Robinson et al. 2013a).

For both 2,4-D and dicamba, BL injury evaluations at 14 and 28 DAT followed similar trends to standard phytotoxicity ratings (Tables 2.2 and 2.3). Although this scale was specifically designed for evaluating plant response to auxin exposure, the use of this scale did not offer any increased utility in the evaluation of soybean response to synthetic auxin herbicides throughout the season in our research, especially with the associated time necessary to train personnel and perform ratings using this scale. Additionally, the use of this rating scale requires the presence of active growth at the terminal bud, which is not present during later reproductive growth stages when ratings were conducted. Our research involved several investigators evaluating these experiments across multiple states and traditional plant injury response estimates (0 to 100 scale) were more consistent than the BL injury scale when several individuals were involved with the evaluations.

Soybean plant height reduction following dicamba exposure at 14 DAT was greater when exposure occurred at V2 compared with R1, with a 3X difference in sensitivity (Table 2.2). In contrast, soybean were more sensitive to 2,4-D exposure at the R1 growth stage compared with the V2 growth stage at 14 DAT, with a 2X difference in sensitivity (Table 2.3). For both 2,4-D and dicamba, height reduction at 28 DAT was not influenced by exposure timing. At maturity, soybean height in response to dicamba exposure was highly influenced by exposure timing, with a 17X higher ED_{10} value at V2 compared with R1 (Table 2.2). For 2,4-D, the timing of exposure did not have an effect on soybean height at maturity (Table 2.3).

2.4.2 Harvest Index Parameters.

The ED_{10} values for soybean grain yield following dicamba exposure at V2 and R1 were 4.61 and 1.66 g ha^{-1}, respectively (Table 2.2). Similar to the mature soybean plant height data, grain yield was also more sensitive to dicamba exposure at the R1 growth stage than the V2 growth stage. These trends are consistent with approximately a 2X difference in sensitivity between soybean exposure to dicamba during early vegetative and flowering growth stages reported by Kniss (2018). This demonstrates the developmental plasticity of soybean and the potential for soybean recovery following dicamba exposure early in the growing season if favorable growing conditions persist, as suggested by Kniss (2018). Similarly to height, yield response to 2,4-D exposure was not determined by the timing of exposure, with a combined ED_{10} value of 34 g ha^{-1} (Table 2.3).

The 100-seed mass of seed collected from plants in this experiment did not differ from the nontreated following 2,4-D or dicamba exposure (Table 2.5). The ED_{10} values for seeds m^{-2} were 410 g ha^{-1} for 2,4-D and 31 g ha^{-1} for dicamba (Tables 2.2 and 2.3). Combined across doses within a herbicide, seeds m^{-2} were reduced 13 and 11% for 2,4-D and dicamba, respectively, when exposure occurred at the R1 growth stage compared with the V2 growth stage (Table 2.7). Seeds per pod was also affected by the timing of exposure, with 9% fewer seeds per pod for both 2,4-D and dicamba when exposure occurred at the R1 growth stage compared with the V2 growth stage (Table 2.7). For both 2,4-D and dicamba, pods m^{-2} was reduced by the highest dose of both 2,4-D

(560 g ha^{-1}) and dicamba (56 g ha^{-1}) compared with all other doses and the nontreated control, which were similar to each other (Table 2.5). Pods per node were not affected by 2,4-D. In contrast, dicamba exposure at doses of 5.6 and 56 g ha^{-1} resulted in a decrease in pods per node compared with the nontreated (Table 2.6). The ED_{10} value of reproductive nodes m^{-2} for 2,4-D was 399 g ha^{-1}, indicating a similar sensitivity to seeds m^{-2} (Table 2.3). Reproductive nodes m^{-2} was not highly influenced by dicamba exposure, with no doses different from the nontreated (Table 2.6). The percentage of reproductive nodes was influenced only by the highest dose of each respective herbicide at the R1 exposure timing (Table 2.8). For 2,4-D, the percent reproductive nodes was 81% for the nontreated compared with 72% for exposure to 560 g ha^{-1} of 2,4-D at R1. Exposure to 56 g ha^{-1} of dicamba at R1 resulted in 63% reproductive nodes compared with the nontreated with 81% reproductive nodes. The ED_{10} value for total nodes m^{-2} was 409 g ha^{-1} for 2,4-D, indicating a similar sensitivity of this growth parameter to seeds m^{-2} and reproductive nodes m^{-2} (Table 2.3). Dicamba exposure at any dose did not influence total nodes m^{-2} compared with the nontreated (Table 2.6). Across the harvest index parameters evaluated, soybean response to both dicamba and 2,4-D was greatest at the highest doses tested (56 and 560 g ha^{-1}, respectively) and the subsequent decreases in herbicide dose did not result in a proportional effect on soybean response. In other words, harvest index parameters were not highly influenced until a 1/10X or 1/2X rate of dicamba or 2,4-D, respectively, were directly applied to the soybean plants.

Cold germination tests revealed no differences in germination following exposure to 2,4-D compared with the nontreated controls (Table 2.8). There was, however, a significant effect of exposure timing to 2,4-D in the accelerated aging germination test, with 52 and 44% germination when exposure occurred at the V2 and R1 growth stages, respectively (Table 2.7). Greenhouse growth experiments uncovered no effect of 2,4-D on germination at any time and percentage of malformed plants at 7 and 14 days after planting (DAP). At 21 DAP, 11% of plants were malformed compared with 8% when exposure to 2,4-D occurred at R1 and V2, respectively (Table 2.7).

For dicamba, cold germination and accelerated aging germination tests revealed that only exposure at the 56 g ha^{-1} dose at the R1 growth stage resulted in reduced germination compared with the nontreated controls with 21 and 1% germination, respectively (Table 2.8). Greenhouse germination studies did not, however, produce similar results, with no treatment effects across dicamba doses or exposure times. Malformed plant counts for exposure at R1 resulted in an increase of 5, 4, and 4% more plants that were malformed compared with exposure at V2 at the 7, 14, and 21 DAP rating timings, respectively (Table 2,8). The greatest number of malformed plants was observed at 7 DAP from seed that was exposed to dicamba at the R1 growth stage, with 17% of plants being malformed.

While these results indicate that glyphosate-resistant soybean responses to 2,4-D and dicamba are similar to other published research, they served as a benchmark for our research methods and sites on dicamba/glyphosate-resistant soybean. It was paramount to establish a baseline in order to ensure that this research would be relevant across multiple geographies and soybean genetics.

2.5 Results and Discussion – Dicamba/Glyphosate-Resistant Soybean

2.5.1 Vegetative Response

Dicamba-resistant soybean expressed similar sensitivity to 2,4-D at 14 DAT for exposure at V2 when a full rate of dicamba was present in the tank, with ED_{10} values of 30 and 24 g ha^{-1} for 2,4-D and 2,4-D plus dicamba, respectively (Table 2.4). Sensitivity was also similar when exposure occurred at the R1 soybean growth stage. Furthermore, at the 28 DAT evaluation for both the V2 and R1 exposure timings soybean were more sensitive to 2,4-D alone compared with 2,4-D plus a full rate of dicamba (Table 2.4). Although injury response to vegetative exposure to 2,4-D may be numerically greater when a full rate of dicamba is present 14 days after exposure, ED values indicate that, with time, 2,4-D plus dicamba will actually result in less injury at a given dose of 2,4-D. Estimates of BL injury lacked the resolution necessary to discern differences in soybean response between 2,4-D or 2,4-D plus dicamba exposure at either rating time (Table 2.4). This observation again suggests a limited utility in using the BL injury scale compared to traditional plant injury estimates for auxin herbicides in our research.

At 14 DAT, soybean height reduction was not affected by the combination of 2,4-D and dicamba plus glyphosate, thus no difference in height reduction should be expected if a full rate of dicamba plus glyphosate is applied with 2,4-D contamination. Soybean growth stage at the time of exposure was not significant for injury at 28 DAT, but soybean sensitivity to 2,4-D was over 1.5X greater than 2,4-D plus dicamba, with ED_{10} values of 46 and 76 g ha^{-1} of 2,4-D, respectively (Table 2.4). Soybean height at maturity was affected to a greater extent by 2,4-D alone at both the V2 and R1 exposure timings compared with dicamba plus 2,4-D tank-contamination (Table 2.4). These results indicate that the effect of 2,4-D on soybean height will not be exacerbated by the presence of dicamba in the tank.

2.5.2 Harvest Index Parameters

Dicamba/glyphosate-resistant soybean yield loss in response to 2,4-D exposure was not influenced by the presence of dicamba in the spray tank at the time of exposure, similar to the height reduction data. Previous research has documented that in three of four site-years, the presence of a labeled herbicide did not adversely affect soybean grain yield response to an auxin herbicide (Kelley et al. 2005). In the aforementioned study, herbicide resistance in the soybean was conferred by an insensitive target site (glyphosate-resistance), while resistance to dicamba in soybean is conferred by metabolism. Based on the previous report and results herein, the intrinsic herbicide resistance mechanism for dicamba will not affect the sensitivity of soybean to off-target auxin herbicides with or without the herbicides enabled by the specific crop-herbicide resistance traits. Compared with the range of dicamba/glyphosate-resistant soybean varieties tested, glyphosate-resistant soybean had a numerically similar injury response at 14 DAT, with ED_{10} values at 35 and 31 g ha^{-1} for glyphosate-resistant soybean at V2 and R1, respectively, and 30 and 35 g ha^{-1} for dicamba/glyphosate-resistant soybean at V2 and R1, respectively. Yield results were also similar, with an ED_{10} value of 34 g ha of 2,4-D for both soybean varieties (glyphosate-resistant versus dicamba/glyphosate-resistant) pooled over exposure timings.

Aside from visual injury, no additional crop response should be expected following dicamba/glyphosate-resistant soybean exposure to 2,4-D from tank contamination when a full rate of dicamba is applied. In regard to yield components described by Board and Modali (2005), the presence of a full rate of dicamba did not affect soybean response to 2,4-D. Additionally, cold germination, accelerated aging germination, greenhouse emergence, and emerged plant malformation in the greenhouse were not affected by the addition of a full rate of dicamba to 2,4-D tank-contamination.

No isogenic soybean cultivars are available to directly evaluate the influence of the dicamba resistance trait on soybean response to 2,4-D. In the absence of isogenic soybean cultivars, we conducted a study across a broad geography to establish the sensitivity of glyphosate-resistant soybean and dicamba/glyphosate-resistant soybean independently. Overall, herbicide dose estimates provided herein for glyphosate-resistant soybean response to dicamba and 2,4-D generally agree with published literature. These results encompass a wide range of environmental conditions across a large portion of the soybean producing region of United States for a broad scope of inference when assessing soybean response to off-target synthetic auxins. Under those conditions that provided largely consistent results with those published on glyphosate-resistant soybean, our results on dicamba/glyphosate-resistant soybean suggest similar responses to a dose range of 2,4-D which confirms the dicamba trait has no influence on sensitivity to 2,4-D. In addition, we observed minor to no influence of full rates of dicamba and glyphosate application with tank-contaminant levels of 2,4-D. Thereby confirming that the resistance trait for dicamba-resistant soybean is extremely robust with rapid dicamba metabolism that prevents any consistent interaction between 2,4-D and dicamba. A continued importance must be placed on preventing herbicide contamination during the mixing and loading procedures, as well as sprayer cleanout methods, to avoid tank contamination of auxin herbicides. Furthermore, significant caution should be practiced prior to, during, and following an application of these herbicides in order to prevent off-target movement of these herbicide from becoming a greater commercial liability.

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Table 2.1. Site characteristics of field trials conducted in 2016 and 2017.

		Soil properties					
State	Year	Texture	OM[a]	pH	CEC	Planting Date[b]	Harvest Date
			%		mEq/100 g soil		
AR	2016	Captina silt loam	1.5	5.9	NA	June 8	NA
	2017	Captina silt loam	1.5	5.9	NA	June 13	NA
IL	2016	Silty clay loam	3.4	6.4	38.5	May 31	Oct 24
	2017	Silty clay loam	3.4	6.1	36.6	May 30	Oct 21
IN	2016	Toronto-Milbrook complex	2.8	6.6	10.8	May 25	Nov 3
	2017	Toronto-Milbrook complex	2.8	6.6	10.8	June 6	Oct 31
MO	2016	Mexico silt loam	2.8	5.7	10	June 1	Nov 2
	2017	Mexico silt loam	2.3	6	10.5	May 15	Oct 18
MS	2016	Brooksville silty clay	1.7	6.3	24.1	July 1	Oct 7
WI	2016	Silt loam	2.6	6.6	NA	May 18	Oct 19

[a] Abbreviations: CEC, cation exchange capacity; NA, not applicable; OM, organic matter.

[b] Planting date and harvest date were the same for both glyphosate- and dicamba/glyphosate-resistant soybean trials during each year.

Table 2.2. Response of glyphosate-resistant soybean to dicamba exposure at the V2 or R1 soybean growth stage across all locations in 2016 and 2017.

Parameter[a]	Growth Stage	ED_5 (± SE)[c]	ED_{10} (± SE)	ED_{20} (± SE)
		--------------------------- g ae ha^{-1}	---------------------------	
Injury 14 DAT[b]	V2	0.005 (0.002)	0.03 (0.01)	0.19 (0.04)
	R1	0.04 (0.02)	0.18 (0.06)	0.93 (0.21)
Injury 28 DAT	V2	0.12 (0.04)	0.42 (0.11)	1.67 (0.29)
	R1	0.01 (0.01)	0.07 (0.02)	0.46 (0.09)
BL injury 14 DAT	V2	0.002 (0.001)	0.01 (0.005)	0.1 (0.03)
	R1	0.005 (0.003)	0.03 (0.01)	0.24 (0.06)
BL injury 28 DAT	V2	0.03 (0.02)	0.17 (0.06)	1.00 (0.21)
	R1	0.003 (0.002)	0.03 (0.01)	0.31 (0.08)
Height reduction 14 DAT	V2	0.13 (0.04)	0.50 (0.12)	2.13 (0.33)
	R1	0.55 (0.18)	1.74 (0.43)	6.03 (0.93)
Height reduction 28 DAT	V2 and R1	0.16 (0.04)	0.57 (0.11)	2.27 (0.30)
Mature height	V2	1.16 (1.65)	4.87 (4.60)	23.0 (11.6)
	R1	0.05 (0.06)	0.28 (0.28)	1.96 (1.26)
Grain yield	V2	1.61 (1.65)	4.61 (3.39)	14.4 (6.29)
	R1	0.72 (0.42)	1.66 (0.77)	4.11 (1.38)
Seeds m^{-2}	V2 and R1	22.9 (83.5)	30.9 (75.1)	42.3 (49.0)
Nodes m^{-2}	V2 and R1	35.3 (35.6)	42.9 (25.1)	52.7 (8.26)

[a] Parameters: Visual injury (0 to 100%), Behrens and Lueschen scale injury (0 to 100), height reduction (0 to 100%), soybean height at maturity, grain yield, seeds m^{-2}, and total nodes m^{-2}.

[b] Abbreviations: BL, Behrens and Lueschen scale; DAT, days after treatment; ED, effective dose; SE, standard error;

[c] ED_5, ED_{10}, and ED_{20}, effective doses resulting in 5, 10 and 20% soybean response.

Table 2.3. Response of glyphosate-resistant soybean to 2,4-D exposure at the V2 or R1 soybean growth stage across all locations in 2016 and 2017.

Parameter[a]	Growth stage	ED_5 (± SE)[c]	ED_{10} (± SE)	ED_{20} (± SE)
		---------------------------- g ae ha^{-1}	---------------------------	
Injury 14 DAT[b]	V2	20 (2)	35 (3)	64 (4)
	R1	16 (2)	31 (3)	64 (5)
Injury 28 DAT	V2 and R1	41 (7)	72 (9)	133 (12)
BL injury 14 DAT	V2	20 (2)	34 (3)	64 (5)
	R1	17 (3)	34 (4)	72 (6)
BL injury 28 DAT	V2 and R1	28 (7)	63 (12)	149 (17)
Height reduction 14 DAT	V2	23 (4)	43 (6)	84 (8)
	R1	9 (2)	23 (4)	65 (8)
Height reduction 28 DAT	V2 and R1	31 (5)	61 (8)	127 (11)
Mature height	V2 and R1	20 (10)	57 (20)	177 (36)
Grain yield	V2 and R1	16 (6)	34 (10)	80 (17)
Seeds m^{-2}	V2 and R1	348 (1319)	410 (1016)	488 (537)
Reproductive nodes m^{-2}	V2 and R1	321 (373)	399 (284)	501 (125)
Nodes m^{-2}	V2 and R1	332 (392)	409 (291)	508 (118)

[a] Parameters: Visual injury (0 to 100%), Behrens and Lueschen scale injury (0 to 100), height reduction (0 to 100%), soybean height at maturity, grain yield, seeds m^{-2}, reproductive nodes m^{-2}, and total nodes m^{-2}.

[b] Abbreviations: BL, Behrens and Lueschen scale; DAT, days after treatment; ED, effective dose; SE, standard error;

[c] ED_5, ED_{10}, and ED_{20}, effective doses resulting in 5, 10 and 20% soybean response.

Table 2.4. Response of dicamba/glyphosate-resistant soybean to 2,4-D plus dicamba or 2,4-D alone exposure at the V2 or R1 growth stage across all locations in 2016 and 2017.

Parameter[a]	Exposure Timing	Herbicide	ED_5 (± SE)[c]	ED_{10} (± SE)	ED_{20} (± SE)
			---------------------- g ae ha^{-1}	----------------------	
Injury 14 DAT[b]	V2	2,4-D	17 (2)	30 (3)	54 (4)
		Both[d]	13 (2)	24 (3)	47 (4)
	R1	2,4-D	18 (3)	35 (4)	70 (6)
		Both	27 (4)	49 (6)	94 (9)
Injury 28 DAT	V2	2,4-D	43 (13)	79 (18)	156 (23)
		Both	55 (18)	97 (23)	179 (29)
	R1	2,4-D	37 (9)	68 (13)	133 (18)
		Both	63 (20)	109 (26)	196 (30)
BL injury 14 DAT	V2	-	11 (1)	21 (2)	44 (3)
	R1	-	27 (3)	48 (4)	89 (6)
BL injury 28 DAT	V2	-	31 (11)	74 (19)	190 (27)
	R1	-	30 (8)	63 (11)	141 (18)
Height reduction 14 DAT	V2	-	19 (2)	36 (3)	72 (5)
	R1	-	12 (2)	29 (4)	78 (7)
Height reduction 28 DAT	-	2,4-D	21 (4)	46 (6)	108 (10)
	-	Both	38 (8)	76 (11)	158 (15)
Mature plant height	V2	2,4-D	27 (14)	67 (25)	185 (40)
		Both	34 (17)	79 (28)	197 (41)
	R1	2,4-D	8 (4)	24 (9)	79 (19)
		Both	24 (11)	58 (19)	153 (32)
Grain yield	-	2,4-D	17 (4)	34 (6)	70 (9)
	-	Both	32 (8)	59 (12)	113 (16)

[a] Parameters: Visual injury (0 to 100%), Behrens and Lueschen scale injury (0 to 100), Height reduction (0 to 100%), soybean height at maturity, grain yield.

[b] Abbreviations: BL, Behrens and Lueschen scale; DAT, days after treatment; ED, effective dose; SE, standard error;

[c] ED_5, ED_{10}, and ED_{20}, effective doses resulting in 5, 10 and 20% soybean response.

[d] Both refers to the combination of 2,4-D across the rate structure with a dose of 560 g ae ha^{-1} dicamba in the tank mixture.

Table 2.5. Response of glyphosate-resistant soybean to 2,4-D or dicamba exposure at the V2 or R1 soybean growth stage across all locations in 2016 and 2017.

Herbicide	Dose	100-seed mass[a]	Seeds m^{-2}	Pods m^{-2}
	g ae ha^{-1}	-----g-----		
Dicamba	0	15.27 ab	2325 a	1261 a
	0.056	15.83 a	2333 a	1145 a
	0.56	15.61 a	2525 a	1337 a
	5.6	15.76 a	2400 a	1188 a
	56	14.61 b	1601 b	802 b
2,4-D	0	15.26 ab	2325 a	1261 a
	0.56	15.44 ab	2385 a	1215 a
	5.6	15.80 a	2289 a	1153 a
	56	15.61 a	2447 a	1219 a
	560	14.63 b	1573 b	828 b

[a] Treatment means within a herbicide or response followed by the same letter are not statistically different according to Tukey's HSD (honestly significant difference) test ($P \leq 0.05$).

Table 2.6. Response of glyphosate-resistant soybean to 2,4-D or dicamba exposure at the V2 or R1 soybean growth stage across all locations in 2016 and 2017.

Herbicide	Dose	Pods per node[a]	Reproductive nodes m^{-2}	Nodes m^{-2}
	g ae ha^{-1}			
Dicamba	0	2.32 a	547.5 ab	677.2 ab
	0.056	2.26 a	541.2 ab	672.5 ab
	0.56	2.17 ab	646.6 a	799.8 a
	5.6	2.02 bc	618.3 a	764.0 a
	56	1.93 c	464.3 b	550.6 b
2,4-D	0	- -	547.5 a	677.2 a
	0.56	- -	565.1 a	714.7 a
	5.6	- -	549.9 a	686.7 a
	56	- -	607.3 a	761.6 a
	560	- -	411.5 b	520.0 b

[a] Treatment means within a herbicide or response followed by the same letter are not statistically different according to Tukey's HSD (honestly significant difference) test ($P \leq 0.05$).

Table 2.7. Response of glyphosate-resistant soybean to 2,4-D or dicamba exposure at the V2 or R1 soybean growth stage across all locations in 2016 and 2017.

Herbicide	Timing	Seeds m^{-2} [a]	Seeds per pod	Accelerated aging germination	Malformed plants		
					7 DAP[b]	14 DAP	21 DAP
				------------------------------	-%	------------------------------	
Dicamba	V2	2370 a	2.15 a	- -	11.6 b	10.3 b	8.7 b
	R1	2103 b	1.96 b	- -	16.8 a	14.2 a	12.5 a
2,4-D	V2	2352 a	2.20 a	52.0 a	- -	- -	8.1 b
	R1	2056 b	2.01 b	43.5 b	- -	- -	10.6 a

[a] Treatment means within a herbicide or response followed by the same letter are not statistically different according to Tukey's HSD (honestly significant difference) test ($P \leq 0.05$).

[b] Abbreviation: DAP, days after planting;

Table 2.8. Response of glyphosate-resistant soybean to 2,4-D or dicamba exposure at the V2 or R1 soybean growth stage across all locations in 2016 and 2017.

Herbicide	Timing	Dose	Reproductive nodes[a]	Cold germination	Accelerated aging germination
		g ae ha^{-1}	----------------------------------	%----------------------------------	
Dicamba	V2	0	80.0 a	74.9 bc	36.8 b
		0.056	79.7 a	82.5 ab	57.4 ab
		0.56	76.8 a	82.1 ab	57.6 ab
		5.6	80.2 a	83.3 ab	53.8 ab
		56	79.7 a	91.0 a	68.8 a
	R1	0	80.5 a	81.4 ab	42.6 ab
		0.056	80.8 a	75.2 bc	35.9 b
		0.56	82.0 a	84.3 ab	46.8 ab
		5.6	80.1 a	71.4 c	35.8 b
		56	62.8 b	21.0 d	0.2 c
2,4-D	V2	0	78.0 a	74.9 c	- -
		0.56	79.1 a	81.3 abc	- -
		5.6	78.3 a	80.8 abc	- -
		56	79.1 a	81.5 abc	- -
		560	83.0 a	87.4 a	- -
	R1	0	80.5 a	81.4 abc	- -
		0.56	79.6 a	81.8 abc	- -
		5.6	81.8 a	80.8 abc	- -
		56	79.3 a	85.2 ab	- -
		560	71.8 b	78.3 bc	- -

[a] Treatment means within a herbicide or response followed by the same letter are not statistically different according to Tukey’s HSD (honestly significant difference) test ($P \leq 0.05$).

CHAPTER 3. GLYPHOSATE-RESISTANT SOYBEAN RESPONSE TO DICAMBA EXPOSURE AND LABELED POSTEMERGENCE HERBICIDES

3.1 Abstract

Soybean injury from herbicides can occur from the direct application of labeled herbicides as well as accidental exposure to herbicides that move off-target. Both routes of herbicide injury have been extensively investigated. However, the combined influence of injury from herbicides labeled for postemergence (POST) applications in soybean and from off-target exposure from a herbicide such as dicamba has remained unclear. Field experiments were conducted in 2017 and 2018 to evaluate the influence of dicamba exposure before or after labeled POST herbicides on dicamba-sensitive soybean. Labeled POST herbicide combinations known to cause soybean injury were applied at either the V3 or R1 growth stage of soybean. A reduced rate of dicamba (5.6 g ae ha^{-1}) was applied at R1 following the planned POST herbicides applied at V3, or dicamba was applied at V3 prior to the planned POST herbicides applied at R1 in order to simulate off-target dicamba exposure. Soybean injury at 28 days after the R1 application was influenced primarily by the timing of dicamba exposure rather than the labeled POST herbicide, with up to 14% injury from POST herbicides alone and up to 37% injury from dicamba exposure alone at R1. Plant height reduction 28 days after the R1 application was also influenced by dicamba exposure and timing, with up to 1% height reduction from labeled POST herbicides and up to 17 and 44% height reduction from dicamba exposure at V3 and R1, respectively. Soybean height at physiological maturity was reduced by 20 and 42% when dicamba exposure occurred at the V3 and R1 growth stages, respectively, across labeled POST herbicides. Soybean yield reduction in response to dicamba alone was greater during the 2017 growing season with a 37% reduction in yield from

dicamba exposure at R1 in 2017 compared with a 17% reduction in 2018. Regardless of the difference in yield response between years, the primary factor that influenced yield was the timing of dicamba exposure. Furthermore, the labeled POST herbicides evaluated in this study did not affect soybean response to dicamba in regards to yield. These findings indicate that soybean growers should not be overly concerned with the potential for soybean injury from labeled postemergence herbicides when implementing the necessary herbicides for weed control if injury from dicamba exposure already exists, or is a potential threat later in the growing season.

3.2 Introduction

The commercialization of dicamba-resistant soybean with the use of approved formulations of dicamba in 2017 was followed by the rapid adoption of this technology by soybean growers throughout the United States. Of the 36 million ha of soybean planted in the United States in 2017, around 22% were dicamba-resistant (Lingenfelter 2017). At the start of this research, dicamba herbicide labels allowed for applications to occur extending up to and including the R1 soybean growth stage (Anonymous 2018a, 2018b). With a wide range of soybean planting dates, dicamba applications can be made to soybeans throughout a relatively long period during the growing season.

Growers of dicamba-sensitive crops have expressed concerns with the implementation of this technology due to the potential for off-target movement of dicamba. Off-target herbicide movement can occur from drift and/or sprayer contamination (Boerboom 2003, Maybank et al. 1978). In order to minimize the potential for off-target movement, products containing dicamba that are currently labeled for application in dicamba-resistant soybean provide restrictions regarding how they may be applied. These restrictions include buffer zones between application areas and sensitive plants, wind speed limits, and application equipment constraints (Anonymous

2018a, 2018b). Even with significant label restrictions for applying dicamba, off-target dicamba exposure was a major concern for dicamba-sensitive soybean growers during the 2017 and 2018 growing seasons (Bradley 2018).

Soybean response to low dose dicamba exposure can be characterized by leaf cupping and crinkling, whereas higher doses of dicamba can result in the death of the apical meristem (Wax et al. 1969, Zimmer et al. 2019). Soybean injury has been reported following exposure to doses as low as 0.01% of the labeled use rate of 560 g ae ha^{-1} at both vegetative and reproductive exposure timings (Solomon and Bradley 2014).

Although foliar injury symptoms may develop at these low doses of dicamba exposure, higher doses are often necessary to cause significant yield reduction. A meta-analysis by Kniss (2018) reported that the average dose necessary to cause 5% yield loss was 1.9, 5.7, and 0.89 g ae ha^{-1} at early vegetative (V1 to V3), late vegetative (V4 to V7), and reproductive (R1 to R2) growth stages, respectively. This meta-analysis draws similar conclusions to a previous meta-analysis conducted by Egan et al. (2014), both indicating the dose necessary to cause a soybean yield reduction during vegetative growth is often higher than the dose necessary to cause similar yield reduction at reproductive timings. Environmental conditions throughout the growing season were identified as important factors in both studies. Increased dicamba injury was observed when air temperatures were higher during and following application (Al-Khatib and Peterson 1999). The dicamba doses necessary to cause a 10% soybean grain yield reduction were 15 and 1.3 g ae ha^{-1} in 1980 and 1981, respectively (Weidenhamer et al. 1989). This 11.5-fold difference in dose was attributed to dry conditions in 1981 resulting in a 240 mm difference in rainfall during June through August between the two years. When environmental conditions are optimal for soybean growth, yield losses as a result of dicamba exposure at a given dose may be far less severe than when environmental conditions are limiting yield potential.

Another abiotic stress factor for soybean can be the application of herbicides labeled for POST applications (Loux et al. 2019). Thus, the soybean stress induced by labeled herbicide applications prior to or after dicamba exposure may influence the response of the soybean. Current postemergence herbicide options for soybean may include acetochlor, chlorimuron-ethyl, lactofen, and/or 2,4-DB, all of which may cause soybean injury following application (Barker et al. 1984, Jhala et al. 2015, Young et al. 2003). Although foliar injury symptoms may develop, yield losses from typically injurious POST soybean herbicides are rare (Young et al. 2003). The application timings for these herbicides encompasses early vegetative through early reproductive stages encompassing a period when off-target dicamba exposure may occur. Symptoms of off-target dicamba exposure may take up to two weeks to develop, during which time labeled POST herbicide applications may be made to soybean that have been exposed to dicamba unbeknownst to the applicator. For example, soybean injury was increased and grain yield was reduced from a simulated dicamba/diflufenzopyr drift event followed by a chlorimuron-ethyl application two to four days later compared with dicamba/diflufenzopyr drift alone (Brown et al. 2009). In a study with dicamba as a tank contaminant, soybean injury was increased when imazethapyr, imazamox, or fomesafen was applied at a labeled field use rate in combination with 5.6 g ae ha^{-1} of dicamba, compared with dicamba alone applied at either the V3 or V7 growth stage (Kelley et al. 2005). Soybean grain yield was also reduced when imazethapyr, imazamox, or fomesafen was applied at the V7 growth stage in combination with dicamba compared with dicamba alone.

Of the available herbicides for dicamba-sensitive soybean growers, several options are known to cause foliar injury such as lactofen, acetochlor, chlorimuron, and/or 2,4-DB. With the potential for injury from unintentional dicamba exposure, dicamba-sensitive soybean growers may be faced with the decision of which potentially-injurious herbicide program to use in order to maximize herbicide efficacy and minimize the risk of any additional yield loss if a dicamba exposure event occurs. The objective of this study was to determine if soybean injury and yield reduction were influenced by an application of a labeled POST herbicide combination known to cause injury prior to or following a dicamba exposure event.

3.3 Materials and Methods

Field trials were conducted at the Throckmorton Purdue Agricultural Center (40.2969°N, 86.9036°W) in 2017 and 2018 on a Toronto-Millbrook complex soil (fine-silty, mixed, superactive, mesic Udollic Epiaqualfs). The soil pH was 6.6 and the site was managed under fertilizer recommendations from Vitosh et al. (1995). The trial site was prepared by using a chisel plow in the fall followed by a field cultivator prior to spring planting. Glyphosate-resistant soybean (Asgrow® 2933, Bayer Crop Science, Research Triangle Park, NC) were planted in 76-cm rows at a seeding rate of 350,000 seeds ha^{-1} on June 12 and May 22 in 2017 and 2018, respectively, with a plot size of 3 m wide by 9 m long. The entire trial area was treated with a preemergence application of *s*-metolachlor (1504 g ai ha^{-1}) (Dual II Magnum®, Syngenta Crop Protection, LLC, Greensboro, NC) and sulfentrazone (278 g ai ha^{-1}) + cloransulam-methyl (36 g ai ha^{-1}) (Authority® First DF, FMC Corp., Philadelphia, PA) to maintain the trial area as weed-free. A single postemergence application of glyphosate (1120 g ae ha^{-1}) with ammonium sulfate (5% v v^{-1}) (N-Pak® AMS Liquid, Winfield Solutions, LLC, St. Paul, MN) was made to the entire trial area between the V3 and R1 experimental treatments to control weeds that emerged prior to canopy closure.

Herbicide treatments (Table 3.2) and simulated off-target dicamba exposure of 5.6 g ae ha^{-1} were applied to the entire 4-row plot width with a 3.0-m wide, CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} operating at 207 kPa using eight TT 110015 nozzles (Teejet Technologies, Springfield, IL) and a 38-cm nozzle spacing. Labeled POST herbicides were applied at the V3 growth stage followed by dicamba exposure at the R1 growth stage or dicamba was applied at the V3 growth stage followed by the labeled POST herbicide at the R1 growth stage.

Visual injury estimates (phytotoxicity and Behrens and Lueschen scale) (Behrens and Lueschen 1979), plant height reduction, and growth stage were recorded 14 and 28 days after treatment. Plant population and overall plant height from the soil surface to the top of the main stem were recorded at physiological maturity. Five plants from each of the center two rows of each plot were collected at maturity for a total of ten plants to measure harvest index parameters. Nodes per plant, reproductive nodes per plant, pods per plant, 100-seed mass, and total seed mass were recorded from these selected plants. Grain yield was determined by harvesting the center two rows of each plot and adjusted to 13% moisture content. The experiment was conducted as a randomized complete block design with four replications in a factorial arrangement with dicamba exposure, dicamba exposure timing, and labeled POST herbicide combination as factors. Data were subjected to ANOVA using PROC GLIMMIX in SAS 9.4 (SAS Institute, Inc., Cary, NC) with dicamba exposure, dicamba exposure timing, and labeled POST herbicide combination as fixed factors and replicate (block) nested within year as a random factor. Means separation was performed using Tukey's HSD test at α=0.05. Data for soybean height and Behrens and Lueschen scale injury estimates 28 days after "B" (R1) application timing (DATB) were combined across years and overall soybean injury 28 DAT, soybean injury 28 DATB, height reduction 28 DATB, and yield were not due to a significant year interaction.

3.4 Results and Discussion

3.4.1 Soybean Injury

Dicamba exposure alone at the V3 growth stage resulted in less soybean injury (11 to 13%) than exposure at R1 (25 to 37%) at 28 days after treatment during the 2017 and 2018 seasons, respectively (data not presented). In 2017, soybean injury from dicamba 28 days after the R1 application timing was not influenced by labeled POST herbicide applications prior to or following dicamba exposure (Table 3.3). Similarly, in 2018, soybean injury from dicamba exposure at the R1 growth stage was not influenced by the application of labeled POST herbicides at V3. When dicamba exposure occurred at V3 prior to POST herbicide application at R1, treatments containing lactofen resulted in greater soybean injury (9 to 25%) compared with dicamba alone (4%) (Table 3.3). The increased injury from lactofen + glyphosate and acetochlor + lactofen + glyphosate at R1 following dicamba exposure at V3 was similar to the injury from lactofen + glyphosate and acetochlor + lactofen + glyphosate alone at R1 in the absence of dicamba exposure. Additionally, soybean exposure to dicamba at V3 followed by 2,4-DB + lactofen + glyphosate at R1 resulted in an 11% increase in injury compared with the 2,4-DB + lactofen + glyphosate alone at R1 (Table 3.3). These results indicate that aside from an application of 2,4-DB + lactofen + glyphosate at R1 following dicamba exposure at V3, labeled POST herbicides did not have an influence on soybean injury in response to accidental dicamba exposure.

3.4.2 Behrens and Lueschen Scale Injury

Similar to the previous discussion on overall soybean injury, dicamba exposure alone at the R1 growth stage resulted in greater injury (39%) using the Behrens and Luschen scale (BL injury) compared with the V3 growth stage (20%) 28 days after application (data not presented). Data for BL injury at 28 days after the R1 application indicated that the timing of dicamba exposure had the greatest effect on soybean injury. Dicamba exposure at R1 caused 40% BL injury, while exposure at V3 caused only 10% BL injury (Table 3.4). The Behrens and Lueschen scale is specific to auxin herbicide injury; thus, injury from additional labeled POST herbicides is not reflected in ratings when this scale is used. Additionally, this rating scale depends on vegetative characteristics such as terminal leaflet cupping that do not apply after soybean cease terminal growth during reproductive growth stages. The labeled POST herbicides did not contribute to the auxin-specific injury evaluated with this scale. This rating scale did not provide any additional utility in the evaluation of the interaction of labeled POST herbicides and accidental dicamba exposure.

3.4.3 Soybean Height

Reductions in soybean plant height at 28 days after the R1 application were influenced by dicamba exposure and the timing of exposure, as well as the labeled POST herbicide combination. In 2017, dicamba exposure at R1 resulted in 33% height reduction compared with 14% from dicamba exposure at V3 (Table 3.4). Independent of dicamba exposure, treatments containing lactofen reduced soybean plant height compared with no labeled POST herbicide at all (Table 3.5). In 2018, dicamba exposure at V3 resulted in 17% soybean plant height reduction across treatments including labeled POST applications, while exposure at R1 caused 44% height reduction (Table 3.4). The only labeled POST herbicide combination that caused height reduction in 2018 was 2,4-DB + lactofen + glyphosate, regardless of dicamba exposure or timing (Table 3.5). The aforementioned results demonstrate that, similarly to soybean injury, reductions in soybean height, at 28 days after the R1 application, were influenced primarily by timing of dicamba exposure, and, to a lesser extent, the application of a labeled POST herbicide combination.

Soybean height at physiological maturity was influenced by the interaction of dicamba exposure and soybean growth stage, as well as the labeled POST herbicide combination. Soybean height reduction from dicamba exposure at V3 was 20% in this study (Table 3.4), which is similar to a previous report of 23% height reduction (Kelley et al. 2005). Dicamba exposure at R1 resulted in the greatest height reduction of 42% (Table 3.4). Labeled POST herbicide combinations alone applied at either V3 or R1 resulted in 5% height reduction overall (Table 3.4). Soybean height at maturity was reduced by 18, 21, and 22% compared with no labeled POST herbicide when lactofen + glyphosate, acetochlor + lactofen + glyphosate, and 2,4-DB + lactofen + glyphosate were applied, respectively, regardless of the timing of dicamba exposure (Table 3.5).

3.4.4 Soybean Grain Yield

In 2017, soybean yield potential was limited by a later planting date compared with 2018. The record-breaking county average soybean yield in 2018 was 13% greater than 2017 in the county where the research was conducted (USDA-NASS 2019). The average yield of nontreated control plots in 2017 was 9.4% less than in 2018, following the trend of county average soybean yield. The later planting date in 2017 can account for some disparity in yields between the two years (Pedersen and Lauer 2003). Yield reductions occurred as a result of POST herbicides alone during the 2017 growing season in contrast to studies documenting yield response of soybean to the individual herbicides used in this study (Barker et al. 1984, Beam et al. 2018, Jhala et al. 2015, Lich et al. 1997, Wichert and Talbert 1993). In 2017, soybean yield was reduced 37% when dicamba exposure occurred at R1 in the absence of labeled POST herbicide application (Table 3.6). When labeled POST herbicides were applied at V3 prior to dicamba exposure at R1, no additional yield reduction was observed. When dicamba exposure alone occurred at V3, soybean yield was reduced by 13% with no further reduction in yield as a result of labeled POST herbicide application at R1 (Table 3.6). Although yield reductions were observed following labeled POST herbicide applications alone in 2017, these effects were masked when dicamba exposure occurred.

In 2018, soybean yield reduction following dicamba exposure alone at R1 was 17% compared with the 37% yield reduction observed in 2017 (Table 3.6). Similar to 2017, soybean yield was not influenced by labeled POST herbicide applications made at V3 prior to dicamba exposure at R1. When labeled POST herbicides were applied at R1 following dicamba exposure at V3, no yield reduction was observed compared with the dicamba-only control. These results could be expected, as soybean injury from the labeled POST herbicides used in this experiment is often transient in nature and does not result in yield reduction (Barker et al. 1984, Beam et al. 2018, Jhala et al. 2015, Lich et al. 1997, Wichert and Talbert 1993, Young et al. 2003). The results presented herein indicate that soybean yield response to dicamba was dictated by the timing at which exposure occurred, not the labeled POST herbicide combination used.

In conclusion, soybean injury was slightly influenced by labeled POST herbicides, with up to 14% injury resulting from POST herbicides alone at the R1 application timing. However, soybean injury was mostly influenced by dicamba exposure and the soybean growth stage at the time of exposure, with dicamba exposure at R1 resulting in the greatest observed injury. Soybean yield loss from dicamba exposure was not influenced by labeled POST herbicides applied prior to or following dicamba exposure. Soybean yield response to dicamba was determined by the timing at which dicamba exposure occurred. As a result, the choice of labeled POST herbicides should not be limited based on the potential for soybean exposure to dicamba and any concerns for accentuating soybean injury from a previous exposure to dicamba. Soybean response to dicamba was independent of the labeled POST herbicide applied prior to or following exposure, and more related to the combined effects of the environment, timing of exposure, and dose. Further research evaluating these interactions in 2,4-D- and dicamba-resistant soybean in which metabolism confers herbicide resistance is necessary to determine if a similar response could be expected independent of the intrinsic herbicide resistance mechanism. The ability of 2,4-D- or dicamba-resistant soybean to metabolize 2,4-D or dicamba may be reduced by exposure to off-target dicamba or 2,4-D prior to, during, or following applications of labeled 2,4-D or dicamba tank-mixes.

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Table 3.1. Sources of commercial herbicides used in field experiments.

Trade Name	Active Ingredient	Manufacturer and website
Butoxone® 7500	2,4-DB	S.R.F.A., LLC Lake Success, NY. N/A
Classic®	Chlorimuron-ethyl	Corteva Agriscience. Wilmington, DE. www.corteva.us
Cobra®	Lactofen	Valent USA Corp. Walnut Creek, CA. www.valent.com
Roundup PowerMAX®	Glyphosate	Bayer Crop Science. Research Triangle Park, NC. www.cropscience.bayer.com
Warrant®	Acetochlor	Bayer Crop Science. Research Triangle Park, NC. www.cropscience.bayer.com
XtendiMax®	Dicamba	Bayer Crop Science. Research Triangle Park, NC. www.cropscience.bayer.com

Table 3.2. Description of herbicide treatments and application rates used in tank-mixtures.

Herbicide[a]	Rate (g ai or ae ha^{-1})	Adjuvant[b]
None	-	-
Dicamba	5.6	-
Glyphosate	1120	AMS
Chlorimuron-ethyl + glyphosate	8.8 + 1120	AMS
Lactofen + glyphosate	220 + 1120	AMS, MSO
Acetochlor + lactofen + glyphosate	1260 + 220 + 1120	AMS, MSO
2,4-DB + lactofen + glyphosate	35 + 220 + 1120	AMS, MSO

[a] Dicamba applied at the V3 growth stage followed by labeled POST herbicide at the R1 growth stage or labeled POST herbicide applied at the V3 growth stage followed by dicamba at the R1 growth stage

[b] AMS – ammonium sulfate was added at 5% v v^{-1} (N-Pak® AMS Liquid, Winfield Solutions, LLC, St. Paul, MN); MSO – methylated seed oil was added at 1% v v^{-1} (MSO Ultra™, Precision Laboratories, LLC, Waukegan, IL)

Table 3.3. Soybean injury as influenced by the interaction of herbicide and application timing at 28 days after the R1 application in 2017 and 2018.

		Dicamba exposure R1		Dicamba exposure V3	
Year[a]	Herbicide combination	Labeled POST only V3	Labeled POST V3 fb[b] dicamba R1	Labeled POST only R1	Dicamba V3 fb labeled POST R1
		%			
2017	none	0 f	25 a-d	0 f	9 e
	gly	0 f	26 a-c	0 f	8 e
	chlor + gly	0 f	30 a	1 f	9 e
	lac + gly	0 f	28 ab	7 e	13 de
	aceto + lac + gly	0 f	32 a	9 e	15 c-e
	2,4-DB + lac + gly	0 f	34 a	12 e	16 b-e
2018	none	0 g	37 ab	0 g	4 f
	gly	0 g	36 b	0 g	5 ef
	chlor + gly	0 g	39 ab	0 g	5 ef
	lac + gly	0 g	41 ab	9 e	9 e
	aceto + lac + gly	0 g	41 ab	14 d	16 d
	2,4-DB + lac + gly	5 ef	46 a	14 d	25 c

[a] Means within a year followed by the same letter are not statistically different according to Tukey's HSD (honestly significant difference) test ($P \leq 0.05$).

[b] Abbreviations: aceto, acetochlor; chlor, chlorimuron-ethyl; fb, followed by; gly, glyphosate; lac, lactofen. Refer to Table 3.2 for herbicide rates and adjuvants.

Table 3.4. Soybean height reduction and Behrens and Lueschen scale estimate 28 days after the R1 application and soybean height at physiological maturity as a percentage of the nontreated control by dicamba exposure and dicamba exposure timing in 2017 and 2018.

Response	Year[a]	Dicamba exposure timing	Dicamba exposure	
			Yes	No
			------------- % -------------	
Behrens and Lueschen scale estimate	pooled	V3	10 b	0 c
		R1	40 a	0 c
Height in season	2017	V3	14 b	1 c
		R1	33 a	0 c
	2018	V3	17 b	0 c
		R1	44 a	0 c
Height at maturity	pooled	V3	80 b	95 a
		R1	58 c	95 a

[a] Treatment means within a year or response followed by the same letter are not statistically different according to Tukey's HSD (honestly significant difference) test ($P \leq 0.05$).

Table 3.5. Soybean height reduction at 28 days after the R1 application in 2017 and 2018 and soybean height at physiological maturity as a percentage of the nontreated control by labeled POST herbicide combination regardless of dicamba exposure or timing.

Labeled herbicide applied with dicamba[b]	Height reduction at 28 days after R1[a]		Height at maturity
	2017	2018	
	--------------- % ---------------		% of nontreated
None (dicamba alone)	3 c	7 b	86 a
Glyphosate	6 bc	7 ab	83 ab
Chlorimuron-ethyl + glyphosate	5 bc	7 ab	86 ab
Lactofen + glyphosate	9 b	9 ab	82 bc
Acetochlor + lactofen + glyphosate	10 ab	10 ab	79 cd
2,4-DB + lactofen + glyphosate	16 a	12 a	78 d

[a] Treatment means within a year followed by the same letter are not statistically different according to Tukey's HSD (honestly significant difference) test ($P \leq 0.05$).

Table 3.6. Soybean grain yield as influenced by herbicide and application timing in 2017 and 2018.

Year[a]	Labeled herbicide applied with dicamba	Dicamba exposure R1		Dicamba exposure V3	
		Labeled POST only V3	Labeled POST V3 fb[b] dicamba R1	Labeled POST only R1	Dicamba V3 fb labeled POST R1
		------------------------------ % of nontreated ----------------------------			
2017	none	100 a	63 f-i	100 a	87 a-e
	gly	94 ab	64 f-i	91 a-c	88 a-e
	chlor + gly	95 ab	62 g-i	95 ab	88 a-d
	lac + gly	86 a-e	65 f-i	78 b-g	70 e-i
	aceto + lac + gly	84 a-e	53 i	74 b-h	80 b-f
	2,4-DB + lac + gly	78 b-g	56 hi	72 d-i	76 c-g
2018	none	100 a-e	83 c-e	100 a-e	99 a-e
	gly	101 a-d	82 de	105 a	103 a-c
	chlor + gly	104 ab	84 a-e	100 a-e	94 a-e
	lac + gly	105 a	81 e	97 a-e	97 a-e
	aceto + lac + gly	93 a-e	83 de	97 a-e	82 a-e
	2,4-DB + lac + gly	98 a-e	83 b-e	96 a-e	85 a-e

[a] Treatment means followed by the same letter within each year are not statistically different according to Tukey's HSD (honestly significant difference) test ($P \leq 0.05$).

[b] Abbreviations: aceto, acetochlor; chlor, chlorimuron-ethyl; fb, followed by; gly, glyphosate; lac, lactofen. Refer to Table 3.2 for herbicide rates and adjuvants.

CHAPTER 4. THE EFFECTS OF APPLICATION FACTORS ON DICAMBA VOLATILIZAITON IN A CONTROLLED ENVIRONMENT

4.1 Abstract

Soybean exposure to the off-target movement of dicamba has been a major concern of dicamba-sensitive soybean growers during the 2017, 2018, and 2019 growing seasons. Although restrictions continue to expand on how dicamba may be applied postemergence to dicamba-resistant soybean, the issue related to off-target movement of dicamba is still prevalent among state regulatory agencies. No conclusion has been reached regarding the extent that dicamba volatilization contributes to this off-target movement; however, the factors that contribute to dicamba volatilization continue to be explored to identify potential causes that can be managed. Controlled environment experiments were conducted to quantify the effects of the following on the relative volatilization of three dicamba formulations from dicamba-resistant soybean: 1) spray additives sold as drift reduction agents, 2) spray solution ions that may be found in water supplies used as spray carrier, 3) a range of spray solution pH, and 4) suspended soil in carrier water. Dicamba diglycolamine (DGA), diglycolamine with VaporGrip® (DGA + VG), or N,N-Bis-(3-aminpropyl) methylamine) (BAPMA) was applied to dicamba-resistant soybean at a rate of 560 g ae ha^{-1} and placed into a closed chamber where air sampling was used to collect dicamba that volatilized for a duration of 48 h. Drift reduction additives resulted in no increase of dicamba volatilization compared with dicamba alone across all three formulations. Additionally, suspended high organic matter or high clay soil in the spray solution did not result in increased volatilization compared with dicamba alone. At an adjusted spray solution pH of 3.0, dicamba volatilization was increased 2.8X and 3.9X for the DGA + VG and BAPMA formulations, respectively, compared with each respective dicamba formulation applied alone at a native pH of 5.4 and 6.4, respectively.

Spray solution pH levels of 4, 5, and 6 were not different from dicamba alone with a native pH for the BAPMA and DGA + VG formulations. The presence of diammonium sulfate (AMS) and ferrous sulfate in the carrier water resulted in volatilization increases of 5X and 9X for the DGA + VG formulation, respectively, and 11X for the BAPMA formulation compared with dicamba alone. This increase in volatilization was not caused by pH, as the average native pH across BAPMA and DGA + VG dicamba alone in the ions trials was 5.96 while the average for AMS and ferrous sulfate across the same formulations were at pH 6.14 and 5.32, respectively, much higher than either formulation at pH 3 which caused nearly a 3X and 4X increase, respectively. This increase in volatilization cannot be attributed to sulfate anion, either, as numerous salts containing the sulfate anion were tested without similar increases in volatilization. Application factors, especially spray solution pH and ion content, can cause increased dicamba volatilization and care should be taken by applicators to understand the characteristics of their spray solution when applying dicamba.

4.2 Introduction

The increase in dicamba applications for weed management in soybean production in the United States has heightened concern for the off-target movement of dicamba to sensitive crops. Numerous factors can contribute to off-target movement, including spray tank contamination, physical spray drift, spraying into temperature inversions, and herbicide volatilization (Anonymous 2019a, Maybank et al. 1978, Osborne et al. 2015). Regardless of the source, dicamba off-target movement has been the cause of numerous, annual complaints to state regulatory agencies by dicamba-sensitive crop growers ever since the first year of registration for over-the-top use of dicamba in dicamba-resistant soybean in 2017 (Bradley 2018).

The use of a drift reduction agent (DRA) when certain tank-mix partners are applied with dicamba is one strategy that has been mandated by the registrants of current dicamba formulations to reduce the potential for off-target movement (Anonymous 2019a, 2019b). The DRA products approved for use with dicamba function by increasing the viscosity of the spray solution, ultimately decreasing the proportion of drift-prone droplets (less than 150 μm in diameter) while increasing the overall volume median diameter (VMD) of the spray cloud (VanGessel and Johnson 2005). The three basic types of adjuvant chemistry used in these DRA products to alter the spray solution in this manner include hydroxypropyl guar, polyacrylamide, and polyvinyl polymer (Young et al. 2016). As a result of modifying the physical spray characteristics of the spray solution, the spray pattern may be altered by the use of a DRA (Fietsam et al. 2004). In addition to affecting the spray pattern, Petersen et al. (1985) reported that a polyvinyl polymer-based DRA increased the absorption of dicamba in some instances.

An emphasis on the spray solution pH has also been identified as a factor that may contribute to the off-target movement of dicamba (Anonymous 2019a, 2019b). Dicamba herbicide labels indicate that a low spray solution pH (pH < 5.0) may increase the potential for dicamba volatilization. The premise for this caution is the lower spray pH may favor the formation of dicamba acid, which is the volatile species, and may result in greater levels of secondary off-target movement. Mueller and Steckel (2019) reported that the pH level of dicamba spray solutions could be influenced by the initial pH of the carrier water as well as the addition of glyphosate to the spray solution.

Water quality factors such as dissolved cations and suspended soil particles have been documented to influence herbicide efficacy. Nalewaja and Matysiak (1993) reported that hard water containing 0.02 M calcium chloride reduced dicamba dimethylamine efficacy on kochia

(*Kochia scoparia*). The formation of a herbicide complex with dissolved cations in the spray solution has reduced foliar absorption of herbicides (Thelen et al. 1995). Herbicides may also become adsorbed to soil particles in water and form complexes that reduce the availability of herbicides to plants (Oschwald 1972). With up to 15% of dicamba dimethylamine remaining on soybean leaves 60 h after treatment (Petersen et al. 1985), any additional reduction in foliar absorption into target plants caused by hard water contamination or suspended soil particles may result in an increase in dicamba remaining on the leaf surface.

The volatilization of dicamba has been characterized by numerous studies, evaluating parameters such as dicamba salt formulation, spray solution pH, treated surface characteristics, and air temperature (Behrens and Lueschen 1979, Egan and Mortensen 2012, Mueller et al. 2013, Oseland et al. 2018, Sciumbato et al. 2004, Strachan et al. 2010). These studies have employed various techniques to investigate dicamba volatilization, including field, greenhouse, and growth chamber studies utilizing air sampling equipment and/or sensitive bio-indicator plants. While all of these techniques can provide information to help better understand dicamba volatilization, research conducted by Ouse et al. (2018) indicated that growth chamber studies in combination with air sampling can provide a relatively rapid and quantitative evaluation of relative differences in herbicide volatilization.

The objectives of this research were to determine the effect of DRAs, spray solution pH, spray solution ions, and suspended soil in solution on dicamba volatilization from three dicamba formulations applied to dicamba-resistant soybean plants in a controlled environment.

4.3 Materials and Methods

4.3.1 Plant Propagation and Herbicide Application

Controlled environment studies were conducted to quantify the effects of dissolved ions in spray solution, DRAs, spray solution pH, and suspended soil in the spray solution on dicamba volatilization from dicamba-resistant soybean plants. Dicamba-resistant soybeans (Asgrow® 30X8, Bayer Crop Science, Research Triangle Park, NC) were grown in a greenhouse with a 16-h light period that was maintained at an average temperature of 27 C and watered daily. Soybeans were planted at a depth of 1.5 cm in a 10- by 10-cm pot in a 2:1 potting soil (Pro-Mix FLX, Premier Horticulture Inc., Quakertown, PA) sand medium with a final stand of one plant per pot. Pots were fertilized once (Jack's Professional 20-20-20 General Purpose Fertilizer, JR Peters Inc Allentown, PA) when the soybeans reached the unifoliate stage. At the V2 soybean growth stage (two fully developed trifoliate leaves), plants were sprayed using a single-nozzle, track-mounted research sprayer calibrated to deliver 140 L ha^{-1} using a Turbo Teejet Induction 11002 nozzle (Teejet Technologies, Springfield, IL) at a height of 51 cm above the soybean canopy. A total of six plants per application pass were placed in a line at the center of the spray pattern to ensure uniform coverage and sprayed with dicamba at a rate of 560 g ae ha^{-1}.

The three dicamba formulations used in these experiments were the DGA, BAPMA, and DGA + VG and were mixed with deionized water as the spray carrier. The pH of each spray solution was measured prior to application with a laboratory pH meter (model FiveEasy™, Mettler-Toledo, LLC, Columbus, OH). Immediately after application, the doors of the spray booth were opened slightly for 15 s to evacuate any fine particles through a charcoal filter exhaust system. Plants were then transferred to a separate room where aluminum foil was used to cover the soil surface, preventing excessive water evaporation and dicamba volatilization from the soil during the experiment. After pots were covered, they were transferred into vapor chambers (Figures 4.1

and 4.2). Vapor chambers were then carried to the growth chamber (model FXR-37, BioChambers, Winnipeg, Manitoba, Canada) and placed on a shelf at which time the air sampler was immediately attached to the chamber and vacuum source (Figure 4.3). The growth chamber was set to a 14-h day light cycle at a temperature of 35 C and 40% relative humidity.

4.3.2 Vapor Chambers

Vapor chambers were constructed using clear, weatherproof polypropylene storage containers (model USB-LD, IRIS USA, Inc, Pleasant Prairie, WI) modified to facilitate an air sampler and allow air flow across treated plants (Figures 4.1 and 4.2). Polypropylene was selected for the chambers since this is the same material composition for containers used to collect dicamba samples in field experiments to minimize interactions with dicamba sorption. The container lid was constructed with a polyethylene foam gasket to create an airtight and waterproof seal. A total of eight air inlet holes were drilled near the bottom of the chamber at one end, opposite the air outlet in order to provide airflow across the entire width of the chamber. The single air outlet was fitted with an air sampler housed in a nitrile rubber grommet for a secure, airtight closure near the top of the chamber (Figures 4.1 and 4.2).

Air samplers for vapor chambers were assembled using glass tubes with an inside diameter of 1.918 cm and an overall length of 10 cm (SKC catalog no. P22692G, SKC, Inc, Eighty Four, PA) containing, in order from air inlet to air outlet, a glass fiber filter (SKC catalog no. 225-702), 1080 mg of XAD-2 sorbent (SKC catalog no. P226201), a 1.9 cm-long polyurethane foam (PUF) plug (SKC catalog no. 226-92), 560 mg of XAD-2 sorbent, and a 3.8 cm-long PUF plug. A central vacuum source was used to provide air flow which would bring the conditioned air from the growth chamber through each individual vapor chamber. Each vapor chamber was connected from the air sampler through 6.35-mm inside diameter chemical-resistant tubing to an individual airflow meter (model RMA-23-BV, Dwyer Instrument, Michigan City, IN) set to 2.9 L min^{-1} on a vacuum manifold (Figure 4.3). The air flow rate was set to allow for a complete turnover of air in the vapor chamber every 20 min (Long 2017).

4.3.3 Drift Reduction Agents

Three DRA products were selected for testing, representing the three major chemical classes of commercially available products that are approved for application with dicamba in dicamba-resistant crops: hydroxypropyl guar, polyvinyl polymer, and polyacrylamide. The polyacrylamide adjuvant also included an alkyl polyglucoside (APG) surfactant. The DRA products tested were applied at the maximum use rate according to the DRA label and mixed in order according to the dicamba product label. Each of the three DRAs were tested with each of the three dicamba formulations in a separate experiment along with dicamba alone. No comparisons across dicamba formulations were made for any experiment due to space limitations in the growth chamber preventing simultaneous testing. After spraying plants with DRA + dicamba spray solutions, four 90-mm qualitative filter paper discs were placed on inverted petri dishes in the center of the spray path, in the same position as the plants, and sprayed with the same solution as plants in order to determine the total applied herbicide per unit area. Filter papers were removed from the spray chamber and placed into 50-ml centrifuge tubes that were subsequently filled with 50 ml of methanol and stored at -20 C until processing.

4.3.4 Suspended Soil

In order to generate water for suspended soil experiments, two samples of field soil were collected, representative of a high clay (45% clay) and high organic matter (6.2% organic matter) soil type. Field soil was dried, ground, and passed through a 0.71-mm screen in order to produce a uniform sample. The target for the high turbidity sample was 110 nephelometric turbidity units, which was chosen based on the measured turbidity level of the Wabash River at West Lafayette, IN on June 1, 2019. High turbidity water for high organic matter (OM) and high clay soils were generated by adding 0.396 g and 0.308 g soil, respectively, to 400 mL of deionized water and mixing with a magnetic stir bar for 5 min. Low turbidity samples were mixed with 100 mL of high turbidity sample and 100 mL of deionized water, resulting in a 50% concentration of soil in the low turbidity mixtures.

4.3.5 Spray Solution pH

The spray solution pH levels tested were 3.0 to 8.0 in increments of 1.0 pH unit, in addition to dicamba alone at its unadjusted pH (native) for a total of seven experimental treatments. Each of the three dicamba formulations were tested in separate experiments. The adjustment of spray pH was achieved by adding a solution of reagent-grade 0.1 M HCl or 0.07 M NaOH into the spray solution and titrating until the desired solution pH was achieved (Roskamp and Johnson 2013). A preliminary experiment indicated that the pH of each solution prepared with this method was stable for a period of 2 h, which exceeded the maximum time from mixing to spraying during any experiment.

4.3.6 Spray Solution Ions

Spray solution ions tested (Table 4.4) were selected based on ions tested by Nalewaja and Matysiak (1991) in order to examine the effects of both hard water cations and anions on dicamba volatilization. Five spray solution ion treatments were tested along with dicamba alone for a total of six experimental treatments per dicamba formulation. A separate experiment was conducted for each of the three dicamba formulations. Ammonium sulfate (AMS) was included in this experiment to quantify the increase in dicamba volatilization known to occur with this adjuvant (Anonymous 2019a, 2019b). Ions tested in water quality experiments were American Chemical Society-grade and thoroughly mixed into the carrier water at a concentration of 0.02 M cation prior to the addition of dicamba (Nalewaja and Matysiak 1991). The cation concentration in this experiment was held constant in order to focus on the influence of cations on dicamba volatilization, independent of concentration.

4.3.7 Sample Processing and Quantification

Air sample tube processing began with rinsing the sampler exterior with methanol to remove any potential dicamba contamination during sampler handling. Sample tube media were removed from the sampler housing by pressing the material into a 50-ml polypropylene centrifuge tube (model 352070, Corning Inc, Corning, NY) using a wooden dowel that was disposed of after use for a single sampler. A total volume of 40 ml of methanol was used to rinse the inside of the air sampler housing into the centrifuge tube and samples were stored at -20 C prior to processing for liquid chromatography – mass spectrometric (LC-MS) analysis. At the time of sample processing for the LC-MS analysis, 50 µl of 100 µg ml^{-1} deuterium-labeled (D3) dicamba was added to each sample as an internal standard and samples were shaken overnight at 4 C. After shaking, the total volume of methanol from each sample was carefully transferred into a new 50-ml centrifuge tube, concentrated to dryness under N_2 gas at room temperature, and re-suspended to a final volume of 100 or 400 µl in methanol. In contrast to air samplers, it was not necessary to concentrate deposition samples due to the high concentration of dicamba in the solution. For the filter paper deposition samples collected during DRA experiments, 25 µl of 100 µg ml^{-1} internal standard was added to 475 µl of each sample. Both sample types were subjected to the same isotope-ratio based internal quantification.

The LC-MS analysis was performed using an Agilent 1290 Infinity II liquid chromatography (LC) system with diode array detection coupled to an Agilent 6135 single quadrupole mass spectrometer with a jetstream electrospray ionization (ESI) source (Agilent USA, Santa Clara, CA). A total of 20 µl of sample volume was injected into the system. The LC separation was performed on an SB C18 column (1.8 µm, 2.1 by 50 mm; Agilent USA). Mobile phase solvents were LC-MS grade water (A) and acetonitrile (B) both with 0.1% formic acid at a flow rate of 0.3 ml min^{-1}. The gradient program started with 20% B with a 2 min hold, followed by a linear gradient to 75% B over 10 min with a hold of 1 min. The gradient program was then reversed back to the initial condition of 20% B at 12 min with a final hold of 1 min. Total runtime of the method was 13 min, in which dicamba was eluted at 4.6 min. The column temperature was maintained at 30 C. The jetstream ESI source was operated in negative ion and selected ion monitoring (SIM) mode. The fragment ions m/z 175 and 178 showing the loss of M-COOH- were monitored for dicamba and D3-dicamba, respectively, and the peak areas used for quantification. These ions were selected for maximum sensitivity. Sheath gas temperature was set at 360 C with the flow at 13 ml min^{-1} and drying gas temperature was kept at 350 C with a flow of 12 ml min^{-1}.

For the internal quantification, 190 µl of dicamba at five concentrations prepared by serial dilution (10, 5, 2.5, 1.25, and 0.625 µg ml^{-1}) were mixed with 10 µl of 100 µg ml^{-1} D3-dicamba solution in order to calculate average relative response factor (RRF). All standards were analyzed with same LC-MS method used for the analysis of experimental samples. The response factor (RF) of dicamba and D3-dicamba were calculated separately by dividing peak area by concentration and the RRF was calculated by dividing RF of dicamba by the RF of D3 dicamba. The RRFs were calculated for all five calibration levels and the average RRF was used in the calculation of the dicamba quantity in experimental samples.

4.3.8 Statistical Analysis

Each experiment was designed as a randomized complete block design with four replications. Each experiment was conducted twice. Dicamba amounts from deposition samples collected during DRA experiments were used to normalize the amount of dicamba volatilization per amount of dicamba applied relative to the dicamba alone deposition sample. Dicamba applied per filter paper and air concentration (µg m^{-3}) were subjected to single factor ANOVA using Proc GLIMMIX in SAS ver. 9.4 (SAS Institute, Cary, NC). Data were checked for normality and homogeneity of variance prior to analysis. Replication was considered random and interactions between treatment and run were evaluated and found to be non-significant. Means separation was performed using Tukey's HSD test at α=0.05.

4.4 Results and Discussion

4.4.1 Drift Reduction Agents

The amount of dicamba detected from deposition samples on filter paper varied between DRAs added to the spray mixture. Notably, polyacrylamide + APG resulted in an average of 2.7X more deposition due to a reduced width of the spray pattern concentrating more spray solution into the center of the spray path. Across the three dicamba formulations and DRAs, only the polyacrylamide + APG product caused this pronounced increase in deposition.

The volatilization of dicamba DGA alone resulted in a dicamba air concentration of 4590 ng m^{-3} (Table 4.2). Concentrations of 2569, 3309, and 3946 ng m^{-3} resulted from the polyacrylamide + APG, hydroxypropyl guar, and polyvinyl polymer DRA treatments, respectively. With respect to the DGA formulation, all DRAs resulted in similar air concentrations compared with the dicamba alone treatment. For the DGA + VG formulation alone the air concentration was 1696 ng m^{-3}. While the inclusion of a DRA did not alter the volatilization of DGA + VG dicamba compared with dicamba alone, differences were apparent among the DRAs themselves. The addition of polyacrylamide + APG reduced volatilization resulting in an air concentration of 879 ng m^{-3} compared with hydroxypropyl guar and polyvinyl polymer DRAs (1974 and 2071 ng m^{-3}, respectively). Similar to the DGA formulation, BAPMA dicamba alone resulted in the highest concentration of dicamba at 2301 ng m^{-3} compared with the applications containing a DRA. While the air concentrations of dicamba with hydroxypropyl guar and polyvinyl polymer DRAs was similar to dicamba alone at 1456 and 1765 ng m^{-3}, respectively, the use of polyacrylamide + APG DRA decreased volatilization to a concentration of 798 ng m^{-3} compared with dicamba alone and the polyvinyl polymer DRA.

The polyacrylamide + APG product used in this experiment also contains a surfactant, which may have resulted in increased absorption of dicamba into the soybean plants (Petersen et al. 1985). In theory, if less dicamba remains on the leaf surface, less will be capable of volatilizing from the leaf surface. The effects of the surfactant in the polyacrylamide + APG DRA may have resulted in increased absorption of the BAPMA salt to a greater extent than the DGA salt found in the DGA and DGA + VG formulations. Regardless, our research provides no evidence that the commercial use of DRA products with dicamba formulations would contribute to greater volatilization of dicamba.

4.4.2 Suspended Soil

Dicamba volatilization resulted in an air concentration of 4217 ng m^{-3} for the dicamba DGA formulation. This dicamba concentration was similar across both the high OM and high clay soil types (Table 4.3). Although at a lower concentration (2860 ng m^{-3} for dicamba alone), the DGA + VG formulation exhibited similar characteristics to the DGA formulation with no differences across soil types or concentrations. The dicamba-alone concentration for the BAPMA formulation was 5362 ng m^{-3}. This concentration was similar to all soil types and concentrations tested. However, the high concentration of the high OM soil increased dicamba volatilization (7052 ng m^{-3}) compared with the high concentration of the high clay soil (4251 ng m^{-3}). Although herbicides may interact with suspended soil in the spray solution (Oschwald 1972), these potential interactions did not demonstrate any increase in dicamba volatilization compared with the respective dicamba formulation in clean, deionized water.

4.4.3 Spray Solution pH

The air concentration resulting from DGA dicamba alone at a native pH was 1933 ng m^{-3} (Table 4.4). Across the pH range of 4 to 8, including the native pH, dicamba volatilization was similar for the DGA formulation. The effect of solution pH was less pronounced for the DGA formulation than the DGA + VG and BAPMA formulations. The application of DGA + VG dicamba resulted in an air concentration of 4394 ng m^{-3}. Applying DGA + VG dicamba at a spray solution pH of 3 resulted in a 2.8X increase in volatilization (12189 ng m^{-3}) compared with dicamba DGA + VG at native pH. From the pH range of 4 to 6, including at native pH, dicamba DGA + VG was similar. The air concentration of dicamba following the application of BAPMA dicamba was 2649 ng m^{-3}. The volatilization of BAPMA dicamba was increased by 3.9X to 10437 ng m^{-3} compared with BAPMA at native pH. As pH increased from 4 to 6.4 (native pH of BAPMA), no difference in dicamba volatilization was observed. Across all three formulations tested, the pH range of 4 to 6 had no influence on volatilization and was similar to dicamba at the native pH for each formulation. Only pH 3 resulted in consistently greater volatilization than other levels, regardless of dicamba formulation tested. The BAPMA and DGA + VG formulations demonstrated the lowest amount of volatilization with the higher pH levels of 7 and 8, but these levels would rarely be observed in commercial applications (Mueller and Steckel 2019). Our research, using dicamba-resistant soybean as the primary spray target, indicates that even though a lower spray solution pH would theoretically favor the formation of dicamba acid and a concomitant increase in volatilization, the spray pH would need to approach 3.0 before an increase in volatilization occurs. The pH of spray solutions in commercial applications would rarely be this acidic unless strong acids or buffering agents are intentionally used to reduce the pH. Additionally, the pH of the spray target can influence relative dicamba volatilization. Following a dicamba application to field soil adjusted to a range of pH levels from 4.3 to 8.3, as pH decreased the level of volatilization increased (Oseland et al. 2018). It is possible that the leaf surface of a dicamba-resistant soybean plant is capable of influencing dicamba volatilization in a similar manner. If the leaf surface has a buffering capacity which can increase the pH of an acidic spray deposit, this could prevent the reduced pH spray solution from evolving dicamba acid and subsequent volatilization.

4.4.4 Spray Solution Ions

Of the ions tested, both AMS and ferrous sulfate resulted in drastic increases in dicamba volatilization across all formulations. The increase in volatility from the addition of ferrous sulfate to deionized water was 9 to 16x across all three dicamba formulations (Table 4.5). Likewise, the increase in volatility with the addition of AMS to deionized water was 5 to 14X across all formulations of dicamba. The presence of calcium sulfate, magnesium sulfate, and calcium chloride did not increase dicamba volatility. However, calcium chloride did reduce volatility for the DGA + VG formulation (Table 4.5). Based on the average pH levels of AMS (6.3), ferrous sulfate (5.1), and dicamba only (6.1) spray solutions across all three dicamba formulations, spray solution pH could not be the main factor driving dicamba volatilization for these ions. Even with reductions in pH when ferrous sulfate was present in the spray solution, these pH levels were within the label requirements the BAPMA and DGA + VG formulations (Anonymous 2019a, 2019b). Furthermore, the sulfate anion cannot be implicated for the increase in dicamba volatility since calcium sulfate and magnesium sulfate did not influence volatility.

Although a constant cation concentration (0.2M) was used in this experiment to reduce confounding across treatments, the concentrations of ions in this experiment are generally high compared with those found in Indiana groundwater sources. For all cations and anions except ammonium, iron, and magnesium, the concentration used in this experiment was within 2X of maximum values reported by a survey conducted by the Indiana Department of Natural Resources (IDNR 1999). The maximum iron concentration reported by this survey was 12.6 mg L^{-1}, while the iron concentration in this experiment was 1117 mg L^{-1}, an 89X increase compared with water conditions throughout the state.

Although the tested concentration of iron was high compared with ambient water sources, iron salts can be found in foliar-applied nutrient products used in crop production. One such iron-containing product (Brandt® Smart Fe) that is currently labeled for use with both DGA + VG and BAPMA dicamba contains 827 mg L^{-1} of iron derived from ferrous sulfate at the labeled rate (Anonymous 2020). Thus, some commercial applications of dicamba have the potential to include these higher rates of iron in which our research demonstrated a marked increase in dicamba volatilization. Future research on the effects of iron in the spray solution should be targeted towards plant nutritional products containing iron. In contrast to iron, AMS was tested at a much lower concentration (1.3 g L^{-1}) compared with a commercial standard rate for conditioning hard water (2% w w^{-1} or 20.4 g L^{-1}). The low AMS concentration compared to commercial use rates used herein demonstrates that even a small amount of AMS can cause a drastic increase in dicamba volatilization across dicamba formulations if present during a commercial application of dicamba.

More research is needed to determine if the effects of a full rate of AMS would have any additional effect on dicamba volatilization.

Application factors can have an influence on dicamba volatilization in a controlled environment. The use of drift reduction agents does not appear to contribute towards any increase in dicamba volatilization from soybean plants. Similarly, no increase in dicamba volatilization was observed following an application of dicamba with high clay or high OM soil suspended in the spray solution. This study of spray solution pH indicated that a spray solution pH of 3.0 can cause an increase in dicamba volatilization, indicating that pH does in fact play a role in the volatilization of dicamba. In this study only dicamba and a pH modifier were added to the spray solution, while in reality, dicamba would rarely be the only herbicide in a spray solution. The addition of tank-mix partners to dicamba spray solutions can influence the solution pH (Mueller and Steckel 2019). In addition to an effect of pH, certain spray solution ions had a pronounced effect on dicamba volatilization. Ferrous sulfate and AMS increased dicamba volatilization and cannot be explained by a change in the spray solution pH or the sulfate anion. The relative differences in volatilization at pH 3 and native pH for BAPMA and DGA + VG were 4X and 3X, respectively, while the relative differences between AMS spray solutions and BAPMA or DGA + VG alone were 11X and 5X, respectively. Thus, the influence of a pH as low as 3 did not increase dicamba volatilization as much as AMS.

Future research on the mechanism of the interaction between AMS and ferrous sulfate with dicamba should be investigated. Testing of different concentrations and different salts containing ammonium and iron ions could yield results further elucidating this mechanism. Aluminum sulfate is also used in soil management and may interact with dicamba similar to iron sulfate. Thus, testing dicamba volatilization in the presence of aluminum sulfate would help discern if the interaction with iron sulfate is specific to iron, or is similar across different metal cations. Since the addition of glyphosate to spray solutions with dicamba formulations can reduce the spray pH (Mueller and Steckel 2019), the impact of glyphosate on dicamba volatilization when applied to soybean leaf surfaces should be conducted. Furthermore, the volatilization of dicamba over a spray solution pH gradient and in the presence of spray solution ions should be tested on glass slides to separate chemical interactions from a biological effect on the leaf surface observed in this research. Although direct comparisons between formulations were not allowed due to the experimental design, the trends in the experimental factors for DRAs, a pH gradient, spray water turbidity, and spray solution ions were generally consistent across the three formulations. In other words, the same factors that influence volatility for an older formulation, such as dicamba DGA, are still relevant to the new dicamba DGA + VG and BAPMA formulations. Thus, the development of these formulations were based on overcoming some other factors associated with dicamba volatilization rather than the factors tested in our research.

4.5 Literature Cited

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Table 4.1. Sources of commercial herbicides used in controlled environment experiments.

Trade Name	Active Ingredient (s)	Manufacturer and website
Clarity®	Dicamba	BASF Corp. Research Triangle Park, NC. www.basf.com
Engenia®	Dicamba	BASF Corp. Research Triangle Park, NC. www.basf.com
Intact™	Polyethylene glycol, choline chloride, guar gum	Precision Laboratories LLC, Waukegan, IL www.precisionlab.com
Leeway II™	Trisodium citrate dihydrate, alkyl polyglucoside C9-11, diethylene glycol	KALO, Inc, Overland Park, KS www.kalo.com
Reign®	Polyvinyl polymer	Loveland Products, Inc Greenley, CO www.lovelandproducts.com
XtendiMax®	Dicamba	Bayer Crop Science. Research Triangle Park, NC. www.cropscience.bayer.com

Table 4.2. Effect of drift reduction agents on relative dicamba air concentration over 48 hours at 35 C and 40% relative humidity following application of three dicamba salt formulations at 560 g ae ha^{-1} to dicamba-resistant soybeans.[a]

Drift reduction agent	DGA[b]	DGA + VG	BAPMA
	----------------------------	ng m^{-3}	----------------------------
None	4590 a	1696 ab	2301 a
Polyacrylamide + APG	2569 a	879 b	798 b
Hydroxypropyl guar	3309 a	1974 a	1456 ab
Polyvinyl polymer	3946 a	2071 a	1765 a

[a] Treatment means within a formulation (column) followed by the same letter are not statistically different according to Tukey's HSD (honestly significant difference) test ($P \leq 0.05$).

[b] Abbreviations: APG, alkyl polyglucoside; BAPMA, N,N-Bis-(3-aminopropyl) methylamine; DGA, diglycolamine; DGA+VG, diglycolamine + "VaporGrip®";

Table 4.3. Effect of suspended soil type and concentration on relative dicamba air concentration over 48 hours at 35 C and 40% relative humidity following application of three dicamba salt formulations at 560 g ae ha^{-1} to dicamba-resistant soybeans.[a]

Soil type	Soil concentration	DGA[b]	DGA + VG	BAPMA
		-------------------------	ng m^{-3}	-------------------------
None	-	4217 a	2860 a	5362 ab
High OM	high	5170 a	3209 a	7052 a
	low	5140 a	3080 a	5744 ab
High clay	high	4523 a	2798 a	4251 b
	low	4890 a	3714 a	7001 ab

[a] Treatment means within a formulation (column) followed by the same letter are not statistically different according to Tukey's HSD (honestly significant difference) test ($P \leq 0.05$).

[b] Abbreviations: BAPMA, N,N-Bis-(3-aminopropyl) methylamine; DGA, diglycolamine; DGA+VG, diglycolamine + "VaporGrip®"; OM, organic matter;

Table 4.4. Effect of solution pH on relative dicamba air concentration over 48 hours at 35 C and 40% relative humidity following application of three dicamba salt formulations at 560 g ae ha^{-1} to dicamba-resistant soybeans.[a]

pH level	DGA[b]	DGA + VG	BAPMA
	--------------------------------	ng m^{-3}	---------------------------------
Native pH[c]	1933 ab	4394 bc	2649 bc
3.0	3468 a	12189 a	10437 a
4.0	1702 ab	4791 b	4177 b
5.0	1735 ab	5716 b	4420 b
6.0	1580 ab	5486 b	3746 bc
7.0	1013 b	3259 c	2314 c
8.0	1631 ab	3343 c	2264 c

[a] Treatment means within a formulation (column) followed by the same letter are not statistically different according to Tukey's HSD (honestly significant difference) test ($P \leq 0.05$).

[b] Abbreviations: BAPMA, N,N-Bis-(3-aminopropyl) methylamine; DGA, diglycolamine; DGA+VG, diglycolamine + "VaporGrip®";

[c] Dicamba pH levels without adjustment were as follows: DGA, 6.3; DGA + VG, 5.4; BAPMA, 6.4;

Table 4.5. Effect of spray solution salts on relative dicamba air concentration over 48 hours at 35 C and 40% relative humidity following application of three dicamba salt formulations at 560 g ae ha^{-1} to dicamba-resistant soybeans.[a]

Spray solution ion	Salt concentration Cation / Anion	DGA[b]	DGA + VG	BAPMA
	M	-------------------------	ng m^{-3}	-------------------------
None	N/A	2006 b	4887 c	4212 b
Ferrous sulfate	0.02 / 0.02	32034 a	44055 a	47363 a
Diammonium sulfate	0.02 / 0.01	27963 a	25841 b	48180 a
Calcium sulfate	0.02 / 0.02	2447 b	4581 c	3613 b
Magnesium sulfate	0.02 / 0.02	1556 b	3849 cd	3880 b
Calcium chloride	0.02 / 0.04	1062 b	3319 d	3555 b

[a] Treatment means within a formulation (column) followed by the same letter are not statistically different according to Tukey's HSD (honestly significant difference) test ($P \leq 0.05$).

[b] Abbreviations: BAPMA, N,N-Bis-(3-aminopropyl) methylamine; DGA, diglycolamine; DGA+VG, diglycolamine + "VaporGrip®";

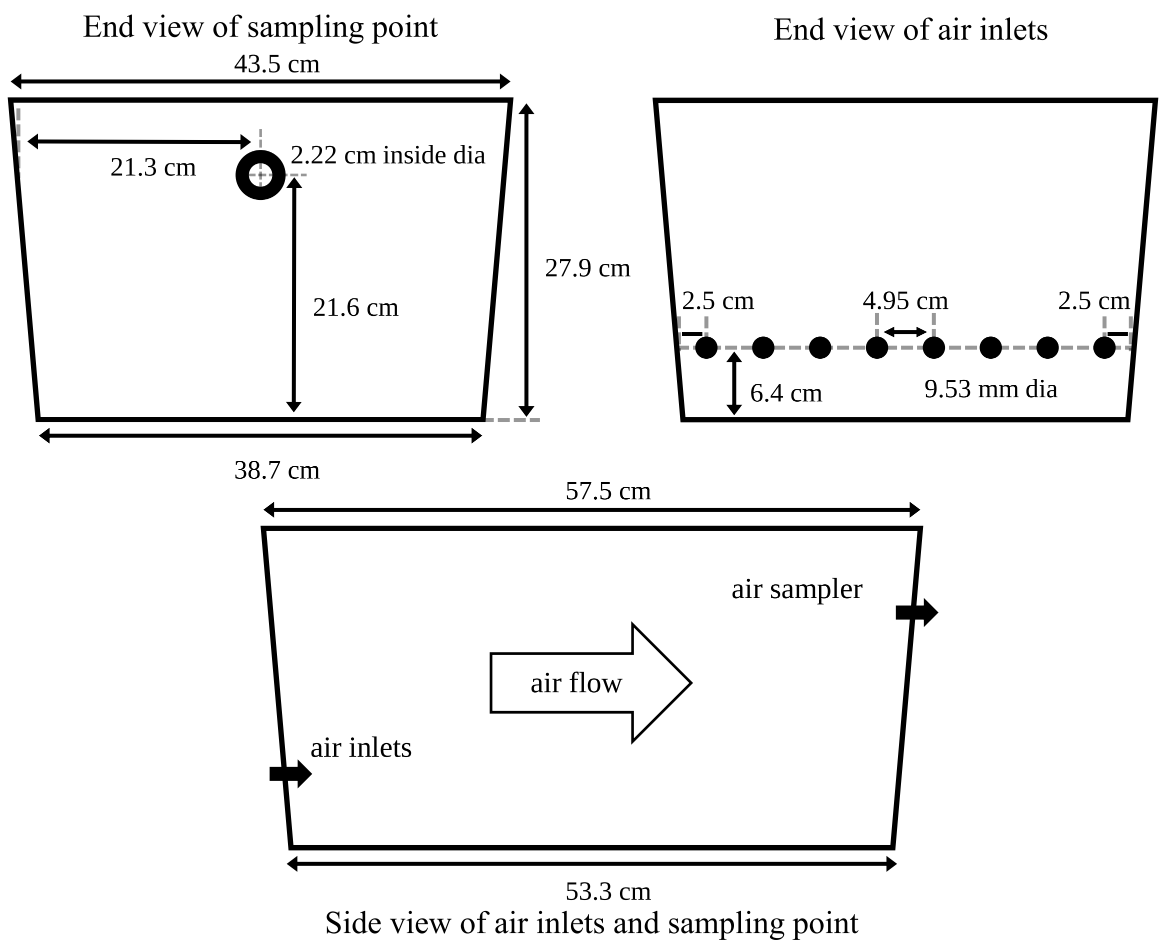

Figure 4.1. Vapor chamber diagram with side and end views with associated dimensions on size and placement of air sampler and inlet orifices.

Figure 4.2. Picture of vapor chamber with treated soybean plants following an experiment.

Figure 4.3. Picture of vapor chambers arranged in growth chamber during an experiment.

CHAPTER 5. CONCLUSIONS

In conclusion, glyphosate-resistant soybean response to 2,4-D and dicamba was largely similar to published literature regarding the subject. Dicamba-resistant soybean response to 2,4-D was also similar to the response of glyphosate-resistant soybean. If 2,4-D tank-contamination occurs when dicamba and glyphosate are being applied to dicamba-resistant soybean, applicators should be concerned primarily about the 2,4-D tank-contamination that occurred rather than the fact that dicamba was present during the application. Dicamba did not exacerbate the symptoms of 2,4-D on dicamba-resistant soybean. Future research should be conducted on the response of 2,4-D-resistant soybean to dicamba tank-contamination to determine if the 2,4-D resistance trait confers a differential soybean response to dicamba off-target movement compared with glyphosate- and dicamba/glyphosate-resistant soybean.

As the adoption of dicamba-resistant and 2,4-D-resistant soybeans continues increase, there is still a large demand for conventional soybeans that do not possess a herbicide-resistance trait along with many other types of soybean that are sensitive to both 2,4-D and dicamba. For many of these growers, herbicides that may cause crop response such as lactofen and acetochlor are critical to weed management. With the potential for injury from herbicides that are intentionally applied and the possibility of off-target dicamba movement, these growers are left facing a dilemma on what herbicides can be applied to achieve adequate weed control without compromising profitability by reducing grain yield. The results of this research determined that soybean response to dicamba in terms of grain yield would not be affected by the other labeled POST herbicide options. If a grower has dicamba injury present in their field or expects dicamba exposure to occur later in the season, they should not hesitate to apply the necessary herbicides to control the weeds present in their field. Further research evaluating these interactions in 2,4-D-and dicamba-resistant soybean in which metabolism confers herbicide resistance is necessary to determine if a similar response could be expected independent of the intrinsic herbicide resistance mechanism. The ability of 2,4-D- or dicamba-resistant soybean to metabolize 2,4-D or dicamba may be reduced by exposure to off-target dicamba or 2,4-D prior to, during, or following applications of labeled 2,4-D or dicamba tank-mixes.

Herbicide application factors can have an influence on dicamba volatilization in a controlled environment. The use of drift reduction agents does not appear to contribute towards any increase in dicamba volatilization from soybean plants. Similarly, no increase in dicamba volatilization was observed following an application of dicamba with high clay or high OM soil suspended in the spray solution. However, applicators should still consider these spray parameters as potential areas of concern for optimizing weed control. Spray solution pH of 3.0 can cause an increase in dicamba volatilization, but this low of a solution pH would rarely occur in a commercial application and would likely only be the result of intentional pH modification by the applicator. In this study only dicamba and a pH modifier were added to the spray solution, while in reality, dicamba would rarely be the only herbicide in a spray solution. Since the addition of glyphosate to spray solutions with dicamba formulations can reduce the spray pH, the impact of glyphosate on dicamba volatilization when applied to soybean leaf surfaces should be investigated. Furthermore, the volatilization of dicamba over a spray solution pH gradient and in the presence of spray solution ions should be tested on glass slides to separate chemical interactions from a biological effect on the leaf surface.

Ions in the spray solution with dicamba can be a result of the water quality used as the carrier, and can originate from surface or ground water supplies. Ions may also be added to the spray solution by the applicator when using products intended to enhance herbicide efficacy or to supply essential nutrients to the crop or soil. The addition of AMS and ferrous sulfate had a pronounced effect on dicamba volatilization that cannot be explained by a change in the spray solution pH or the sulfate anion. The influence of a spray solution pH as low as 3 did not increase dicamba volatilization as much as AMS. Future research on the mechanism of the interaction between AMS and ferrous sulfate with dicamba should be conducted. Testing of different concentrations and different salts containing ammonium and iron ions, or even aluminum, could yield results that further elucidate the chemical mechanism underlying the increased dicamba volatilization. Commercially, spray solutions may be created that inadvertently increase the potential for dicamba volatilization, such as the use of plant nutritional products containing iron. Adherence to current dicamba labels in regard to drift reduction agents, spray solution pH, and the prohibition of ammonium sulfate can effectively reduce the potential for dicamba volatilization, but other spray solution parameters may also need to be considered.

APPENDIX A. SUPPLEMENTAL DATA ANALYSIS FOR DICAMBA-RESISTANT SOYBEAN RESPONSE TO 2,4-D TANK-CONTAMINATION DURING A DICAMBA APPLICATION.

Results of data analysis for tank-contamination study for glyphosate-resistant soybean exposure to dicamba.

Parameter	Source	DF	F	P
Injury 14 DAT	rate	4	450.59	<.0001
	timing	1	33.98	<.0001
	rate*timing	4	7.49	<.0001
Injury 28 DAT	rate	4	431.22	<.0001
	timing	1	19.79	<.0001
	rate*timing	4	2.12	0.0776
BL injury 14 DAT	rate	4	427.48	<.0001
	timing	1	14.01	0.0002
	rate*timing	4	2.34	0.0551
BL injury 28 DAT	rate	4	303.42	<.0001
	timing	1	5.39	0.0208
	rate*timing	4	2.45	0.0456
Height reduction 14 DAT	rate	4	367.66	<.0001
	timing	1	19.46	<.0001
	rate*timing	4	5.72	0.0002
Height reduction 28 DAT	rate	4	305.49	<.0001
	timing	1	0.11	0.425
	rate*timing	4	1.13	0.3412
Mature height	rate	4	42.65	<.0001
	timing	1	17.29	<.0001
	rate*timing	4	3.75	0.0063
Grain yield	rate	4	94.93	<.0001
	timing	1	27.09	<.0001
	rate*timing	4	8.18	<.0001
Hundred seed mass	rate	4	5.12	0.0005
	timing	1	2.07	0.1515
	rate*timing	4	2.19	0.0716

Seeds m^{-2}	rate	4	7.58	<.0001
	timing	1	5.10	0.0252
	rate*timing	4	0.76	0.5501
Seeds per pod	rate	4	1.63	0.1653
	timing	1	13.49	0.0003
	rate*timing	4	0.83	0.5039
Pods m^{-2}	rate	4	8.67	<.0001
	timing	1	0.52	0.4708
	rate*timing	4	1.02	0.3989
Pods per node	rate	4	8.67	<.0001
	timing	1	0.77	0.3818
	rate*timing	4	0.19	0.9456
Reproductive nodes m^{-2}	rate	4	3.74	0.0061
	timing	1	2.04	0.1552
	rate*timing	4	1.97	0.1013
Percent reproductive nodes	rate	4	8.90	<.0001
	timing	1	2.03	0.1548
	rate*timing	4	11.54	<.0001
Nodes m^{-2}	rate	4	5.98	0.0002
	timing	1	2.44	0.1202
	rate*timing	4	1.11	0.3546
Cold germination	rate	4	29.79	<.0001
	timing	1	117.50	<.0001
	rate*timing	4	63.06	<.0001
Accelerated aging germination	rate	4	11.80	<.0001
	timing	1	68.62	<.0001
	rate*timing	4	22.73	<.0001
Greenhouse emergence				
4 DAP	rate	4	0.30	0.8772
	timing	1	1.09	0.3014
	rate*timing	4	0.22	0.9243
7 DAP	rate	4	0.58	0.6757
	timing	1	3.00	0.0886
	rate*timing	4	0.55	0.7022

14 DAP	rate	4	0.26	0.9022
	timing	1	1.29	0.2600
	rate*timing	4	0.53	0.7148
21 DAP	rate	4	0.44	0.7793
	timing	1	1.35	0.2506
	rate*timing	4	0.63	0.6420
Greenhouse malformed plants				
7 DAP	rate	4	0.95	0.4407
	timing	1	11.34	0.0013
	rate*timing	4	2.13	0.0881
14 DAP	rate	4	1.08	0.3751
	timing	1	7.83	0.0069
	rate*timing	4	1.54	0.2021
21 DAP	rate	4	0.7020	0.7020
	timing	1	10.95	0.0016
	rate*timing	4	1.67	0.1697

Results of data analysis for tank contamination study for glyphosate-resistant soybean exposure to 2,4-D.

Parameter	Source	DF	F	P
Injury 14 DAT	rate	4	1413.73	<.0001
	timing	1	11.30	0.0009
	rate*timing	4	5.19	0.0004
Injury 28 DAT	rate	4	414.36	<.0001
	timing	1	0.00	0.9886
	rate*timing	4	1.62	0.1679
BL injury 14 DAT	rate	4	1093.98	<.0001
	timing	1	16.45	<.0001
	rate*timing	4	6.17	<.0001
BL injury 28 DAT	rate	4	219.25	<.0001
	timing	1	2.65	0.1047
	rate*timing	4	0.64	0.6337
Height reduction 14 DAT	rate	4	604.97	<.0001
	timing	1	4.18	0.0416
	rate*timing	4	10.53	<.0001
Height reduction 28 DAT	rate	4	433.34	<.0001
	timing	1	0.00	0.9828
	rate*timing	4	1.38	0.2396
Mature height	rate	4	77.81	<.0001
	timing	1	2.43	0.1212
	rate*timing	4	1.76	0.1401
Grain yield	rate	4	118.26	<.0001
	timing	1	0.13	0.7225
	rate*timing	4	0.31	0.8685
Hundred seed mass	rate	4	3.71	0.0058
	timing	1	0.44	0.5076
	rate*timing	4	0.59	0.6717
Seeds m^{-2}	rate	4	6.84	<.0001
	timing	1	5.84	0.0167
	rate*timing	4	0.34	0.8473
Seeds per pod	rate	4	0.34	0.8476
	timing	1	14.90	0.0001
	rate*timing	4	0.69	0.5967

Pods m^{-2}	rate	4	5.96	0.0002
	timing	1	0.33	0.5665
	rate*timing	4	0.29	0.8837
Pods per node	rate	4	2.38	0.0516
	timing	1	2.76	0.0979
	rate*timing	4	1.05	0.3835
Reproductive nodes m^{-2}	rate	4	4.54	0.0016
	timing	1	0.04	0.8323
	rate*timing	4	0.33	0.8581
Percent reproductive nodes	rate	4	0.99	0.4121
	timing	1	1.10	0.2958
	rate*timing	4	8.82	<.0001
Nodes m^{-2}	rate	4	5.45	0.0004
	timing	1	0.00	0.9633
	rate*timing	4	0.51	0.7253
Cold germination	rate	4	2.44	0.0566
	timing	1	0.06	0.8032
	rate*timing	4	5.06	0.0014
Accelerated aging germination	rate	4	0.13	0.9726
	timing	1	9.02	0.0038
	rate*timing	4	2.31	0.0675
Greenhouse emergence				
4 DAP	rate	4	0.95	0.4391
	timing	1	1.85	0.1787
	rate*timing	4	0.49	0.7453
7 DAP	rate	4	1.31	0.2764
	timing	1	1.20	0.2771
	rate*timing	4	0.83	0.5114
14 DAP	rate	4	0.43	0.7832
	timing	1	2.35	0.3101
	rate*timing	4	0.51	0.7265
21 DAP	rate	4	0.57	0.6826
	timing	1	2.53	0.1170
	rate*timing	4	0.52	0.7192

Greenhouse malformed plants				
7 DAP	rate	4	0.73	0.5776
	timing	1	0.25	0.6221
	rate*timing	4	2.09	0.0939
14 DAP	rate	4	0.52	0.7213
	timing	1	3.53	0.0651
	rate*timing	4	1.29	0.2840
21 DAP	rate	4	0.19	0.9412
	timing	1	4.23	0.0440
	rate*timing	4	0.99	0.4208

Results of data analysis for tank contamination study for dicamba-resistant soybean exposure to 2,4-D or 2,4-D plus dicamba.

Parameter	Source	DF	F	P
Injury 14 DAT	herbicide	1	0.01	0.9230
	rate	4	1426.91	<.0001
	timing	1	85.33	<.0001
	herb*rate	4	1.51	0.1968
	herb*time	1	5.90	0.0154
	rate*timing	4	4.47	0.0014
	herb*rate*timing	4	1.23	0.2976
Injury 28 DAT	herbicide	1	4.26	0.0394
	rate	4	762.78	<.0001
	timing	1	5.41	0.0203
	herb*rate	4	1.00	0.4089
	herb*time	1	0.95	0.3299
	rate*timing	4	2.28	0.0594
	herb*rate*timing	4	0.44	0.7784
BL injury 14 DAT	herbicide	1	0.29	0.5924
	rate	4	1202.38	<.0001
	timing	1	100.19	<.0001
	herb*rate	4	1.84	0.1193
	herb*time	1	1.78	0.1829
	rate*timing	4	10.92	<.0001
	herb*rate*timing	4	0.31	0.8739
BL injury 28 DAT	herbicide	1	1.63	0.2020
	rate	4	350.89	<.0001
	timing	1	0.16	0.6913
	herb*rate	4	0.33	0.8566
	herb*time	1	0.18	0.6682
	rate*timing	4	3.05	0.0167
	herb*rate*timing	4	0.66	0.6171
Height reduction 14 DAT	herbicide	1	0.65	0.4210
	rate	4	1145.61	<.0001
	timing	1	11.82	0.0006
	herb*rate	4	0.88	0.4742
	herb*time	1	2.20	0.1384
	rate*timing	4	6.00	<.0001
	herb*rate*timing	4	0.20	0.9404

Height reduction 28 DAT	herbicide	1	4.57	0.0329
	rate	4	767.08	<.0001
	timing	1	0.87	0.3522
	herb*rate	4	2.83	0.0240
	herb*time	1	0.96	0.3264
	rate*timing	4	1.57	0.1807
	herb*rate*timing	4	0.18	0.9491
Mature height	herbicide	1	5.06	0.0253
	rate	4	277.96	<.0001
	timing	1	2.19	0.1403
	herb*rate	4	0.96	0.4306
	herb*time	1	8.54	0.0038
	rate*timing	4	1.96	0.1004
	herb*rate*timing	4	0.66	0.6187
Grain yield	herbicide	1	8.86	0.0030
	rate	4	687.61	<.0001
	timing	1	0.35	0.5555
	herb*rate	4	3.05	0.0165
	herb*time	1	2.34	0.1265
	rate*timing	4	0.33	0.8588
	herb*rate*timing	4	0.66	0.6191
Hundred seed mass	rate	4	20.92	<.0001
	timing	1	0.07	0.7886
	rate*time	4	0.47	0.7591
	herbicide	1	1.03	0.3106
	herb*rate	4	1.68	0.1534
	herb*time	1	1.45	0.2284
	herb*rate*timing	4	0.32	0.8678
Seeds m^{-2}	rate	4	63.94	<.0001
	timing	1	0.34	0.5591
	rate*time	4	1.16	0.3279
	herbicide	1	0.12	0.7305
	herb*rate	4	0.38	0.8244
	herb*time	1	0.00	0.9801
	herb*rate*timing	4	0.43	0.7833
Seeds per pod	rate	4	6.84	<.0001
	timing	1	21.35	<.0001
	rate*time	4	2.18	0.0717
	herbicide	1	0.00	0.9740
	herb*rate	4	0.52	0.7206
	herb*time	1	0.41	0.5237
	herb*rate*timing	4	0.71	0.5840

Pods m^{-2}	rate	4	23.25	<.0001
	timing	1	4.24	0.0403
	rate*time	4	0.83	0.5081
	herbicide	1	1.12	0.2919
	herb*rate	4	0.88	0.4756
	herb*time	1	0.97	0.3251
	herb*rate*timing	4	0.40	0.8054
Pods per node	rate	4	11.33	<.0001
	timing	1	10.67	0.0012
	rate*time	4	4.84	0.0008
	herbicide	1	0.79	0.3749
	herb*rate	4	0.55	0.6975
	herb*time	1	0.66	0.4177
	herb*rate*timing	4	0.31	0.8708
Reproductive nodes m^{-2}	rate	4	23.19	<.0001
	timing	1	5.22	0.0231
	rate*time	4	1.52	0.1960
	herbicide	1	1.17	0.2804
	herb*rate	4	0.75	0.5583
	herb*time	1	1.33	0.2494
	herb*rate*timing	4	0.48	0.7478
Percent reproductive nodes	rate	4	4.77	0.0009
	timing	1	18.46	<.0001
	rate*time	4	20.35	<.0001
	herbicide	1	0.00	0.9848
	herb*rate	4	0.97	0.4232
	herb*time	1	2.47	0.1167
	herb*rate*timing	4	0.54	0.7051
Nodes m^{-2}	rate	4	21.29	<.0001
	timing	1	14.85	0.0001
	rate*time	4	3.73	0.0056
	herbicide	1	1.30	0.2554
	herb*rate	4	0.49	0.7414
	herb*time	1	0.56	0.4534
	herb*rate*timing	4	0.41	0.7981
Cold germination	herbicide	1	2.85	0.0940
	rate	4	11.90	<.0001
	rate*herb	4	0.76	0.5563
	timing	1	0.57	0.4517
	timing*herb	1	0.02	0.8784
	rate*time	4	2.49	0.0467
	herb*rate*timing	4	0.91	0.4628

Accelerated aging germination	herbicide	1	0.49	0.4843
	rate	4	4.49	0.0020
	rate*herb	4	1.89	0.1161
	timing	1	0.81	0.3709
	timing*herb	1	0.06	0.8010
	rate*time	4	1.78	0.1358
	herb*rate*timing	4	0.66	0.6205
Greenhouse emergence				
4 DAP	herbicide	1	1.28	0.2607
	rate	4	0.61	0.6527
	rate*herb	4	0.48	0.7480
	timing	1	1.11	0.2950
	timing*herb	1	0.05	0.8187
	rate*time	4	2.00	0.0983
	herb*rate*timing	4	0.20	0.9354
7 DAP	herbicide	1	0.86	0.3541
	rate	4	0.34	0.8492
	rate*herb	4	0.81	0.5218
	timing	1	0.03	0.8621
	timing*herb	1	0.08	0.7732
	rate*time	4	1.97	0.1033
	herb*rate*timing	4	0.18	0.9488
14 DAP	herbicide	1	2.54	0.1137
	rate	4	0.32	0.8660
	rate*herb	4	0.40	0.8056
	timing	1	0.85	0.3596
	timing*herb	1	0.03	0.8661
	rate*time	4	2.08	0.0872
	herb*rate*timing	4	0.46	0.7682
21 DAP	herbicide	1	2.44	0.1205
	rate	4	0.38	0.8259
	rate*herb	4	0.32	0.8649
	timing	1	0.53	0.4664
	timing*herb	1	0.05	0.8153
	rate*time	4	1.84	0.1244
	herb*rate*timing	4	0.27	0.8999

Greenhouse malformed plants				
7 DAP	herbicide	1	1.95	0.1653
	rate	4	1.43	0.2283
	rate*herb	4	0.58	0.6808
	timing	1	0.30	0.5872
	timing*herb	1	0.02	0.8911
	rate*time	4	0.26	0.9014
	herb*rate*timing	4	0.29	0.8824
14 DAP	herbicide	1	2.14	0.1456
	rate	4	0.82	0.5157
	rate*herb	4	0.62	0.6519
	timing	1	2.14	0.1456
	timing*herb	1	1.68	0.1974
	rate*time	4	0.25	0.9116
	herb*rate*timing	4	1.29	0.2772
21 DAP	herbicide	1	0.77	0.3812
	rate	4	2.27	0.0656
	rate*herb	4	0.71	0.5871
	timing	1	2.25	0.1362
	timing*herb	1	0.50	0.4790
	rate*time	4	1.27	0.2863
	herb*rate*timing	4	0.30	0.8790

APPENDIX B. SUPPLEMENTAL DATA ANALYSIS FOR GLYPHOSATE-RESISTANT SOYBEAN RESPONSE TO DICAMBA EXPOSURE AND LABELED POSTEMERGENCE HERBICIDES.

Results of data analysis for multiple herbicide injury trial.

Parameter	Source	DF	F	P
Injury 28 DAT	treatment	23	238.58	<.0001
	year	1	20.89	<.0001
	year*treatment	23	6.69	<.0001
Height reduction 28 DAT	treatment	23	66.48	<.0001
	year	1	0.26	0.6316
	year*treatment	23	1.64	0.0433
BL injury 28 DAT	treatment	23	9518.46	<.0001
	year	1	2.25	0.1354
	year*treatment	23	0.95	0.5389
Mature height	treatment	23	70.23	<.0001
	year	1	1.47	0.2708
	year*treatment	23	1.54	0.0689
Grain yield	treatment	23	16.31	<.0001
	year	1	9.64	0.021
	year*treatment	23	2.61	0.0003
Injury 28 DAT - 2017	dicamba	1	1068.38	<.0001
	dicamba timing	1	2.32	0.1325
	dicamba*dica_time	1	298.48	<.0001
	herbicide	5	18.96	<.0001
	dicamba*herbicide	5	2.17	0.0674
	dica_time*herbicide	5	11.05	<.0001
	dicamba*dica_time*herb	5	6.45	<.0001
Injury 28 DAT - 2018	dicamba	1	3922	<.0001
	dicamba timing	1	288	<.0001
	dicamba*dica_time	1	1521	<.0001
	herbicide	5	196.97	<.0001
	dicamba*herbicide	5	17.1	<.0001
	dica_time*herbicide	5	60.01	<.0001
	dicamba*dica_time*herb	5	18.87	<.0001
Height reduction 28 DAT - 2017	dicamba	1	498.97	<.0001
	dicamba timing	1	19.69	<.0001
	dicamba*dica_time	1	45.24	<.0001

	herbicide	5	14.11	<.0001
	dicamba*herbicide	5	1.62	0.1651
	dica_time*herbicide	5	1.57	0.1812
	dicamba*dica_time*herb	5	0.71	0.6199
Height reduction 28 DAT - 2018	dicamba	1	800.56	<.0001
	dicamba timing	1	48.61	<.0001
	dicamba*dica_time	1	50.3	<.0001
	herbicide	5	2.79	0.0236
	dicamba*herbicide	5	0.68	0.6422
	dica_time*herbicide	5	0.67	0.6461
	dicamba*dica_time*herb	5	0.12	0.9872
BL injury 28 DAT	dicamba	1	2155.93	<.0001
	dicamba timing	1	1657.39	<.0001
	dicamba*dica_time	1	1657.39	<.0001
	herbicide	5	0.94	0.4557
	dicamba*herbicide	5	0.94	0.4557
	dica_time*herbicide	5	0.75	0.5879
	dicamba*dica_time*herb	5	0.75	0.5879
Mature Height	dicamba	1	1051.75	<.0001
	dicamba timing	1	193.18	<.0001
	dicamba*dica_time	1	194.03	<.0001
	herbicide	5	11.44	<.0001
	dicamba*herbicide	5	0.77	0.5751
	dica_time*herbicide	5	0.67	0.6467
	dicamba*dica_time*herb	5	0.69	0.629
Grain yield - 2017	dicamba	1	135.92	<.0001
	dicamba timing	1	34.3	<.0001
	dicamba*dica_time	1	82.51	<.0001
	herbicide	5	17.39	<.0001
	dicamba*herbicide	5	2.86	0.021
	dica_time*herbicide	5	2.54	0.036
	dicamba*dica_time*herb	5	1.23	0.3026
Grain yield - 2018	dicamba	1	49.39	<.0001
	dicamba timing	1	14.12	0.0004
	dicamba*dica_time	1	18.29	<.0001
	herbicide	5	2.1	0.0757
	dicamba*herbicide	5	0.3	0.913
	dica_time*herbicide	5	1.28	0.2821
	dicamba*dica_time*herb	5	0.96	0.4511

APPENDIX C. SUPPLEMENTAL DATA ANALYSIS FOR THE EFFECTS OF APPLICATION FACTORS ON DICAMBA VOLATILIZATION IN A CONTROLLED ENVIRONMENT.

Results of data analysis for controlled environment volatilization study.

Parameter	Source	DF	F	P
DRA				
DGA	treatment	3	0.41	0.7487
	run	1	7.91	0.0099
	treatment*run	3	0.52	0.6696
DGA + VG	treatment	3	0.21	0.8866
	run	1	0.55	0.4646
	treatment*run	3	0.05	0.9832
BAPMA	treatment	3	11.32	<.0001
	run	1	124.20	<.0001
	treatment*run	3	2.39	0.0935
Suspended soil				
DGA	treatment	4	0.76	0.5607
	run	1	11.07	0.0025
	treatment*run	4	1.00	0.4235
DGA + VG	treatment	4	26.71	<.0001
	run	1	15.82	0.0004
	treatment*run	4	16.64	<.0001
BAPMA	treatment	4	2.44	0.0686
	run	1	9.97	0.0036
	treatment*run	4	1.64	0.1894
Spray pH				
DGA	treatment	6	0.99	0.4496
	run	1	2.52	0.1210
	treatment*run	6	1.01	0.4312
DGA + VG	treatment	6	8.35	<.0001
	run	1	0.03	0.8595
	treatment*run	6	2.90	0.0204
BAPMA	treatment	6	6.25	0.0001
	run	1	34.82	<.0001
	treatment*run	6	2.10	0.0757

Spray solution ions				
DGA	treatment	5	17.93	<.0001
	run	1	27.16	<.0001
	treatment*run	5	2.51	0.0487
DGA + VG	treatment	5	35.79	<.0001
	run	1	0.14	0.7087
	treatment*run	5	1.39	0.2518
BAPMA	treatment	5	29.99	<.0001
	run	1	11.98	0.0014
	treatment*run	5	1.45	0.2318
Volatilization DRA				
DGA	treatment	3	1.55	0.2280
DGA + VG	treatment	3	5.21	0.0068
BAPMA	treatment	3	6.95	0.0015
Suspended soil				
DGA	treatment	4	0.63	0.6480
DGA + VG	treatment	4	7.88	0.0002
BAPMA	treatment	4	3.06	0.0303
Spray pH				
DGA	treatment	6	3.37	0.0082
DGA + VG	treatment	6	30.98	<.0001
BAPMA	treatment	6	19.96	<.0001
Spray solution ions				
DGA	treatment	5	44.70	<.0001
DGA + VG	treatment	5	297.30	<.0001
BAPMA	treatment	5	162.08	<.0001

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Thesis Title:
Dicamba Volatilization and the Effect of Synthetic Auxin Herbicides on Sensitive Soybean

Major Professor: Dr. Bryan Young

Publications:

Zimmer M, Hayden C, Whitford F, Young BG, Johnson WG (2019) Differentiating 2,4-D and dicamba injury on soybeans. https://ag.purdue.edu/btny/weedscience/Documents/WS-56.pdf.

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