SPECIALTY CROP SENSITIVITY TO DICAMBA

A Thesis

Presented to

The Faculty of the Graduate School

At the University of Missouri

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science, Plant, Insect and Microbial Sciences

By

TIMOTHY C.J. RICE

Dr. Reid J. Smeda, Thesis supervisor

August 2022

The undersigned, appointed by the Dean of the Graduate School,

Have examined the thesis entitled

SPECIALTY CROP SENSITIVITY TO DICAMBA

Presented by Timothy C.J. Rice

A candidate for the degree of

Master of Science, Plant, Insect and Microbial Sciences

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

Reid J Smeda

Professor

Michele R. Warmund

Professor

Chung-Ho Lin

Research Associate Professor

Acknowledgements

Foremost I would like to thank Dr. Reid Smeda for giving me the opportunity to pursue this degree. He has provided me with years of invaluable experience, knowledge and advice. I am grateful for the time I was able to spend as a part of his team and will remember the lessons I have learned here for years to come. I would also like to extend my sincere thanks to my committee members. I am grateful to Dr. Chung-Ho Lin for his unmatched patience and willingness to work with me through the Covid-19 outbreak. By opening his lab to me and training me on the LCMSMS, he went above and beyond as a committee member and mentor. I would also like to thank Dr. Michele Warmund for lending her unparalleled knowledge of specialty crops and providing her insight into our project. My committee members have been incredibly flexible, understanding and helpful over the course of this degree and I am very thankful for the support I received from each of them.

This specialty crop research could not have been possible without the funding from the USDA specialty crop block program. Additionally, the help and insight I received from the staff at Eurofins was indispensable and I would like to thank each and every one of them. Furthermore, Dr. Mark Ellersieck offered invaluable assistance with statistical analysis.

I would like to thank my colleagues for their support and encouragement throughout my three years here. To Shaun Billman, I say thank you for the countless days you have spent grinding away, keeping us graduate students on track and keeping our lab running like clockwork. Without you, there would be no lab. To my fellow graduate students Emma Gaither, Sarah Dixon and Michelle Maile, thank you for always providing good commentary and making the hardest days a bit easier. I could not have completed this degree without the help of each and every person in our lab. From my first day of planting to my final day of writing, you have all been there and helped every inch of the way.

I also extend my unending gratitude to my friends and family for their enduring support and cheer throughout the course of my degree. To my parents, Lynn and Earl Rice, I want to say thank you for all the sacrifices you have made to help me reach where I am today. Every goal I have ever achieved is only made possible by the foundations you have laid for me. To my brother, Christopher Rice, I would like to say thank you for always being a stellar role model and constantly pushing me to be a better version of myself. I never would have strived for this degree without your example. Finally, I would like to thank Taylor Tinnes for her unyielding love, support and encouragement. I appreciate the sacrifices you made to be here with me and everything you have done to keep me strong throughout this process. At the end of every day, you were the one behind the scenes keeping me going. I could not have done this without you.

Table of Contents

List of Tables

Table **Chapter 2** **Page**

List of Figures

Figure Chapter 2 Page

Figure	Chapter 3	Page

List of Appendices

Chapter 1: Literature Review

Introduction

Watermelon (*Citrullus lanatus*), tomatoes (*Solanum lycopersicum*) and potatoes (*Solanum tuberosum*) are important specialty crops, accounting for 4% of the total agricultural crops sales in 2019 (USDA-NASS). Production continues to grow, as the sales these three crops generated increased from 30% of all specialty crop sales in 2012 to 47% in 2019 (USDA-NASS). During this time, dicamba (2-methoxy-3,6-dichlorobenzoic acid) tolerant soybeans (*Glycine max*) and cotton (*Gossypium hirsutum*) were first introduced (2017) and approximately 1.45 million hectares of soybean were injured by the off-target movement of dicamba across the United States (Bradley, 2017). Buffer distances have been implemented to protect sensitive soybeans and current regulations mandate a downwind buffer distance of 73 meters between the last treated row of a field and the nearest downwind field edge (USEPA). This distance was adjusted in 2021 after the previous buffer distance of 37 m proved insufficient. While the new buffer distance has been deemed adequate for sensitive soybean, it is not known whether this buffer distance is sufficient to effectively protect sensitive specialty crops from dicamba for damage, yield loss and fruit contamination. The parameters of these experiments were developed prior to the 2021 buffer distance amendment.

Activity of Dicamba

Following the success of the synthetic auxin herbicide, 2, 4-D, dicamba was discovered in 1942 by Zimmerman and Hitchcock (Ross and Lembi, 2009). Classified as a growth regulator, dicamba can act at multiple sites in a plant to disrupt hormone balance and protein synthesis, ultimately resulting in a variety of plant growth abnormalities (University of Minnesota Extension). More specifically, dicamba is categorized as a synthetic auxin. There are three different chemical families of synthetic auxin herbicides including phenoxyacetic acids, benzoic acids and pyridine acids (Hall et al., 1997). Dicamba is a benzoic acid and was first implemented as an herbicide option in the 1960's (Ross and Lembi, 2009).

Dicamba exhibits non-selective activity on broadleaf plants with little activity on grass species, controlling at least 147 weed species (Monsanto, 2016). Monocots have a greater ability to metabolize dicamba into non-herbicidal compounds compared to broadleaves (Chang et al., 1971). With this selectivity, dicamba was primarily used to control broadleaf weeds in monocot crops such as turf, sorghum, corn and other small grains.

As a synthetic auxin, much research has focused on the mechanism of action for dicamba as an herbicide. The known roles of the hormone auxin (indole-3-acetic acid) include coordinating the development of organs such as lateral roots, expanding cell walls, inducing flower development, fruit set and an abundance of other physiological processes (Bhalerao et al., 2002; Majda and Robert, 2018; Cheng and Zhao, 2007; De Jong et al., 2007; Woodward and Bartel, 2005). Synthetic auxins such as dicamba function by mimicking this plant hormone. In the presence of synthetic auxins, hormone receptors bind with the synthetic hormones which in turn prevents the reception of natural hormones. The herbicidal effect of auxinic herbicides has been attributed to an over induction of the auxin response in susceptible plants (Kelley et al., 2007). There is evidence that dicamba drift concentrations as little as 0.001% of the field application rates can result in significant phenotypic damage in sensitive plants (Bohnenblust et al., 2013). At high concentrations, the effects of synthetic auxins have been described as a natural auxin overdose (Grossman, 2000). This response can be divided into three consecutive phases in the plant: stimulation of abnormal growth and gene expression; inhibition of growth and physiological responses; and tissue senescence, cell and plant death (Gleason et al., 2011).

In the first stage, dicamba activates the ATPase pump, creating a high concentration of hydrogen ions and a low pH environment surrounding cell walls which allows cell walls to loosen and elongate (Shaner, 2014). As a result of this process, common symptomology such as leaf cupping, stem and leaf epinasty, cracked and swollen stems, chlorosis and necrosis appear on sensitive plants after exposure to dicamba (Sciumbato et al., 2004). The second phase of growth-inhibiting effects are caused by auxin-induced ethylene synthesis, which triggers an increase in the biosynthesis of abscisic acid (ABA) (Hansen and Grossmann, 2000). The accumulation of ABA induces stomatal closure which causes a lack of carbon assimilation and biomass production (Hansen et al., 2000). Taiz et al. (1998) found ABA naturally promotes leaf senescence. The unnatural effects of this process can be seen in phase three as the accumulation of ethylene and ABA begin to promote cell death and leaf senescence (Grossmann, 2000).

Adoption of Dicamba

Dicamba was first described in 1958 and then approved for use in the US in 1962 (Hartzler, 2017). At the time, dicamba largely competed with atrazine and 2,4-D as a broadleaf control method in corn. Dicamba was not widely adopted, as less than 10% of United States corn production area was treated with dicamba by 1979 (Hartzler, 2017). Adoption of dicamba remained low until the introduction of herbicide tolerant crops.

In 1996, tolerance to glyphosate was introduced for soybean (*Glycine max*), followed by cotton (1996), canola (1996), corn (1998), alfalfa (2005) and sugarbeet (2007) (Reddy et al., 2012). Agro-economic advantages of glyphosate-tolerant (GT) crops such as low environmental toxicity, reduced risk of failed weed control and fewer herbicide applications drove the rapid diffusion and popularity of GT crops (Bonny, 2008). The rapid adoption of glyphosate tolerant crops led to the development of glyphosate resistant weeds (Bonny, 2008). Glyphosate-resistance first appeared in rigid ryegrass (*Lolium rigidum)* in an apple orchard in Australia in 1996 (Heap and Duke, 2018). Since then, fifty-five weed species have evolved resistance to glyphosate across 38 countries (Heap, 2022). The increase in resistant weed species has decreased the utility of glyphosate in many broadleaf cropping systems. For example, in Georgia cotton (*Gossypium hirsutum*), grower herbicide input costs more than doubled following the evolution and spread of glyphosate resistance in Palmer amaranth (*Amaranthus palmeri S.*) (Sosnoskie et al., 2014). This has left many looking for other methods of burndown weed control.

The adoption of synthetic auxin herbicides as a burndown weed control method became more prevalent due to the decreased efficacy of glyphosate. Hatterman-Valenti et al. (2017) found that sensitive plants applied with dicamba displayed more visual symptomology when compared to sensitive plants applied with a glyphosate. Furthermore, dicamba weed management programs have proven effective against glyphosate-resistant horseweed (*Erigeron canadensis*) (Byker et al. 2013; Flessner et al. 2015) and palmer amaranth (Cahoon et al. 2015; Inman et al. 2016). These are two of the most troublesome weeds in the US (Van Wychen, 2016).

The success of dicamba as a weed management tool led to the genetic engineering of new crops with synthetic auxin tolerance (Behrens et al. 2007). Monsanto developed dicamba-tolerant soybean and cotton, both of which were approved by the EPA for in-crop use in 2016 (Roundup Ready XTEND® Crop System, Bayer Crop Science, 2019). Dicamba-tolerant crops tolerate post emergence applications of dicamba by rapidly metabolizing the herbicide with insertion of dicamba monooxygenase (DMO), which prevents accumulation of toxic levels in the crop (Behrens et al. 2007). Dicamba-tolerant varieties have been widely adopted with approximately 8.1 million hectares of dicamba tolerant soybeans planted in 2017 and rising to more than 16.2 million hectares in 2018 (Roundup Ready XTEND® Crop System, Bayer Crop Science, 2019).

The widespread adoption of dicamba-tolerant crops did not result in a simultaneous increase of dicamba applications. In 2019, three years after the introduction of dicamba-tolerant crops, only 66% of cotton acreage in Missouri was treated with dicamba (USDA-ERS, 2021). However, approximately 85% of cotton acreage in Missouri was planted with dicamba-tolerant varieties in 2019 (USDA-ERS, 2021).

Off-Target Movement of Dicamba

As a hormone mimic capable of inducing herbicidal effects at very low doses, dicamba off-target movement from treated areas has significant repercussions. In 2017, the year dicamba-tolerant soybeans were first introduced, there were 2,708 dicamba-related injury cases in the United States due to off-target movement (Bradley, 2017). Dicamba off-target movement has continued to be a major concern in agriculture and can cause major yield reductions on susceptible non-target crops even at low rates (Osipitan, 2019). Osipitan (2019) found that exposure to dicamba at 56 g a.e. ha^{-1} can reduce the yields for susceptible soybeans up to 96%.

Off-target movement can occur either during application of dicamba when spray droplets are carried to adjacent areas or following volatilization from treated areas (Soltani et al., 2020). Volatilization is the chemical process by which a compound is converted to the gas phase via evaporation, sublimation, or desorption and then enters the atmosphere (Bedos et al., 2002).

Spray drift has been defined as the movement of pesticide dust or droplets through the air beyond the intended area of application at the time of application, or soon after (US EPA, 2014).

The extent of spray drift occurring during application is dependent on mechanical factors including application height, herbicide formulation, spray droplet size, nozzle spacing and application speed (Mohseni-Moghadam et al., 2015). Drift can be reduced if droplet size is increased by reducing spray pressure, increasing nozzle orifice size, using special drift reduction nozzles and using additives that increase spray viscosity (Dexter, 1993). Environmental factors such as wind speeds on the day of application and soybean production hectarage can be equally impactful to physical drift (Oseland et al., 2020). Additionally, spray droplets released above a target area at a reduced nozzle height are affected by lower wind velocities which also minimizes drift (Dexter, 1993).

Dicamba volatility can result in losses of up to 80% of applied dicamba (DMA) but is also dependent on a number of chemical and environmental factors (Sharkey et al., 2020; Behrens and Lueschen, 1979). Dicamba has a high vapor pressure which is defined as the maximum solubility of a substance in the air (Redemann et al., 1948). Dicamba, as the unformulated acid, has a vapor pressure of 2.0×10^{-5} mm Hg at 25 C while a less volatile herbicide such as glyphosate, as an unformulated acid, has a vapor pressure of 1.84×10^{-7} mm Hg at 25 C (Behrens and Lueschen, 1979; National Pesticide Information Center, 2011). Higher vapor pressure values indicate higher volatility (Mackay et al., 1982). While the vapor pressure of a chemical is important in characterizing volatility in stable conditions, actual dicamba volatility rates are dependent on both chemical formulation and environmental conditions during and after application (Hee et al., 1974).

Environmental conditions that affect dicamba volatility include temperature, relative humidity, and wind speeds (Egan et al., 2012). Environmental periods of high temperature and low humidity specifically are conducive to high volatility (Egan et al., 2012). While a majority of volatility occurs in the first 8 hours after application, dicamba can volatilize up to 72 hours after treatment (Bish et al., 2019).

Furthermore, volatility is impacted by chemical formulation as disassociation of a formulated salt from the dicamba acid results in greater volatility (Grover and Smith, 1974). Sharkey et al. (2020) found that dicamba volatilization is primarily influenced by the number of amine functional groups that can participate in hydrogen bonding with a dicamba acid. Current salt formulations for dicamba fall into three basic categories: dimethylamine (DMA), diglycolamine (DGA) or N,N-bis-aminopropyl methylamine (BAPMA) salts (Jones, 2015). The first commercial formulation of dicamba, sold under the trade name Banvel®, was comprised of the DMA salt. More recent formulations such as Xtendimax with VaporGrip® and FeXapan plus VaporGrip® contain the DGA salt while other formulations such as (Engenia®) contain the BAPMA salt. Newer formulations are being sought to minimize the natural tendency of dicamba to volatilize and move off-target, potentially causing damage to nearby sensitive crops. The use of drift reduction agents (DRAs) can further increases the stability of dicamba formulations (Long, 2017).

Latorre et al. (2017) found significant differences in volatility among the DMA, DGA and BAPMA formulations with the DMA formulation resulting in the greatest risk for volatilization. The DGA salt of dicamba is 8-fold less volatile than the DMA salt (Egan et al., 2012). Additionally, Mueller et al. (2013) found that less dicamba (DGA formulation) was detected within the first 12 hours after application compared to the DMA salt of dicamba. Further reductions of volatility were made with the development of dicamba containing BAPMA salts which have a higher affinity for the dicamba acid molecule due to its molecular weight, type and number of amine functional groups (Sharkey et al 2020). Sharkey et al. (2020) found that BAPMA salts had a greater ability to reduce dicamba volatilization than both DMA and DGA formulations. In 2016, the BAPMA and DGA formulations of dicamba were approved by the EPA for application to dicamba-resistant soybean and cotton in the U.S. (US EPA 2016). With less volatile dicamba formulations available, adoption of DT crops has increased, although the accompanying off-target movement continues to threaten sensitive specialty crops.

Economic Importance of Specialty Crops

Increasing adoption of DT crops with accompanying off-target movement threatens sensitive specialty crops. Specialty crops, or minor crops, refer to fruits, vegetables, and ornamentals that require higher labor input and more expensive weed management programs than major crops such as corn and soybean (Fennimore et al., 2008). In 2017, 6,300,000 hectares of specialty crops were planted across the United States (USDA Census of Agriculture, 2017). Sales for vegetables such as watermelon, tomatoes and potatoes made up 38% of sales from all specialty crops in 2017 (USDA Census of Agriculture, 2017).

Tomatoes and potatoes are two of the leading specialty crops in the nation while watermelon is the most abundant vegetable crop in Missouri (USDA Census of Agriculture, 2017; Missouri Specialty Crop Survey, 2017). In 2021, $530 million worth of watermelon were harvested across 41,000 hectares in the United States (USDA-NASS, 2022). Approximately 70% of

United States watermelon production occurs in Texas, Florida, Georgia and California (Agricultural Marketing Resource Center, 2018). Across 110,000 heactares, $1.5 billion of tomatoes were harvested in the United States in 2021 (USDA-NASS, 2022). Over the past three years, California and Florida have accounted for 90% of tomato production in the United States (USDA, 2019 Vegetables summary, 2020). In 2021, $4 billion worth of potatoes were harvested across 380,000 hectares in the United States (USDA-NASS, 2022). Idaho and Washington produce more than half of the annual supply of potatoes in the United States (Agricultu ral Marketing Resource Center, 2018). With such a large economic impact, there is reasonable concern that these specialty crops may be negatively impacted by dicamba off-target movement in states where dicamba is used extensively.

California, Florida, Georgia, Idaho, Texas and Washington produce the largest yields for potatoes, tomatoes and watermelon across the United States (Agricultural Marketing Resource Center, 2018). Conservative estimates for dicamba use are about 1.1 million kg., 6,000 kg., 105,000 kg., 4,500 kg., 27,000 kg. and 13,000 kg for Texas, Florida, Georgia, California, Idaho and Washington respectively in 2017 (USGS, 2019) with the label rate being about 0.56 kg ae/ ha^{-1}. With such large amounts of dicamba being used in these major vegetab le production states, there is potential for damage to occur in watermelons, tomatoes and potatoes through yield reductions or produce contamination.

Response of Sensitive Non-Target Crops

Many specialty crops are grown in close proximity to major product ion crops. In 2021, 37 million hectares of corn, 4 million hectares of cotton and 35 million hectares of soybean were planted in the US (USDA NASS, 2022). With such a large portion of US production area devoted to crops that may be treated with dicamba, specialty crops are likely to be in close proximity to dicamba, and subject to damage. For example, herbicide drift from neighboring fields has been the primary reason for synthetic auxin damage to tomato plants (Fagliari et al. 2005; Lovelace et al. 2007). Furthermore, post-emergence dicamba applications for tolerant crops can occur while specialty crops are at sensitive growth stages, such as the early vegetative stage (Kruger et al. 2012). These factors, in addition to the lack of natural dicamba tolerance in specialty crops can result in stunted growth, yield reductions and potential crop loss for these vegetables, highlighting the need for diligence in the application of dicamba in proximity to susceptible crops (Hatterman-Valenti et al., 2017).

Tomato

In 2021, approximately 110,000 hectares of tomatoes with a value of approximately $1.5 billion were planted across the United States ranging from California to Florida (USDA NASS, 2022). In Missouri, no current production statistics are available from the USDA, but tomatoes are common fruit sold at local farmers markets and grown in home gardens. Tomatoes are highly sensitive to synthetic auxin herbicides (Fagliari et al. 2005; Kruger et al. 2012; Knezevic et al. 2018; Hermes 2020) and can sustain damage from exposure to dicamba at rates as low as 0.32 g ae ha^{-1} (Knezevic et al., 2018).

Tomatoes are most sensitive to dicamba during the early bloom stage compared to vegetative stages of growth. Jordan et al. (1974) found that plants exposed to 100 g ae ha^{-1} of dicamba during early bloom yielded 91% less than controls compared to only 14% when dicamba was applied during fruit set. Kruger et al. (2012) found that a 2.4 g ha^{-1} rate resulted in a 5%

flower loss at the early vegetative stage, whereas a 1.5 g ha^{-1} rate caused a 5% flower loss at the early bloom stage. Additionally, it was determined that an estimated 10% loss in marketable red fruits would require a rate of 4.5 or 3.9 g ha^{-1} for the first and early bloom stage (Kruger et al., 2012). Knezevic et al. (2018) also reported that taller tomatoes (45 cm) applied with dicamba appeared more sensitive to biomass reductions than shorter tomatoes (25 cm) applied with dicamba.

Dicamba damage on tomato plants can vary depending on the method in which tomatoes make contact with dicamba. In a study by Knezevic et al. (2018), it was found that doses of 0.82, 4.49 and 56 g ae ha^{-1} (1/683, 1/125 and 1/10 of the label rate) of dicamba caused 10%, 50% and 80% visual injury, respectively at 21 DAT. However, when dicamba was applied to field soil at a 1× rate, volatility of dicamba resulted in visual injury ranging from 20-24% for tomato (Bauerle et al., 2015). This demonstrates how dicamba concentration may become diluted in drift scenarios and result in lower damage when compared to tomatoes directly applied with the same rate.

Larger leaf area and smaller trichome densities have also been found to increase tomato susceptibility to dicamba damage due to the increased opportunity for dicamba to reach a leaf surface where it can be absorbed by the plant (Zangoueinejad et al., 2020). Schmoll et al. (2000) found that reductions in total fruit fresh weight of hydroponically grown tomatoes were highly correlated (r^2 = 0.93) with leaf area reductions caused by dicamba. Fruit fresh weight per plant was reduced by 6% at 1 µg/L dicamba and 73% at 10 µg/L dicamba (r^2 = 0.87).

Cultivar differences may also play a role in the overall susceptibility of a tomato plant to dicamba damage. Warmund et al. (2022) demonstrated that damage severity from dicamba drift on tomato was cultivar dependent in a field setting. The tomato cultivar Florida 91 was identified as the least dicamba susceptible cultivar tested (Warmund et al., 2022). Zangoueinejad et al. (2019) also tested 3 wild tomato ascensions: TOM199, TOM198, and TOM300 and found that all 3 survived exposure to dicamba at potential drift rates (2.8 g a.e. ha^{-1}). A follow up study determined that the dicamba tolerance trait in the three tomato varieties used aforementioned, might be associated with the drought stress gene, 1e16 (Zangoueinejad et al., 2020).

Watermelon

In 2021, approximately 41,000 hectares of tomatoes with a value of approximately $530 million were planted across the United States ranging from California to Georgia (USDA NASS, 2022). In Missouri, 1,250 hectares of watermelon were planted in 2017 (USDA NASS, 2022). Dicamba drift on watermelon poses a serious risk for visual injury, reduced vine growth, yield loss, and detectable residue in marketable fruit (Culpepper et al., 2018). Culpepper et al. (2018) looked at the effects of low dose applications of dicamba on watermelon which is the only published research at the time of this writing. This study reported that dicamba applied at 1/250th of the label rate was a high enough concentration for watermelon to display detectable visual injury (Culpepper et al., 2018).

The greatest factor influencing the level of damage observed from dicamba is the stage of melon growth at the time of exposure (Culpepper et al., 2018). Total biomass of marketable melons was 69, 89, and 103 kg $plot^{-1}$ when a 1/75 rate of dicamba herbicide was applied at 20, 40, and 60 days after planting (DAP) (Culpepper et al., 2018). Furthermore, Culpepper et al. (2018) found that watermelon treated with the 1/75 rate of dicamba at 20, 40, and 60 DAP exhibited 40, 16, and 11% visual injury, respectively and had vine lengths 49, 61, and 84% the length of the non-treated check, respectively. Culpepper et al. (2018) demonstrated that watermelons damaged during the vegetative stages of growth will suffer from increased visual injury, decreased vine growth and larger yield reductions when compared to more mature watermelons applied with the same rates.

In addition to growth reductions, watermelon exposed to dicamba may also be susceptible to fruit contamination from herbicide residues. Across their two year study, Culpepper et al. (2018) reported dicamba residues in some of their treated plots. In 2014, watermelon fruit treated with the 1/250 and 1/75 use rates of dicamba contained residue concentrations of 0 and 0.01 ppm, respectively (Culpepper et al., 2018). In 2015, watermelon fruit treated with the 1/250 and 1/75 use rates of dicamba contained residue concentrations of 0.01 and 0.03 ppm, respectively (Culpepper et al., 2018). Furthermore, across both years, residues were more likely to reside in fruit in larger quantities when damage occurred closer to harvest (Culpepper et al., 2018).

While watermelon are susceptible to dicamba, they may be less susceptible when compared to tomato. Culpepper et al. (2018) reported that watermelons treated with the 1/75 rate dicamba exhibited 40% visual injury, while Knezevic et al. (2018) found that tomatoes applied with 1/125 rate of dicamba exhibited 50% visual injury.

Potato

In 2021, approximately 380,000 hectares of potatoes with an approximate value of $4 billion were planted across the United States ranging from Washington to Texas (USDA NASS, 2022). In Missouri, 3,150 hectares of potatoes were planted in 2018 (USDA NASS, 2022). When exposed to dicamba, potatoes can suffer negative affects to both growth and yield (Geary et al., 2019). Hatterman-Valenti et al. (2017) reported that 0.1 kg ha^{-1} of dicamba could result in a visual injury rating of up to 49% in North Dakota at 20 DAT. Furthermore, it was concluded that even low doses of dicamba could result in more damage to potatoes compared to glyphosate. Hatterman-Valenti et al. (2017) also found that potato plants exhibit up to 20% visual injury without negative effects on total or marketable tuber production. The same study found that exposure to 0.1 kg ha^{-1} dicamba resulted in 20% and 47% yield reductions for total and marketable potatoes, respectively. Wall et al. (1994) found that potatoes applied with dicamba (22 g ai ha^{-1}) 2-3 weeks after planting resulted in a reduced tuber and marketable yield by 40% and 74% respectively, which further supports the findings of Hatterman.

Aside from growth reductions, a second concern for potato growers is whether or not dicamba residues can be found in marketable tubers, even if tuber yield remains unaltered. While research on residues in potato tubers exposed to foliar applied dicamba is limited, Seefeldt et al. (2014) found that potatoes are very susceptible to injury from dicamba soil residues. Residues can be readily taken up from the soil causing foliar injury to the mother crop and subsequently, being stored in the daughter tuber (Seefeldt et al., 2014). Daughter tubers are those that form throughout the growing season from the mother tuber that was initially planted at the beginning of the growing season. While daughter tubers remained visually unaltered, the yield of daughter tubers was reduced up to 90% when their mother tubers were exposed to soil applied dicamba at a dose of 140 g ae ha^{-1} (Seefeldt et al., 2014). Furthermore, when these daughter tubers were replanted, the new emerging shoots displayed 6% visual injury and plant height reductions of approximately 20% were observed five weeks after planting (WAP). This highlights the ability of dicamba residues to persist inside of marketable daughter tubers after exposure to dicamba (Seefeldt et al., 2014).

Residue Analysis of Dicamba

The quantification of pesticide residues in marketable fruit is paramount to grower success and the creation of consumer health and safety legislation (USEPA Dicamba Memorandum, 2020; Fong et al., 1999; Melo et al., 2013). For growers, it is essential to minimize residue concentrations relative to the oral reference dose (RfD), as high value crops may suffer quality losses and organic growers could lose organic certification due to fruit contamination (USEPA Dicamba Memorandum, 2020). The U.S. EPA has set the RfD for dicamba at 300 µg/kg /day (USEPA Dicamba; Pesticide Tolerances, 2016; NPIC-Dicamba, 2011). Residue analysis can help growers determine the conditions in which their produce may be contaminated above the legal RfD.

The sample preparation and residue analysis process for environmental and food samples is well established for many pesticides (Pico et al., 2004). The process for identifying and quantifying dicamba residues in watermelon via LCMSMS is well documented in the literature with residues being detected at concentrations as small as 0.01 ppm (Culpepper et al., 2014). Additionally, a process for quantifying dicamba in tomato has been documented with recovery rates as high as 100% (Melo et al. 2013). Residue analysis of other pesticides, such as carbendazim, have also been documented in tomatoes with detectable concentrations as small as 0.014 ppm (Pujeri et al., 2016). Documentation on the analysis of dicamba residues in potato is less available and requires more research.

Objectives

The objectives of research in this thesis are:

1) To determine whether current EPA buffer distance guidelines, currently set at 73 m, are sufficient to preclude measurable damage to nearby sensitive potatoes, tomatoes and watermelon.

2) To quantify dicamba residues in potentially exposed tomato and watermelon fruit as well as potato tubers.

3) To determine whether there are varietal differences among tomatoes and watermelon to increasing doses of dicamba.

Literature Cited

Agricultural Marketing Resource Center. (2018) Available at: https://www.agmrc.org/

Bauerle, M. J., Griffin, J. L., Alford, J. L., Curry, A. B., & Kenty, M. M. (2015). Field evaluation of auxin herbicide volatility using cotton and tomato as bioassay crops. Weed Technology, 29(2), 185-197.

Bedos, C., Cellier, P., Calvet, R., Barriuso, E., & Gabrielle, B. (2002). Mass transfer of pesticides into the atmosphere by volatilization from soils and plants: overview. Agronomie, 22(1), 21-33.

Behrens MR, Mutlu N, Chakraborty S, Dumitru R, Jiang WZ, LaVallee BJ, Herman PL, Clemente TE, Weeks DP (2007) Dicamba resistance: enlarging and preserving biotechnology-based weed management strategies. Science 316:1185-1188

Behrens, R., & Lueschen, W. E. (1979). Dicamba volatility. Weed Science, 27(5), 486-493.

Bhalerao, R. P., Eklöf, J., Ljung, K., Marchant, A., Bennett, M., & Sandberg, G. (2002). Shoot-derived auxin is essential for early lateral root emergence in Arabidopsis seedlings. The Plant Journal, 29(3), 325-332.

Bish, M. D., Farrell, S. T., Lerch, R. N., & Bradley, K. W. (2019). Dicamba losses to air after applications to soybean under stable and nonstable atmospheric conditions. Journal of Environmental Quality, 48(6), 1675-1682.

Bohnenblust, E., Egan, J. F., Mortensen, D., & Tooker, J. (2013). Direct and indirect effects of the synthetic-auxin herbicide dicamba on two lepidopteran species. Environmental Entomology, 42(3), 586-594.

Bonny, S. (2008). Genetically modified glyphosate-tolerant soybean in the USA: adoption factors, impacts and prospects. A review. Agronomy for Sustainable Development, 28(1), 21-32.

Bradley, K.W. (2017) A final report on dicamba-injured soybean acres. Available at: https://ipm.missouri.edu/IPCM/2017/10/final_report_dicamba_injured_soybean/

Byker HP, Soltani N, Robinson DE, Tardif FJ, Lawton MB, Sikkema PH (2013) Control of glyphosate-resistant horseweed (*Conyza canadensis*) with dicamba applied preplant and postemergence in dicamba-resistant soybean. Weed Technol 27:492-496

Cahoon CW, York AC, Jordan DL, Everman WJ, Seagroves RW, Culpepper AS, Eure PM (2015) Palmer amaranth (Amaranthus palmeri) management in dicamba-resistant cotton. Weed Technol 29:758-770

Chang, F. Y., & Born, W. V. (1971). Dicamba uptake, translocation, metabolism, and selectivity. Weed Science, 19 (1), 113-117.

Cheng, Y., & Zhao, Y. (2007). A role for auxin in flower development. Journal of Integrative Plant Biology, 49(1), 99-104.

Culpepper, A. S., Sosnoskie, L. M., Shugart, J., Leifheit, N., Curry, M., & Gray, T. (2018). Effects of low-dose applications of 2, 4-D and dicamba on watermelon. Weed Technol, 32 (3), 267-272.

De Jong, M., Mariani, C., & Vriezen, W. H. (2009). The role of auxin and gibberellin in tomato fruit set. Journal of Experimental Botany, 60(5), 1523-1532.

Dexter, A. G. (1993). Herbicide spray drift. North Dakota State University Extension Service, NDSU, Fargo, ND 58105.

Egan, J. F., & Mortensen, D. A. (2012). Quantifying vapor drift of dicamba herbicides applied to soybean. Environmental Toxicology and Chemistry, 31(5), 1023-1031.

Fagliari, J. R., Oliveira Jr.*, R. S. D., & Constantin, J. (2005). Impact of sublethal doses of 2, 4-D, simulating drift, on tomato yield. Journal of Environmental Science and Health, Part B, 40(1), 201-206.

Fennimore, S. A., & Doohan, D. J. (2008). The challenges of specialty crop weed control, future directions. Weed Technol, 22(2), 364-372.

Flessner ML, McElroy JS, McCurdy JD, Toombs JM, Wehtje GR, Burmester CH, Price AJ. and Ducar JT (2015) Glyphosate-resistant horseweed (*Conyza canadensis*) control with dicamba in Alabama. Weed Technol 29:633-640

Fong WG, Moye HA, Seiber JN, Toth JP. 1999. Pesticide residues in foods. Methods, techniques, and regulations. New York: John Wiley & Sons. p 339.

Geary, N. D., Hatterman-Valenti, H., Secor, G. A., Zollinger, R. K., & Robinson, A. P. (2019). Response of ‘russet burbank’seed tubers containing dicamba and glyphosate. Weed Technol, 33(1), 9-16.

Gish, T. J., Prueger, J. H., Daughtry, C. S., Kustas, W. P., McKee, L. G., Russ, A. L., & Hatfield, J. L. (2011). Comparison of field-scale herbicide runoff and volatilization losses: An eight-year field investigation. Journal of Environmental Quality, 40(5), 1432-1442.

Gleason, C., Foley, R. C., & Singh, K. B. (2011). Mutant analysis in Arabidopsis provides insight into the molecular mode of action of the auxinic herbicide dicamba. PloS one, 6(3), e17245.

Grossmann, K. (2000). Mode of action of auxin herbicides: a new ending to a long, drawn out story. Trends in Plant Science, 5(12), 506-508.

Grover, R. A. J., & Smith, A. E. (1974). Adsorption studies with the acid and dimethylamine forms of 2, 4-D and dicamba. Canadian Journal of Soil Science, 54(2), 179-186.

Hall, T. S. J., Burton, J. D., Kuhr, R. J., Roe, R. M., & Sterling, T. M. (1997). Mechanism of action of natural auxins and the auxinic herbicides. Herbicide Activity: Toxicology, Biochemistry and Molecular Biology, 111-141.

Hansen, H., & Grossmann, K. (2000). Auxin-induced ethylene triggers abscisic acid biosynthesis and growth inhibition. Plant Physiology, 124(3), 1437-1448.

Hartzler B (2017) A historical perspective on dicamba. Iowa State University Extension and Outreach. Available at: https://crops.extension.iastate.edu/blog/bobhartzler/historical-perspective-dicamba.

Hatterman-Valenti, H., Endres, G., Jenks, B., Ostlie, M., Reinhardt, T., Robinson, A., ... & Zollinger, R. (2017). Defining glyphosate and dicamba drift injury to dry edible pea, dry edible bean, and potato. HortTechnology, 27(4), 502-509.

Heap, I., & Duke, S. O. (2018). Overview of glyphosate-resistant weeds worldwide. Pest Management Science, 74(5), 1040-1049.

Heap, I. The International Herbicide-Resistant Weed Database. Online. Tuesday, April 19, 2022

Hee, S. Q., & Sutherland, R. G. (1974). Volatilization of various esters and salts of 2, 4-D. Weed Science, 22(4), 313-318.

Hermes, Morgan Elizabeth (2020). Effects of simulated 2,4-D and dicamba drift on field-grown tomato plants. Master's thesis, Texas A&M University. Available electronically from https://hdl.handle.net/1969.1/192312.

Inman MD, Jordan DL, York AC, Jennings KM, Monks DW, Everman WJ, Bollman SL, Fowler JT, Cole RM, Soteres JK (2016) Long-term management of Palmer amaranth (*Amaranthus palmeri*) in dicamba-tolerant cotton. Weed Science 64:161-169

Jones, G. T., et al. Comparison of two dicamba formulations for risk of off-target movement to soybean. Soybean Research Studies 2015: 124.

Jordan, T.N., Romanowski, R.R. (1974) Comparison of dicamba and 2,4-D injury to field grown tomatoes. Hort Science (9) pp.74-75.

Kelley, K. B., & Riechers, D. E. (2007). Recent developments in auxin biology and new opportunities for auxinic herbicide research. Pesticide Biochemistry and Physiology, 89(1), 1-11.

Knezevic SZ, Osipitan O A, Scott JE (2018) Sensitivity of grape and tomato to micro-rates of dicamba-based herbicides. Journal of Horticulture 5: 229. doi: 10.4172/2376-0354.1000229

Kruger, G. R., Johnson, W. G., Doohan, D. J., & Weller, S. C. (2012). Dose response of glyphosate and dicamba on tomato (*Lycopersicon esculentum*) injury. Weed Technol, 26(2), 256-260.

Latorre D, Reynolds D, Young B, Norsworthy J, Culpepper S, Bradley K, Bish M, Kruger G, and Stephenson D (2017) Evaluation of volatility of dicamba formulations in soybean crop. North Cent Weed Sci Soc Absr 213. North Cent Weed Sci Soc (Dec. 2017)

Long, J. L. (2017). Influence of application factors on dicamba volatility (Doctoral dissertation, Purdue University).

Lovelace, M. L., Talbert, R. E., Scherder, E. F., & Hoagland, R. E. (2007). Effects of multiple applications of simulated quinclorac drift rates on tomato. Weed Science, 55(2), 169-177.

Mackay, D., Bobra, A., Chan, D. W., & Shiu, W. Y. (1982). Vapor-pressure correlations for low-volatility environmental chemicals. Environmental Science & Technology, 16(10), 645-649.

Majda, M., & Robert, S. (2018). The role of auxin in cell wall expansion. International Journal of Molecular Sciences, 19(4), 951.

Melo, A., Mansilha, C., Pinho, O., & MPLVO Ferreira, I. (2013). Analysis of pesticides in tomato combining QuEChERS and dispersive liquid–liquid microextraction followed by high-performance liquid chromatography. Food Analytical Methods, 6(2), 559-568.

Mohseni-Moghadam, M., & Doohan, D. (2015). Response of bell pepper and broccoli to simulated drift rates of 2, 4-D and dicamba. Weed Technol, 29(2), 226-232.

Monsanto Corporation. (2016) Xtendimax® product label. St. Louis, Monsanto. Available at: http://cdms.net/ldat/ldDF9000.pdf

Mueller, T. C., Wright, D. R., & Remund, K. M. (2013). Effect of formulation and application time of day on detecting dicamba in the air under field conditions. Weed Science,61(4), 586-593.

National Pesticide Information Center. (2011) Dicamba Technical Fact Sheet. Available at: http://npic.orst.edu/factsheets/archive/dicamba_tech.html

National Pesticide Information Center. (2011) Glyphosate Technical Fact Sheet. Available at: http://npic.orst.edu/factsheets/archive/glyphotech.html

Oseland, E., Bish, M., Steckel, L., & Bradley, K. (2020). Identification of environmental factors that influence the likelihood of off-target movement of dicamba. Pest Management Science, 76(9), 3282-3291.

Osipitan, O. A., Scott, J. E., & Knezevic, S. Z. (2019). Glyphosate-resistant soybean response to micro-rates of three dicamba-based herbicides. Agrosystems, Geosciences & Environment, 2(1), 1-8.

Picó, Y., Blasco, C., & Font, G. (2004). Environmental and food applications of LC–tandem mass spectrometry in pesticide-residue analysis: An overview. Mass Spectrometry Reviews, 23(1), 45-85.

Pujeri, U. S., Pujar, A. S., Pujari, K. G., Kumbar, M. I., & Yadawe, M. S. (2016). Quantitative analysis of pesticide residues in vegetables. International Journal of Scientific & Engineering Research, 7(5), 386-392.

Reddy, K. N., & Nandula, V. K. (2012). Herbicide resistant crops: History, development and current technologies. Indian Journal of Agronomy, 57(1), 1-7.

Redemann, C. E., Chaikin, S. W., Fearing, R. B., & Benedict, D. (1948). The volatility and vapor pressure of nine organic arsines. Journal of the American Chemical Society, 70(2), 637-639.

Ross M.A, C.A. Lembi. (2009) Applied weed science, including the ecology and management of invasive plants. 3rd edition. Pearson Prentice Hall. Pp 209-225.

Roundup Ready Xtend Crop System. (2019) History Available at: https://www.roundupreadyxtend.com/About/History/Pages/default.aspx

Schmoll, J. T., Harrison, S. K., Regnier, E. E., & Bennett, M. A. (2000). Hydroponic tomato (*Lycopersicon esculentum*) response to dicamba in the nutrient media 1. Weed Technology, 14(3), 562-568.

Sciumbato, A. S., Chandler, J. M., Senseman, S. A., Bovey, R. W., & Smith, K. L. (2004). Determining exposure to auxin-like herbicides. I. Quantifying injury to cotton and soybean. Weed Technology, 18(4), 1125-1134.

Seefeldt, S. S., Boydston, R. A., & Kaspari, P. N. (2014). Clopyralid and dicamba residue impacts on potatoes and weeds. American Journal of Potato Research, 91(6), 625-631.

Shaner D (2014) Herbicide handbook. Weed Science Society of America, Champaign, IL:315

Sharkey, S. M., Stein, A., & Parker, K. M. (2020). Hydrogen bonding site number predicts dicamba volatilization from amine salts. Environmental Science & Technology, 54(21), 13630-13637.

Soltani N, Oliveira MC, Alves GS, Werle R, Norsworthy JK, Sprague CL, Young BG, Reynolds DB, Brown A, Sikkema PH (2020) Off-target movement assessment of dicamba in North America. Weed Technol. 34: 318–330. doi: 10.1017/wet.2020.17

Sosnoskie LM, Culpepper AS (2014) Glyphosate-resistant Palmer amaranth (Amaranthus palmeri) increases herbicide use, tillage, and hand-weeding in Georgia cotton. Weed Science 62:393- 402

Taiz L, Zeiger E. (eds) 1998. Plant physiology. Sunderland: Sinauer Associates Publishers.

Taylor, A. W., & Spencer, W. F. (1990). Volatilization and vapor transport processes. Pesticides in the Soil Environment: Processes, Impacts and Modeling, 2, 213-269.

University of Minnesota Extension (2018) Growth regulator herbicides. Available at: https://extension.umn.edu/herbicide-mode-action-and-sugarbeet-injury-symptoms/growth-regulator-herbicides

USDA- National Agricultural Statistics Service (USDA NASS). (2022) Statistics by Subject. Available at: https://quickstats.nass.usda.gov/

USDA- Economic Research Service (USDA ERS). (2021) Available at: https://www.ers.usda.gov/

USDA- Vegetable Summary. (2019) Available at: https://www.nass.usda.gov/Publications/Todays_Reports/reports/vegean20.pdf

USEPA (2002) A review of the reference dose and reference concentration processes. In Risk Assessment Forum, U. The Environmental Protection Agency.

USEPA (2016) Dicamba; Pesticide Tolerances. Available at: https://www.govinfo.gov/content/pkg/FR-2016-12-08/pdf/2016-29245.pdf

USEPA (2020) Memorandum Supporting Decision to Approve Registration for the Uses of Dicamba on Dicamba Tolerant Cotton and Soybean. Available at: https://www.epa.gov/sites/default/files/2020-10/documents/dicamba-decision_10-27-2020.pdf

United States Geological Survey (USGS). (2019) Available at: https://www.usgs.gov/

Van Wychen L (2016) 2015 Baseline Survey of the Most Common and Troublesome Weeds in the United States and Canada. http://wssa.net/wp-content/uploads/2015_Weed_Survey_Final.xlsx Accessed: 9 July, 2017

Wall, D. A. (1994). Potato (*Solanum tuberosum*) response to simulated drift of dicamba, clopyralid, and tribenuron. Weed Science, 42(1), 110-114.

Warmund, M., Ellersieck M. & Smeda R. J. (2022). Sensitivity of tomato cultivars to sublethal rates of dicamba and 2,4-D. Horticulturae, In Review.

Woodward, A. W., & Bartel, B. (2005). Auxin: regulation, action, and interaction. Annals of Botany, 95(5), 707-735.

Zangoueinejad, R., Alebrahim, M. T., & Tseng, T. M. (2019). Evaluation of auxin tolerance in selected tomato germplasm under greenhouse and field conditions. Weed Technol, 33(6), 815-822.

Zangoueinejad, R., Alebrahim, M. T., & Tseng, T. M. (2020). Absorption and translocation of dicamba in dicamba-tolerant wild tomato. Canadian Journal of Plant Science, 101(1), 30-38.

Zangoueinejad, R., Alebrahim, M. T., & Tseng, T. M. (2020). Molecular and physiological response to dicamba in dicamba-tolerant wild tomato. Journal of the Mississippi Academy of Science, 65, 358-373.

Chapter 2: Buffer distance impact on dicamba damage to potato, tomato and watermelon.

Timothy C. Rice, Sarah E. Dixon, Emma L. Gaither, Jerri L. Henry and Reid J. Smeda

Abstract

Dicamba off-target movement can result in severe plant damage to sensitive crops in close proximity to applied areas. Mandatory buffer distances between applied areas and sensitive crops have been implemented to protect these crops, but it is unknown if current buffer distance guidelines are sufficient to preclude damage to sensitive vegetable crops. In central Missouri, a field experiment in 2020 and 2021 was established using watermelon (*Citrullus lanatus*), tomato (*Solanum lycopersicum*) and potato (*Solanum tuberosum*) plants to estimate the impact of buffer distance, in both the upwind and downwind direction, following a dicamba application to adjacent dicamba tolerant (DT) soybeans (*Glycine max*). Replicated strips of each transplanted crop (20.1 m row per crop, totaling 61 m per strip) were planted at 8.4, 16.8, 33.6, 50.3, and 67 m west from a 1 ha block of DT soybeans. An adjacent area of land was planted with the vegetables established east of the DT soybeans. DT soybeans were sprayed with dicamba Xtendimax® at 0.56 kg ae-ha^{-1} with a prevailing eastern and western wind in 2020 and 2021, respectively. Assessment data included watermelon vine growth and visual injury of tomato, potato, watermelon and dicamba sensitive (DS) soybean tissue from 3-28 days after treatment (DAT) of DT soybeans. Fruit samples of tomato, potato and watermelon were harvested at 14 and 28 DAT for residue analysis. Visual responses of tomato, potato and watermelon were minimal (<3%) in both years at a 33.6 m buffer distance and beyond in both the upwind and downwind directions. Vine growth of watermelon was reduced up to 50% for plants at 8.4 m downwind of DT soybeans when compared to watermelon at 33.6 m or further. Potato and tomato yields remained largely unaffected by buffer distance. DS soybean visual

injury was highest (18%) at 8.4 m downwind. Dicamba residues were detected (≤ 18 μg/kg) in fruit samples from all three crops at a distance of 8.4 m. Results from this experiment indicate that a buffer distance of 16.8 m is required to prevent fruit contamination, but 33.6 m is needed to prevent significant damage and growth reduction to nearby tomatoes, potatoes and watermelon.

Introduction

Dicamba injury on sensitive crops has been a contested issue since the introduction of dicamba-tolerant (DT) soybeans and cotton (*Gossypium hirsutum*). Following the introduction of glyphosate-tolerant soybeans in 1996, the number of glyphosate-resistant weed species has continued to rise and result in less effective broadleaf weed control from glyphosate (Heap, 2018; Heap, 2010). DT soybeans and cotton were introduced to help manage glyphosate-resistant weeds (Soltani et al., 2020). In 2017, the year DT crops were introduced, there were 2,708 dicamba-related injury cases in the United States (Bradley, 2017). In 2020, applicators continued to widely use dicamba as more than 900,000 kg of dicamba were applied to 1.6 million hectares of DT soybeans (usage rate is 0.56 kg ae ha^{-1}) in Missouri (USDA NASS, 2022).

Dicamba damage to sensitive non-target crops occurs through off-target movement via particle or vapor drift. Particle drift may be induced during application by high wind speeds which prevent dicamba from landing on its intended target (Egan et al., 2012). Other factors that influence particle drift include mechanical factors such as application height, herbicide formulation, spray droplet size, nozzle spacing and application speed in addition to environmental factors such as usage area (Mohseni-Moghadam et al., 2015; Oseland et al., 2020).

In addition to particle drift, dicamba can also move off-target after application via volatilization. High air temperatures and low relative humidity may cause dicamba particles to volatilize into small aerosol particles (Egan et al., 2012). In vapor form, dicamba is highly susceptible to off target movement via wind or temperature inversions (Bish et al., 2019). The chemical composition of dicamba products also impacts volatility. The original formulations of dicamba possessed a high risk to volatilize due to the dimethylamine (DMA) salt used in those formulations (Latorre et al., 2017). New formulations of dicamba include different salts, such as BAPMA (N,N-bis-aminopropyl methylamine), which are significantly less volatile than older formulations due to the salts increased affinity for the dicamba acid (Sharkey et al 2020). However, even these formulations do not eliminate off-target movement; required buffers further reduce off-target movement (Jones et al., 2019).

Mandatory buffer distances between dicamba-treated areas and nearby sensitive crops have been implemented to protect susceptible crops from off-target movement. In 2020, the EPA amended dicamba application guidelines to include a new mandatory buffer distance of 73 m between any potentially sensitive crop and a dicamba treated area (US-EPA, 2020). Despite this amendment, dicamba damage was still reported to the EPA for tomato, potato and watermelon in 2021 (Academics, 2021; USEPA, 2021; Dunbar, 2021). It is not clear if this buffer distance is sufficient to preclude dicamba injury to sensitive crops.

Dicamba off-target movement can result in severe plant damage to sensitive crops at doses lower than the recommended rate of dicamba (Bohnenblust et al., 2016). Osipitan (2019) found that exposure to dicamba at 56 g ha^{-1} can reduce the yields for susceptible soybeans up to 96%. Jones et al. (2019) looked at the relationship between damage to dicamba sensitive

(DS) soybean following a dicamba application and buffer distance. It was found that a 5% height reduction occurred at more than 80 m downwind and yield losses for DS soybean were measured up to 33.6 m downwind (Jones et al., 2019). Furthermore, dicamba damage (5%) was noted up to 153 m away from the applied area. With DS soybean exhibiting damage at distances further than the EPA mandated buffer distance, it is important to consider the impacts of dicamba on specialty crops.

Specialty crops such as tomatoes, potatoes and watermelon make up a large percent of specialty crops grown in Missouri and are susceptible to herbicide drift from major production areas. Across the United States approximately 370,000, 110,000 and 40,000 hectares of potato, tomato and watermelon were planted respectively, in 2020 (USDA NASS, 2022). Over 35 million hectares of soybeans were planted across the United States and 2.3 million were planted in Missouri in 2021 (USDA NASS, 2022). This large production area subject to treatment with post-emergence applications of dicamba can subject specialty crops to damage from off-target movement. In fact, herbicide drift from neighboring fields has been the primary reason for synthetic auxin damage to tomato plants (Fagliari et al. 2005; Lovelace et al. 2007b).

In addition to tomato; potato and watermelon are also sensitive to damage from dicamba off-target movement. Knezevic et al. (2018) found that doses of 0.82, 4.49 and 56 g ae ha^{-1} (1/683, 1/125 and 1/10 of the label rate) of dicamba caused an estimated 10%, 50% and 80% visual injury to tomatoes, respectively at 21 DAT. Culpepper et al. (2018) found that watermelon treated with the 1/75 rate of dicamba at 20 days after planting (DAP) exhibited 40% visual injury and had vine lengths reduced by 51% compared to non-treated control plants.

Hatterman-Valenti et al. (2017) reported that 0.1 kg ha^{-1} of dicamba resulted in visual injury of up to 49% in North Dakota at 20 DAT.

Although visual injury to tissue of sensitive plants can be problematic, there are limited reports if residue of dicamba can be found in tomato and watermelon fruit or potato tubers. Culpepper et al. (2018) reported dicamba residue concentrations of 0.01 and 0.03 ppm in watermelon fruit treated with the 1/250 and 1/75 use rates of dicamba. Sirons et al. (1982) found 30 and 10 µg/kg of dicamba in tomato plants app1ied with 2.2 g ae ha^{-1} at 7 and 14 days after treatment, respectively. The presence of other pesticides, such as carbendazim, have also been documented in tomatoes with detectable concentrations as small as 0.014 ppm (Pujeri et al., 2016). Documentation on the analysis of dicamba residues in potato has not been reported and requires more research. The presence of these pesticide residues in horticultural products is of concern to human health (Melo et al., 2013).

In order to minimize damage and contamination in specialty crops, it is essential to understand the impact buffer distance has on dicamba off-target movement and crop susceptibility. The objectives of this field study were to determine whether current EPA buffer distance guidelines, currently set at 73 m, are sufficient to preclude measurable damage to nearby sensitive potatoes, tomatoes and watermelon. Additionally, this study aimed to quantify dicamba residues in potentially exposed tomato and watermelon fruit as well as potato tubers.

Materials and Methods

A field experiment was established in 2020 and 2021 at the Bradford Research Center near Columbia, MO (38° 53’43.12”N 92°12’21.06”W). Crop varieties included ‘Crimson Sweet’ watermelon, ‘Mountain Merit’ tomato (Harris Seeds Company, Rochester, NY, USA) and ‘Atlantic’ potato (Black Gold Farms, Grand Forks, ND, USA). Varieties of each crop were selected due to their individual disease resistances and prevalent use by Missouri specialty crop growers. In both years, transplants of tomato and watermelon were germinated in 4 cm peat pots (Harris Seeds Company, Rochester, NY, USA) containing a 30:70 mixture of Mexico silt loam (fine, smectitic, mesic vertic epiaqualfs) (USDA Official Soil Series Descriptions, 2022) and peat-based greenhouse media (Pro-Mix BX Mycorrhizae; Hummert International, Earth City, MO, USA) at a University of Missouri greenhouse. Tomato and watermelon seedlings were fertilized weekly with a 10-10-10 N-P-K all-purpose fertilizer (Hummert International, Earth City, MO, USA) until transplanted into the field. Tomatoes were transplanted at a height of ~ 30 cm and watermelon were transplanted at first vine development. In 2020, tomato and watermelon transplants were started on April 20 and transplanted into the field on May 29. The seed potatoes were directly sown into the ground on May 22. In 2021, tomato and watermelon transplants were started on April 21 and transplanted into the field on June 4. The seed potatoes were directly sown into the ground on June 4. Prior to planting in both years, signature series pelletized lime fertilizer (MK Minerals Inc., Wathena, KS, USA) was applied to every plot at 20 kg per 100 m^2. All plants were then fertilized (200 ppm Nitrogen) with Jacks Proffesional LX calcium-rich fertilizer (JR Peters Inc., Allentown, PA, USA) at planting and then as needed until the end of the growing season. Single rows of DS soybeans (Liberty Link S170082, BASF, Ludwigshafen, Germany) and bulk DT soybeans (Roundup Ready 2 Xtend AG43X0, Bayer, Leverkusen, Germany) were planted on June 2 and June 8 in 2020 and 2021 respectively. Soybeans were planted with 76 cm spacing at a population of 345,000 seeds ha^{-1}.

Two adjacent fields (61 m X 244 m & 61 m X 210 m) were selected for use in both 2020 and 2021. One area consisted of a Mexico silt loam soil type with a pH of 6.3 and 2.1% organic matter. The second area consisted of a Mexico silt loam soil type with a pH of 6.2 and 1.8% organic matter. Each field contained both a vegetable area and production area of DT soybean. The vegetable area contained five, 1 m x 61 m rows of specialty crops planted in a 1.25 mm white plastic mulch (Morgan County Seed, Barnett, MO, USA). White plastic mulch and 8 mm irrigation drip tape (Rain-Flo Irrigation, East Earl, PA, USA) was installed using a Model 480 plastic mulch layer (Rain-Flo Irrigation, East Earl, PA, USA) which created a 10 cm raised bed. White plastic mulch was not applied to the 67 m distances of both fields in 2021 due to constant over-saturation. Each row contained four, 5.1 m long plots of each vegetable crop, randomly placed along the row, for a total of 12 plots per row. Each 5.1 m plot contained 8 tomatoes plants, 10 potatoes plants or 4 watermelon seedlings spaced 0.635 m, 0.5 m or 2.54 m, respectively (2 watermelons were planted per space). Rows were planted at distances of 8.4, 16.8, 33.6, 50.3 and 67 m from the 1.25 hectare block of DT soybeans. One field was oriented with the specialty crops to the east of the DT soybeans while the other was oriented in the opposite direction, with the specialty crops to the west of the DT soybeans. All crops which were downwind of the dicamba application will be hereafter termed downwind, while crops that were upwind of the dicamba application will be hereafter referred to as upwind. A single row of DS soybeans was also planted in front of each row of specialty crops (the side closest to the DT soybean block) as an indicator species.

In both 2020 and 2021, dicamba was applied to the DT soybean blocks of both fields with a tractor sprayer using TTI11003 spray nozzles (TeeJet Technologies, Wheaton, IL, USA) with dicamba at a rate of 0.56 kg ae ha^{-1}. The tank mixture also contained Impetro 2 (MFA Agri Service, Columbia, MO, USA) at 1% v/v as a drift reducing agent. In 2020, the wind was blowing from the east at 4.8 km h^{-1} during application, which allowed one block to simulate a downwind off-target movement study and the adjacent block to simulate an application upwind of the sensitive crops. The air temperature was 31° C with a relative humidity of 78%. In 2021, the wind was blowing from the west at 6.4 km h^{-1} during application which allowed for the simultaneous simulation of both the upwind and downwind studies. The air temperature was 30°C with a relative humidity of 59%.

Prior to planting the vegetable crops in both years, an application of S-metolachlor (1.45 kg ai ha^{-1}) and metribuzin (0.34 kg ai ha^{-1}) herbicides were made next to the tomato and potato plots for residual weed control. S-metolachlor alone was applied at a rate of 1.4 kg ai ha^{-1} to the watermelon plots. Supplemental applications of fungicide were made as needed to reduce the potential for disease on all three crops. Azoxystrobin was applied at a rate of 0.22 kg ai ha^{-1} to the watermelon and a rate of 0.11 kg ai ha^{-1} to the potatoes and tomatoes. Two fungicide applications were made during each year. No other pest management products were needed. To minimize weed competition, hand-weeding was used to control the weed population in the sensitive crop areas throughout the growing season.

In 2021, a cover crop of rye (*Secale cereale*) was planted in the specialty crop blocks of both fields to promote weed suppression. Rye was planted at a population of 2.6 million seeds ha^{-1} in October, 2020. Rye growth was terminated with glyphosate (0.84 kg ae ha^{-1}) in May, 2021.

Tomato cages (0.9 m) were assembled and secured around each individual tomato plant via rebar (Ahrens Steel, Columbia, MO, USA). As a determinate variety, the Mountain Merit tomatoes needed this support for optimal growth. Additionally, an electric fence (SGT Knots Supply Co., Statesville, NV, USA) was assembled around both specialty crop blocks in both years to prevent wildlife foraging.

Data were collected on watermelon, tomatoes, potatoes and DS soybean injury at 7, 14, 21 and 28 days after treatment (DAT). Visual ratings (0-100%) were recorded where a rating of 0% meant no visible damage and 100% meant complete plant death. When the DT soybeans were treated with dicamba, 3 random watermelon vines per plot were tagged with a zip tie in order to measure vine length growth at 7, 14, 21 and 28 DAT. Tomato and potato weight data were taken by weighing the total fruit or below ground tuber weight of 3 random plants per plot. At 14 and 28 DAT, three tomato fruit, three potato tubers and one watermelon were collected from each plot at random for residue analysis.

The experiment was a split plot arranged in a randomized complete block design. The main plot factor was wind direction (n=2) and buffer distance was the subplot factor (n=5). Vegetable types were randomly assigned to one of 12 plots within each buffer distance. Biological replicates for each vegetable plot were eight tomato plants, ten potato plants, or four watermelon plants. Visual injury, watermelon vine length, vegetable fresh weights at harvest and dicamba residue data were subjected to ANOVA using PROC GLMMIX in SAS 9.4 (SAS Institute Inc., Cary, NC, USA) with main and subplot factors as fixed effects and blocks as random. Significant means were separated at α=0.05 using Tukey's Honestly Significant Difference (HSD).

Residues of dicamba from all 3 crops were analyzed using similar preparation and detection methods. The samples from each plot were homogenized in a blender and stored in a 0.56 cubic m chest freezer (Frigidaire, Augusta, GA, USA) at -18°C until delivered to Eurofins Agroscience Services (Columbia, MO, USA) for analysis. Once received, the samples were placed into a Robot Coupe model R10 chopper (Robot Coupe Inc., Jackson, MS, USA). The tissue was blended with dry ice until the whole sample reached uniform size and texture. The samples were then placed in a T-49F sublimation freezer (True Manufacturing Company, O'Fallon, MO, USA) for 2 days, allowing the dry ice to evaporate from the samples. Between samples, the processing equipment was triple rinsed and washed with distilled water and Versa Clean™ multi-purpose cleaner (Fisher Scientific, Hampton, NH, USA) and the counters were cleaned with methanol.

Following homogenization, the samples were prepared for residue analysis following the method described by Guo et al., (2016) with modifications and specific details described below. Each sample (5 g) was placed into a 50-mL polypropylene centrifuge tube (VWR International, Radnor, PA, USA). Standards were fortified with proper fortification solutions. All control and treated samples were given 0.1 mL of 1N HCl solution followed by 0.2 mL of the 1.0 µg/mL mixed IS working solution 34598-MIS01 (Eurofins Agroscience Services, Columbia, MO, USA). Two 6.35 mm steel grinding balls and 24.7 mL of 40.6% ACN in acidified water solution were added to all samples. Next, samples were placed in a Genogrinder (SPEX SamplePrep LLC, Metuchen, NJ, USA) for 20 minutes at 1200 strokes per min. After grinding, samples were centrifuged using a Sorvall Legend XTR (Thermo Scientific, Waltham, MA, USA) at 4000 rpm for 10 minutes at 10 °C. Supernatant was transferred into a clean 50 mL polypropylene tube. From there, 0.25 mL of supernatant was transferred from each 50 mL tube to a clean 1.4 mL polypropylene tube. Samples were hydrolyzed in an Isotemp 500 series oven (Fisher Scientific, Hampton, NH, USA) for approximately 1 hour at approximately 95 °C and then allowed to cool to room temperature. Upon cooling, each tube received 0.25 mL of 20:80 ethyl acetate:isooctane. Next, samples were mixed on a multi-tube vortexer (VWR International, Radnor, PA, USA) for 2 minutes and then centrifuged again at 4000 rpm for 5 minutes at 10 °C to separate organic and aqueous phases. The organic layer (0.15 mL) was transferred to a clean 1-mL glass-lined 96-well plate. Samples were evaporated to dryness under nitrogen (N^2) using a Biotage SPE Dry sample concentrator 96 (Biotage, Uppsala, Sweden) with settings at 30°C and 30 L N^2/min. Samples were re-constituted with 0.3 mL of Sample Reconstitution Solution (25% MeOH in acidified water). Finally, samples were mixed on a multi-tube vortexer for 5 minutes followed by sonication in a Branson 2210 (Branson Ultrasonics, Brookfield, CT, USA) for approximately 5 minutes and then mixed one more time on a multi-tube vortexer for 5 minutes.

Residue detection for each tissue sample was performed utilizing a Shimadzu Prominence CBM-20A Series LC (Shimadzu Scientific Instruments, Columbia, MD, USA) with an AB Sciex Q-Trap 5500 Mass Spectrometer detector (AB Sciex LLC, Framingham, MA, USA). Chromatography utilized 0.01% formic acid in water and 0.01% formic acid in methanol for the mobile phases with a Phenomenex Kinetex Biphenyl 100 mm x 2.1 mm, 1.7 µm column (Phenomenex, Torrance, CA, USA). Analytical data were processed using Analyst software (AB Sciex LLC, Framingham, MA, USA). The LOQ was established at 0.01 ppm of dicamba. For accurate quantification, a standard curve was generated at each injection using eight control samples spiked with a dicamba internal standard at concentrations ranging from 0.003 to 0.4 PPM.

Results and Discussion

Visual Injury. Although visual symptoms from dicamba occurred at all observation dates, optimum symptomology demonstrating the relationship between buffer distance and visual injury was noted at 21 DAT. Therefore, only this date was selected to show results. Other timings and data not discussed are available in the Appendix.

Visual damage to all four crops remained low (≤ 20%) which resulted in minimal symptomology. Stem splitting and twisting were uncommon in this experiment due to the low exposure rates. Leaf cupping was the most notable symptom observed across all plant species. The plants exhibiting the most injury generally had a larger number of leaves cupping when compared to plants with less injury.

Downwind, the indicator rows of DS soybean exhibited up to 18% visual injury by 21 DAT, with the highest damage occurring at 8.4 m and decreasing with increasing distance (Figure 2.1). Upwind damage was minimal with a maximum of 2% visual injury observed at 8.4 m and decreasing to 1% at greater distances (Figure 2.1). Indicator soybeans demonstrate that dicamba off-target movement did occur in the field study in both 2020 and 2021.

Potato injury showed a similar trend to the DS soybean. At 21 DAT, the highest damage was observed downwind at 8.4 m (5.4%). Damage generally decreased with increasing distance and by 33.6 m, damage downwind was below 2% for all timings (Figure 2.2). Upwind damage remained below 2% for all distances. No damage occurred beyond 33.6 m for both upwind and downwind potatoes, 21 DAT (Figure 2.2).

Downwind tomato visual injury was highest (7%) at 8.4 m (Figure 2.3). Observable damage for tomatoes decreased stepwise with increasing buffer distance. At 33.6 m, damage was reduced to 3% and 1% by 67 m. Upwind, the highest damage observed was 4.5% at 8.4 m. Tomato damage remained below 3% for the remaining distances which was statistically similar (Figure 2.3).

Despite a canopy close to the ground, patterns for watermelon plants were similar compared to the other crops (Figure 2.4). The highest damage recorded was 6% at 8.4 m downwind. While overall damage was low, damage for watermelon persisted above 2% through 33.6 m, similar to potato and tomato. This trend was observed both upwind and downwind. Damage dropped to a mean of approximately 1% at 50.3 m and was absent at 67 m (Figure 2.4).

The visual injury ratings of the DS soybean, potato, tomato and watermelon all indicate that 33.6 m was sufficient to minimize visible dicamba injury to sensitive crops under the conditions of this experiment. The potato data demonstrates how damage may be minimized when sensitive crops are upwind from a dicamba application. Furthermore, it demonstrates how damage may be prevented entirely, even in the downwind direction, after 33.6 m. The tomato data reinforces the potato data by demonstrating how a majority of damage is prevented in the upwind direction. Additionally, data shows that damage is minimal, even in the downwind direction, after 33.6 m. The watermelon data further supports these findings by demonstrating that a majority of watermelon damage is also prevented beyond 33.6 m.

Jones et al. (2019) found that dicamba damage (5%) was detectable 153 m away from an applied area for DS soybean. A different study by Soltani et al. (2020) recorded dicamba off-target movement damage on DS soybean 250 m away. However, this study reported that a majority of damage to DS soybean was prevented at a buffer distance of 20 m. The data from this study agrees with the findings of Jones et al. (2019) and Soltani et al. (2020). Damage can occur at the furthest distances (67 m), but a shorter distance (33.6 m) was sufficient to prevent a majority of the damage recorded.

Response of watermelon vine growth. At 28 DAT vine lengths for all buffer distances and directions were not significantly different (Figure 2.5). However, downwind vine lengths at 8.4 m were 50% shorter when compared to vine lengths at 33.6 m. This indicates that visual injury to watermelon (Figure 2.4) may impact vine growth. Upwind, watermelon vine length was not impacted at any buffer distance (Figure 2.5). These data suggest that watermelon is not particularly sensitive to low rates of dicamba.

Culpepper et al. (2018) found that watermelon treated with the 1/75 rate of dicamba exhibited vine length reductions of up to 50% when compared to the non-treated controls. Watermelon in this study had a similar reduction at 8.4 m downwind (50%) when compared to the longest recorded vine length at 33.6 m downwind. Although watermelon in this study was exposed to unknown rates of dicamba or none, visual injury data for watermelon suggests plants at 8.4 m downwind were exposed to dicamba (Figure 2.4). Therefore, the data from this study agrees with the findings of Culpepper et al. (2018), which indicate that exposure to dicamba can negatively impact watermelon vine length growth. However, no differences in vine growth were significant and therefore the data indicates that buffer distance does not impact the effects of dicamba off-target movement on watermelon vine length under the conditions of this experiment.

Fruit and tuber response of tomato and potato. For both tomato and potato weights, data from 2020 and 2021 were analyzed separately because there was a significant difference in the data across years.

In 2020, potato tuber weight was significantly reduced (70%) at 8.4 downwind compared to 33.6 m downwind (Figure 2.6). Yields remained high after 33.6 m. Upwind, the lowest yield was recorded (0.2 kg) at 50.3 m and was significantly lower than the highest yield recorded upwind (0.6 kg) at 8.4 m. While tuber weight increased with buffer distance downwind, upwind potatoes demonstrated the opposite effect (Figure 2.6). This effect could be the result of a potato stress response following exposure to dicamba. Plants exposed to higher concentrations of dicamba may have entered reproduction earlier and had more time to develop large tubers when compared to plants that were exposed to less dicamba and underwent less stress. The dicamba visual injury data for potatoes reinforces this hypothesis as the downwind plants suffered more damage but produced higher yields (Figure 2.2). Alternatively, the upwind reductions may be attributed to early season stunting due to flooding and other environmental conditions in that field in 2020.

In 2021, there were no significant differences for potato tuber weights across all distances and fields (Figure 2.7). However, weights tended to increase with increasing buffer distance both upwind and downwind. The exception to this was at 67 m which did not receive white plastic both upwind and downwind in 2021. The lack of plastic mulch increased weed competition and flooding at these positions which likely resulted in minimal tuber development (Figure 2.7). Significant yield differences observed in 2020 which were absent in 2021 could be the result of differences in dicamba concentrations that moved off-target in each year.

For tomato fruit harvested downwind in 2020, yields were lowest (1.8 kg) at 8.4 m but increased with buffer distance until 33.6 m where weights remained around 3.75 kg for the remaining distances (Figure 2.8). Upwind, the lowest weight recorded (0.9 kg) was at 50.3 m and was significantly smaller than the heaviest weight recorded upwind (3.75 kg) at 8.4 m. Similar to potato tubers in 2020, tomato fruit weight increased with buffer distance downwind, but upwind potatoes demonstrated the opposite effect (Figure 2.8). This could also be due to an increased stress response in plants exposed to higher concentrations of dicamba, similar to potato. The dicamba visual injury data for tomatoes reinforces this hypothesis as the downwind plants suffered more damage but produced higher yields (Figure 2.3). Alternatively, the upwind reductions recorded may be attributed to early season stunting as a result of flooding and other environmental conditions in that field, in 2020.

In 2021, there were no significant differences for tomato fruit weights across all distances in both fields (Figure 2.9). Major fruit reductions measured for tomatoes at 67 m did not receive white plastic both upwind and downwind in 2021. This increased weed competition and flooding at these positions which likely resulted in the minimal fruit growth demonstrated (Figure 2.9). Significant yield differences observed in 2020 which were absent in 2021 could be the result of differences in dicamba concentrations that moved off-target in each year.

Potato and tomato data from 2020 demonstrate that losses to fresh weight are largely negated at a buffer distance of 33.6 m in the downwind direction. Upwind data for both crops demonstrated that fresh weights decreased with buffer distance, in 2020. Weights recorded in 2021 remained largely unaffected by buffer distance, both upwind and downwind of the dicamba treated area. The downwind data from 2020 and all of the 2021 data indicate that a buffer distance of 33.6 m may be sufficient to prevent fresh weight losses to tomato fruits and potato tubers. This agrees with the findings of Jones et al. (2019) who only noted yield losses in DS soybean ≤ 33.6 m. The upwind data for 2020 disagrees with these findings, but reductions documented for this data are likely attributed to other environmental factors, such as flooding, rather than dicamba damage.

Residue Analysis. Dicamba was detected (≤ 18 µg/kg) in fruit and tuber samples from all three crops (Table 2.1). However, no concentrations above the limit of quantification (10 µg/kg) were detected beyond 8.4 m for any crops in both years. Dicamba residues (18 µg/kg) were only detected in potato 28 DAT at 8.4 m in the upwind direction. Tomato fruit only contained dicamba residues (13 µg/kg) at 8.4 m in the upwind direction, 14 DAT. Watermelon fruit only contained dicamba residues (13 µg/kg) at 8.4 m in the downwind direction, 14 DAT.

While all of the dicamba residues detected in these samples were below the USEPA dicamba oral reference dose (RfD) of 300 µg/kg/day (USEPA Dicamba; Pesticide Tolerances, 2016; NPIC-Dicamba, 2011), the data suggests that dicamba residues are not consistently below detectable limits until a distance of 16.8 m for all three crops.

Visual injury, watermelon vine length, tomato fruit fresh weight and potato tuber fresh weight all suggest that a buffer distance of 33.6 m is likely sufficient to prevent damage and injury to the three specialty crops tested in this study. Jones et al. (2019) found dicamba damage at a buffer distance of 153 m, height reductions at ≥80 m and yield losses at 33.6 m in DS soybeans. While some of the findings from Jones et al. (2019) agrees with the data of this study, it was found that potato, tomato and watermelon tended to require a shorter buffer distance than DS soybean to prevent losses and damage. This may indicate that these crops have a higher tolerance to dicamba when compared to DS soybean. However, it also could be an indication of other environmental factors that minimized damage to the specialty crops in our study. It is difficult to determine what rates of dicamba these crops are exposed to when off-target movement occurs which highlights the importance of quantifying residues found in the fruit. While the aforementioned data in this study suggested that a buffer distance of 33.6 m was sufficient to protect specialty crops, residue analysis indicates that a distance of 16.8 m is sufficient to prevent detectable dicamba contamination. Additionally, all residues detected were below the USEPA RfD of 300 µg/kg for all samples.

Growers will likely prevent a majority of fruit contamination at 16.8 m, however, preventing visual reductions, damage and losses to potatoes, tomatoes and watermelon require implementing a buffer distance of 33.6 m. The data from this study indicate that the current EPA buffer distance of 73 m is sufficient to protect potato, tomato and watermelon under the conditions of this field experiment.

Literature Cited

Academics. 2021. EPA-OPP notes from an August 30th, 2021 meeting with academics involved with weed science about dicamba.

Bish, M. D., Farrell, S. T., Lerch, R. N., & Bradley, K. W. (2019). Dicamba losses to air after applications to soybean under stable and nonstable atmospheric conditions. Journal of Environmental Quality, 48(6), 1675-1682.

Bohnenblust EW, Vaudo AD, Egan F, Mortensen DA, Tooker JF (2016) Effects of the herbicide dicamba on nontarget plants and pollinator visitation. Environmental Toxicology and Chemistry, 35:144-151

Bradley, K.W. (2017) A final report on dicamba-injured soybean acres. Available at: https://ipm.missouri.edu/IPCM/2017/10/final_report_dicamba_injured_soybean/

Culpepper, A. S., Sosnoskie, L. M., Shugart, J., Leifheit, N., Curry, M., & Gray, T. (2018). Effects of low-dose applications of 2, 4-D and dicamba on watermelon. Weed Technol, 32 (3), 267-272.

Dunbar, K. 2021a. Alleged Dicamba for EPA by Category as of 7-30-21 at 2 pm.xlsx. Email to K. Tindall dated 30 July 2021.

Dunbar, K. 2021b. ADA- Dicamba Case Status Update Summary 08-31-2021 [Arkansas]. Email to K. Tindall dated 1 Sept 2021.

Egan, J. F., & Mortensen, D. A. (2012). Quantifying vapor drift of dicamba herbicides applied to soybean. Environmental Toxicology and Chemistry, 31(5), 1023-1031.

Fagliari, J. R., Oliveira Jr.*, R. S. D., & Constantin, J. (2005). Impact of sublethal doses of 2, 4-D, simulating drift, on tomato yield. Journal of Environmental Science and Health, Part B, 40(1), 201-206.

Guo, H., Riter, L. S., Wujcik, C. E., & Armstrong, D. W. (2016). Quantitative analysis of dicamba residues in raw agricultural commodities with the use of ion-pairing reagents in LC–ESI–MS/MS. Talanta, 149, 103-109.

Hatterman-Valenti, H., Endres, G., Jenks, B., Ostlie, M., Reinhardt, T., Robinson, A., ... & Zollinger, R. (2017). Defining glyphosate and dicamba drift injury to dry edible pea, dry edible bean, and potato. HortTechnology, 27(4), 502-509.

Heap, I. M. 2010. International survey of herbicide resistant weeds. Online. WeedScience.org, Corvallis, OR.

Heap, I., & Duke, S. O. (2018). Overview of glyphosate-resistant weeds worldwide. Pest Management Science, *74*(5), 1040-1049.

Jones, G. T., Norsworthy, J. K., Barber, L. T., & Scott, R. C. (2015). Comparison of two dicamba formulations for risk of off-target movement to soybean. Soybean Research Studies 2015, 124.

Jones, G. T., Norsworthy, J. K., & Barber, T. (2019). Off-target movement of diglycolamine dicamba to non-dicamba soybean using practices to minimize primary drift. Weed Technol, 33(1), 24-40.

Knezevic SZ, Osipitan O A, Scott JE (2018) Sensitivity of grape and tomato to micro-rates of dicamba-based herbicides. Journal of Horticulture 5: 229. doi: 10.4172/2376-0354.1000229

Latorre D, Reynolds D, Young B, Norsworthy J, Culpepper S, Bradley K, Bish M, Kruger G, and Stephenson D (2017) Evaluation of volatility of dicamba formulations in soybean crop. North Cent Weed Sci Soc Absr 213. North Cent Weed Sci Soc (Dec. 2017)

Lovelace, M. L., Talbert, R. E., Scherder, E. F., & Hoagland, R. E. (2007). Effects of multiple applications of simulated quinclorac drift rates on tomato. Weed Science, 55(2), 169-177.

Melo, A., Mansilha, C., Pinho, O., & MPLVO Ferreira, I. (2013). Analysis of pesticides in tomato combining QuEChERS and dispersive liquid–liquid microextraction followed by high-performance liquid chromatography. Food Analytical Methods, 6(2), 559-568.

Mohseni-Moghadam, M., & Doohan, D. (2015). Response of bell pepper and broccoli to simulated drift rates of 2, 4-D and dicamba. Weed Technol, 29(2), 226-232.

National Pesticide Information Center. (2011) Dicamba Technical Fact Sheet. Available at: http://npic.orst.edu/factsheets/archive/dicamba_tech.html

Oseland, E., Bish, M., Steckel, L., & Bradley, K. (2020). Identification of environmental factors that influence the likelihood of off-target movement of dicamba. Pest Management Science, 76(9), 3282-3291.

Osipitan, O. A., Scott, J. E., & Knezevic, S. Z. (2019). Glyphosate-resistant soybean response to micro-rates of three dicamba-based herbicides. Agrosystems, Geosciences & Environment, 2(1), 1-8.

Pujeri, U. S., Pujar, A. S., Pujari, K. G., Kumbar, M. I., & Yadawe, M. S. (2016). Quantitative analysis of pesticide residues in vegetables. International Journal of Scientific & Engineering Research, 7(5), 386-392.

Sharkey, S. M., Stein, A., & Parker, K. M. (2020). Hydrogen bonding site number predicts dicamba volatilization from amine salts. Environmental Science & Technology, 54(21), 13630-13637.

Sirons, G. J., Anderson, G. W., Frank, R., & Ripley, B. D. (1982). Persistence of hormone-type herbicide residue in tissue of susceptible crop plants. Weed Science, 30(6), 572-578.

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Official Soil Series Descriptions. Available online. Accessed [05/06/2022].

Soltani N, Oliveira MC, Alves GS, Werle R, Norsworthy JK, Sprague CL, Young BC, Reynolds DB, Brown A, Sikkema PH (2020) Off-target movement assessment of dicamba in North America. Weed Technol 34:318-330

USDA- National Agricultural Statistics Service (USDA NASS). (2022) Statistics by Subject. Available at: https://quickstats.nass.usda.gov/

USEPA (2016) Dicamba; Pesticide Tolerances. Available at: https://www.govinfo.gov/content/pkg/FR-2016-12-08/pdf/2016-29245.pdf

USEPA (2020) Memorandum Supporting Decision to Approve Registration for the Uses of Dicamba on Dicamba Tolerant Cotton and Soybean. Available at: https://www.epa.gov/sites/default/files/2020-10/documents/dicamba-decision_10-27-2020.pdf

USEPA (2021) Dicamba incident report memorandum. Available at: https://downloads.regulations.gov : EPA-HQ-OPP-2020-0492-0021_content.pdf

Warmund, M., Ellersieck M. & Smeda R. J. (2022). Sensitivity of tomato cultivars to sublethal rates of dicamba and 2,4-D. Horticulturae, In Review.

Table 2.1 Mean of 2020 and 2021 dicamba residues detected in potato tubers, tomato fruit and watermelon fruit harvested 14 and 28 DAT, per distance and field. D1-D5 represent the five distances per field where D1 to D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Residues were quantified as µg/kg. The limit of quantification (LOQ) was set at 10 µg/kg and any concentration detected below this limit cannot be accurately detected as dicamba.

Field	Timing	Distance (m)	Dicamba Concentration (µg/kg)		
			Potato	Tomato	Watermelon
Downwind	14 DAT	8.4	ND	ND	13
		16.8	ND	ND	ND
		33.7	ND	ND	ND
		54.6	ND	ND	ND
		67	ND	ND	ND
	28 DAT	8.4	ND	ND	ND
		16.8	ND	ND	ND
		33.7	ND	ND	ND
		54.6	ND	ND	ND
		67	ND	ND	ND
Upwind	14 DAT	8.4	ND	13	ND
		16.8	ND	ND	ND
		33.7	ND	ND	ND
		54.6	ND	ND	ND
		67	ND	ND	ND
	28 DAT	8.4	18	ND	ND
		16.8	ND	ND	ND
		33.7	ND	ND	ND
		54.6	ND	ND	ND
		67	ND	ND	ND

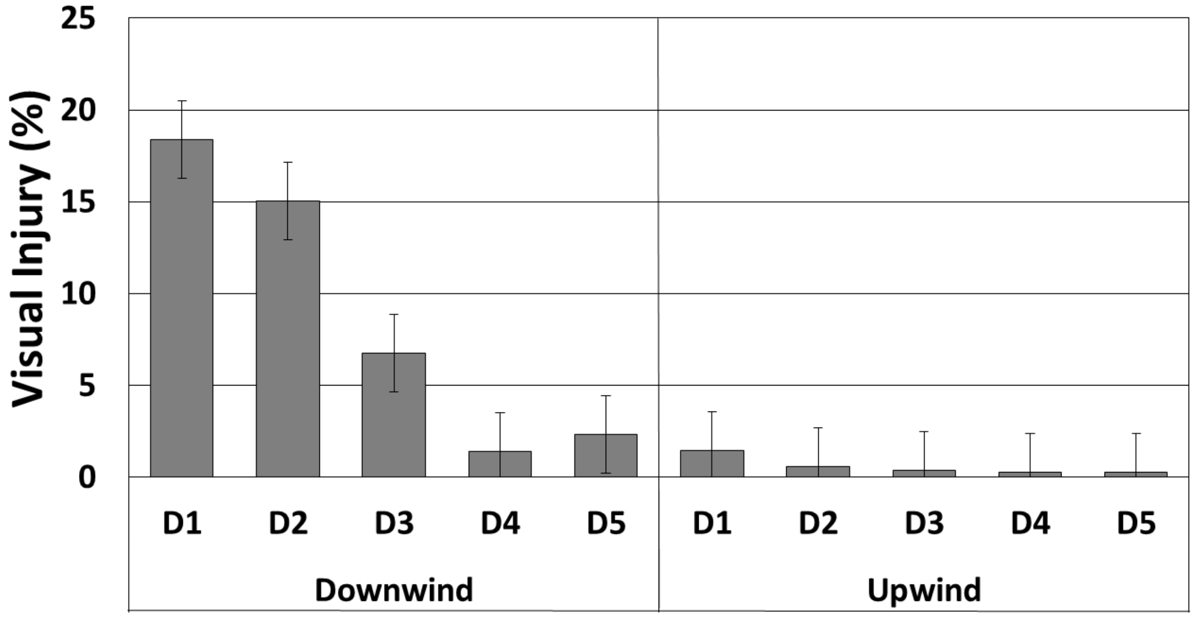


Figure 2.1 Mean of 2020 and 2021 dicamba susceptible (DS) soybean visual injury per distance and field, 21 DAT. D1-D5 represent the five distances per field where D1 to D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Visual Injury (%) is a damage rating scale where 0 = no visible damage and 100 = complete plant death. The visual injury ratings for DS soybeans were not significantly different at α = 0.05 using Tukey's HSD. Vertical bars indicate standard error of the means.

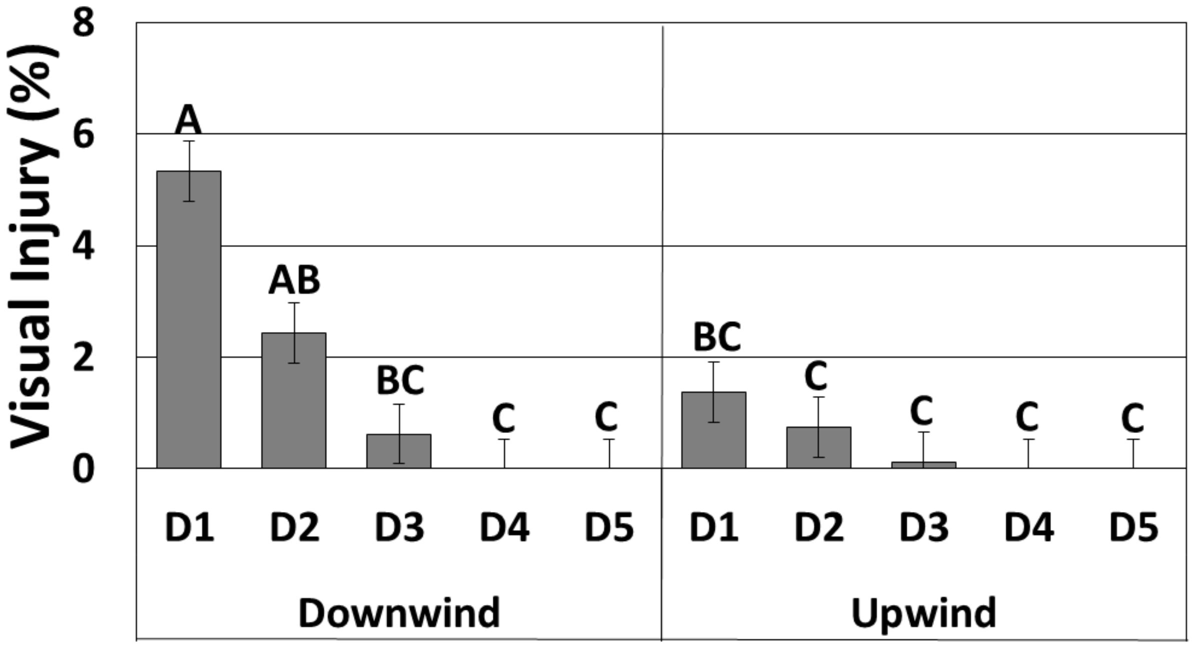


Figure 2.2 Mean of 2020 and 2021 potato visual injury per distance and field, 21 DAT. D1-D5 represent the five distances per field where D1 to D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Visual Injury (%) is a damage rating scale where 0 = no visible damage and 100 = complete plant death. Means with the same letter are not significantly different at α = 0.05 using Tukey's HSD. Vertical bars indicate standard error of the means.

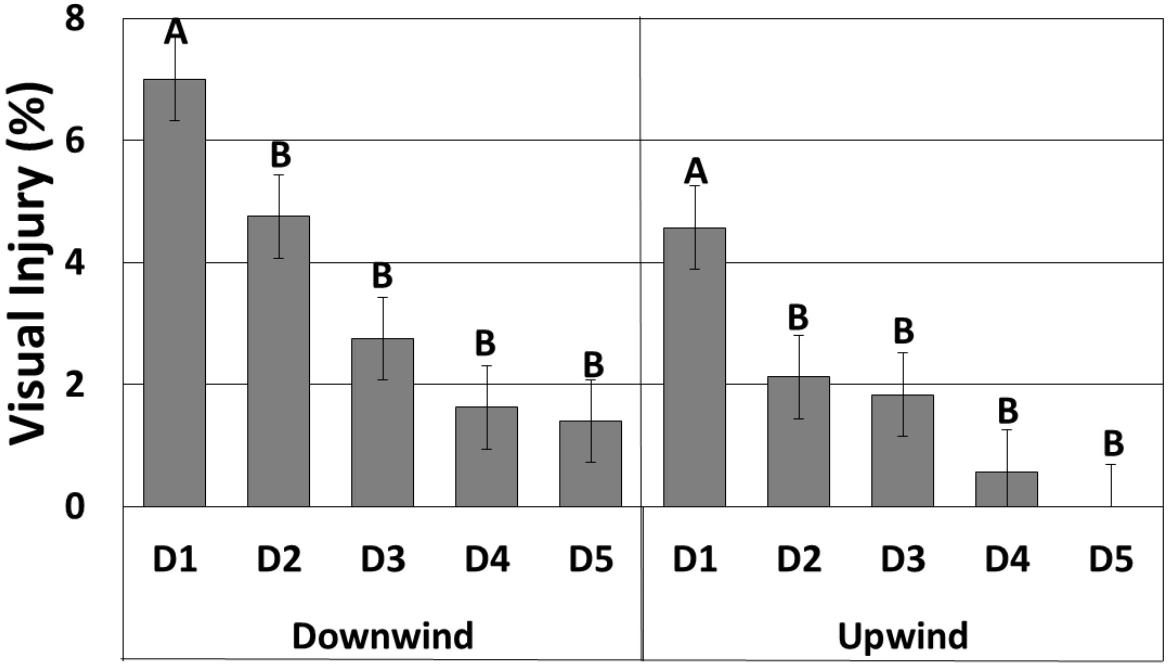


Figure 2.3 Mean of 2020 and 2021 tomato visual injury per distance and field, 21 DAT. D1-D5 represent the five distances per field where D1 to D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Visual Injury (%) is a damage rating scale where 0 = no visible damage and 100 = complete plant death. Means with the same letter are not significantly different at α = 0.05 using Tukey's HSD. Vertical bars indicate standard error of the means.

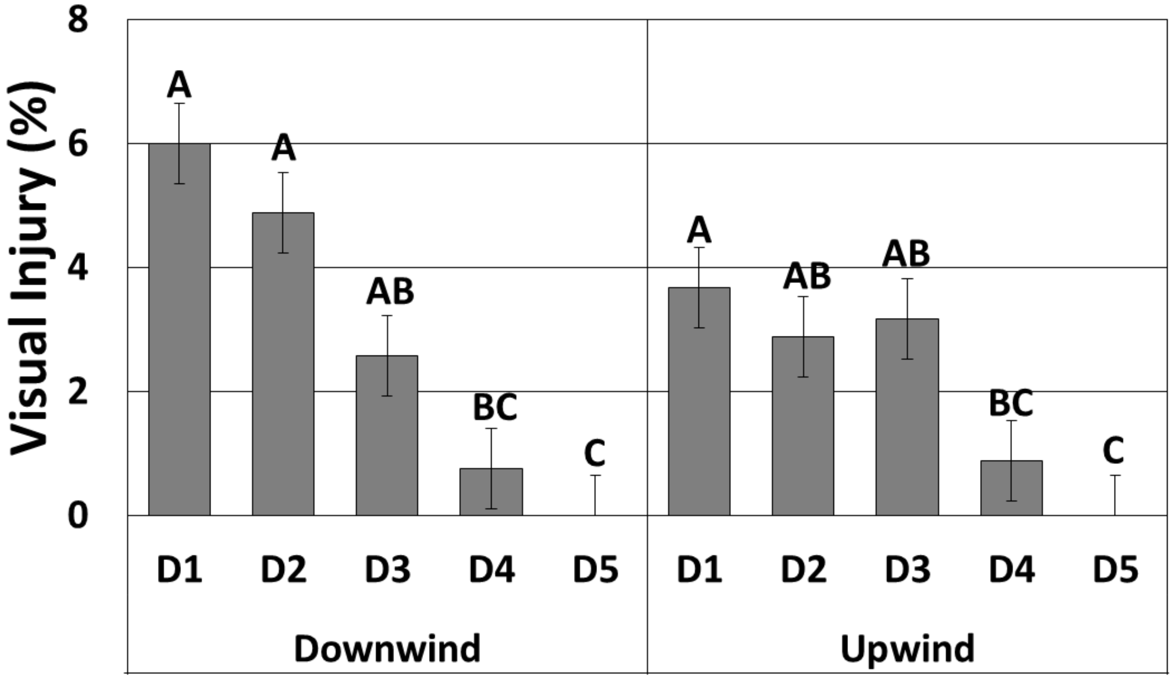


Figure 2.4 Mean of 2020 and 2021 watermelon visual injury per distance and field, 21 DAT. D1-D5 represent the five distances per field where D1 to D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Visual Injury (%) is a damage rating scale where 0 = no visible damage and 100 = complete plant death. Means with the same letter are not significantly different at $\alpha = 0.05$ using Tukey's HSD. Vertical bars indicate standard error of the means.

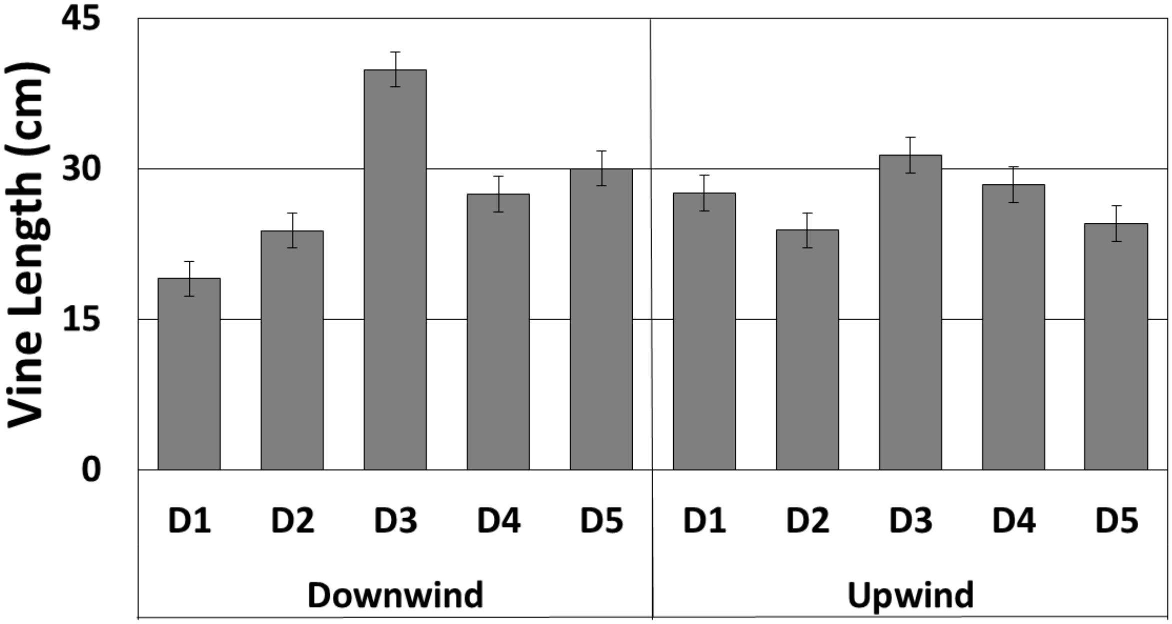


Figure 2.5 Mean of 2020 and 2021 watermelon vine length per distance and field, 28 DAT. D1-D5 represent the five distances per field where D1 to D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Watermelon vine lengths were not significantly different at α = 0.05 using Tukey's HSD. Vertical bars indicate standard error of the means.

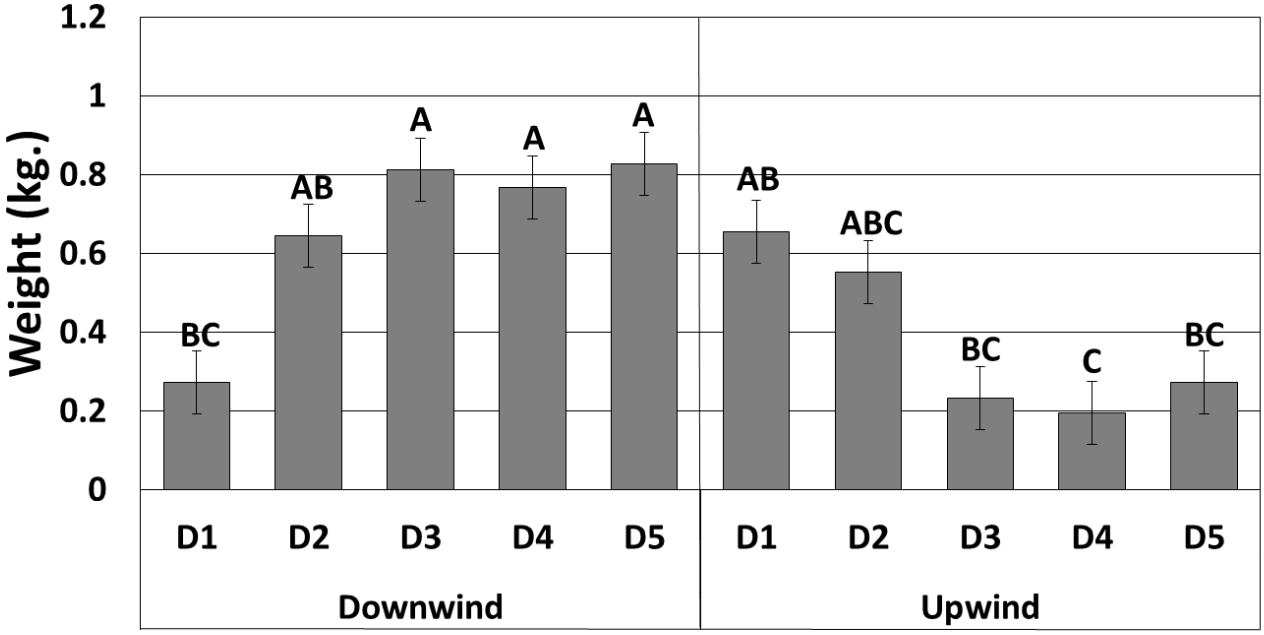


Figure 2.6 Mean of 2020 end of season potato tuber fresh weight per distance and field. D1-D5 represent the five distances per field where D1 to D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Means with the same letter are not significantly different at α = 0.05 using Tukey's HSD. Vertical bars indicate standard error of the means.

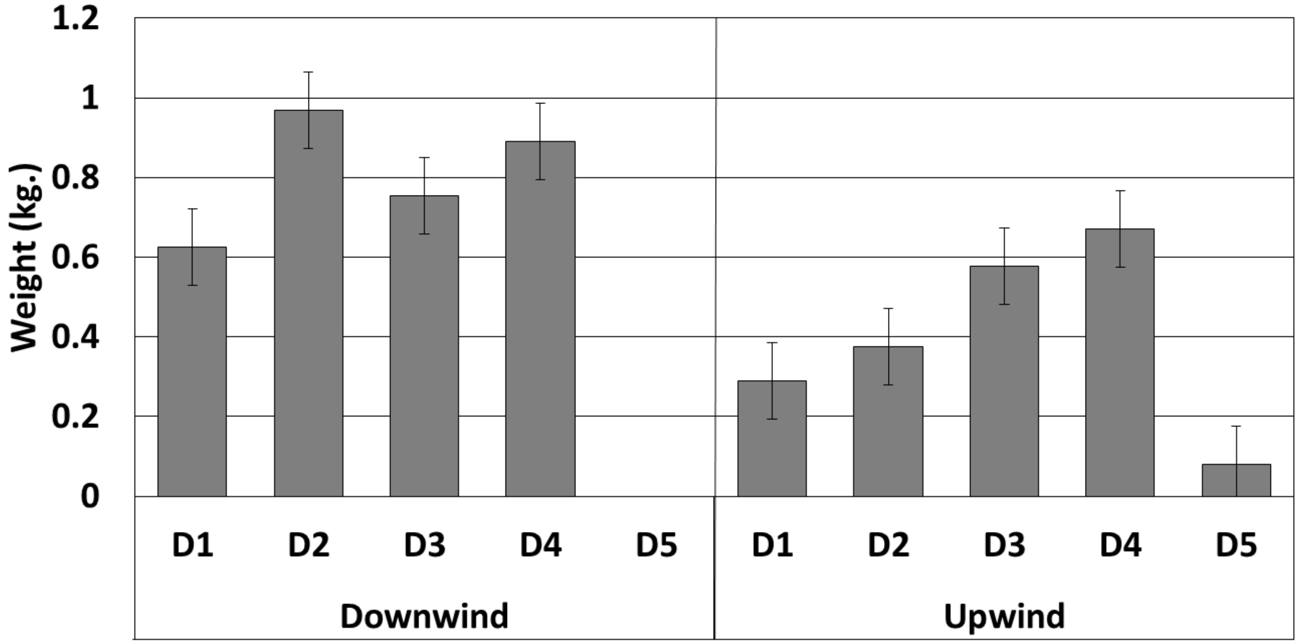


Figure 2.7 Mean of 2021 end of season potato tuber fresh weight per distance and field. D1-D5 represent the five distances per field where D1 to D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Rows at 67 m did not receive white plastic mulch in 2021, resulting in high weed pressure and minimal tuber formation. Potato tuber fresh weights for 2021 were not significantly different at α = 0.05 using Tukey's HSD. Vertical bars indicate standard error of the means.

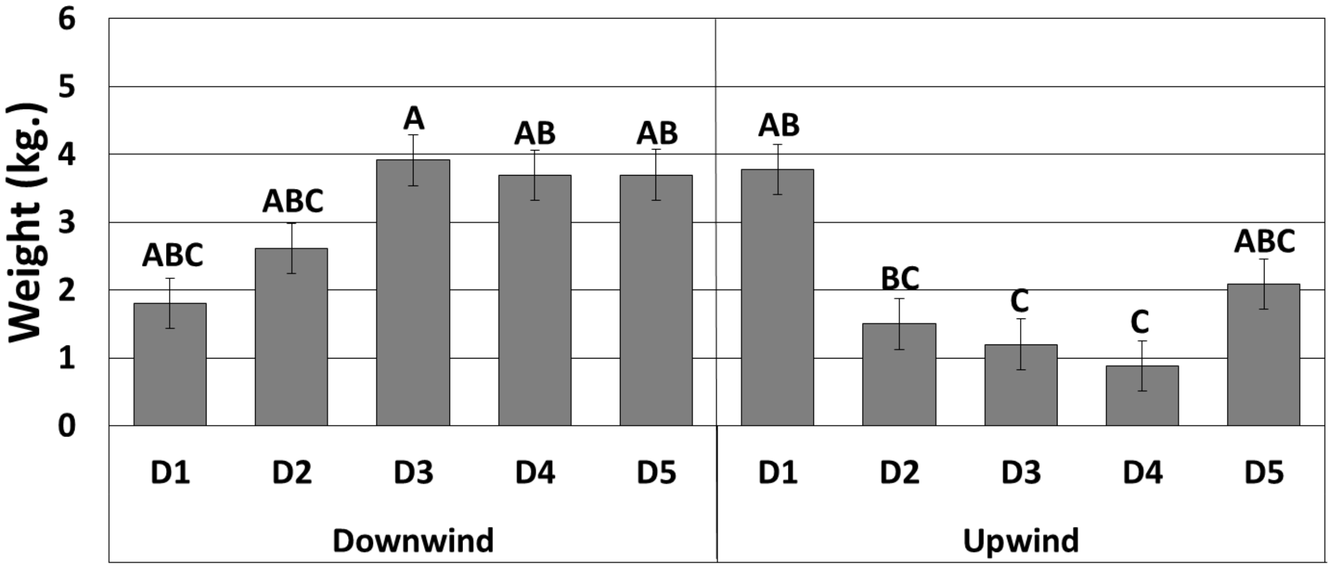


Figure 2.8 Mean of 2020 end of season tomato fruit fresh weight per distance and field. D1-D5 represent the five distances per field where D1 to D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Means with the same letter are not significantly different at $\alpha = 0.05$ using Tukey's HSD. Vertical bars indicate standard error of the means.

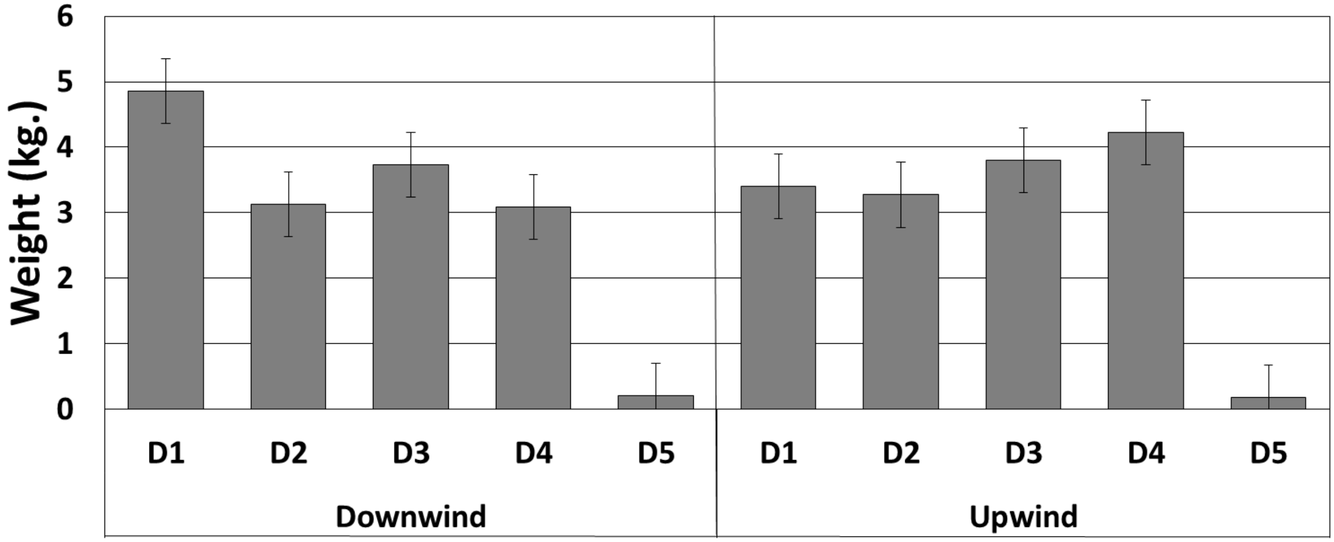


Figure 2.9 Mean of 2021 end of season tomato fruit fresh weight per distance and field. D1-D5 represent the five distances per field where D1 to D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Rows at 67 m did not receive white plastic mulch in 2021, resulting in high weed pressure and minimal fruit formation. Tomato fruit fresh weights for 2021 were not significantly different at $\alpha = 0.05$ using Tukey's HSD. Vertical bars indicate standard error of the means.

Chapter 3: Differences in Tomato and Watermelon Cultivar Responses to Increasing Doses of Dicamba.

Timothy C. Rice, Sarah E. Dixon and Reid J. Smeda

Abstract

Dicamba can result in severe plant damage to sensitive crops, but differential tolerance to dicamba may be present among cultivars of these sensitive crops. Identifying tolerant cultivars of sensitive crops can limit the potential damage for growers if incidents of dicamba off-target movement occur. In central Missouri, a greenhouse study using seven tomato (*Solanum lycopersicum*) and five watermelon (*Citrullus lanatus*) cultivars was established to compare the responses of different specialty crop cultivars to sub-lethal doses of dicamba. A preliminary dose response with dicamba revealed 50% inhibiting doses (ID_{50}) of 76 g ae ha^{-1} and 255 g ae ha^{-1} for 'Mountain Merit' tomato and 'Crimson Sweet' watermelon, respectively. Tomato cultivars included 'Better Boy F1', 'Brandywine Pink', 'Carolina Gold F1', 'Mountain Merit F1', 'Red Grape F1', 'Roma VF' and 'Supersweet 100 F1' while watermelon cultivars included 'Black Tail Mountain Organic', 'Crimson Sweet', 'Millionaire F1', 'Sangria F1' and 'Yellow Doll F1'. Plants were grown until four true leaves developed and then treated with a range of dicamba doses (25.6 g ae ha^{-1} to 766.8 g ae ha^{-1}). Visual injury was assessed at 7, 14 and 21 days after treatment (DAT), with plant dry weight determined at 21 DAT. Dry weight and visual injury data indicate that the range of tomato responses clearly fell into three groups: highly sensitive ('Roma'); moderately sensitive ('Better Boy', 'Brandywine Pink' and 'Supersweet') and sensitive ('Carolina Gold', 'Grape' and 'Mountain Merit'). Visual injury data for watermelon revealed that all cultivars overall responded similarly. However, plant biomass of 'Millionaire' and 'Yellow Doll' were reduced by 25% compared to their untreated controls at

a dose of 28 g ae ha^{-1}, while 'Black Tail Mountain', 'Crimson Sweet' and 'Sangria' dry weights required a dose of 250 g ae ha^{-1} to reduce biomass by 25%. Tomato and watermelon producers may consider selection of more tolerant cultivars in areas where the risk of off-target damage from dicamba may exists$_5$.

Introduction

The introduction of dicamba tolerant (DT) cropping systems in 2017 resulted in 43% of all soybean (*Glycine max*) production area being planted with DT varieties in 2018 and 69% of cotton (*Gossypium hirsutum*) by 2019 (Wechsler et al., 2019; Dodson et al., 2021). However, this level of adoption also led to reports of dicamba damage to adjacent sensitive crops. In 2017 alone, 2,708 dicamba-related injury cases on sensitive crops were reported across the United States (Bradley, 2017). Potato (*Solanum tuberosum*), tomato and watermelon are a few crops proven to be susceptible to dicamba at low doses (Bohnenblust et al., 2016; Culpepper et al. 2018; Jones et al. 2015; Knezevic et al. 2018; Wall et al. 1994).

Exposure of sensitive crops to low doses of dicamba mainly occurs through off-target movement (Fagliari et al. 2005; Lovelace et al. 2007). Off-target movement allows dicamba to move large distances and damage has been detected up to 250 m from an applied area (Jones et al., 2019; Soltani et al., 2020). Off-target movement can occur during the application of dicamba when spray droplets are carried to adjacent areas or following application via volatilization from treated areas (Soltani et al., 2020). Factors that influence particle drift include mechanical factors such as application height, herbicide formulation, spray droplet size, nozzle spacing and application speed in addition to environmental factors such as size of production area and high wind speeds (Mohseni-Moghadam et al., 2015; Oseland et al., 2020).

After application, high temperatures and low humidity may cause dicamba particles to volatilize into vapor particles (Egan et al., 2012). In vapor form, dicamba is even more susceptible to off target movement via high wind speeds or temperature inversions (Bish et al., 2019).

Although dicamba is capable of moving long distances via off-target movement (Jones et al. 2015), significant damage to tomato, potato and watermelon has also been recorded at representative drift rates. Culpepper et al. (2018) found that watermelon treated 20 days after planting (DAP) with 7.5 g ae ha^{-1} of the label rate of dicamba exhibited 40% visual injury and had vine lengths 49% the length of the non-treated check. Knezevic et al. (2018) found that tomatoes treated with 4.5 g ae ha^{-1} of the label rate of dicamba caused 50% visual injury 21 DAT. Wall et al. (1994) found up to 30% visual injury on potatoes with 40% total tuber reductions at a rate of 2.8 g ae ha^{-1}.

Specialty crops such as tomato, potato and watermelon are a major concern for off-target movement due to their dicamba sensitivity, but also for their high economic value and impact on human health (Kruger et al., 2012; Culpepper et al., 2018). Across the United States approximately 110,000, 380,000 and 41,000 hectares of tomatoes, potatoes and watermelon were planted, respectively in 2021. These crops were worth $1.5 billion, $4 billion and $530 million, respectively in 2021 (USDA NASS, 2022). Additionally, these vegetables are also important food sources highly beneficial to human health (Oguntibeju et al., 2013). They contain valuable food ingredients such as carbohydrates, vitamins and minerals which are vital to maintaining and repairing the body, in addition to preventing diseases (Hanif et al., 2006). In order to protect these valuable crops, it is essential to find new management strategies that minimize dicamba damage to these crops.

Although growers cannot control unwanted chemical trespassing from dicamba, one potential method to minimize dicamba impact is production of less susceptible cultivars. In soybeans, cultivar differences have been widely recorded for metribuzin sensitivity (Hardcastle et al., 1974; Wax et al., 1976). Wax et al. (1976) recorded that some soybean cultivars exposed to soil applied metribuzin (1.7 kg ai ha^{-1}) exhibited 100% visual injury while others exhibited as low as 35% visual injury at 4 weeks after planting. Soybean has also demonstrated cultivar differences in susceptibility to other herbicides including: bentazon, bromoxynil, chloroxuron, and 2,4-DB (Wax et al., 1974). Significant cultivar differences in other crops such as eggplant ('Black Beauty' and 'Santana'), snap bean ('Bush Blue Lake 274' and 'Caprice') and cucumber ('Burpless Beauty' and 'Python') were noted in a dicamba dose response by Wasacz (2022).

In tomato, potato and watermelon there is limited evidence that variability in herbicide response exists between cultivars. Dedola et al. (2014) compared the residue quantity and decay rates of 28 pesticides on two different cultivars of tomato ('Birikino' and 'Tombola') that exhibited different surface/weight ratios. The study found that the cultivar with a higher exposed leaf surface area contained more residues but also demonstrated higher residue decay rates for 18 herbicides (Dedola et al., 2014). The impact of tomato leaf surface area on herbicide susceptibility was also noted by Zangoueinejad et al. (2020) who recorded a correlation between leaf surface area and dicamba exposure. A study by Warmund et al. (2022) further demonstrated cultivar differences in tomato where the severity of injury from dicamba drift was cultivar dependent in a field setting. Limited information is available on watermelon cultivar differences to herbicides. However, a study by Song et al. (2006) demonstrated differences between watermelon and closely related cucurbit species, such as muskmelon, after exposure to acetochlor. Concerning potatoes, little research is available on cultivar differences to herbicide exposure. One study by Gawronski et al. (1985) did demonstrate cultivar differences in the ability of potato to translocate and distribute metribuzin. This may indicate differences in cultivar metabolism which is a known method of herbicide tolerance for crops and weeds (Ohkawa, 1999; Smeda and Vaughn, 1997; Hatzios, 1997). With broad genetic variability for traits such as fruit size, color, leaf shape and taste, tomato, potato and watermelon cultivars may exhibit inherent differences in tolerance to dicamba.

This study aimed to determine the sensitivities of seven different tomato cultivars and five different watermelon cultivars to low dose applications of dicamba. This information will help growers understand the impact dicamba has on their specialty crops. Additionally, it may reveal cultivars of these crops that demonstrate natural tolerance to dicamba at low rates.

Materials and Methods

Greenhouse experiments with tomato and watermelon were conducted in Columbia, MO between August, 2021 and March, 2022. Research focused on seven tomato cultivars including 'Better Boy F1', 'Brandywine Pink', 'Carolina Gold F1', 'Mountain Merit F1', 'Red Grape F1', 'Roma VF' and 'Supersweet 100 F1' and five watermelon cultivars including 'Black Tail Mountain Organic', 'Crimson Sweet', 'Millionaire F1', 'Sangria F1' and 'Yellow Doll F1'. Cultivars were selected due to their differences in representative traits such as fruit size, color, growth rate, leaf shape or seed type. Seed was purchased from Harris Seeds Company (Rochester, NY, USA). Plastic pots (10 cm) were filled with a 30:70 mixture of Mexico silt loam (fine, smectitic, mesic vertic epiaqualfs) (USDA Official Soil Series Descriptions, 2022) and peat-based greenhouse media (Pro-Mix BX Mycorrhizae; Hummert International, Earth City, MO, USA).

Approximately 60 seeds of each cultivar were planted across 40 pots. As seedlings emerged, plants were thinned as needed and 36 plants were selected for treatment based on the desired growth stage. Plants were exposed to a 15-hour photoperiod supplemented by high-pressure sodium lights and were hand-watered daily. A macronutrient fertilizer (Peters Professional 20-20-20 General-Purpose Water-Soluble Fertilizer; Hummert International) was applied bi-weekly at 200 ppm N starting 3 weeks after planting until the end of the experiment. Tomatoes were treated when the second set of true leaves had developed and plants were 12 to 16 cm in height. Watermelon were treated at 10 to 12 cm in height, with vining just initiating.

Dicamba application rates were determined from a pre-screening trial using the tomato cultivar 'Mountain Merit' and the watermelon cultivar 'Crimson Sweet' applied with dicamba at rates of 25.6 to 511.1 g ae ha^{-1}. These representative cultivars revealed that inhibiting doses of approximately 76 and 255 g ha^{-1} were required to reduce the dry weights and visual injury of tomato and watermelon by 50% (ID_{50}), respectively. Dicamba doses used in the cultivar experiment were derived from these ID_{50}s. Seedlings from both crops and all cultivars were exposed to nine treatments including eight different doses of dicamba ranging from 25.6 to 383.5 g ae ha^{-1} (tomato) or 76.7 to 766.8 g ha^{-1} (watermelon), along with an untreated control (Table 3.1). All dicamba treatments contained the diglycolamine salt of dicamba (3,6-dichloro-o-anisic acid) and Impetro® 2 (MFA Agri Service, Columbia, MO, USA) at 1% v/v. Treatments were applied using an air pressurized track sprayer calibrated to deliver 140 L ha^{-1} using an 8001E nozzle (TeeJet Technologies, Urbandale, IA, USA).

Assessment of tomato and watermelon response to dicamba included visual evaluation of damage. Damage was evaluated using a scale of 0 to 100% where a rating of 0% indicated no visible damage and 100% indicated complete plant death due to dicamba exposure. Evaluations were conducted 7, 14 and 21 days after treatment (DAT). Each plant was cut at the soil level 21 DAT and placed in paper bags, dried for seven days at 32 °C and weight recorded. Once dried, each plant was weighed individually. To facilitate comparison of different cultivars, dry weights for treated plants were converted to percentage of control plants using the mean for untreated control for each respective cultivar.

The experimental design was a randomized complete block design with four replications. Each experiment with all cultivars was repeated. A total of 8 plants was used to generate each data point. Dicamba dose and cultivars were considered fixed variables, whereas replications within treatments were considered random. Visual injury and dry weight data were analyzed using a log-logistic analysis (Seefeldt et al., 1995) of non-linear regression models in GraphPad-Prism 9.1.0 (Graphpad Software Inc., San Diego, CA, USA).

Results and Discussion

Tomato. A log-logistic equation was used to determine the best line of fit for the visual injury responses of each tomato cultivar. A pairwise comparison of models for each cultivar was done using a lack-of-fit F-test to test the hypothesis that the curves have the same slope when α = 0.05. Alternatively, this test determines if the more complex model (nonparallel curves) can be reduced to a simpler model (parallel curves). Nonsignificant pairs were identified and combined into models, and the lack-of-fit F-test was repeated. The 7 tomato cultivars were separated into 3 model groups considered to be significantly different ($P < 0.0001$): highly sensitive (‘Roma’); moderately sensitive (‘Better Boy’, ‘Brandywine Pink’ and ‘Supersweet’) and sensitive (‘Carolina

Gold', 'Grape' and 'Mountain Merit') (Table 3.2). This process was repeated for dry weights which resulted in the same grouping based on relative sensitivity (Table 3.3).

Although visual injury from dicamba occurred on treated tomatoes at all observation dates with optimum symptomology for cultivars noted at 21 DAT. Therefore, only this date was selected to show results. Dicamba injury to tomato was primarily manifested as leaf cupping, stem thickening and stem twisting with callous formation across all timings. By 21 DAT, leaf defoliation and necrosis was observed at higher rates. At a dose of 51.1 g ha^{-1}, highly sensitive tomatoes exhibited almost complete plant death (85% visual injury) while moderately sensitive and sensitive tomatoes sustained damage of 35% and 25%, respectively (Figure 3.1). At a dose of 102.3 g ae ha^{-1}, highly sensitive, moderately sensitive and sensitive tomatoes displayed visual damage of 88.3%, 72.5% and 45%, respectively. The visual injury ID_{50} of each group were 35.61, 66.2 and 130.6 g ha^{-1} for highly sensitive, moderately sensitive and sensitive tomatoes, respectively. At the highest dose of 383.5 g ha^{-1}, plants from all three groups died.

Tomato dry weight data resulted in the same sensitivity groupings as the visual injury (Figure 3.2). At a dose of 102.3 g ha^{-1}, highly sensitive, moderately sensitive and sensitive tomatoes had mean dry weights of 25.3, 34.5 and 48.9% of the untreated control, respectively. The dry weight ID_{50} of each group was 20.4, 28.3 and 28.7 g ha^{-1} for highly sensitive, moderately sensitive and sensitive tomatoes, respectively. At the highest dose of 383.5 g ha^{-1}, highly sensitive, moderately sensitive and sensitive tomatoes had dry weights of 19.5, 24.7 and 37.4% of the untreated control, respectively.

Watermelon. A log-logistic equation was used to determine the best line of fit for the dry weight responses of each watermelon cultivar (Table 3.4). A pairwise comparison of models for each cultivar was done using a lack-of-fit F-test to test the hypothesis that the curves have the same slope when $\alpha = 0.05$. Alternatively, this test determines if the more complex model (nonparallel curves) can be reduced to a simpler model (parallel curves). Non-significant pairs were identified and combined into models, and the lack-of-fit F-test was repeated. The 5 watermelon cultivars were separated into 2 model groups considered to be significantly different ($P < 0.0001$): highly sensitive (Millionaire and Yellow Doll) and sensitive (Black Tail Mountain, Crimson Sweet and Sangria) (Table 3.4). This process was repeated for visual injury but there were no significant differences between watermelon cultivars (Table 3.5).

Dry weight data suggest that highly sensitive watermelon had consistently lower dry weights when compared to sensitive watermelon (Figure 3.3). At a dose of 204.5 g ha^{-1}, highly sensitive and sensitive watermelon had dry weights of 47.7 and 74.4% of the untreated control, respectively. The dry weight ID_{50} of each group was 104.5 and 262.7 g ha^{-1} for highly sensitive and sensitive watermelon, respectively. At the highest dose of 766.7 g ha^{-1}, highly sensitive and sensitive watermelon had dry weights of 30.7% and 48.1% of the untreated control, respectively.

Similar to tomato, the visual injury data from 21 DAT proved most effective at highlighting cultivar differences for watermelon and injury was primarily observed as stem thickening and twisting with callous formation across all timings. By 21 DAT, leaf defoliation and necrosis were also observed at higher rates. The extra sum-of-squares F test indicated that one curve fit all 5 watermelon cultivars ($P < 0.1026$) (Table 3.5). This curve indicates that a dose of approximately 262.4 g ha^{-1} dicamba was sufficient to result in a 50% visual injury rating for all 5 cultivars (Figure 3.4).

Results from this study reinforce that tomato and watermelon plants are sensitive to low dose applications of dicamba, although, the ID_{50} concentration was approximately four-fold higher for watermelon when compared to tomato. Knezevic et al. (2018) and Culpepper et al. (2018) recorded damage on tomatoes and watermelon treated with 4.5 and 7.5 g ae ha^{-1} of dicamba at 21 and 20 DAT, respectively. Results from this study determined that multiple cultivars of tomatoes and watermelon exhibited injury with dicamba rates as low as 25.6 and 76.7 g ha^{-1}, respectively. These doses also reduced dry weights of tomato and watermelon by approximately 25% when compared to the untreated controls. Knezevic et al. (2018) and Culpepper et al. (2018) used rates lower than those used in this experiment but doses remained low when compared to the recommended use rate of dicamba (560 g ae ha^{-1}).

This study also reinforces that herbicide sensitivities can vary between cultivars. Warmund et al. (2022) found cultivar differences in tomato response to dicamba, while earlier studies demonstrated differences in herbicide sensitivities between cultivars of soybean, cucumber, eggplant and other crops (Hardcastle et al., 1974; Wax et al., 1976; Wasacz, 2022).

The seven tomato cultivars tested in this study were shown to have 3 different levels of sensitivity: highly sensitive (‘Roma’); moderately sensitive (‘Better Boy’, ‘Brandywine Pink’ and ‘Supersweet’) and sensitive (‘Carolina Gold’, ‘Grape’ and ‘Mountain Merit’) (Table 3.2 & 3.3). These groupings were reinforced by both the visual injury and dry weight data (Figures 3.1 & 3.2). These data indicate that cultivars grouped as ‘sensitive’ may be good cultivars to be used in areas where dicamba off-target movement may occur. Furthermore, the data indicates that the highly sensitive cultivar 'Roma' should potentially be avoided in the same high risk areas.

The five watermelon cultivars tested in this study were shown to have 2 different levels of sensitivity: highly sensitive ('Millionaire' and 'Yellow Doll') and sensitive ('Black Tail Mountain', 'Crimson Sweet' and 'Sangria') (Table 3.4). However, these groupings were only reinforced by dry weight data (Figure 3.3). Visual injury data indicated no cultivar differences in sensitivity to dicamba (Figure 3.4). Differences in dry weight for watermelon may be attributed to differences in growth rates observed in untreated controls. Even though all watermelon cultivars were applied with dicamba at the same heights, untreated controls continue grow at normal rates. Cultivars with naturally slower growth rates may experience reduced effects from dry weight reductions due to dicamba exposure. Additionally, the gradual decline of the dose response curves for watermelon dry weights indicates that there is less acute sensitivity for all watermelon cultivars towards dicamba (Figure 3.3). Considering these factors and the visual injury data, it is possible that these five watermelon cultivars do not significantly differ in dicamba sensitivity. However, dry weight data still indicates that watermelon grouped as 'sensitive' may provide a small amount protection in areas where dicamba off-target movement may occur. Additionally, the 'highly sensitive' cultivars may be more susceptible and should potentially be avoided in high-risk areas.

The differences in sensitivity noted in this study could be related to natural growth rates as discussed above, leaf surface area as demonstrated by Dedola et al. (2014) and Zangoueinejad et al. (2020) or due to increased herbicide metabolism in some cultivars (Ohkawa, 1999; Smeda and Vaughn, 1997; Hatzios, 1997). However, more work is needed to accurately identify tolerant cultivars of both crops and reduce the impact of dicamba damage on affected specialty crops in production areas.

The data from this study reinforce that watermelon show decreased sensitivity to dicamba when compared to tomato. Additionally, cultivar differences in both tomato and watermelon sensitivity to dicamba were observed under the conditions of this greenhouse experiment.

Literature Cited

Bohnenblust EW, Vaudo AD, Egan F, Mortensen DA, Tooker JF (2016) Effects of the herbicide dicamba on nontarget plants and pollinator visitation. Environ. Toxicol. Chem. 35:144-151

Bradley, K.W. (2017) A final report on dicamba-injured soybean acres. Available at: https://ipm.missouri.edu/IPCM/2017/10/final_report_dicamba_injured_soybean/

Culpepper, A. S., Sosnoskie, L. M., Shugart, J., Leifheit, N., Curry, M., & Gray, T. (2018). Effects of low-dose applications of 2, 4-D and dicamba on watermelon. Weed Technol, 32(3), 267-272.

Dedola, F., Cabizza, M., & Satta, M. (2014). Determination of 28 pesticides applied on two tomato cultivars with a different surface/weight ratio of the berries, using a multiresidue GC-MS/MS method. Journal of Environmental Science and Health, Part B, 49(9), 671-678.

Dodson, L., Wechsler, S. J., Williamson, S., McFadden, J., & Smith, D. (2021). Adoption of genetically engineered dicamba-tolerant cotton seeds is prevalent throughout the United States. Amber Waves: The Economics of Food, Farming, Natural Resources, and Rural America, 2021(1490-2021-1200).

Fagliari, J. R., Oliveira Jr.*, R. S. D., & Constantin, J. (2005). Impact of sublethal doses of 2, 4-D, simulating drift, on tomato yield. Journal of Environmental Science and Health, Part B, 40(1), 201-206.

Gawronski, S. W., Haderlie, L. C., Callihan, R. H., & Dwelle, R. B. (1985). Metribuzin absorption, translocation, and distribution in two potato (Solanum tuberosum) cultivars. Weed Science, 33(5), 629-634.

Hanif, R., Iqbal, Z., Iqbal, M., Hanif, S., & Rasheed, M. (2006). Use of vegetables as nutritional food: role in human health. Journal of Agricultural and Biological Science, 1(1), 18-22.

Hardcastle, W. S. (1974). Differences in the tolerance of metribuzin by varieties of soybeans. Weed Research, 14(3), 181-184.

Hatzios, K. K. (Ed.). (1997). Regulation of enzymatic systems detoxifying xenobiotics in plants (Vol. 37). Springer Science & Business Media.

Heap, I. M. 2010. International survey of herbicide resistant weeds. Online. WeedScience.org, Corvallis, OR.

Johnson B, Young B, Matthews J, Marquardt, P, Slack C, Bradley K, York A, Culpepper S, Hager A, Al-Khatib K, Steckel L, Moechnig M, Loux M, Bernards M, Smeda R (2010) Weed control in dicamba-resistant soybeans. Crop Manag 9(1), 10.1094/CM- 2010-0920-01-RS

Jones, G. T., Norsworthy, J. K., Barber, L. T., & Scott, R. C. (2015). Comparison of two dicamba formulations for risk of off-target movement to soybean. Soybean Research Studies 2015, 124.

Knezevic SZ, Osipitan O A, Scott JE (2018) Sensitivity of grape and tomato to micro-rates of dicamba-based herbicides. J Hortic 5: 229. doi: 10.4172/2376-0354.1000229

Kruger, G. R., Johnson, W. G., Doohan, D. J., & Weller, S. C. (2012). Dose response of glyphosate and dicamba on tomato (Lycopersicon esculentum) injury. Weed Technol, 26(2), 256-260.

Lovelace, M. L., Talbert, R. E., Scherder, E. F., & Hoagland, R. E. (2007). Effects of multiple applications of simulated quinclorac drift rates on tomato. Weed Science, 55(2), 169-177.

Monsanto Corporation. (2016) Xtendimax® product label. St. Louis, Monsanto. Available at: http://cdms.net/ldat/ldDF9000.pdf

Oguntibeju, O. O., Truter, E. J., & Esterhuyse, A. J. (2013). The role of fruit and vegetable consumption in human health and disease prevention. Diabetes Mellitus-Insights and Perspectives, 3(2), 172-180.

Ohkawa, H., Tsujii, H., & Ohkawa, Y. (1999). The use of cytochrome P450 genes to introduce herbicide tolerance in crops: a review. Pesticide Science, 55(9), 867-874.

Smeda, R. J., & Vaughn, K. C. (1997). Mechanisms of resistance to herbicides. Molecular Mechanisms of Resistance to Agrochemicals, 79-123.

Seefeldt, S. S., Jensen, J. E., & Fuerst, E. P. (1995). Log-logistic analysis of herbicide dose-response relationships. Weed Technol, 9(2), 218-227.

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Official Soil Series Descriptions. Available online. Accessed [05/06/2022].

Soltani N, Oliveira MC, Alves GS, Werle R, Norsworthy JK, Sprague CL, Young BC, Reynolds DB, Brown A, Sikkema PH (2020) Off-target movement assessment of dicamba in North America. Weed Technol 34:318-330

Song, C., Teng, C., Tian, L., Ma, H., & Tao, B. (2006). Seedling growth tolerance of Cucurbits crops to herbicides Stomp and Acetochlor. Gen. Appl. Plant Physiology, 32(3-4), 165-174.

USDA- National Agricultural Statistics Service (USDA NASS). (2022) Statistics by Subject. Available at: https://quickstats.nass.usda.gov/

Wall, D. A. (1994). Potato (*Solanum tuberosum*) response to simulated drift of dicamba, clopyralid, and tribenuron. Weed Science, 42(1), 110-114.

Warmund, M., Ellersieck M. & Smeda R. J. (2022). Sensitivity of tomato cultivars to sublethal rates of dicamba and 2,4-D. Horticulturae, In Review.

Wasacz, M. H. (2022). Crop tolerance and yield response of select mid-Atlantic vegetables to sub-lethal dicamba rates (Doctoral dissertation, Rutgers University-School of Graduate Studies).

Wax, L. M., Bernard, R. L., & Hayes, R. M. (1974). Response of soybean cultivars to bentazon, bromoxynil, chloroxuron, and 2, 4-DB. Weed Science, 22(1), 35-41.

Wax, L. M., Stoller, E. W., & Bernard, R. L. (1976). Differential response of soybean cultivars to metribuzin 1. Agronomy Journal, 68(3), 484-486.

Wechsler, S. J., Smith, D., McFadden, J., Dodson, L., & Williamson, S. (2019). The use of genetically engineered dicamba-tolerant soybean seeds has increased quickly, benefiting adopters but damaging crops in some fields. Amber Waves: The Economics of Food, Farming, Natural Resources, and Rural America, 2019(1490-2020-845).

Young, B. G. 2006. Changes in herbicide use patterns and production practices resulting from glyphosate-resistant crops. Weed Technol. 20:301-307.

Zangoueinejad, R., Alebrahim, M. T., & Tseng, T. M. (2020). Absorption and translocation of dicamba in dicamba-tolerant wild tomato. Canadian Journal of Plant Science, 101(1), 30-38.

Table 3.1 Rates of dicamba used to treat all tomato and watermelon cultivars.

	Dicamba Dose (g ae ha^{-1})	
Treatment	Tomato	Watermelon
1	0	0
2	25.56	76.69
3	51.13	153.39
4	76.69	204.52
5	102.26	255.56
6	153.39	383.47
7	204.52	511.12
8	255.65	639.03
9	383.47	766.68

Table 3.2 List of visual injury sensitivity groups for tomato cultivars 'Better Boy', 'Brandywine Pink', 'Carolina Gold', 'Grape', 'Mountain Merit', 'Roma', and 'Supersweet' subjected to dicamba at doses ranging from 0 to 383 g ae ha^{-1} in a greenhouse study. A log-logistic equation was used to determine the best line of fit for each tomato cultivar. A pairwise comparison of models for each cultivar was conducted and nonsignificant pairs were combined into models. 3 model groups considered to be significantly different were identified (P < 0.0001): highly sensitive; moderately sensitive and sensitive. Equations of best fit for each group are shown with accompanying R squared value. Groups were separated using the lack-of-fit F test at α = 0.05.

Group (Cultivars)	**Best Fit Equation** $f(x) = C + \frac{D-C}{1+(\frac{x}{ID_{50}})^b}$	**Goodness of Fit** (R Squared)
Moderately Sensitive ('Better Boy', 'Brandywine' and 'Supersweet')	$f(x) = -0.2325 + \frac{102.3 + 0.2325}{1 + (\frac{x}{66.2})^{2.480}}$	0.998
Sensitive ('Carolina Gold', 'Grape' and 'Mountain Merit')	$f(x) = -1.272 + \frac{118.1 + 1.272}{1 + (\frac{x}{130.6})^{1.414}}$	0.992
Highly Sensitive ('Roma')	$f(x) = -0.352 + \frac{96.38 + 0.352}{1 + (\frac{x}{35.61})^{5.202}}$	0.9917

Table 3.3 List of dry weight sensitivity groups for tomato cultivars 'Better Boy', 'Brandywine Pink', 'Carolina Gold', 'Grape', 'Mountain Merit', 'Roma', and 'Supersweet' subjected to dicamba at doses ranging from 0 to 383 g ae ha^{-1} in a greenhouse study. A log-logistic equation was used to determine the best line of fit for each tomato cultivar. A pairwise comparison of models for each cultivar was conducted and nonsignificant pairs were combined into models. 3 model groups considered to be significantly different were identified (P < 0.0001): highly sensitive; moderately sensitive and sensitive. Equations of best fit for each group are shown with accompanying R squared value. Groups were separated using the lack-of-fit F test at α = 0.05.

Group (Cultivars)	**Best Fit Equation** $f(x) = C + \frac{D-C}{1+(\frac{x}{ID_{50}})^{b}}$	**Goodness of Fit** (R Squared)
Moderately Sensitive ('Better Boy', 'Brandywine' and 'Supersweet')	$f(x) = 23.97 + \frac{100.7 - 23.97}{1 + (\frac{x}{28.26})^{-1.403}}$	0.9971
Sensitive ('Carolina Gold', 'Grape' and 'Mountain Merit')	$f(x) = 37.42 + \frac{106.8 - 37.42}{1 + (\frac{x}{28.72})^{-1.388}}$	0.9773
Highly Sensitive ('Roma')	$f(x) = 19.62 + \frac{102.7 - 19.62}{1 + (\frac{x}{20.36})^{-1.392}}$	0.9803

Table 3.4 List of dry weight sensitivity groups for watermelon cultivars 'Black Tail Mountain', 'Crimson Sweet', 'Millionaire', 'Sangria' and 'Yellow Doll' subjected to dicamba at doses ranging from 0 to 767 g ae ha-1 in a greenhouse study. A log-logistic equation was used to determine the best line of fit for each tomato cultivar. A pairwise comparison of models for each cultivar was conducted and nonsignificant pairs were combined into models. 2 model groups considered to be significantly different were identified (P < 0.0001): highly sensitive and sensitive. Equations of best fit for each group are shown with accompanying R squared value. Groups were separated using the lack-of-fit F test at α = 0.05.

Group (Cultivars)	**Best Fit Equation** $f(x) = C + \frac{D-C}{1+(\frac{x}{ID_{50}})^{b}}$	**Goodness of Fit** (R Squared)
Sensitive ('Black Tail Mountain', 'Crimson Sweet' and 'Sangria')	$f(x) = -91.93 + \frac{100.1 + 91.93}{1 + (\frac{x}{3997})^{-6753}}$	0.9260
Highly Sensitive ('Millionaire' and 'Yellow Doll')	$f(x) = 14.84 + \frac{97.89 - 14.84}{1 + (\frac{x}{104.5})^{-0.7731}}$	0.9885

Table 3.5 List of visual injury sensitivity groups for watermelon cultivars 'Black Tail Mountain', 'Crimson Sweet', 'Millionaire', 'Sangria' and 'Yellow Doll' subjected to dicamba at doses ranging from 0 to 767 g ae ha^{-1} in a greenhouse study. A log-logistic equation was used to determine the best line of fit for each tomato cultivar. A pairwise comparison of models for each cultivar was conducted and non-significantly different pairs were combined into models. No significant differences were identified and one model fit all cultivars (P < 0.0001). Equation of best fit for the group is shown with accompanying R squared value. Group separation was attempted using the lack-of-fit F test at α = 0.05.

Group (Cultivars)	**Best Fit Equation** $f(x) = C + \frac{D-C}{1+(\frac{x}{ID_{50}})^{b}}$	**Goodness of Fit** (R Squared)
Sensitive ('BTM', 'Crimson Sweet', 'Millionaire', 'Sangria' and 'Yellow Doll')	$f(x) = 5.385 + \frac{109.7 + 5.385}{1 + (\frac{x}{262.4})^{1.948}}$	0.9731

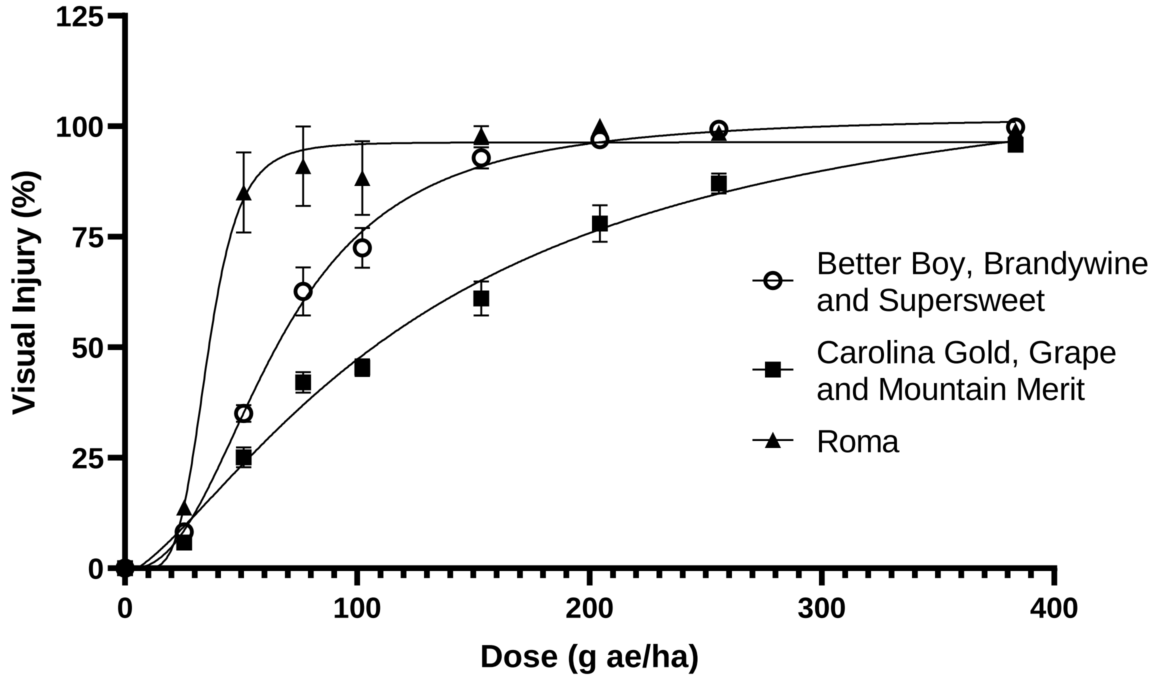


Figure 3.1 Visual injury dose-response models for tomato cultivars 'Better Boy', 'Brandywine Pink', 'Carolina Gold', 'Grape', 'Mountain Merit', 'Roma', and 'Supersweet' subjected to dicamba at doses ranging from 0 to 383 g ae ha^{-1} in a greenhouse study, 21 DAT. Damage was evaluated using a scale of 0 to 100% where a rating of 0% meant no visible damage was noted and 100% meant complete plant death due to dicamba exposure. Dicamba was applied to plants using an automated track sprayer calibrated to deliver 140 L ha^{-1} when plants reached 16 cm in height. The lack-of-fit F-test was performed in pairwise comparisons to identify cultivars that could be combined into a single model.

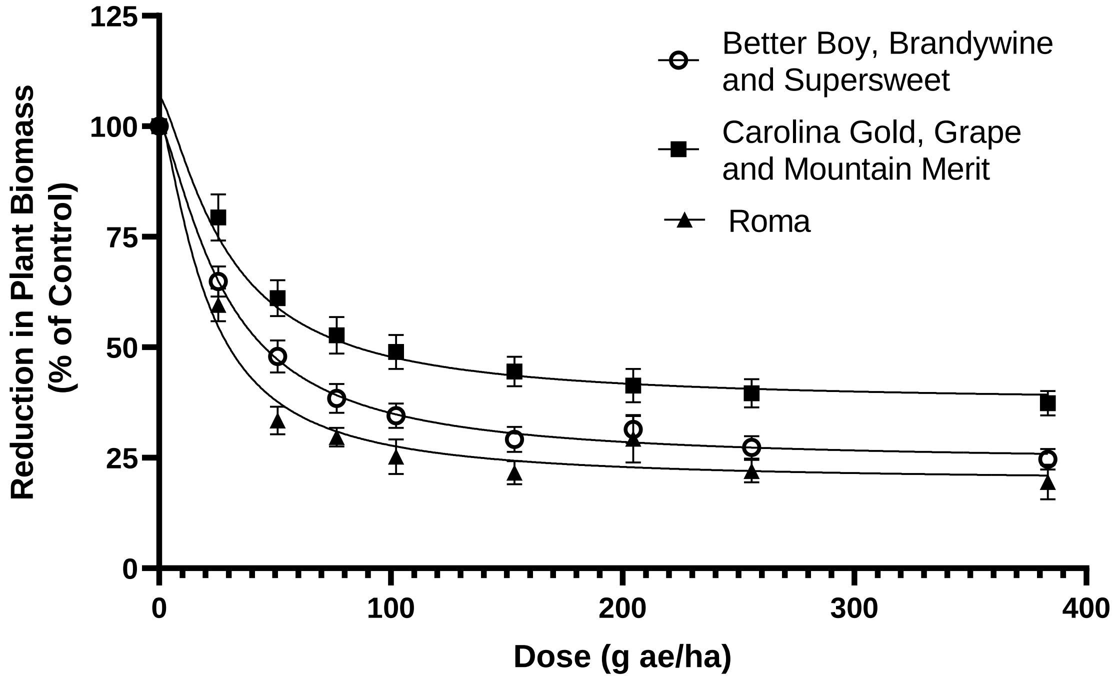


Figure 3.2 Dry weight dose-response models for tomato cultivars 'Better Boy', 'Brandywine Pink', 'Carolina Gold', 'Grape', 'Mountain Merit', 'Roma', and 'Supersweet' subjected to dicamba at doses ranging from 0 to 383 g ae ha^{-1} in a greenhouse study. Dicamba was applied to plants using an automated track sprayer calibrated to deliver 140 L ha^{-1} when plants reached 16 cm in height. Plants were harvested 21 DAT, and dry biomass data was converted to a percent of the mean control. The lack-of-fit F-test was performed in pairwise comparisons to identify cultivars that could be combined into a single model.

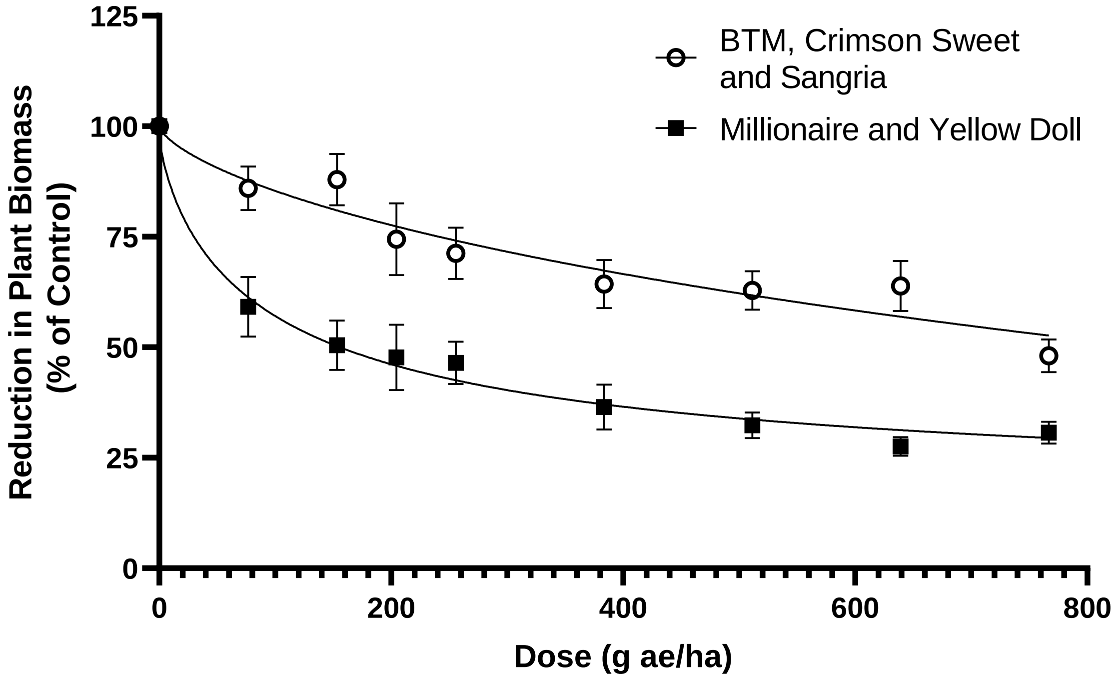


Figure 3.3 Dry weight dose-response models for watermelon cultivars 'Black Tail Mountain', 'Crimson Sweet', 'Millionaire', 'Sangria' and 'Yellow Doll' subjected to dicamba at doses ranging from 0 to 767 g ae ha^{-1} in a greenhouse study. Dicamba was applied to plants using an automated track sprayer calibrated to deliver 140 L ha^{-1} when plants reached 12 cm in height. Plants were harvested 21 DAT, and dry biomass data was converted to a percent of the mean control. The lack-of-fit F-test was performed in pairwise comparisons to identify cultivars that could be combined into a single model.

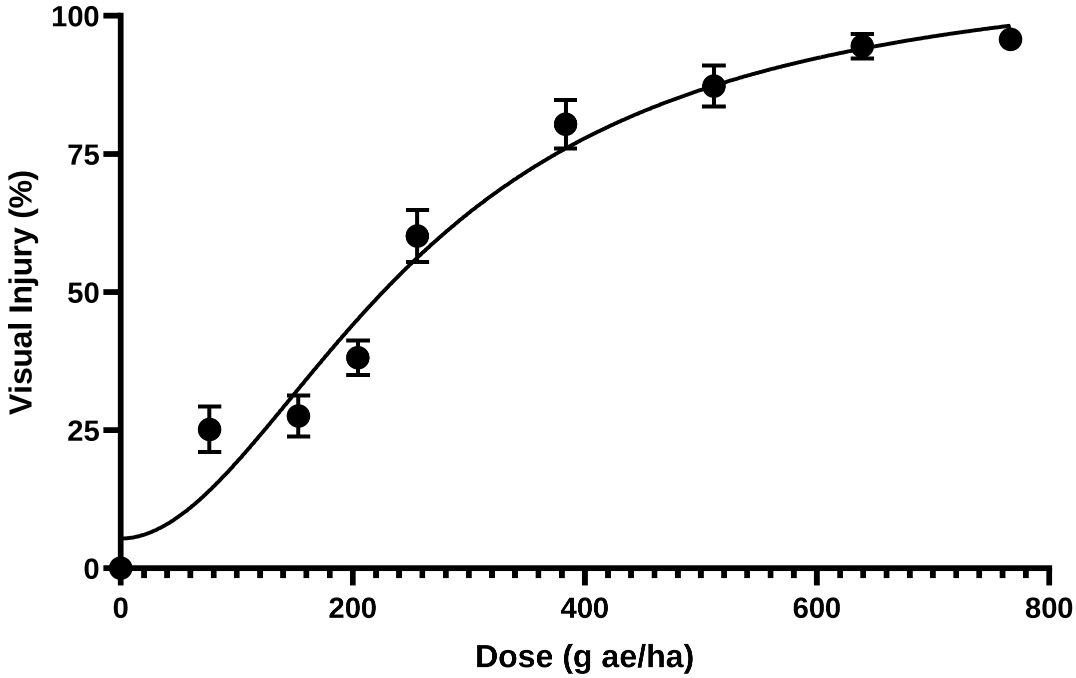


Figure 3.4 Visual injury dose-response models for watermelon cultivars 'Black Tail Mountain', 'Crimson Sweet', 'Millionaire', 'Sangria' and 'Yellow Doll' subjected to dicamba at doses ranging from 0 to 767 g ae ha^{-1} in a greenhouse study, 21 DAT. Damage was evaluated using a scale of 0 to 100% where a rating of 0% meant no visible damage was noted and 100% meant complete plant death due to dicamba exposure. Dicamba was applied to plants using an automated track sprayer calibrated to deliver 140 L ha^{-1} when plants reached 12 cm in height. The lack-of-fit F-test was performed in pairwise comparisons to identify cultivars that could be combined into a single model.

APPENDIX

A.1 Mean of 2020 and 2021 dicamba susceptible (DS) soybean visual injury per distance and field, 14 DAT. D1-D5 represent the five distances per field where D1 –D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Visual Injury (%) is a damage rating scale where 0 = no visible damage and 100 = complete plant death.

Field	Distance	2020	2021
Downwind	D1	12.07	29.87
	D2	2.80	17.67
	D3	0.60	10.36
	D4	1.00	2.67
	D5	6.80	3.67
Upwind	D1	0.73	2.80
	D2	0.80	0.27
	D3	1.07	0.20
	D4	0.73	0.00
	D5	0.13	0.60

A.2 Mean of 2020 and 2021 dicamba susceptible (DS) soybean visual injury per distance and field, 28 DAT. D1-D5 represent the five distances per field where D1 –D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Visual Injury (%) is a damage rating scale where 0 = no visible damage and 100 = complete plant death.

Field	Distance	2020	2021
Downwind	D1	4.07	20.73
	D2	2.87	23.27
	D3	0.93	10.21
	D4	1.33	2.00
	D5	2.00	2.40
Upwind	D1	0.13	1.53
	D2	0.20	0.20
	D3	0.33	0.13
	D4	0.13	0.07
	D5	0.00	0.13

A.3 Mean of 2020 and 2021 potato visual injury per distance and field, 7 DAT. D1-D5 represent the five distances per field where D1 –D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Visual Injury (%) is a damage rating scale where 0 = no visible damage and 100 = complete plant death.

Field	Distance	2020	2021
Downwind	D1	6.67	0.00
	D2	4.00	2.00
	D3	0.75	0.00
	D4	2.75	0.00
	D5	0.00	5.00
Upwind	D1	0.75	1.25
	D2	1.50	1.25
	D3	0.00	2.00
	D4	0.00	0.00
	D5	0.00	0.00

A.4 Mean of 2020 and 2021 potato visual injury per distance and field, 14 DAT. D1-D5 represent the five distances per field where D1 –D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Visual Injury (%) is a damage rating scale where 0 = no visible damage and 100 = complete plant death.

Field	Distance	2020	2021
Downwind	D1	2.5	14.5
	D2	4.5	4
	D3	1.25	1.5
	D4	0	0
	D5	0	0
Upwind	D1	3.75	2.25
	D2	5	2.5
	D3	2	0
	D4	1.5	0
	D5	0	0

A.5 Mean of 2020 and 2021 potato visual injury per distance and field, 28 DAT. D1-D5 represent the five distances per field where D1 –D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Visual Injury (%) is a damage rating scale where 0 = no visible damage and 100 = complete plant death.

Field	Distance	2020	2021
Downwind	D1	2.5	5.75
	D2	6	1
	D3	0	1
	D4	0	0.75
	D5	0	0
Upwind	D1	1.25	0
	D2	0.75	0.75
	D3	0	0
	D4	0	0
	D5	0	0

A.6 Mean of 2020 and 2021 tomato visual injury per distance and field, 7 DAT. D1-D5 represent the five distances per field where D1 –D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Visual Injury (%) is a damage rating scale where 0 = no visible damage and 100 = complete plant death.

Field	Distance	2020	2021
Downwind	D1	8.00	4.75
	D2	7.25	6.75
	D3	2.25	4.25
	D4	1.25	0.50
	D5	0.00	0.00
Upwind	D1	1.00	1.00
	D2	0.00	4.00
	D3	1.75	1.75
	D4	0.00	0.50
	D5	0.00	0.00

A.7 Mean of 2020 and 2021 tomato visual injury per distance and field, 14 DAT. D1-D5 represent the five distances per field where D1 –D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Visual Injury (%) is a damage rating scale where 0 = no visible damage and 100 = complete plant death.

Field	**Distance**	**2020**	**2021**
Downwind	D1	15.75	14.00
	D2	2.25	6.00
	D3	3.25	3.25
	D4	0.50	1.00
	D5	0.00	0.00
Upwind	D1	3.75	1.00
	D2	1.50	1.25
	D3	1.50	1.50
	D4	2.67	0.50
	D5	2.50	0.00

A.8 Mean of 2020 and 2021 tomato visual injury per distance and field, 28 DAT. D1-D5 represent the five distances per field where D1 –D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Visual Injury (%) is a damage rating scale where 0 = no visible damage and 100 = complete plant death.

Field	**Distance**	**2020**	**2021**
Downwind	D1	2.50	12.00
	D2	0.00	4.75
	D3	1.50	2.50
	D4	0.00	1.50
	D5	0.00	0.00
Upwind	D1	2.00	0.00
	D2	0.00	2.00
	D3	2.00	1.25
	D4	0.00	0.00
	D5	0.00	0.00

A.9 Mean of 2020 and 2021 watermelon visual injury per distance and field, 7 DAT. D1-D5 represent the five distances per field where D1 –D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Visual Injury (%) is a damage rating scale where 0 = no visible damage and 100 = complete plant death.

Field	Distance	2020	2021
Downwind	D1	2.00	0.50
	D2	5.00	1.50
	D3	3.00	1.25
	D4	1.25	0.00
	D5	0.00	0.00
Upwind	D1	3.75	0.00
	D2	0.75	0.00
	D3	3.50	0.00
	D4	0.00	0.50
	D5	0.00	0.00

A.10 Mean of 2020 and 2021 watermelon visual injury per distance and field, 14 DAT. D1-D5 represent the five distances per field where D1 –D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Visual Injury (%) is a damage rating scale where 0 = no visible damage and 100 = complete plant death.

Field	Distance	2020	2021
Downwind	D1	4.25	6.50
	D2	4.75	8.50
	D3	3.67	4.25
	D4	1.00	1.75
	D5	0.00	0.00
Upwind	D1	3.75	8.33
	D2	1.75	6.75
	D3	5.25	0.00
	D4	0.00	1.50
	D5	0.00	0.00

A.11 Mean of 2020 and 2021 watermelon visual injury per distance and field, 28 DAT. D1-D5 represent the five distances per field where D1 –D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Visual Injury (%) is a damage rating scale where 0 = no visible damage and 100 = complete plant death.

Field	Distance	2020	2021
Downwind	D1	5.50	6.50
	D2	0.00	2.00
	D3	2.67	2.67
	D4	0.00	0.00
	D5	0.00	0.00
Upwind	D1	4.00	1.00
	D2	4.33	0.00
	D3	3.00	0.00
	D4	1.50	0.00
	D5	0.00	0.00

A.12 Mean of 2020 and 2021 watermelon vine length per distance and field, 7 DAT. D1-D5 represent the five distances per field where D1 –D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Visual Injury (%) is a damage rating scale where 0 = no visible damage and 100 = complete plant death.

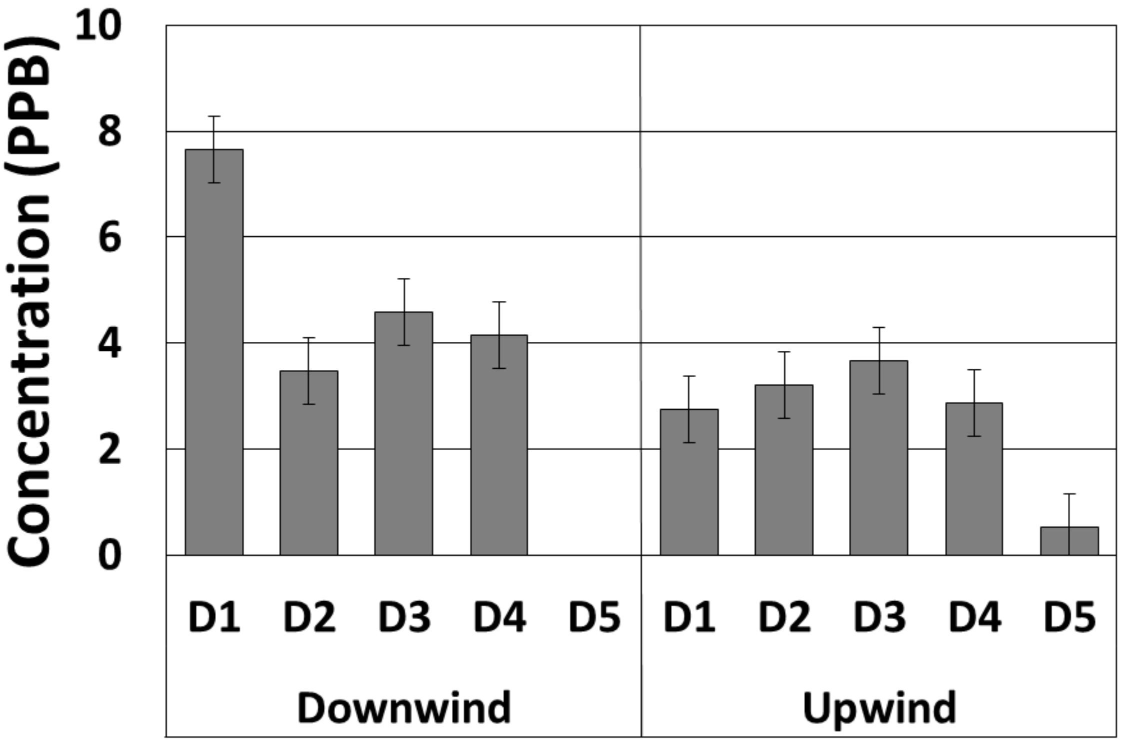
Field	Distance	2020	2021
Downwind	D1	12.08	8.63
	D2	6.36	11.79
	D3	8.45	9.50
	D4	10.13	6.25
	D5	4.18	2.50
Upwind	D1	14.29	8.47
	D2	4.43	9.58
	D3	3.70	9.50
	D4	9.33	9.42
	D5	15.00	4.22

A.13 Mean of 2020 and 2021 watermelon vine length per distance and field, 14 DAT. D1-D5 represent the five distances per field where D1 –D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Visual Injury (%) is a damage rating scale where 0 = no visible damage and 100 = complete plant death.

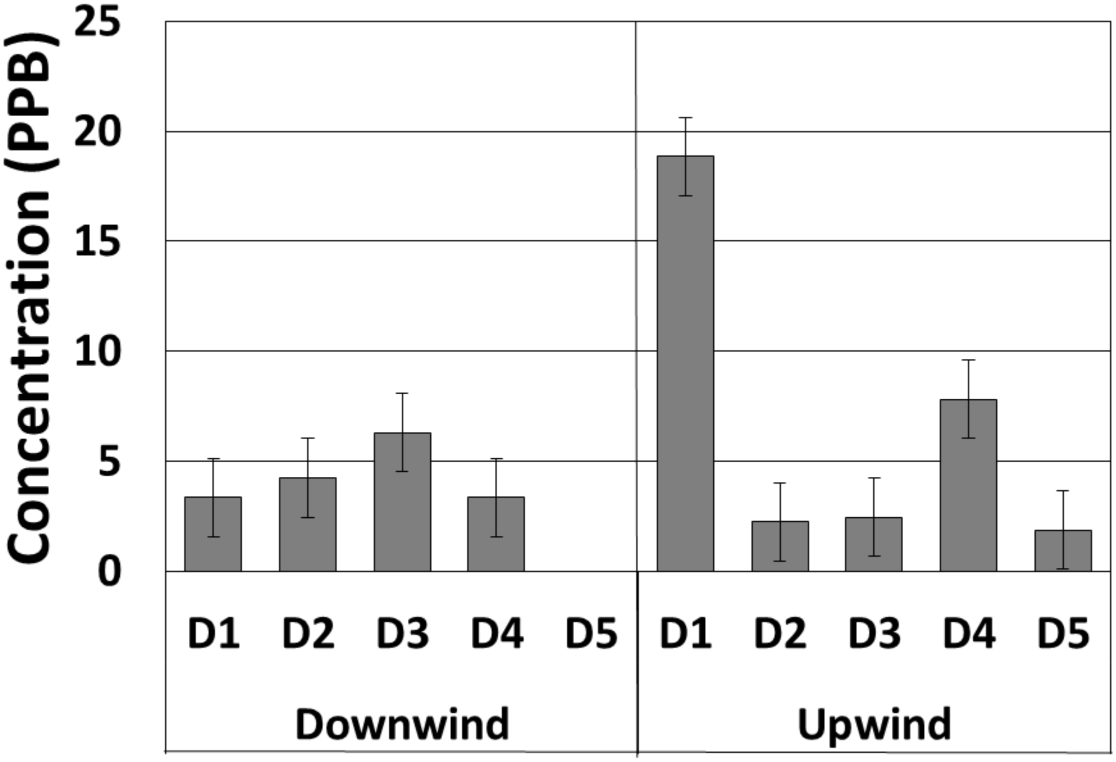
Field	Distance	2020	2021
Downwind	D1	12.75	15.56
	D2	10.08	16.53
	D3	26.57	20.54
	D4	21.50	10.83
	D5	6.13	-
Upwind	D1	12.67	15.97
	D2	8.00	14.07
	D3	6.82	14.30
	D4	16.25	18.60
	D5	12.67	10.95

A.14 Mean of 2020 and 2021 watermelon vine length per distance and field, 21 DAT. D1-D5 represent the five distances per field where D1 –D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Visual Injury (%) is a damage rating scale where 0 = no visible damage and 100 = complete plant death.

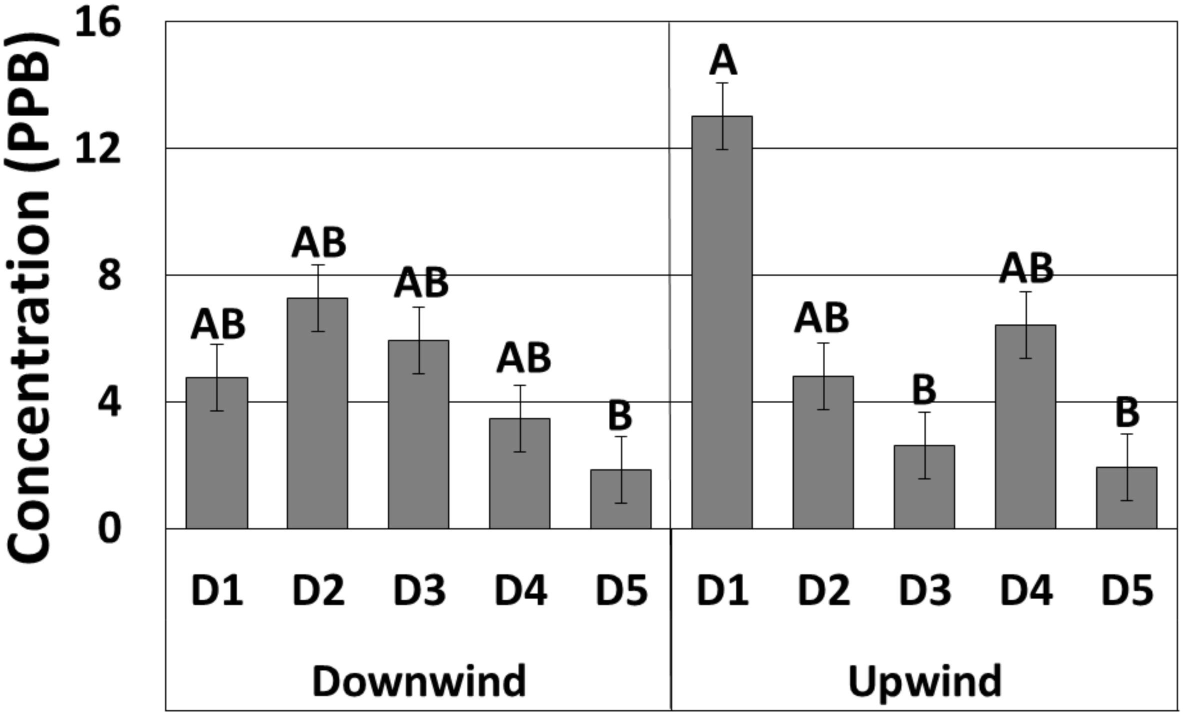
Field	Distance	2020	2021
Downwind	D1	16.11	22.94
	D2	22.60	17.27
	D3	38.33	30.29
	D4	28.50	15.57
	D5	12.80	27.50
Upwind	D1	26.86	23.29
	D2	33.50	17.70
	D3	3.20	20.97
	D4	22.20	26.00
	D5	15.75	14.30



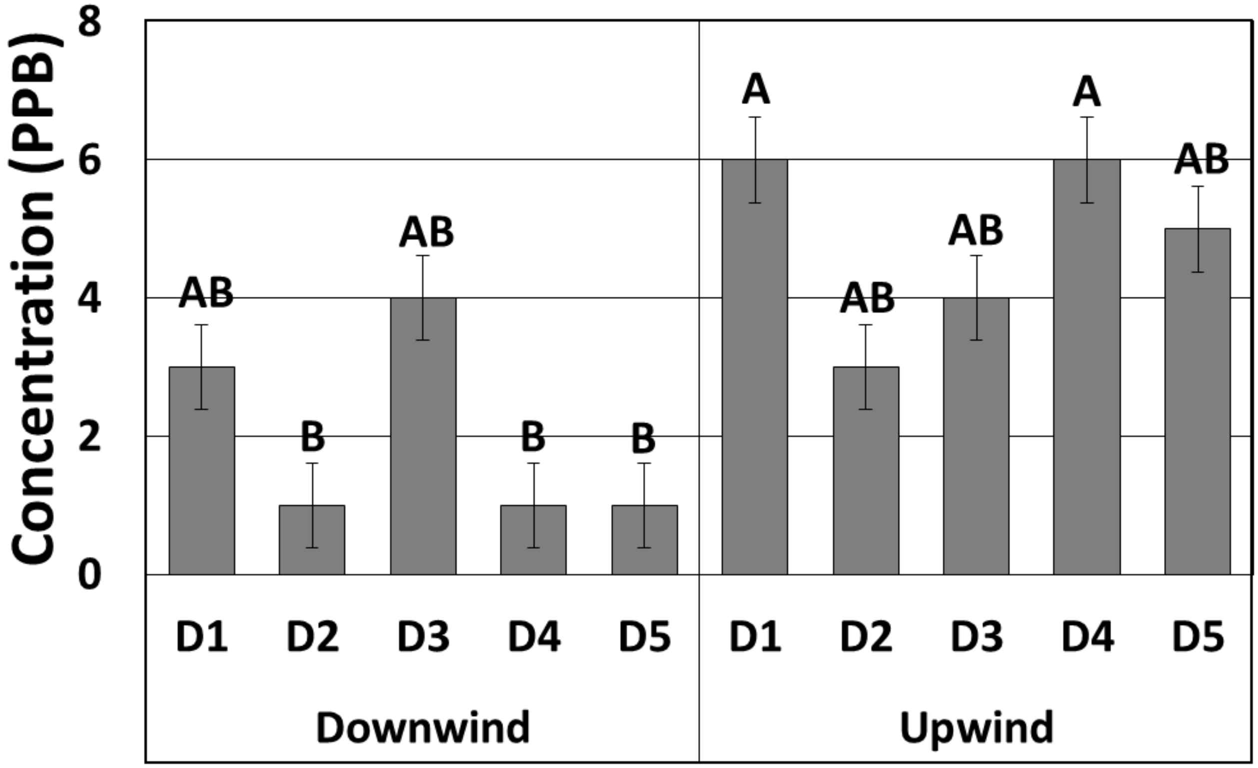
A.15 Mean of 2020 and 2021 dicamba residues detected in potato tubers harvested 14 DAT, per distance and field. D1-D5 represent the five distances per field where D1 to D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Residues were quantified as µg/kg. The limit of quantification (LOQ) was set at 10 µg/kg and any concentration detected below this limit cannot be accurately detected as dicamba. Potato residues from tubers harvested 14 DAT were not significantly different at $\alpha = 0.05$ using Tukey's HSD. Vertical bars indicate standard error of the means.



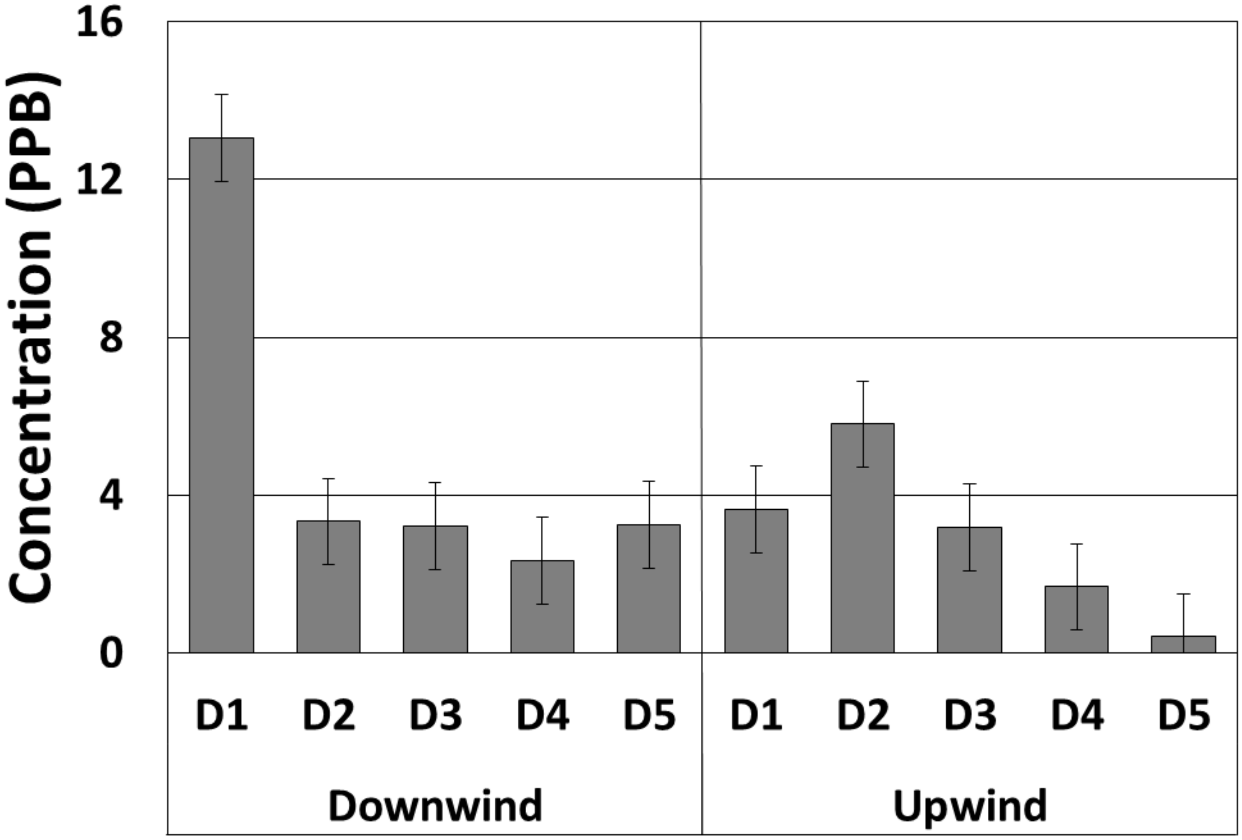
A.16 Mean of 2020 and 2021 dicamba residues detected in potato tubers harvested 28 DAT, per distance and field. D1-D5 represent the five distances per field where D1 to D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Residues were quantified as µg/kg. The limit of quantification (LOQ) was set at 10 µg/kg and any concentration detected below this limit cannot be accurately detected as dicamba. Potato residues from tubers harvested 28 DAT were not significantly different at $\alpha = 0.05$ using Tukey's HSD. Vertical bars indicate standard error of the means.



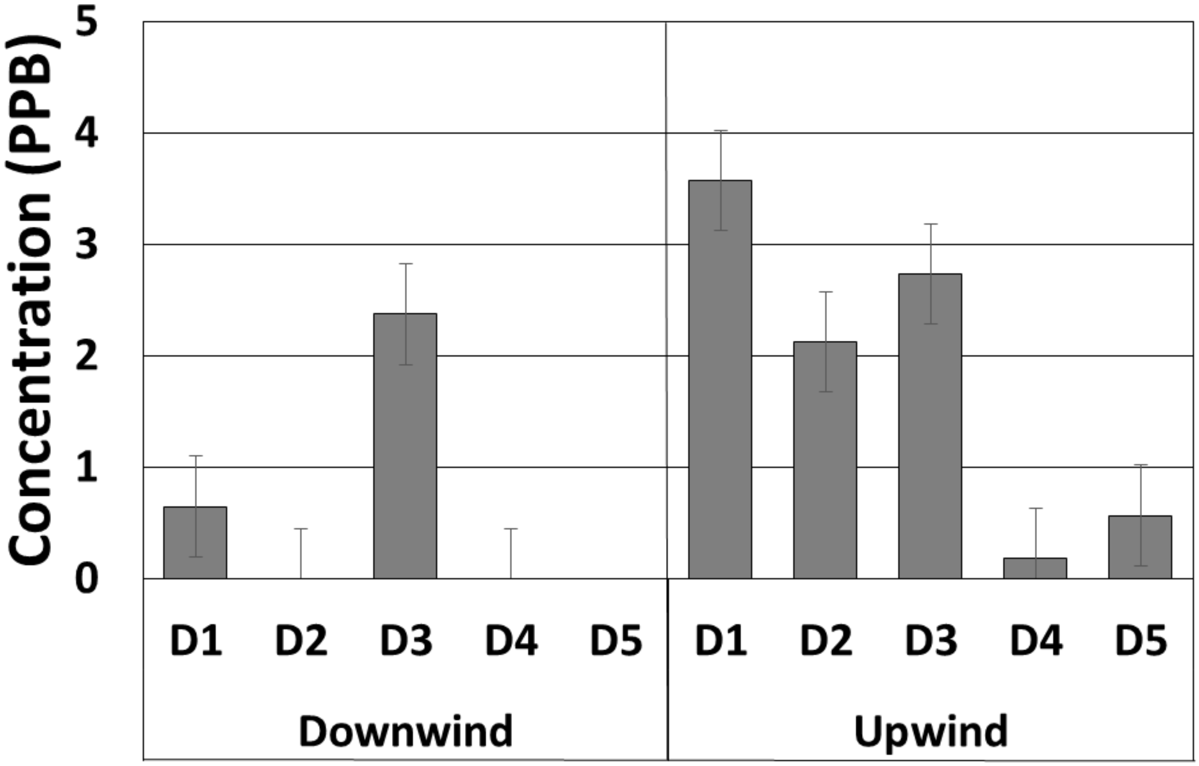
A.17 Mean of 2020 and 2021 dicamba residues detected in tomato fruit harvested 14 DAT, per distance and field. D1-D5 represent the five distances per field where D1 to D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Residues were quantified as µg/kg. The limit of quantification (LOQ) was set at 10 µg/kg and any concentration detected below this limit cannot be accurately detected as dicamba. Means followed by the same letter are not significantly different at $\alpha = 0.05$ using Tukey's HSD. Vertical bars indicate standard error of the means.



A.18 Mean of 2020 and 2021 dicamba residues detected in tomato fruit harvested 28 DAT, per distance and field. D1-D5 represent the five distances per field where D1 to D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Residues were quantified as µg/kg. The limit of quantification (LOQ) was set at 10 µg/kg and any concentration detected below this limit cannot be accurately detected as dicamba. Means followed by the same letter are not significantly different at $\alpha = 0.05$ using Tukey's HSD. Vertical bars indicate standard error of the means.



A.19 Mean of 2020 and 2021 dicamba residues detected in watermelon fruit harvested 14 DAT, per distance and field. D1-D5 represent the five distances per field where D1 to D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Residues were quantified as µg/kg. The limit of quantification (LOQ) was set at 10 µg/kg and any concentration detected below this limit cannot be accurately detected as dicamba. Residues from watermelon fruit harvested 14 DAT were not significantly different at $\alpha = 0.05$ using Tukey's HSD. Vertical bars indicate standard error of the means.



A.20 Mean of 2020 and 2021 dicamba residues detected in watermelon fruit harvested 28 DAT, per distance and field. D1-D5 represent the five distances per field where D1 to D5 = 8.4, 16.8, 33.6, 50.3 and 67 m from the applied area, respectively. Residues were quantified as µg/kg. The limit of quantification (LOQ) was set at 10 µg/kg and any concentration detected below this limit cannot be accurately detected as dicamba. Residues from watermelon fruit harvested 28 DAT were not significantly different at $\alpha = 0.05$ using Tukey's HSD. Vertical bars indicate standard error of the means.

A.21 Visual injury of tomato cultivars 'Better Boy', 'Brandywine Pink', 'Carolina Gold', 'Grape', 'Mountain Merit', 'Roma', and 'Supersweet' subjected to dicamba at doses ranging from 0 to 383 g ae ha^{-1} in a greenhouse study, 7 DAT. Damage was evaluated using a scale of 0 to 100% where a rating of 0% meant no visible damage was noted and 100% meant complete plant death due to dicamba exposure. Dicamba was applied to plants using an automated track sprayer calibrated to deliver 140 L ha^{-1} when plants reached 16 cm in height.

Dose (g ae ha^{-1})	Better Boy	Brandywine	Carolina Gold	Grape	Mountain Merit	Roma	Supersweet
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25.56	5.88	4.25	6.63	3.00	4.63	6.50	8.63
51.13	25.63	26.88	16.13	20.63	15.75	33.50	25.38
76.69	33.75	35.71	24.38	31.00	30.00	51.25	31.88
102.26	37.50	35.00	26.88	38.75	33.75	56.25	41.25
153.39	43.75	48.75	35.00	41.88	43.75	64.29	50.63
204.52	53.75	53.57	43.75	49.38	45.00	68.13	63.57
255.65	58.75	59.17	45.00	58.13	49.38	66.63	76.25
383.47	65.63	61.25	55.63	55.63	51.88	68.75	72.50

A.22 Visual injury of tomato cultivars 'Better Boy', 'Brandywine Pink', 'Carolina Gold', 'Grape', 'Mountain Merit', 'Roma', and 'Supersweet' subjected to dicamba at doses ranging from 0 to 383 g ae ha^{-1} in a greenhouse study, 14 DAT. Damage was evaluated using a scale of 0 to 100% where a rating of 0% meant no visible damage was noted and 100% meant complete plant death due to dicamba exposure. Dicamba was applied to plants using an automated track sprayer calibrated to deliver 140 L ha^{-1} when plants reached 16 cm in height.

Dose (g ae ha^{-1})	Better Boy	Brandywine	Carolina Gold	Grape	Mountain Merit	Roma	Supersweet
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25.56	7.13	6.63	6.25	4.13	4.88	7.88	9.88
51.13	28.75	29.29	18.13	33.13	18.50	59.29	35.63
76.69	51.88	55.00	27.57	37.50	33.75	75.00	49.38
102.26	54.38	58.33	35.00	42.50	34.38	90.83	73.50
153.39	78.13	85.00	53.75	60.00	37.50	89.00	72.14
204.52	87.50	86.67	74.29	60.83	49.38	95.00	82.86
255.65	91.88	95.00	73.13	81.88	65.00	95.00	98.75
383.47	98.75	100.00	93.75	83.75	175.00	98.13	98.13

A.23 Visual injury of watermelon cultivars 'Black Tail Mountain', 'Crimson Sweet', 'Millionaire', 'Sangria' and 'Yellow Doll' subjected to dicamba at doses ranging from 0 to 767 g ae ha^{-1} in a greenhouse study, 7 DAT. Damage was evaluated using a scale of 0 to 100% where a rating of 0% meant no visible damage was noted and 100% meant complete plant death due to dicamba exposure. Dicamba was applied to plants using an automated track sprayer calibrated to deliver 140 L ha^{-1} when plants reached 12 cm in height.

Dose (g ae ha^{-1})	Black Tail Mountain	Crimson Sweet	Millionaire	Sangria	Yellow Doll
0.00	0.00	0.00	0.00	0.00	0.00
25.56	8.00	8.00	12.50	3.38	12.50
51.13	11.17	9.75	10.00	17.50	12.50
76.69	21.67	16.25	22.50	16.63	21.88
102.26	24.17	20.00	22.50	17.50	26.25
153.39	33.33	26.88	23.75	26.88	25.63
204.52	39.00	23.13	31.25	24.38	24.38
255.65	33.00	30.63	47.50	40.00	39.38
383.47	50.00	48.13	40.00	41.88	38.75

A.24 Visual injury of watermelon cultivars 'Black Tail Mountain', 'Crimson Sweet', 'Millionaire', 'Sangria' and 'Yellow Doll' subjected to dicamba at doses ranging from 0 to 767 g ae ha^{-1} in a greenhouse study, 14 DAT. Damage was evaluated using a scale of 0 to 100% where a rating of 0% meant no visible damage was noted and 100% meant complete plant death due to dicamba exposure. Dicamba was applied to plants using an automated track sprayer calibrated to deliver 140 L ha^{-1} when plants reached 12 cm in height.

Dose (g ae ha^{-1})	Black Tail Mountain	Crimson Sweet	Millionaire	Sangria	Yellow Doll
0.00	0.00	0.00	0.00	0.00	0.00
25.56	16.60	12.88	9.50	9.50	18.38
51.13	15.83	27.50	11.67	17.86	20.00
76.69	34.00	25.71	21.67	24.17	30.83
102.26	57.50	31.88	67.50	31.43	60.63
153.39	61.67	53.75	87.50	66.25	63.75
204.52	98.00	53.75	90.00	58.13	66.25
255.65	90.00	53.75	100.00	72.50	85.63
383.47	99.00	71.88	85.00	73.75	84.38