EVALUATIONS OF DRIFTABLE FRACTIONS OF DICAMBA AND 2,4-D TO SELECTED HERBACEOUS AND WOODY PLANT SPECIES

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

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

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ABSTRACT

Commercialization of 2,4-D and dicamba-resistant cotton and soybean has given producers another mechanism of action to control herbicide resistant weeds. As a result of the adoption of this technology, off-target movement of 2,4-D and dicamba onto nearby sensitive broadleaf species is a major concern for specialty crop growers, homeowners, organic producers, and commercial growers of ornamental plant species. Two separate studies were conducted in 2017 and 2018 to determine the sensitivity of select herbaceous and woody plant species to driftable fractions of 2,4-D or dicamba with or without glyphosate. In the herbaceous plant study, three driftable fractions of 1/10, 1/100, and 1/300th of the fully labeled rate of 2,4-D, 2,4-D plus glyphosate, dicamba, and dicamba plus glyphosate were applied to begonia, coleus, geranium, impatiens, marigold, petunia, vinca, and zinnia. Based on visual injury assessments recorded 28 days after treatment (DAT), coleus was found to be the most sensitive species to driftable fractions of all herbicides. It was also found that marigold and geranium had a greater sensitivity to treatments containing 2,4-D but, coleus and zinnia had a greater sensitivity to treatments containing dicamba. In the woody plant study, the same herbicides were applied but at different fractions corresponding to 1/2, 1/20, and 1/200th of the full labeled rates. Herbicide treatments were applied to apple, crabapple, dogwood, elderberry, elm, grape, hydrangea, maple, oak, pecan, redbud, rose, raspberry, strawberry, sweetgum, viburnum, and walnut plants. Visual injury assessments along with shoot length measurements and tree trunk diameter indicate that grapes were the most sensitive species to all herbicide treatments while hydrangea was the least sensitive. In addition, walnut, grape and elm were found to have a greater sensitivity to 2,4-D while apple, maple, and peach had a greater sensitivity to dicamba. Both studies found that as the herbicide rate increased,

greater visual injury, and greater reductions in height and dry biomass or trunk diameter and shoot length were observed. Furthermore, when glyphosate was applied with either 2,4-D or dicamba at the high rates, visual injury increased for most plant species. While the lowest rates of herbicides applied in these studies did not cause significant reductions in height, dry biomass, shoot length or tree trunk diameter, visual injury was still observed for some species. Since many of the species investigated in these studies are fruiting or ornamental plants, it is likely that even the low levels of injury would not be tolerated by homeowners or commercial growers.

CHAPTER I

LITERATURE REVIEW

Justification

The spread of herbicide resistance weeds in row crop production systems has left farmers with fewer weed control options (Norsworthy et al. 2012). To combat herbicide-resistant weeds, agrochemical companies have developed soybean (*Glycine max* (L.) Merr.) and cotton (*Gossypium hirsutum* L.) plants that are resistant to 2,4-D and dicamba (Behrens et al. 2007; Wright et al. 2010). Recent deregulation of dicamba and 2,4-D tolerant crop cultivars in the U.S. allows farmers to utilize these new technologies to control herbicide resistant weeds (USDA-APHIS 2014; USDA-APHIS 2015). Dicamba and 2,4-D are effective on difficult to control weeds such as waterhemp (*Amaranthus tuberculatus* (Moq.) J. D. Sauer), horseweed (*Conyza canadensis* (L.) Cronq.), Palmer amaranth (*Amaranthus palmeri* S. Wats.), and giant ragweed (*Ambrosia trifida* L.) (Johnson et al., 2010; Kruger et al. 2010; Robinson et al. 2012; Spaunhorst et al. 2014; Shergill et al. 2017).

The adoption of 2,4-D and dicamba resistant crops will likely result in more frequent applications of 2,4-D and dicamba and therefore, increased risk of off-target movement of these herbicides to nearby sensitive crops like soybean, cotton, grape (*Vitis vinifera* L.), and a variety of vegetable crops (Egan et al. 2014; Solomon and Bradley 2014; Mohseni-Moghadam and Doohan 2015; Mohseni-Moghadam et al. 2015). The sensitivity of certain agronomic crops to these herbicides has been well documented. For example, soybeans are much more sensitive to dicamba compared with 2,4-D (Al-Khatib and Peterson 1999; Anderson et al. 2004; Johnson et al. 2012; Egan et al. 2014; Solomon and Bradley 2014). Solomon and Bradley (2014) showed that 2.8 g dicamba ae ha^{-1} applied to soybean at the V3 stage of growth resulted in 32% visual injury, however the same rate of 2,4-D caused only 3% injury. Similar sensitivity differences have been documented with wine grapes; applications of 2,4-D at 1/30th of the normal use rate resulted in 66% visual injury 42 days after treatment (DAT), while 1/30th of the normal use rate of dicamba caused only 47% injury (Mohseni-Moghadam et al. 2015). Sensitivity differences between 2,4-D and dicamba for various crop and plant species are important to help understand the susceptibility of a species to off-target movement (Johnson et al. 2012). However, few studies have examined the sensitivity differences of other non-target speciality crops to 2,4-D and dicamba. Therefore, the objectives of this M.S. thesis research are to: 1) determine the sensitivity of selected woody and herbaceous species to driftable fractions of 2,4-D and dicamba, and 2) document the differences in symptomology between 2,4-D and dicamba on selected species.

2,4-D

The synthetic auxin herbicide 2,4-D was first discovered during the World War II era and was originally proposed for use in biological warfare (Peterson 1967). However, the potential for 2,4-D to be used as an agricultural herbicide was realized as the war came to an end. Scientists were interested in the ability of the chemical to translocate throughout a plant and it's selective post-emergent (POST) activity on broadleaf plants only, while leaving grasses unaffected (Peterson 1967). Initially, 2,4-D was utilized in cereal crops for selective control of broadleaf weeds, but 2,4-D was also useful in pre-plant no-till applications (Wilson and Worsham 1988), lawns, pastures, rights-of-way, roadsides and other non-crop areas (Peterson 1967; Peterson et al. 2016). Currently, 2,4-D is labeled for the control of at least 95 annual and perennial weed species including waterhemp, horseweed, morningglory (*Ipomoea spp*.), common ragweed (*Ambrosia artemisiifolia* L.), giant ragweed, and field bindweed (*Convolvulus arvensis* L.) (Anonymous 2017b).

The three most common formulations of 2,4-D that have been commercialized are the amine salt, ester, and most recently the choline salt (Peterson et al. 2016). One of the primary differences between these formulations is their volatility. Volatilization is defined as the physical change of a liquid or solid compound into a gaseous state. Volatility can result in the herbicide evaporating and moving away from the target (Ross and Lembi 2009). Sosnoskie et al. (2015) observed greater injury on cotton after being exposed to volatiles of 2,4-D ester compared with the amine and choline formulations. In addition, the ester formulation traveled the greatest distance in a field setting and resulted in greater injury to cotton up to 48 meters (m) from the treated plot (Sosnoskie et al. 2015). The newest formulation, 2,4-D choline, has recently been approved by the Environmental Protection Agency and is available as a stand-alone product (Enlist One ®) or as a prepackaged mix with glyphosate (Enlist Duo ®) from Dow AgroSciences (US EPA 2014).

Recently, 2,4-D-resistant corn, cotton, and soybean has been developed and commercialized by Dow AgroSciences (Wright et al. 2010). These genetically modified crops contain a gene that encodes for an aryloxyalkanoate dioxygenase enzyme that cleaves 2,4-D and makes it non-toxic to broadleaf plants (Wright el al. 2010). Recent deregulation of 2,4-D-resistant crops allows farmers to apply 2,4-D choline POST to cotton and soybeans to control problematic weeds, which has never been possible before (USDA-APHIS 2014). If the adoption of 2,4-D-resistant crops follows a similar trend as the adoption of glyphosate-resistant crops, there are likely to be increased applications of 2,4-D in U.S. agriculture in the near future (Mortensen et al. 2012).

Dicamba

Following the breakthrough with 2,4-D, an additional synthetic auxin herbicide was discovered in 1942 by Zimmerman and Hitchcock (Ross and Lembi 2009). Dicamba was the first benzoic acid herbicide commercially available. However, it was not until the early 1960’s that dicamba was first developed for herbicide use in the United States (Ross and Lembi 2009). The first formulation of dicamba was the dimethylamine (DMA) salt of dicamba and was sold under the trade name of Banvel ®. Since that time, dicamba has been labeled for use in small grains, corn, grass pastures, and a variety of other crops to control emerged broadleaf weeds (Burnside and Lavy 1966; Egan and Mortensen 2012). Both dicamba and 2,4-D mimic the endogenous auxin within plants, indole-3-acetic acid (IAA). The mechanism of action of synthetic auxin compounds such as 2,4-D and dicamba are not well understood, but these compounds appear to activate the ATPase pump, which creates a high concentration of hydrogen ions in the cell wall region, creating a low pH environment which causes cell walls to become loosened and allows cell elongation. At high concentrations, these herbicides cause vascular tissue damage and inhibit cell division and growth, eventually leading to plant death (Shaner 2014). Currently, dicamba is labeled for the control of approximately 150 annual, biennial, and perennial broadleaf weeds including horseweed, common ragweed, giant ragweed, waterhemp, and Palmer amaranth (Anonymous 2017a).

Several different formulations of dicamba have been commercialized within the agricultural market, some of which include the DMA salt, diglycolamine (DGA) salt, and most recently the DGA salt plus VaporGrip technology (Xtendimax with VaporGrip ®, FeXapan plus VaporGrip ®) and the N,N-bis-(aminopropyl) methylamine (BAPMA) salt (Engenia ®). Like 2,4-D, one of the primary differences in formulations of dicamba is their ability to volatilize and cause injury to nearby sensitive plant species (Behrens and Lueschen 1979; Egan and Mortensen 2012; Mueller et al. 2013; Latorre et al. 2017). For example, Behrens and Lueschen (1979) found that soybean placed in a field three days following an application of the DMA salt of dicamba resulted in as much as 48% visible injury (Behrens and Lueschen 1979). In some of the first work of its kind, these early results revealed that the DMA salt of dicamba is volatile and has the ability to cause significant injury to sensitive soybean plants (Behrens and Lueschen 1979). In a more recent experiment, Mueller et al. (2013) found that less dicamba was detected within the first 12 hours after application of the DGA compared to the DMA salt of dicamba. However, after 12 hours, there were no differences in dicamba air concentrations between the two formulations (Mueller et al. 2013). Latorre et al. (2017) also found that significant differences in volatility among the DMA, DGA, BAPMA and DGA plus VaporGrip formulations with that the DMA formulation resulting in the greatest risk for volatilization. The BAPMA and DGA plus VaporGrip formulations of dicamba were approved in 2016 by the EPA for application to dicamba-resistant cotton and soybean in the U.S. (US EPA 2016).

The approval of these new dicamba formulations was driven by the recent deregulation of dicamba-resistant cotton and soybean. Dicamba-resistant cotton and soybean contains an enzyme, dicamba monooxygenase (DMO), that detoxifies dicamba into 3,6-dichlorosalicylic acid (DCSA), which has no herbicidal activity (Behrens et al. 2007). The adoption of dicamba-resistant crops will likely lead to increased applications of dicamba and therefore a greater likelihood of its off-target movement to sensitive species such as soybean, cotton, grapes and vegetables (Everitt and Keeling 2009; Egan and Mortensen 2012; Mohseni-Moghadam and Doohan 2015; Mohseni-Moghadam et al. 2015). In fact, in 2017 an estimated 3.6 million acres of soybeans were injured throughout the Midwestern and Southern United States as a result of off-target movement of dicamba. Additionally, 2,708 investigations were conducted by state departments of agriculture as a result of suspected dicamba injury (Bradley 2017). While soybean injury made up the majority of the injury reports in 2017, it was not the only crop impacted. Specialty crops like trees, grapes, and vegetables also experienced injury from off-target movement of dicamba as well.

Glyphosate

Although the early breakthrough and discovery of synthetic herbicide compounds such as 2,4-D and dicamba proved to be useful in agriculture, the commercialization of glyphosate in 1974 forever revolutionized agriculture. Glyphosate was first synthesized in 1950 by Henri Martin, but the herbicidal properties of glyphosate were not realized until 1970 by John Franz. Later, in 1974, glyphosate was commercialized and sold under the trade name Roundup ® by Monsanto Company (Franz et al. 1997). Glyphosate was initially used in non-crop areas, right-a-ways, industrial areas, understory vegetation control, and residential areas (Franz et al. 1997; Duke 2018). Glyphosate’s utility as a non-selective, broad spectrum herbicide that is translocated to roots and shoots within plants made it the first of its kind. Thus, making glyphosate a great herbicide for annual or perennial grass or broadleaf species. (Franz et al. 1997; Duke 2018). Glyphosate also has a unique mechanism of action, in which it inhibits 5-enolypyruvyl-shikimate-3-phosphate synthase (EPSPS). Inhibition of EPSPS results in a depletion of aromatic amino acids, which are essential for protein synthesis and biosynthetic pathways responsible for plant growth (Franz et al. 1997; Shaner 2014).

Following commercialization of glyphosate in the 1970's, glyphosate resistant (GR) soybeans became available in 1996. GR soybeans contain a CP4-EPSPS enzyme which was derived from *Agrobacterium*. The CP4-EPSPS enzyme is insensitive to glyphosate and thus confers glyphosate resistance in crops, such as soybean (Dill 2005). The expression of this enzyme confers high level GR in crops. Ten years following release of GR technology, greater than 90% of soybean, corn, and cotton acres in the United States were GR (Duke 2017). However, recently many agronomic weed species including waterhemp (Legleiter and Bradley 2008), Palmer amaranth (Culpepper et al. 2006), horseweed (VanGessel 2001), and giant ragweed (Norsworthy et al. 2010) have developed GR, which has limited the ability of glyphosate's effectiveness on these weed species.

Off-target Movement of Herbicides

The ability of herbicides like 2,4-D and dicamba to move off target and cause damage to nearby sensitive plants can be influenced by a variety of factors. For example, wind speed has a significant impact on spray drift (Nordby and Skuterud 1974; Wang and Rautmann 2008; Alves et al. 2017). Nordby and Skuterud (1974) showed that strong wind velocities of 4.0 meters per second (m/s) occurring during an application of aminotriazole, caused damage to sensitive barley (*Hordeum vulgare* L.) plants up to 200 m from the treated area, and that higher boom heights and strong wind velocities were two of the most important factors responsible for spray drift. Similarly, Wang and Rautmann (2008) found that wind speed was the most important factor that influenced spray drift, accounting for nearly one-third of the variability of the total spray drift observed in their study. Wolf et al. (1993) reported that with wind speeds of 9 to 30 kilometers hour^{-1}, drift rates could range from 1.8 to 16%, of the full labeled rate, respectively. Alves et al. (2017) also showed that as wind speed increased from 1 to 5 m/s, greater drift of dicamba was detected.

Nozzles are also known to impact the amount of drift that can occur during pesticide applications (Wang and Rautmann 2008; Alves et al. 2017). Nozzles that produce larger droplet size tend to result in less drift because the coarser spray droplets are heavier and fall to the target faster. Alves et al. (2017) showed that when wind speeds were 0.9 m/s, Extended Range nozzles, which produce medium droplet size, resulted in 25 times more drift compared with Turbo TeeJet Induction nozzles that produce an ultra coarse droplet. However, when wind speeds were 4.9 m/s, the Extended Range nozzles only resulted in 4 times more drift compared with the Turbo TeeJet Induction nozzles, indicating that high wind speeds can influence ultra coarse droplets (Alves et al. 2017). Wang and Rautmann (2008) also reported that nozzles can influence 12 to 30% of the variability of spray drift measured. Results from these studies indicate the importance of using correct nozzles, especially when making applications of herbicides such as 2,4-D and dicamba.

Ground speed of the spray equipment is another factor that influences drift (Holterman et al. 1997; Wang and Rautmann 2008). Increased sprayer speed accounted for approximately 8% of the variability observed during spray drift. (Wang and Rautmann 2008). Holterman et al. (1997) found that when sprayer speed was increased from 0.10 to 1.5 m/s there was approximately a 0.5 and 1% of dose detected downwind, respectively. Labels for the newly formulated dicamba products limit ground speed to 24 kilometers per hour in an attempt to reduce spray drift (Anonymous 2017a). However, a ground speed restriction has not been set for the newly formulated 2,4-D choline (Anonymous 2017b).

Boom height can also influence spray drift and deposition (Nordby and Skuterud 1974; Holterman et al. 1997). Nordby and Skuterud (1974) found that spray drift deposition was increased from 1 to 3.2% when changing the boom height from 40 to 80 cm. The authors concluded that pesticides sprayed from 80 cm above the ground will be strongly influenced by air currents, which would then move the spray droplets off-target (Nordby and Skuterud 1974). Holterman et al. (1997) also found that higher wind speeds and greater boom heights had a similar impact on drift; as the boom height increased from 0.35 to 1 m, there was approximately 1 and 10% of a dose detected 1.75 m away, respectively. Therefore, a higher number of spray droplets were not deposited on the target and moved elsewhere (Holterman et al. 1997).

Volatilization is another pathway in which herbicides can move off-target. Volatilization is defined as the physical change of a liquid or solid compound into a gaseous state. It can occur when the herbicide evaporates and moves away from the target (Ross and Lembi 2009). Volatilization of herbicides such as 2,4-D and dicamba can be influenced by formulation, temperature, rainfall, and relative humidity (Behrens and Lueschen 1979; Egan and Mortensen 2012; Sosnoskie et al. 2015). Increased volatilization of dicamba was positively correlated with increasing temperatures (Behrens and Lueschen 1979; Egan and Mortensen 2012). Additionally, Behrens and Lueschen (1979) found that dicamba volatilization was drastically decreased following a rainfall event while Egan and Mortensen (2012) found that dicamba volatility was positively correlated with increases in relative humidity. Formulation also influences the degree of dicamba volatility with the DGA salt of dicamba resulting in 94% less vapor drift as compared with the DMA salt of dicamba (Egan and Mortensen 2012). Formulation of 2,4-D also has an effect on volatilization. Volatiles from the ester formulation of 2,4-D resulted in 76% cotton injury, while the choline salt formulation of 2,4-D resulted in only 5% injury (Sosnoskie et al. 2015).

Soybean Injury and Yield Loss in Response to Dicamba

Dicamba injury to soybeans can manifest itself as stunted growth and delayed development, as well as cupped and malformed leaves, twisting and bent stems and petioles, chlorotic and necrotic tissue, suppression of the terminal bud, stem swelling and cracking, and axillary branching. These symptoms are typically observed within two weeks after the initial application and are common on the newest growth (Wax et al. 1969; Auch and Arnold 1978; Egan and Mortensen 2012; Johnson et al. 2012; Griffin et al. 2013; Robinson et al. 2013; Solomon and Bradley 2014). Soybeans are particularly sensitive to driftable fractions of dicamba (Wax et al. 1969; Behrens and Lueschen 1979; Al-Khatib and Peterson 1999; Johnson et al. 2012; Griffin et al. 2013; Egan et al. 2014; Solomon and Bradley 2014). Several studies have been conducted since the 1970's to determine the effects of off target movement of dicamba on soybean injury and yield loss. A meta-analysis conducted by Egan et al. (2014) summarized the effects of 12 studies that investigated the effects of dicamba injury to soybeans at various growth stages. Results from this meta-analysis revealed that soybeans are far more sensitive to dicamba during the flowering compared to the vegetative growth stages. and that soybean exposed to 0.56, 5.6, and 56 g dicamba ha^{-1} during the flowering stages resulted in approximately 1, 9, and 48% yield loss, respectively (Egan et al. 2014). Additionally, when soybean were injured with 0.56 and 5.6 g dicamba ha^{-1} during the vegetative growth stages, there was 0 and approximately 4% yield loss, respectively (Egan et al. 2014). Kniss (2018) also reported in an updated meta-analysis conducted in 2018 that the dicamba dose required to cause a 2.5% yield loss ranged from 0.15 to 16.0 g ha^{-1}, depending on the soybean stage of growth at the timing of exposure.

2,4-D and Dicamba Injury to Other Plant Species

***Grapes*.**

Development and commercialization of 2,4-D and dicamba-resistant crops has led to concerns with off-target movement of these herbicides not only to sensitive row crops like soybean, but also to other economically-important plant species like grapes. In 2012, there were over 1.1 million acres of grapes grown in the United States (USDA-NASS 2014). Herbicide injury to grapevines has been extensively studied (Ogg et al. 1991; Al-Khatib et al. 1993; Bhatti et al. 1996; Mohseni-Moghadam et al. 2015). Mohseni-Moghadam et al. (2015) found that 2,4-D applied at $1/30^{th}$ (28 g ha^{-1}) of the normal use rate resulted in 66 and 35% injury 42 and 357 days after treatment (DAT), respectively, while dicamba applied at 1/30th of the normal use rate resulted in 47 and 0% injury at the same time intervals after treatment. These rates of 2,4-D and dicamba also resulted in an average shoot length of 22 and 87 cm 42 DAT, respectively (Mohseni-Moghadam et al. 2015). These results show that grapevines have a much higher sensitivity to 2,4-D in comparison to dicamba. Similar studies have also found 2,4-D to be very injurious to grapes (Al-Khatib et al. 1993; Bhatti el al. 1996).

Grape growers also face concerns such as yield loss, herbicide persistence, and successive drift occurrences during the growing season. Ogg et al. (1991) investigated several of the factors listed above with 2,4-D injury to grapevine. When 2,4-D was applied three and four times at a rate of 25 parts per million (ppm) by weight, grape yield was 60 and 55% of the non-treated control, respectively. Ogg et al. (1991) also showed that yield losses could even occur the year following application. When the 25 ppm concentration of 2,4-D was applied three and four times during the growing season, yield in the next season was reduced by 53 and 46% of the non-treated control yield, respectively . These results suggest that if grapevines experience a high dose of 2,4-D or are exposed to multiple drift events in a growing season, significant grape yield reductions can occur in sequential growing seasons.

Cotton.

Cotton injury as a result of off-target movement of 2,4-D or dicamba has been widely investigated (Marple et al. 2007; Everitt and Keeling 2009; Johnson et al. 2012; Egan et al. 2014). Results from a meta-analysis conducted by Egan et al. (2014) indicate that cotton is more sensitive to 2,4-D than dicamba and is most sensitive to 2,4-D during vegetative growth stages. When cotton was injured with 0.56, 5.6, and 56 g 2,4-D ha^{-1} during vegetative growth stages, cotton yield was reduced by 19, 32, and 49%, respectively. During the pre-flowering stages, these same rates of 2,4-D resulted in cotton yield reductions of 9, 33, and 71%, respectively (Egan et al. 2014). Conversely, dicamba applied to cotton at 0.56 g ha^{-1} during vegetative or pre-flowering growth stages resulted in less than 1.3% yield loss while dicamba applied at 56 g ha^{-1} during all growth stages resulted in no more than a 10% cotton yield loss (Egan et al. 2014).

Trees.

The impact of off-target movement of 2,4-D and dicamba on tree species has not been studied extensively. Samtani et al. (2008) examined the effects of multiple herbicides, including dicamba and 2,4-D, on white oak (*Quercus alba* L.) seedlings. Herbicide treatments were applied at 1, 10, and 25% of recommended field use rates to simulate drift. 2,4-D rates ranged from 15 to 375 g ha^{-1} and dicamba rates ranged from 7 to 175 g ha^{-1}. Visual injury was observed and rated on a scale from 1 (no injury) to 10 (all plant tissue injured). Observed symptomology for the 2,4-D and dicamba treatments included leaf cupping, downward leaf margin rolling, elongation of leaf tips, necrosis, chlorosis, and parallel venation (Samtani et al. 2008). Dicamba and 2,4-D applied during the expanded leaf stage at 10% of the field use rate (70 and 150 g ha^{-1}, respectively) resulted in 2.6 and 4.4 visual injury 25 DAT, respectively (Samtani et al. 2008). Al-Khatib et al. (1992) also observed 3, 15, and 37% visual injury to sweet cherry (*Prunus avium* L.) trees 30 DAT at 1/33, 1/10, and 1/3X rates of 2,4-D, respectively. However, by 120 DAT, sweet cherry trees appeared to recover from lower rates of 2,4-D and the authors also noted that two-year old trees were generally less sensitive to the herbicides tested than to one-year old trees (Al-Khatib et al. 1992). Otta (1974) found that Siberian elm (*Ulmus pumila* L.) exhibited leaf cupping symptoms when more than 5 ppm were applied to three-year old established elm trees during the year of application and the subsequent growing season. Bark formation and trunk cross sectional area were also affected by as little as 10 ppm of 2,4-D (Otta 1974).

Tomato.

Several studies have demonstrated that tomatoes (*Solanum lycopersicum* Mill.) are very sensitive to driftable fractions of 2,4-D and dicamba (Hemphill and Montgomery 1981; Gilreath et al. 2001a; Fagliari et al. 2005; Kruger et al. 2012; Bauerle et al. 2015). Kruger et al. (2012) found that tomatoes were most sensitive to dicamba and glyphosate during the early bloom stage compared to the vegetative stages of growth. Their work also found that it took an estimated 1.5 g dicamba ae ha^{-1} to cause a 5% loss in flowering and a 10% loss in marketable fruit was observed when 4.5 or 3.9 g dicamba ae ha^{-1} estimated was applied at the vegetative and reproductive stages, respectively (Kruger at al. 2012). Hemphill and Montgomery (1981) also reported that 20.8 and 208 g 2,4-D ha^{-1} resulted in a 16 and 47% reduction in tomato yield. These results indicate that tomatoes are especially sensitive to low rates of 2,4-D and dicamba, and that extreme care should be taken by applicators when applying herbicides near areas where tomatoes are grown.

Pepper.

In addition to tomatoes, peppers (*Capsicum annuum L.*) have been extensively studied for their sensitivity to 2,4-D and dicamba (Hemphill and Montgomery 1981; Gilreath et al. 2001b; Mohseni-Moghadam and Doohan 2015). Hemphill and

Montgomery (1981) found that applications of 104 and 208 g 2,4-D ha^{-1} on pepper resulted in a 40 and 51% reduction in total yield. Gilreath et al. (2001b) found that peppers had greater visual injury as a result of dicamba compared with 2,4-D, but no differences in marketable yield loss between herbicide treatments were observed. However, overall yield was reduced with bloom applications of either herbicide, but not with post-bloom applications (Gilreath et al. 2001b). These results indicate that peppers are not nearly as sensitive to yield reductions as other crops such as tomatoes and soybean following applications of 2,4-D or dicamba.

Other Vegetable Species.

Some other vegetable plant species that can be damaged as a result of 2,4-D or dicamba drift include potatoes (*Solanum tuberosum* L.), cucumbers (*Cucumis sativus* L.), watermelon (*Citrullus lanatus* Thunb.) and snap beans (*Phaseolus vulgaris* L.). Hemphill and Montgomery (1981) found that cucumbers had reduced yield when 2,4-D was applied at 104 g ha^{-1} or greater. Similar to soybeans, snap beans appear to be more sensitive to dicamba than 2,4-D (Colquhoun et al. 2014). In addition, when dicamba was applied at rates up to 7 g ae ha^{-1} on potatoes, no significant differences in potato yield were recorded but significant visual injury was observed (Colquhoun et al. 2014). Culpepper et al. (2018) found that watermelon transplanted 20 days prior to becoming injured with a $1/75^{th}$ (15 g ha^{-1}) use rate of 2,4-D and dicamba resulted in a yield of 69 kg $plot^{-1}$, which was significantly lower than the non-treated control yield of 94 kg $plot^{-1}$. It was also found that watermelon exhibited greater visual injury to dicamba (24%) than to 2,4-D (20%) (Culpepper et al. 2018).

Annual Flower Species.

Few studies have examined the effects of off-target movement of 2,4-D and dicamba on various annual bedding plants. Hatterman-Valenti and Mayland (2005) applied sub-lethal rates of 2,4-D and dicamba at 0.05, 0.1, and 0.2(dicamba; 28-112 g ha^{-1} and 2,4-D; 78-314 g ha^{-1}) of the labeled rates on various annual flower species including impatiens (*Impatiens wallerana* L.), geraniums (*Pelargonium xhortorum* Bailey), and marigolds (*Tagetes erecta* L.). Impatiens and geraniums were the most tolerant species, with less than 10 and 16% visual injury, respectively, across all rates and herbicides (Hatterman-Valenti and Mayland 2005). Hatterman-Valenti et al. (1995) also found that impatiens and geraniums were fairly tolerant to triclopyr and 2,4-D. However, marigolds exhibited 11 and 21% injury in response to dicamba and 2,4-D treatments, respectively (Hatterman-Valenti and Mayland 2005). In addition to visual injury, reduction in flowering was also observed for these annual flower species, but typically at the highest rates only. Most annual flower species were not found to be exceedingly sensitive to growth regulator herbicides (Hatterman-Valenti et al. 1995; Hatterman-Valenti and Mayland 2005).

Summary and Objectives

The adoption of 2,4-D and dicamba-resistant cotton and soybean will most likely lead to increased applications of 2,4-D and dicamba in U.S. agriculture and in turn, to increased incidences of off-target movement of 2,4-D and dicamba. A wide range of broadleaf plants can experience significant injury symptoms, and in some cases, yield loss from driftable fractions of 2,4-D and dicamba. The sensitivity of species such as soybean, cotton, grapes, and certain vegetable crops to driftable fractions of 2,4-D and dicamba has been extensively studied in previous research. However, limited research has been conducted to determine the differences in sensitivity to 2,4-D and dicamba on other species such as fruit, nut, and ornamental trees, and common annual flower and ornamental species. Therefore, the objectives of this first component of this research are to: 1) determine the sensitivity of selected herbaceous and woody species to driftable fractions of 2,4-D and dicamba and 2) document the differences in symptomology between 2,4-D and dicamba across selected species.

Literature Cited

Anonymous. (2017a) Engenia® herbicide product label. EPA Reg. No.7969-345. Research Triangle Park, NC: BASF Corporation. Pp. 5-8.

Anonymous. (2017b) Enlist One with Colex-D Technology® herbicide product label. EPA Reg. No.62719-695. Indianapolis, IN: Dow AgroSciences LLC. Pp. 6.

Al-Khatib K, Parker R, Fuerst EP (1992) Sweet cherry (*Prunus avium*) response to simulated drift from selected herbicides. Weed Technol 6:975-979

Al-Khatib K, Parker R, Fuerst EP (1993) Wine grape (*Vitis vinifera L.)* response to simulated herbicide drift. Weed Technol 7:97-102

Al-Khatib K, Peterson D (1999) Soybean (*Glycine max*) response to simulated drift from selected sulfonylurea herbicides, dicamba, glyphosate, and glufosinate. Weed Technol 13:264-270

Alves GS, Kruger GR, da Cunha JPA, de Santana DG, Pinto LAT, Guimarães F, Zaric M (2017) Dicamba spray drift as influenced by wind speed and nozzle type. Weed Technol 31:724-731

Andersen SM, Clay S, Wrage L, Matthees D (2004) Soybean foliage residues of dicamba and 2, 4-D and correlation to application rates and yield. Agron J 96:750-760

Auch D, Arnold W (1978) Dicamba use and injury on soybeans (*Glycine max*) in South Dakota. Weed Sci 26:471-475

Bauerle MJ, Griffin JL, Alford JL, Curry AB, Kenty MM (2015) Field evaluation of auxin herbicide volatility using cotton and tomato as bioassay crops. Weed Technol 29:185-197

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 (1979) Dicamba volatility. Weed Sci 27:486-493

Bhatti MA, Al-Khatib K, Parker R (1996) Wine grape (*Vitis vinifera*) response to repeated exposure of selected sulfonylurea herbicides and 2, 4-D. Weed Technol 10:951-956

Burnside O, Lavy T (1966) Dissipation of dicamba. Weeds 14:211-214

Bradley K (2017). A final report on dicamba-injured soybean acres. Integrated Pest Management. University of Missouri. Available at: https://ipm.missouri.edu/IPCM/2017/10/final_report_dicamba_injured_soybean/ Accessed: February 9, 2017.

Colquhoun JB, Heider DJ, Rittmeyer RA (2014) Relationship between visual injury from synthetic auxin and glyphosate herbicides and snap bean and potato yield. Weed Technol 28:671-678

Culpepper AS, Grey TL, Vencill WK, Kichler JM, Webster TM, Brown SM, York AC, Davis JW, Hanna WW (2006) Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) confirmed in Georgia. Weed Sci 54:620-626

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

Derksen, D. A. (1989). Dicamba, chlorsulfuron, and clopyralid as sprayer contaminants on sunflower (*Helianthus annuus*), mustard (*Brassica juncea*), and lentil (*Lens culinaris*), respectively. Weed Sci, 37(4), 616-621.

Dill GM (2005) Glyphosate-resistant crops: history, status and future. Pest Manag Sci: formerly Pesticide Science 61:219-224

Duke SO (2018) The history and current status of glyphosate. Pest Manag Sci 74:1027-1034

Egan JF, Barlow KM, Mortensen DA (2014) A meta-analysis on the effects of 2, 4-D and dicamba drift on soybean and cotton. Weed Sci 62:193-206

Egan JF, Mortensen DA (2012) Quantifying vapor drift of dicamba herbicides applied to soybean. Environ Toxicol and Chem 31:1023-1031

Everitt JD, Keeling JW (2009) Cotton growth and yield response to simulated 2, 4-D and dicamba drift. Weed Technol 23:503-506

Fagliari JR, Oliveira Jr. RSd, Constantin J (2005) Impact of sublethal doses of 2, 4-D, simulating drift, on tomato yield. J of Environ Sci and Heal B 40:201-206

Franz J. E., M. K. Mao, and J. A. Sikorski. 1997. Glyphosate: A Unique Global Herbicide. American Chemical Society. Washington D.C.

Gilreath JP, Chase CA, Locascio SJ (2001a) Crop injury from sublethal rates of herbicide. I. Tomato. HortScience 36:669-673

Gilreath JP, Chase CA, Locascio SJ (2001b) Crop injury from sublethal rates of herbicide. III. Pepper. HortScience 36:677-681

Griffin JL, Bauerle MJ, Stephenson III DO, Miller DK, Boudreaux JM (2013) Soybean response to dicamba applied at vegetative and reproductive growth stages. Weed Technol 27:696-703

Hatterman-Valenti H, Christians NE, Owen MD (1995) Effect of 2, 4-D and triclopyr on annual bedding plants. J of Environ Hort 13:122-125

Hatterman-Valenti H, Mayland P (2005) Annual flower injury from sublethal rates of dicamba, 2, 4-D, and premixed 2, 4-D+ mecoprop+ dicamba. HortScience 40:680-684

Hemphill DD, Montgomery ML (1981) Response of vegetable crops to sublethal application of 2, 4-D. Weed Sci 29:632-635

Holterman H, Van De Zande J, Porskamp H, Huijsmans J (1997) Modelling spray drift from boom sprayers. Comput and Electron in Agric 19:1-22

Johnson AV, Fisher RL, Jordan LD, Edmisten EK, Stewart MA, York CA (2012) Cotton, peanut, and soybean response to sublethal rates of dicamba, glufosinate, and 2, 4-D. Weed Technol 26:195-206

Johnson B, Young B, Matthews J, Marquardt P, Slack C, Bradley K, York A, Culpepper S, Hager A, Al-Khatib K (2010) Weed control in dicamba-resistant soybeans. Crop Manag 9:00-00

Kelley KB, Wax LM, Hager AG, Riechers DE (2005) Soybean response to plant growth regulator herbicides is affected by other postemergence herbicides. Weed Sci 53:101-112

Kniss A (2018) An updated meta-analysis of soybean response to dicamba. Weed Sci Soc of Amer Abstract 29. Weed Sci Soc of Amer (Jan. 2018)

Kruger GR, Davis VM, Weller SC, Johnson WG (2010) Growth and seed production of horseweed (*Conyza canadensis*) populations after exposure to postemergence 2, 4-D. Weed Sci 58:413-419

Kruger GR, Johnson WG, Doohan DJ, Weller SC (2012) Dose response of glyphosate and dicamba on tomato (*Lycopersicon esculentum*) injury. Weed Technol 26:256-260

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)

Legleiter TR, Bradley KW (2008) Glyphosate and multiple herbicide resistance in common waterhemp (*Amaranthus rudis*) populations from Missouri. Weed Sci 56:582-587

Marple ME, Al-Khatib K, Shoup D, Peterson DE, Claassen M (2007) Cotton response to simulated drift of seven hormonal-type herbicides. Weed Technol 21:987-992

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

Mohseni-Moghadam M, Wolfe S, Dami I, Doohan D (2015) Response of wine grape cultivars to simulated drift rates of 2, 4-D, dicamba, and glyphosate, and 2, 4-D or dicamba plus glyphosate. Weed Technol 30:807-814

Mortensen DA, Egan JF, Maxwell BD, Ryan MR, Smith RG (2012) Navigating a critical juncture for sustainable weed management. BioScience 62:75-84

Mueller TC, Wright DR, Remund KM (2013) Effect of formulation and application time of day on detecting dicamba in the air under field conditions. Weed Sci 61:586-593

Nordby A, Skuterud R (1974) The effects of boom height, working pressure and wind speed on spray drift. Weed Res 14:385-395

Norsworthy JK, Jha P, Steckel LE, Scott RC (2010) Confirmation and control of glyphosate-resistant giant ragweed (Ambrosia trifida) in Tennessee. Weed Technol 24:64-70

Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR (2012) Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Sci 60:31-62

Ogg AG, Ahmedullah MA, Wright GM (1991) Influence of repeated applications of 2, 4-D on yield and juice quality of concord grapes (*Vitis labruscana*). Weed Sci 39:284-295

Otta J (1974) Effects of 2, 4-D herbicide on Siberian elm. Forest Sci 20:287-290

Peterson GE (1967) The discovery and development of 2, 4-D. Agricultural History 41:243-254

Peterson MA, McMaster SA, Riechers DE, Skelton J, Stahlman PW (2016) 2, 4-D past, present, and future: a review. Weed Technol 30:303-345

Robinson AP, Simpson DM, Johnson WG (2012) Summer annual weed control with 2, 4-D and glyphosate. Weed Technol 26:657-660

Robinson AP, Simpson DM, Johnson WG (2013) Response of glyphosate-tolerant soybean yield components to dicamba exposure. Weed Sci 61:526-536

Ross MA, Lembi CA (2009) Applied weed science: Including the ecology and management of invasive plants (3rd ed.). Upper Saddle River, NJ: Pearson Prentice Hall.

Samtani JB, Masiunas JB, Appleby JE (2008) Injury on white oak seedlings from herbicide exposure simulating drift. HortScience 43:2076-2080

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

Shergill LS, Bish MD, Biggs ME, Bradley KW (2017) Monitoring the changes in weed populations in a continuous glyphosate-and dicamba-resistant soybean system: A five-year field-scale investigation. Weed Technol:1-8

Solomon CB, Bradley KW (2014) Influence of application timings and sublethal rates of synthetic auxin herbicides on soybean. Weed Technol 28:454-464

Sosnoskie LM, Culpepper AS, Braxton LB, Richburg JS (2015) Evaluating the volatility of three formulations of 2, 4-D when applied in the field. Weed Technol 29:177-184

Spaunhorst DJ, Siefert-Higgins S, Bradley KW (2014) Glyphosate-resistant giant ragweed (Ambrosia trifida) and waterhemp (Amaranthus rudis) management in dicamba-resistant soybean (*Glycine max*). Weed Technol 28:131-141

[USDA-APHIS] US Department of Agriculture-Animal and Plant Health Inspection Service. (2014) Determination of nonregulated status for Dow AgroSciences DAS-68416-4 soybean. Washington, DC: US Department of Agriculture. https://www. aphis.usda.gov/brs/aphisdocs/11_23401p_det.pdf Accessed February 7, 2018

[USDA-APHIS] US Department of Agriculture-Animal and Plant Health Inspection Service. (2015) Determination of nonregulated status for Monsanto Company MON 88708 soybean. Washington, DC: US Department of Agriculture. https://www.aphis.usda.gov/brs/aphisdocs/10_18801p_det.pdf. Accessed February 7, 2018

[USDA-NASS] United States Department of Agriculture-National Agriculture Statistics Service. (2014) 2012 Census of agriculture. Washington, DC: US Department of Agriculture. https://www.agcensus.usda.gov/Publications/2012/Full_Report/Volume_1,_Chapter_1_US/usv1.pdf. Accessed March 5, 2018.

[US EPA] United States Environmental Protection Agency. (2014) Final registration of Enlist Duo herbicide. Washington, DC: US Environmental Protection Agency. https://www.epa.gov/sites/production/files/2014-10/documents/final_registration_-_enlist_duo.pdf. Accessed February 14, 2018

[US EPA] United States Environmental Protection Agency. (2016) Final registration of dicamba on dicamba-tolerant cotton and soybean. Washington, DC: US Environmental Protection Agency. https://www.regulations.gov/document?D=EPA-HQ-OPP-2016-0187-0959 . Accessed February 20, 2018

VanGessel MJ (2001) Glyphosate-resistant horseweed from Delaware. Weed Sci 49:703-705

Wang M, Rautmann D (2008) A simple probabilistic estimation of spray drift—factors determining spray drift and development of a model. Environ Toxicol and Chem 27:2617-2626

Wax L, Knuth L, Slife F (1969) Response of soybeans to 2, 4-D, dicamba, and picloram. Weed Sci 17:388-393

Wilson JS, Worsham AD (1988) Combinations of nonselective herbicides for difficult to control weeds in no-till corn, *Zea mays*, and soybeans, *Glycine max*. Weed Sci 36:648-652

Wolf TM, Grover R, Wallace K, Shewchuk SR, Maybank J (1993) Effect of protective shields on drift and deposition characteristics of field sprayers. Can J of Plant Sci 73:1261-1273

Wright TR, Shan G, Walsh TA, Lira JM, Cui C, Song P, Zhuang M, Arnold NL, Lin G, Yau K (2010) Robust crop resistance to broadleaf and grass herbicides provided by aryloxyalkanoate dioxygenase transgenes. Proceedings of the National Academy of Sciences 107:20240-20245

CHAPTER II

RESPONSE OF COMMON BEDDING PLANTS TO DRIFTABLE FRACTIONS OF 2,4-D AND DICAMBA

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Abstract

Herbicide resistant weed species have limited the ability to control weeds in crops such as soybeans and cotton. However, recent development and commercialization of 2,4-D and dicamba-resistant soybean and cotton will give producers another mode of action to control problematic weed species. As a result of the adoption of these new traits, off-target movement of 2,4-D and dicamba is a major concern, especially for neighbors with sensitive broadleaf annual bedding plant species. An experiment was conducted in 2017 and 2018 to determine the sensitivity of common bedding plant species to driftable fractions of 2,4-D and dicamba with or without glyphosate. Driftable fractions corresponding to 1/10, 1/100 and 1/300th of the full labeled rate (1X) of 2,4-D (1.09 kg ae ha^{-1}), 2,4-D plus glyphosate (1.09 kg ae ha^{-1} plus 1.10 kg ae ha^{-1}), dicamba (0.56 kg ae ha^{-1}) and dicamba plus glyphosate (0.56 kg ae ha^{-1} plus 1.10 kg ae ha^{-1}) were applied to begonia, coleus, geranium, impatiens, marigold, petunia, vinca, and zinnia. Visual injury, plant height, the number of flowers, and plant biomass were recorded at specific time intervals following treatment. Overall, the general order of herbicide-induced visible injury symptoms on the bedding plant species evaluated in this research was coleus > geranium = zinnia = petunia > begonia = marigold = impatiens = vinca. When averaged across all flower species, the 1/10X rate of 2,4-D plus glyphosate resulted in 51% visible

injury 28 days after treatment (DAT) while the 1/10X rate of dicamba plus glyphosate resulted in 43% injury. However, when 2,4-D and dicamba were applied alone at the 1/10X rate, average injury across all species was reduced to 20 and 13%, respectively at 28 DAT. Treatments causing the greatest visual injury also resulted in the greatest reduction of biomass, height and flower production. Marigold and geranium had greater sensitivity to treatments containing 2,4-D compared to dicamba, but coleus and zinnia had greater sensitivity to treatments containing dicamba. Petunia exhibited a high tolerance to 2,4-D or dicamba applied alone. The 1/100 and 1/300X rates that are more likely to equate to driftable fractions in field settings resulted in less than 30% visual injury across all flower species except coleus. However, since these bedding plant species are short-lived and have a high monetary and aesthetic value, it is unlikely that even low levels of injury would be tolerated by homeowners or commercial retailers.

Introduction

The prevalence of herbicide-resistant weeds in U.S. corn, soybean, and cotton production systems has left farmers with a limited number of weed control options (Norsworthy et al. 2012). To combat herbicide-resistant weeds, agrochemical companies have developed soybean (*Glycine max* (L.) Merr.) and cotton (*Gossypium hirsutum* L.) that are resistant to 2,4-D and dicamba (Behrens et al. 2007; Wright et al. 2010). Recent deregulation of dicamba and 2,4-D tolerant crop cultivars in the U.S. allows farmers to utilize these technologies to control herbicide-resistant weeds (USDA-APHIS 2014; USDA-APHIS 2015). Previous research has shown that dicamba and 2,4-D are effective on waterhemp (*Amaranthus tuberculatus* (Moq.) J. D. Sauer), horseweed (*Conyza canadensis* (L.) Cronq.), Palmer amaranth (*Amaranthus palmeri* S. Wats.), and giant ragweed (*Ambrosia trifida* L.), which are some of the most problematic weed species encountered in U.S. crop production systems (Johnson et al. 2010; Kruger et al. 2010; Robinson et al. 2012; Craigmyle et al. 2013; Spaunhorst et al. 2014; Shergill et al. 2017; Van Wychen 2017). It is likely that the adoption of 2,4-D and dicamba resistant crops will result in an increased number of applications of these herbicides in the near future which could, in turn, result in increased off-target movement of these herbicides to neighboring plant species.

The ability of herbicides like 2,4-D and dicamba to move off target and cause damage to nearby sensitive plants can be influenced by a variety of factors. Wind speed, nozzle type, boom height, and herbicide formulation have all been shown to influence off-target movement (Nordby and Skuterud 1974; Holterman et al. 1997; Wang and Rautmann 2008; Egan and Mortensen 2012; Sosnoskie et al. 2015; Alves et al. 2017). Several studies have shown that higher wind speeds can result in greater off-target movement (Nordby and Skuterud 1974; Wolf et al. 1993; Wang and Rautmann 2008; Alves et al. 2017). For example, Alves et al. (2017) found that when wind speeds were increased from one to five meters per second, greater downwind detection of dicamba occurred (Alves et al. 2017). Wolf et al. (1993) reported that wind speeds of 9 to 30 kilometers per hour resulted in 2,4-D drift amounting to 1.8 to 16% of the full labeled herbicide rate. New formulations of 2,4-D choline and dicamba address maximum wind speed within their label, restricting applications to wind speeds less than 16 kilometers per hour (Anonymous 2017a; Anonymous 2017b).

Herbicides such as 2,4-D and dicamba are also susceptible to off-target movement through secondary drift, which includes volatilization. While several factors can influence volatility such as temperature or relative humidity (Behrens and Lueschen 1979; Egan and Mortensen 2012; Sosnoskie et al. 2015), two of the most important factors that influence volatility are the vapor pressure and formulation of a herbicide. Herbicides such as 2,4-D and dicamba are susceptible to volatilization due to the relatively high vapor pressure of these herbicides (Shaner 2014).

Few studies have examined the effects of off-target movement of 2,4-D and dicamba with or without glyphosate on various annual flower or bedding plants. Hatterman-Valenti and Mayland (2005) applied sub-lethal rates of 2,4-D, dicamba, and 2,4-D + dicamba + mecoprop at 5, 10, and 20% of the labeled rates on various annual flower species including impatiens (*Impatiens wallerana* L.), geraniums (*Pelargonium xhortorum* Bailey), and marigolds (*Tagetes erecta* L.) during early flowering stages. Impatiens and geraniums were some of the most tolerant species tested, with less than 10 and 16% visual injury, respectively, across all rates and herbicides (Hatterman-Valenti and Mayland 2005). These authors also found that sub-lethal rates of dicamba caused an increase in flowering on impatiens, while sub-lethal rates of 2,4-D did not. However, some annual flower species tested were more sensitive to sublethal rates of 2,4-D and dicamba including ageratum (*Ageratum houstonianum* Mill.) and alyssum (*Lobularia maritima* Desv.) (Hatterman-Valenti and Mayland 2005). Another study conducted by Hatterman-Valenti et al. (1995) found that impatiens and geraniums were fairly tolerant to driftable fractions of triclopyr and 2,4-D but that marigold, petunia (*Petunia multiflora*), and begonia (*Begonia x semperflorens-cultorum* Hort.) were more sensitive to these herbicide treatments (Hatterman-Valenti et al. 1995). Reduced flowering was also observed with increasing rates of 2,4-D and triclopyr on petunia and marigold, but not on impatiens (Hatterman-Valenti et al. 1995). Most annual flower species were fairly tolerant of sub-lethal rates of growth regulator herbicides (Hatterman-Valenti et al. 1995; Hatterman-Valenti and Mayland 2005). The objective of this research was to determine the sensitivity of common bedding plant species to driftable fractions of 2,4-D and dicamba with and without glyphosate.

Materials and Methods

Eight common bedding plant species; impatiens (*Impatiens walleriana* var. Dazzler), geranium (*Pelargonium x hortorum* var. Pinto), petunia (*Petunia multiflora* var. Hurrah), marigold (*Tagetes patula* var. Bonanza), begonia (*Begonia x semperflorens-cultorum* var. Prelude), vinca (*Catharanthus roseus* var. Titan), zinnia (*Zinnia marylandica* var. Double Zahara), and coleus (*Solenostemon scutellarioides* var. Wizard); were seeded in 7.6 cm pots containing a commercial potting medium (Premier Tech Horticulture, Quakertown, PA) and maintained daily in a greenhouse in the spring of 2017 and 2018. On May 23, 2017 and May 29, 2018, all species were removed from the greenhouse and treated with 1/10, 1/100 and 1/300th of the manufacture's full labeled rate (1X rate) of 2,4-D choline (Enlist One, Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268), 2,4-D choline plus glyphosate (Enlist Duo, Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268), dicamba diglycolamine (DGA) salt (Xtendimax with VaporGrip, Monsanto Company, 800 North Lindbergh Blvd, St. Louis, Missouri 63167), and dicamba DGA plus glyphosate (Xtendimax with VaporGrip plus Roundup Powermax, Monsanto Company, 800 North Lindbergh Blvd, St. Louis, Missouri 63167). All species except coleus were approximately 10 cm in height with 3-4 flowers present. Coleus plants were also 10 cm in height but did not have flowers present at the time of treatment. Full labeled rates that were used to calculate the fraction rates for 2,4-D, 2,4-D plus glyphosate, dicamba and dicamba plus glyphosate were 1.09 kg ae ha^{-1}, 1.09 kg ae ha^{-1} plus 1.10 kg ae ha^{-1}, 0.56 kg ae ha^{-1}, and 0.56 kg ae ha^{-1} plus 1.10 kg ae ha^{-1}, respectively. A non-treated control of each flower species was also included for comparison. New bedding plant species were obtained in 2017 and 2018. All treatments were applied with a CO_2-pressurized backpack sprayer equipped with 8002 XR nozzles (TeeJet®, Spraying Systems Co., PO Box 7900, Wheaton, IL 60187) at 140 L ha^{-1} and 131 kPa. XR nozzles were used to simulate an off-target movement occurrence of these herbicides.

One day following treatment, all flower species were transplanted into raised beds (0.3 m tall and 0.9 m wide) at the University of Missouri Bradford Research Center (38.8929°N, 92.2010°W) with a 1.25 ml white on black plastic mulch layer (FilmTech Corp. 2121 31st Street SW, Allentown, PA 18103) at row spacing's of 15 and 23 cm in 2017 and 2018, respectively. Irrigation was provided via 10 ml sub drip irrigation tape with 10 cm spacing that emitted water at a rate of 3.79 liters per minute (Chapin, Jain Irrigation Inc. 740 Water Street, Watertown, NY 13601) on a biweekly basis. The soil at this location was a Mexico silt loam (fine, smectic, mesic Aeric Vertic Epiaqualfs) with 2.4% organic matter and a pH of 6. The experimental design was a randomized complete block design in a split-plot arrangement with five single-plant replications. Individual species were whole plots while subplots consisted of herbicide treatments.

All common bedding plant species were evaluated for visual injury on a scale from 0 to 100%, where 0 was equal to no injury and 100 was equivalent to complete plant death. Visual injury assessments were taken 28 and 56 days after treatment (DAT) and included an overall evaluation of chlorosis and necrosis of plant tissue as well as leaf cupping, strapping, and overall plant epinasty as a result of herbicide treatments. Plant height measurements were evaluated 28 and 56 DAT by measuring individual plant heights from the soil surface to the top of living plant tissue. Flower production was assessed by counting the number of open, developed flowers per plant 28 DAT. Due to the overall lack of flowering of coleus in these experiments, flower production for this species was not included in the analysis. Additionally, above-ground biomass samples were harvested 56 DAT by clipping at the soil surface, drying in a forced air oven at 49° C for 36 hours, and then weighing. Since each species is distinctly different from one another, height and biomass, expressed as percent of the non-treated, and flower production, expressed as number of open flowers per plant, were analyzed by species. All data were analyzed in SAS (SAS 9.4, SAS® Institute Inc. Cary, NC) using the PROC GLIMMIX procedure. Means were separated using Fishers Protected Least Significant Difference (LSD) at $P \leq 0.05$. Herbicide, rate, and flower species were considered fixed effects, while year and replication were considered to be random effects. Years were classified as random effects so that conclusions about species or treatments can be made over a wide range of environments (Carmer et al. 1989; Blouin et al. 2011).

Results and Discussion

Visual injury 28 DAT. Visual injury symptoms from 2,4-D or dicamba with or without glyphosate included leaf cupping, strapping, epinasty of stems and petioles, callusing and swelling of stem tissue and flowers, stem cracking, necrosis, chlorosis, and plant death. Such symptoms are consistent with those reported by Hatterman-Valenti and Mayland (2005) on various annual flower species. There was a rate by herbicide by plant species interaction ($P<0.0001$) for visual injury 28 DAT. When applied at the 1/10X rate, 2,4-D plus glyphosate resulted in 60 to 78% visual injury on begonia, coleus, geranium, and petunia while dicamba plus glyphosate resulted in 74 and 79% visual injury on petunia and coleus, respectively (Table 2.1). When averaged across all plant species, glyphosate in combination with 2,4-D at the 1/10X rate resulted in greater visual injury (51%) than dicamba plus glyphosate at the 1/10X rate (43%, data not shown). Greater visual injury occurred in response to the 1/10X rate of 2,4-D plus glyphosate compared to the 1/10X rate of 2,4-D alone in all species except impatiens and marigold. Similarly, greater visual injury was observed in response to dicamba plus glyphosate at the 1/10X rate compared to dicamba alone at the 1/10X rate in all species except begonia (Table 2.1). With the exceptions noted above, these results indicate that treatments containing glyphosate in combination with dicamba or 2,4-D at the 1/10X rates result in greater injury than dicamba or 2,4-D alone. Similar results from Mohseni-Moghadam et al. (2015) indicate that combinations of 2,4-D or dicamba with glyphosate caused greater visual injury on grape (*Vitis vinifera*) compared to either of these herbicides alone. Hatterman-Valenti and Mayland (2005) also found that a combination of 2,4-D plus dicamba plus mecoprop resulted in 17% visual injury to salvia while dicamba and 2,4-D applied alone resulted in

7 and 10% injury, respectively. Synergistic effects of 2,4-D plus glyphosate and dicamba plus glyphosate on weed control have also been observed (Flint and Barrett, 1989; Johnson et al. 2010; Craigmyle et al. 2013).

The 1/100X rate of dicamba plus glyphosate resulted in 1 to 39% injury of the eight species evaluated in these experiments, while the 1/100X rate of 2,4-D plus glyphosate resulted only 0 to 7% injury (Table 2.1). On average, dicamba combined with glyphosate caused greater visual injury in comparison to 2,4-D plus glyphosate at the 1/100X rates across all flower species. Species exhibited less than 4% visual injury symptoms in response to the 1/300X rate of all treatments (Table 2.1).

Based on the 1/10X rates, geranium and marigold had a greater sensitivity to 2,4-D than dicamba. Hatterman-Valenti and Mayland (2005) also found marigold to be more sensitive to 2,4-D than dicamba. Coleus and petunia exhibited greater sensitivity to 1/100X treatments of dicamba plus glyphosate compared to 2,4-D in combination with glyphosate. On average, coleus was the most sensitive species to treatments of 2,4-D and dicamba with or without glyphosate while vinca, impatiens, marigold, and begonia were the least sensitive to these herbicides (data not shown). Hatterman-Valenti et al (1995) also found that impatiens and vinca had a low susceptibility to reduced rates of various synthetic auxin herbicide treatments.

Visual injury 56 DAT. Visual injury symptoms for most species declined by 56 DAT for all herbicide treatments, however there was a rate by herbicide by species interaction ($P<0.0001$). Visual injury of species to 1/300X rates of all treatments ranged from 0 to

3% (Table 2.2). However, Hatterman-Valenti et al. (1995) reported that 2,4-D applied at 1 g ae ha^{-1} (approximately 1/1,000X) resulted in injury ranging from 10 to 18% on marigold, petunia, begonia, impatiens, geranium, and vinca eight weeks after treatment. Results from Hatterman-Valenti et al. (1995) may have differed from those found here due to the differences in herbicide formulations and the growth stage of the species in the studies. Less than 2% visual injury was observed in response to 1/100X treatments of 2,4-D and 2,4-D plus glyphosate. However, 0 to 32% visual injury was observed with 1/100X treatments of dicamba and dicamba plus glyphosate across all flower species (Table 2.2). Similar to the responses observed 28 DAT, 1/100X treatments of dicamba plus glyphosate resulted in greater injury than 2,4-D plus glyphosate. By 56 DAT, several species had recovered from the initial injury that occurred 28 DAT. Vinca exhibited 24 and 25% visual injury 28 DAT in response to 1/10X treatments of 2,4-D plus glyphosate and dicamba plus glyphosate, respectively. However, by 56 DAT vinca injury had declined to 8% for both treatments (Table 2.1 and 2.2). Similarly, dicamba and 2,4-D applied to zinnia at the 1/10X rate resulted in 21 and 26% visual injury 28 DAT, but by 56 DAT only 6 and 5% visual injury was observed, respectively (Table 2.1 and 2.2). Other annual flower species like begonia, coleus, geranium, marigold, and zinnia showed little signs of recovery and similar levels of injury from 28 to 56 DAT, especially in response to 1/10X treatments of 2, 4-D plus glyphosate and dicamba plus glyphosate. (Table 2.1 and 2.2). Lastly, petunia injury actually increased by 26 and 12% from 28 to 56 DAT in response to 1/10X treatments of 2,4-D plus glyphosate and dicamba plus glyphosate, respectively. This in large part was due to the increase in tissue death and overall necrosis. Another study found that rose (*Rosa dilecta*) expressed similar levels of injury from 30 to 60 DAT with applications of 1/10 and 1/3X rates of 2,4-D plus glyphosate (Al-Khatib et al 1992).

Plant height. Glyphosate in combination with 2,4-D applied at the 1/10X rate resulted in reduced plant heights compared to the non-treated control for all species except impatiens. Dicamba plus glyphosate applied at the 1/10X rate also reduced plant heights of all species except begonia (Table 2.3). Overall, 2,4-D or dicamba in combination with glyphosate at the 1/10X rate causing the greatest reductions in plant height, and these same treatments also caused the greatest visual injury across all species 28 DAT (Table 2.1). Begonia, geranium, and marigold were the only species to have reductions in plant heights as a result of 1/10X treatments of 2,4-D, while zinnia was the only species to express reduced plant height as a result of dicamba applied alone at the 1/10X rate. Marigold and petunia were the only species that had reductions in plant height as a result of 1/100X rates of 2,4-D plus glyphosate or dicamba plus glyphosate.

When plants heights were recorded again 56 DAT, only the 1/10X rate of 2,4-D plus glyphosate resulted in significant reductions in plant height, ranging from 27 to 83% height of the non-treated. Dicamba combined with glyphosate at the 1/10X rate caused reduced plant height in all species except begonia and impatiens while dicamba alone at the 1/10X rate only reduced plant height in coleus. When 2,4-D was applied at the 1/10X rate only coleus, geranium, and marigold resulted in reduced plant heights ranging from 68 to 80% of the non-treated controls (Table 2.4). Similar results were observed with geranium and marigold 28 DAT, indicating that these species may have a greater sensitivity towards 2,4-D. Except for coleus, no other treatments applied at the 1/100X rate resulted in reduced plant height to species (Table 2.4). Coleus height was 48 and 76% of the non-treated control in response to 1/100X treatments of dicamba plus glyphosate and dicamba alone, respectively. Visual injury assessments 56 DAT also show that coleus was injured most by treatments of dicamba or dicamba plus glyphosate at the 1/100X rates (Table 2.2). These results demonstrate the sensitivity of coleus to 1/100 and 1/10X rates of dicamba or dicamba plus glyphosate. The 1/300X rates of all herbicide treatments did not result in reduced or increased plant heights 28 or 56 DAT. Previous research by Solomon and Bradley (2014) showed that soybean plant heights were generally correlated to visual injury assessments after treatment with a variety of synthetic auxin herbicides. Similar results occurred in these experiments when the heights of bedding species were measured 28 and 56 DAT.

Flower production. In this study, as herbicide rates increased, flower production generally decreased. Hatterman-Valenti et al. (1995) observed a similar trend on impatiens, geranium, vinca, and salvia. The number of flowers per plant was reduced by all 2,4-D plus glyphosate and dicamba plus glyphosate treatments at the 1/10X rate for all species (Table 2.5). Flower production was reduced in several species in response to 1/10X 2,4-D or dicamba alone but not for petunia which exhibited a high tolerance to 2,4-D or dicamba applied alone.

Across all annual flower species, none of the 1/100X rates of 2,4-D with or without glyphosate caused reduced flower production. However, either dicamba or dicamba plus glyphosate at 1/100X rates reduced flower production on all species except for begonia, impatiens, and marigold (Table 2.5). Zinnia was the only species to have lower flower production in response to the 1/100X rate of dicamba therefore, zinnia was considered to be more sensitive to flower loss from treatments containing dicamba than 2,4-D. These results also indicate that flower loss is more likely to occur with 1/100X treatments of dicamba plus glyphosate in comparison to 2,4-D plus glyphosate.

There was not a loss in flower production in response to 1/300X rates of herbicide treatments for any of the species tested (Table 2.5). While some low dose herbicide treatments resulted in greater flower counts, they were not different from the control. Hatterman-Valenti and Mayland (2005) observed increases in flower production for impatiens, salvia, and snapdragons and hypothesized that the increase in flowering was a result of secondary flower stem growth (Hatterman-Valenti and Mayland 2005). However, Hatterman-Valenti and Mayland (2005) found that sublethal rates of 2,4-D and dicamba reduced flowering of ageratum and alyssum more than in dahlia, geranium, impatiens, marigold, salvia, and snapdragon. Generally speaking, species do not appear to be as sensitive to flower loss as other species such as tomatoes. Results from Kruger et al. (2012) indicate that dicamba rates as low as 2.7 g ae ha^{-1} (approximately 1/200X) can cause up to a 10% flower loss in tomatoes.

Biomass. As herbicide rates increased, biomass of coleus, geranium, marigold, petunia, vinca, and zinnia generally decreased (Table 2.6). There were no differences between any of the herbicide treatments and the non-treated control in the biomass of impatiens and begonia, and therefore these species were not included in Table 2.6. Across the six species in Table 2.6, 1/10X treatments of 2,4-D plus glyphosate and dicamba plus glyphosate resulted in biomass ranging from 8 to 53% of the non-treated control. These results are consistent with the previous measurements of visual injury, height, and flower production, and confirm that combinations of 2,4-D plus glyphosate or dicamba plus glyphosate results in the greatest injury to species.

Geranium, marigold, and vinca were the only species to have reduced biomass as a result of 2,4-D applied at the 1/10X rate alone while coleus, vinca, and zinnia were the only flower species to have reduced biomass as a result of applications of 1/10X dicamba. Marigold and geranium did not experience reductions in biomass as a result of 1/10X treatments of dicamba, but biomass was reduced for the equivalent treatments of 2,4-D. A similar trend was observed with plant heights and visual injury 28 and 56 DAT indicating that marigold and geranium are more sensitive to 2,4-D compared to dicamba (Tables 2.1-2.4).

When 2,4-D with or without glyphosate was applied to the species at the 1/100X rate, no loss in biomass occurred. However, dicamba with or without glyphosate at the same rate did cause a loss in biomass in all species tested except geranium and marigold, which was similar to the response observed with flower production (Table 2.5). Of all the species evaluated, coleus experienced the greatest loss in biomass from the dicamba plus glyphosate treatment at the 1/100X rate (Table 2.6). Similar results were observed for coleus plant heights 56 DAT (Table 2.4). Zinnia was the only flower species to have reduced biomass from the 1/100X treatment of dicamba, which also corresponded to the reductions in flowering that were observed 28 DAT.

All herbicides applied at the 1/300X rates did not result in significant loss in biomass to the species evaluated in this research. Additionally, the 1/300X rate of 2,4-D applied to geranium was the only treatment that resulted in greater biomass (139%) than the non-treated control (Table 2.6). These results indicate that 2,4-D at very low concentrations may cause an increase in geranium plant biomass, a phenomenon known as hormesis. Other authors have observed similar effects with low doses of 2,4-D. For example, Hatterman-Valenti et al. (1995) found that 2,4-D applied at 25 g ha^{-1} (approximately 1/50X) resulted in a 4% biomass gain on average for impatiens, marigold, and petunia. Hemphill and Montgomery (1981) also reported that 2.1 g ha^{-1} 2,4 D (approximately 1/500X) resulted in 136% pepper yield of the non-treated. The authors concluded this result was likely due to increased branching and flowering on pepper (Hemphill and Montgomery 1981). Therefore, it seems reasonable that 2,4-D applied at the 1/300X rate caused geranium growth to be stimulated, which caused an increase in biomass compared to the non-treated control.

The results of this research indicate that bedding plant species respond differently from one another to treatments containing 2,4-D or dicamba with or without glyphosate. Coleus was found to be the most sensitive species in this study, while begonia, impatiens, marigold, and vinca had the least sensitivity to the herbicide treatments evaluated. Additionally, marigold and geranium had greater sensitivity to treatments containing 2,4-D compared to dicamba. On the other hand, coleus and zinnia had greater sensitivity to treatments containing dicamba compared to 2,4-D. For begonia, impatiens, petunia, and vinca, there was not a clear trend towards more or less sensitivity towards 2,4-D or dicamba. Petunia expressed the least sensitivity towards all rates of 2,4-D or dicamba applied alone. However, petunia had the greatest levels of injury when glyphosate was combined with 2,4-D or dicamba at the 1/10X rate, suggesting that petunia may be more sensitive to glyphosate than to 2,4-D or dicamba.

When glyphosate was applied with dicamba or 2,4-D at 1/10X rates, visual injury along with reductions in plant height, biomass and flower production were the greatest for all species evaluated in this research. Less injury occurred when 1/10X use rates of 2,4-D or dicamba were applied without glyphosate, and considerably lower levels of injury and growth reduction were observed in response to 1/100 and 1/300X rates of 2,4-D and dicamba, with or without glyphosate. Egan et al. (2014) reported that a driftable fraction of 2,4-D or dicamba corresponding to the 1/10X use rate is a rare off-target movement occurrence but that rates corresponding to 1/100 or 1/300X are more likely to occur in most field settings. In this research, most bedding plant species had low sensitivity to driftable fractions of 2,4-D and dicamba with or without glyphosate. Therefore, these results suggest that, in general, bedding plant species are more tolerant of potential driftable fractions of 2,4-D and dicamba in comparison to other species that have been previously studied such as soybean, snap beans, grapes, cotton, tomato, and watermelon (Al-Khatib and Peterson 1999; Marple et al. 2007; Kruger et al. 2012; Colquhoun et al. 2014; Egan et al. 2014; Solomon and Bradley 2014; Mohseni-Moghadam et al. 2015; Culpepper et al. 2018). However, since these species are short lived and have a high monetary and aesthetic value, it is unlikely that even low levels of injury would be tolerated by homeowners or commercial retailers.

Literature Cited

Anonymous. (2017a) Engenia® herbicide product label. EPA Reg. No.7969-345. Research Triangle Park, NC: BASF Corporation. Pp. 5-8.

Anonymous. (2017b) Enlist One with Colex-D Technology® herbicide product label. EPA Reg. No.62719-695. Indianapolis, IN: Dow AgroSciences LLC. Pp. 6.

Al-Khatib K, Parker R, Fuerst EP (1992) Rose (*Rosa dilecta*) response to simulated herbicide drift. HortTechnol 2:394-398

Al-Khatib K, Peterson D (1999) Soybean (*Glycine max*) response to simulated drift from selected sulfonylurea herbicides, dicamba, glyphosate, and glufosinate. Weed Technol 13:264-270

Alves GS, Kruger GR, da Cunha JPA, de Santana DG, Pinto LAT, Guimarães F, Zaric M (2017) Dicamba spray drift as influenced by wind speed and nozzle type. Weed Technol 31:724-731

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 (1979) Dicamba volatility. Weed Sci 27:486-493

Blouin DC, Webster EP, Bond JA (2011) On the analysis of combined experiments. Weed Technol 25:165-169

Carmer S, Nyquist W, Walker W (1989) Least significant differences for combined analyses of experiments with two-or three-factor treatment designs. Agron J 81:665-672

Colquhoun JB, Heider DJ, Rittmeyer RA (2014) Relationship between visual injury from synthetic auxin and glyphosate herbicides and snap bean and potato yield. Weed Technol 28:671-678

Craigmyle, BD, JM Ellis, KW Bradley (2013) Influence of weed height and glufosinate plus 2,4-D combinations on weed control in soybean with resistance to 2,4-D. Weed Technol 27:271–280

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

Egan JF, Barlow KM, Mortensen DA (2014) A meta-analysis on the effects of 2, 4-D and dicamba drift on soybean and cotton. Weed Sci 62:193-206

Egan JF, Mortensen DA (2012) Quantifying vapor drift of dicamba herbicides applied to soybean. Environ Toxicol and Chem 31:1023-1031

Flint JL, Barrett M (1989) Effects of glyphosate combinations by 2, 4-D or dicamba on field bindweed (*Convolvulus arvensis*). Weed Sci 37:12-18

Hatterman-Valenti H, Christians NE, Owen MD (1995) Effect of 2, 4-D and triclopyr on annual bedding plants. J of Environ Hort 13:122-125

Hatterman-Valenti H, Mayland P (2005) Annual flower injury from sublethal rates of dicamba, 2, 4-D, and premixed 2, 4-D + mecoprop + dicamba. HortScience 40:680-684

Hemphill DD, Montgomery ML (1981) Response of vegetable crops to sublethal application of 2, 4-D. Weed Sci 29:632-635

Holterman H, Van De Zande J, Porskamp H, Huijsmans J (1997) Modelling spray drift from boom sprayers. Comput and Electron in Agric 19:1-22

Johnson B, Young B, Matthews J, Marquardt P, Slack C, Bradley K, York A, Culpepper S, Hager A, Al-Khatib K (2010) Weed control in dicamba-resistant soybeans. Crop Manag 9:00-00

Kruger GR, Davis VM, Weller SC, Johnson WG (2010) Growth and seed production of horseweed (*Conyza canadensis*) populations after exposure to postemergence 2, 4-D. Weed Sci 58:413-419

Kruger GR, Johnson WG, Doohan DJ, Weller SC (2012) Dose response of glyphosate and dicamba on tomato (*Lycopersicon esculentum*) injury. Weed Technol 26:256-260

Marple ME, Al-Khatib K, Shoup D, Peterson DE, Claassen M (2007) Cotton response to simulated drift of seven hormonal-type herbicides. Weed Technol 21:987-992

Mohseni-Moghadam M, Wolfe S, Dami I, Doohan D (2015) Response of wine grape cultivars to simulated drift rates of 2, 4-D, dicamba, and glyphosate, and 2, 4-D or dicamba plus glyphosate. Weed Technol 30:807-814

Nordby A, Skuterud R (1974) The effects of boom height, working pressure and wind speed on spray drift. Weed Res 14:385-395

Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR (2012) Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Sci 60:31-62

Robinson AP, Simpson DM, Johnson WG (2012) Summer annual weed control with 2, 4-D and glyphosate. Weed Technol 26:657-660

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

IL:315

Shergill LS, Bish MD, Biggs ME, Bradley KW (2017) Monitoring the changes in weed populations in a continuous glyphosate-and dicamba-resistant soybean system: A five-year field-scale investigation. Weed Technol:1-8

Solomon CB, Bradley KW (2014) Influence of application timings and sublethal rates of synthetic auxin herbicides on soybean. Weed Technol 28:454-464

Sosnoskie LM, Culpepper AS, Braxton LB, Richburg JS (2015) Evaluating the volatility of three formulations of 2, 4-D when applied in the field. Weed Technol 29:177-184

Spaunhorst DJ, Siefert-Higgins S, Bradley KW (2014) Glyphosate-resistant giant ragweed (Ambrosia trifida) and waterhemp (Amaranthus rudis) management in dicamba-resistant soybean (*Glycine max*). Weed Technol 28:131-141

[USDA-APHIS] US Department of Agriculture-Animal and Plant Health Inspection Service. (2014) Determination of nonregulated status for Dow AgroSciences DAS-68416-4 soybean. Washington, DC: US Department of Agriculture. https://www. aphis.usda.gov/brs/aphisdocs/11_23401p_det.pdf Accessed February 7, 2018

[USDA-APHIS] US Department of Agriculture-Animal and Plant Health Inspection Service. (2015) Determination of nonregulated status for Monsanto Company MON 88708 soybean. Washington, DC: US Department of Agriculture.

https://www.aphis.usda.gov/brs/aphisdocs/10_18801p_det.pdf. Accessed February 7, 2018

Van Wychen L (2017) WSSA survey ranks most common and most troublesome weeds in broadleaf crops, fruits and vegetables. http://wssa.net/2017/05/wssa-survey-ranks-most-common-and-most-troublesome-weeds-in-broadleaf-crops-fruits-and-vegetables/ Accessed Feburary 5, 2019

Wang M, Rautmann D (2008) A simple probabilistic estimation of spray drift—factors determining spray drift and development of a model. Environ Toxicol and Chem 27:2617-2626

Wolf TM, Grover R, Wallace K, Shewchuk SR, Maybank J (1993) Effect of protective shields on drift and deposition characteristics of field sprayers. Can J of Plant Sci 73:1261-1273

Wright TR, Shan G, Walsh TA, Lira JM, Cui C, Song P, Zhuang M, Arnold NL, Lin G, Yau K (2010) Robust crop resistance to broadleaf and grass herbicides provided by aryloxyalkanoate dioxygenase transgenes. Proceedings of the National Academy of Sciences 107:20240-20245

Table 2.1. Visible injury of bedding plant species in response to driftable fractions of 2,4-D and dicamba 28 days after treatment.

Treatment	Fraction of 1X Use Rate	Bedding Plant Species							
		Begonia	Coleus	Geranium	Impatiens	Marigold	Petunia	Vinca	Zinnia
		---Visual Injury (%)---							
2,4-D	1/300	1	0	0	0	0	0	1	0
2,4-D	1/100	1	2	1	1	3	0	0	5
2,4-D	1/10	18	26	42	15	28	2	5	26
2,4-D + glyphosate	1/300	0	1	0	0	0	0	0	2
2,4-D + glyphosate	1/100	1	7	6	3	7	0	4	7
2,4-D + glyphosate	1/10	60	78	65	28	39	65	24	51
Dicamba	1/300	0	2	1	0	0	0	0	0
Dicamba	1/100	3	11	2	3	1	0	2	8
Dicamba	1/10	13	27	13	17	9	5	4	21
Dicamba + glyphosate	1/300	1	3	0	1	1	0	1	3
Dicamba + glyphosate	1/100	1	39	9	9	5	27	5	13
Dicamba + glyphosate	1/10	18	79	50	19	24	74	25	53
[a] LSD $_{(0.05)}$					13				

[a] species x rate x herbicide LSD $_{(0.05)}$.

Table 2.2. Visible injury of bedding plant species in response to driftable fractions of 2,4-D and dicamba 56 days after treatment.

Treatment	Fraction of 1X Use Rate	Bedding Plant Species							
		Begonia	Coleus	Geranium	Impatiens	Marigold	Petunia	Vinca	Zinnia
		--Visual Injury (%) --							
2,4-D	1/300	1	0	0	0	0	0	0	0
2,4-D	1/100	2	0	0	0	1	0	0	0
2,4-D	1/10	8	11	43	10	22	3	2	5
2,4-D + glyphosate	1/300	0	1	0	0	0	0	0	0
2,4-D + glyphosate	1/100	1	2	1	0	0	0	0	2
2,4-D + glyphosate	1/10	60	73	64	32	27	86	8	50
Dicamba	1/300	0	3	0	0	0	0	0	0
Dicamba	1/100	3	12	0	0	0	0	1	1
Dicamba	1/10	11	34	5	16	6	0	2	6
Dicamba + glyphosate	1/300	0	0	0	0	1	0	0	0
Dicamba + glyphosate	1/100	0	25	2	2	4	32	2	7
Dicamba + glyphosate	1/10	17	76	43	17	24	86	8	43
[a] LSD $_{(0.05)}$		12							

[a] species x rate x herbicide LSD $_{(0.05)}$.

Table 2.3. Height of bedding plant species in response to driftable fractions of 2,4-D and dicamba 28 days after treatment.

Treatment	Fraction of 1X Use Rate	Bedding Plant Species [a]							
		Begonia	Coleus	Geranium	Impatiens	Marigold	Petunia	Vinca	Zinnia
		Height (% of Non-Treated)							
Non-treated	--	100	100	100	100	100	100	100	100
2,4-D	1/300	104	109	108	102	99	99	97	110
2,4-D	1/100	98	111	106	105	90	92	96	103
2,4-D	1/10	74	76	70	96	79	91	94	101
2,4-D + glyphosate	1/300	94	108	104	104	99	97	105	102
2,4-D + glyphosate	1/100	94	108	104	107	98	89	102	94
2,4-D + glyphosate	1/10	30	36	60	84	64	51	70	49
Dicamba	1/300	96	119	101	95	91	94	100	99
Dicamba	1/100	100	96	100	102	91	87	97	92
Dicamba	1/10	90	108	93	101	91	91	96	79
Dicamba + glyphosate	1/300	96	103	104	111	106	90	96	100
Dicamba + glyphosate	1/100	99	74	89	91	85	68	95	90
Dicamba + glyphosate	1/10	86	51	78	85	75	49	82	55
$LSD_{(0.05)}$		17	30	18	17	14	11	12	20

[a] Bedding plant species analyzed separately.

Table 2.4. Height of bedding plant species in response to driftable fractions of 2,4-D and dicamba 56 days after treatment.

Treatment	Fraction of 1X Use Rate	Bedding Plant Species [a]							
		Begonia	Coleus	Geranium	Impatiens	Marigold	Petunia	Vinca	Zinnia
		Height (% of Non-Treated)							
Non-treated	--	100	100	100	100	100	100	100	100
2,4-D	1/300	106	93	101	93	105	98	100	100
2,4-D	1/100	105	90	106	94	104	94	97	92
2,4-D	1/10	89	75	68	89	80	95	94	102
2,4-D + glyphosate	1/300	98	90	104	96	99	96	97	103
2,4-D + glyphosate	1/100	106	87	103	101	104	95	98	89
2,4-D + glyphosate	1/10	43	27	56	76	78	51	83	46
Dicamba	1/300	103	90	101	79	94	94	96	95
Dicamba	1/100	106	76	104	104	97	92	94	98
Dicamba	1/10	92	57	103	88	94	98	94	84
Dicamba + glyphosate	1/300	102	81	103	98	92	98	96	100
Dicamba + glyphosate	1/100	105	48	90	78	96	94	94	86
Dicamba + glyphosate	1/10	96	24	77	85	83	64	91	63
$LSD_{(0.05)}$		18	20	18	23	17	17	8	21

[a] Bedding plant species analyzed separately.

Table 2.5 Flower production of bedding plant species in response to driftable fractions of 2,4-D and dicamba 28 days after treatment.

Treatment	Fraction of 1X Use Rate	Bedding Plant Species [a]						
		Begonia	Geranium	Impatiens	Marigold	Petunia	Vinca	Zinnia
		------------------------------Flower Production (number per plant) ------------------------------						
Non-treated		11	6	9	11	15	11	7
2,4-D	1/300	13	6	7	12	17	11	7
2,4-D	1/100	12	4	8	10	16	10	8
2,4-D	1/10	5	0	6	6	14	5	3
2,4-D + glyphosate	1/300	11	8	6	11	14	14	7
2,4-D + glyphosate	1/100	10	4	7	11	12	8	6
2,4-D + glyphosate	1/10	1	0	2	3	0	3	1
Dicamba	1/300	11	6	7	10	16	10	6
Dicamba	1/100	12	2	7	13	12	11	4
Dicamba	1/10	8	1	3	11	14	5	3
Dicamba + glyphosate	1/300	12	6	8	14	13	10	9
Dicamba + glyphosate	1/100	10	1	8	8	4	7	2
Dicamba + glyphosate	1/10	6	0	3	5	0	3	1
LSD $_{(0.05)}$		4	4	5	3	4	4	2

[a] Bedding plant species analyzed separately.

Table 2.6. Biomass of bedding plant species in response to driftable fractions of 2,4-D and dicamba 56 days after treatment.

Treatment	Fraction of 1X Use Rate	Bedding Plant Species [a]					
		Coleus	Geranium	Marigold	Petunia	Vinca	Zinnia
		---------------------------- Biomass (% of Non-Treated) ----------------------------					
Non-treated	--	100	100	100	100	100	100
2,4-D	1/300	113	139	96	99	96	87
2,4-D	1/100	82	120	90	95	83	91
2,4-D	1/10	65	44	63	74	74	88
2,4-D + glyphosate	1/300	68	123	87	83	101	86
2,4-D + glyphosate	1/100	70	122	81	72	81	76
2,4-D + glyphosate	1/10	8	29	53	10	50	18
Dicamba	1/300	99	103	96	106	86	94
Dicamba	1/100	51	133	90	93	82	69
Dicamba	1/10	33	121	84	78	72	67
Dicamba + glyphosate	1/300	70	128	110	86	83	91
Dicamba + glyphosate	1/100	21	90	77	49	71	64
Dicamba + glyphosate	1/10	10	44	43	8	53	22
LSD $_{(0.05)}$		37	34	31	27	19	29

[a] Bedding plant species analyzed separately.

CHAPTER III

INVESTIGATIONS OF THE SENSITIVITY OF ORNAMENTAL, FRUIT, AND NUT PLANT SPECIES TO DRIFTABLE FRACTIONS OF 2,4-D AND DICAMBA

Brian R. Dintelmann, Michele Warmund, Mandy Bish, and Kevin Bradley

Abstract

The recent development and implementation of 2,4-D and dicamba-resistant soybean and cotton has been facilitated by the increased problem of herbicide resistance in weed species. As a result of the adoption of these new traits, off-target movement of 2,4-D and dicamba is a major concern, especially for neighbors with sensitive crops, trees, or ornamental plant species. An experiment was conducted in 2017 and 2018 to determine the sensitivity of driftable fractions of 2,4-D and dicamba with or without glyphosate on common ornamental, fruit, and nut species. Three driftable fractions corresponding to 1/2, 1/20th and 1/200th of the manufacture's full labeled rate (1X rate) of 2,4-D (1.09 kg ae ha^{-1}), 2,4-D plus glyphosate (1.09 kg ae ha^{-1} plus 1.10 kg ae ha^{-1}), dicamba (0.56 kg ae ha^{-1}) and dicamba plus glyphosate (0.56 kg ae ha^{-1} plus 1.10 kg ae ha^{-1}) were applied to apple, crabapple, dogwood, elderberry, elm, grape, hydrangea, maple, oak, peach, pecan, redbud, rose, raspberry, strawberry, sweetgum, viburnum, and walnut plants that were potted in 10 to 20 L containers. Visual injury ratings were recorded 28 and 56 days after treatment (DAT). Tree trunk diameter growth was determined 112 DAT and shoot length measurements were determined 28 and 112 DAT. Across all 18 species, the 1/2X rate of 2,4-D plus glyphosate resulted in 60% injury 28

DAT, while the 1/2X rate of dicamba plus glyphosate resulted in 50% injury. Across all plant species and herbicides, the 1/20X rates cause visual injury ranging from 0 to 66%, while the 1/200X rates resulted in 0 to 19% visual injury. Hydrangea was the least sensitive species tested, while grape was the most sensitive. Shoot length and tree trunk diameter measurements decreased as herbicide rate increased across all plant species. Based on a combination of measurements and visual injury assessments, apple, maple, and peach expressed a greater sensitivity towards treatments containing dicamba while walnut, grape, and elm exhibited greater sensitivity towards 2,4-D. Although the 1/200X rates of 2,4-D and dicamba did not result in significant reductions in shoot length or trunk diameter of these species, obvious signs of injury were observed, which would render these plants unsalable.

Introduction

The predominance of herbicide-resistant weeds in corn (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.), and cotton (*Gossypium hirsutum* L.) have limited the weed control options in these systems (Norsworthy et al. 2012). While there are many ways farmers are beginning to diversify weed control programs such as implementation of cover crops, use of harvest weed seed destruction tools, tillage, and precision weed management (Bajwa et al. 2015; Palhano et al. 2018), most farmers still rely primarily on herbicides for weed control. Recently, agrochemical companies have developed soybean and cotton that are resistant to 2,4-D and dicamba (Behrens et al. 2007; Wright et al. 2010). Recent deregulation of dicamba and 2,4-D resistant crop cultivars in the U.S. allows farmers to utilize these technologies to control herbicide resistant weeds with in-season applications of 2,4-D or dicamba (USDA-APHIS 2014; USDA-APHIS 2015). Previous research indicates that dicamba and 2,4-D are effective on difficult to control weeds such as waterhemp (*Amaranthus tuberculatus* (Moq.) J. D. Sauer), horseweed (*Conyza canadensis* (L.) Cronq.), Palmer amaranth (*Amaranthus palmeri* S. Wats.), and giant ragweed (*Ambrosia trifida* L.), which are some of the most problematic weed species found in soybean and cotton (Johnson et al. 2010; Kruger et al. 2010; Robinson et al. 2012; Craigmyle et al. 2013; Spaunhorst et al. 2014; Shergill et al. 2017; Van Wychen 2017). Therefore, it is likely that the adoption of dicamba and 2,4-D resistant crops will result in a greater number of applications of dicamba and 2,4-D in these crops in the future.

Herbicides like 2,4-D and dicamba are susceptible to off-target movement with any application, and the degree to which this occurs can be influenced by the wind speed, nozzle type, and boom height at the time of application, as well as the herbicide formulation itself (Nordby and Skuterud 1974; Holterman et al. 1997; Wang and Rautmann 2008; Egan and Mortensen 2012; Sosnoskie et al. 2015; Alves et al. 2017). Previous research has shown that increased wind speed results in greater off-target movement of 2,4-D and dicamba (Wolf et al. 1993; Alves et al. 2017). These herbicides are also susceptible to volatilization, which is influenced by temperature, relative humidity, as well as the vapor pressure and formulation of 2,4-D and dicamba applied (Behrens and Lueschen 1979; Egan and Mortensen 2012; Shaner 2014; Sosnoskie et al. 2015).

With increased use of 2,4-D and dicamba in soybean and cotton production systems, there is an increased risk for off-target movement of these herbicides which can cause injury to neighboring sensitive plant species. While a variety of previous research has been conducted on the effects of off-target movement of 2,4-D and dicamba on soybean (Egan et al. 2014; Solomon and Bradley 2014; Kniss 2018), cotton (Marple et al. 2007; Egan et al. 2014), and fruiting/vegetable species (Hemphill and Montgomery 1981; Mohseni-Moghadam and Doohan 2015; Culpepper et al. 2018), limited research has been conducted on perennial and woody plant species. One perennial species that has been extensively studied are grapes (*Vitis vinifera* L.) (Comes et al. 1984; Ogg et al. 1991; Al-Khatib et al. 1993; Bhatti et al. 1996; Bhatti et al. 1997; Mohseni-Moghadam et al. 2015). Mohseni-Moghadam et al. (2015) found that 2,4-D applied at 1/30th of the normal use rate resulted in 66% visual injury 42 days after treatment (DAT), while dicamba applied at 1/30th of the normal use rate resulted in 47% injury at the same time interval following treatment. Similar studies have also found 2,4-D to be injurious to grapes (Al-Khatib et al. 1993; Bhatti el al. 1996; Bhatti el al. 1997).

In addition to grapes, numerous tree species have been found to be sensitive to 2,4-D and dicamba. Samtani et al. (2008) studied the effects of several herbicides, including dicamba and 2,4-D, on white oak (*Quercus alba* L.) seedlings. Following application, the authors observed leaf cupping, downward leaf margin rolling, elongation of leaf tips, necrosis, chlorosis, and parallel venation, and suggested that herbicide applications containing 2,4-D or dicamba near white oaks should be made before the leaf unfolding stage or after the leaf expansion stage in order to minimize injury to trees (Samtani et al. 2008). Al-Khatib et al. (1992) observed 5 to 52% visual injury as a result of 2,4-D treatments ranging from 1/100th to 1/3rd the normal use rates on sweet cherry (*Prunus avium* L.) trees. Al-Khatib et al. (1992) also noted that one-year-old trees were more sensitive to herbicide treatments in comparison to two-year-old trees. Otta (1974) reported that Siberian elms (*Ulmus pumila* L.) exhibited leaf cupping symptoms 10 DAT when 3 to 25 parts per million (ppm) of 2,4-D were applied to three-year old established elm trees. One year following treatment, visual injury assessments indicated that 5 to 25 ppm of 2,4-D caused significant leaf cupping to elm trees. Bark formation and trunk cross sectional area were also affected by as little as 10 ppm of 2,4-D on Siberian elm (Otta 1974). These results demonstrate the sensitivity of a few woody perennial species to 2,4-D and dicamba, however there are many more economically-important tree and ornamental species that have not been studied. The objective of this research was to determine the sensitivity of various common ornamental, fruit, and nut plant species to driftable fractions of 2,4-D and dicamba with and without glyphosate.

Materials and Methods

In 2017 and 2018, 18 perennial plant species including apple (*Malus domestica* ‘Granny Smith’), peach (*Prunus persica* ‘Contender’), grape (*Vitis aestivalis* ‘Norton’), raspberry (*Rubus idaeus* ‘Heritage’), strawberry (*Fragaria x ananassa* ‘Earliglow’), American elderberry (*Sambucus canadesis*), flowering dogwood (*Cornus florida*), red maple (*Acer rubrum*), pin oak (*Quercus palustris*), Sargent crabapple (*Malus sargentii*), American elm (*Ulmus americana*), hydrangea (*Hydrangea macrophylla* ‘Glowing Embers’), nannyberry viburnum (*Viburnum lentago*), eastern redbud (*Cercis canadensis*), American sweetgum (*Liquidambar styraciflua*), pecan (*Carya illinoinensis*), black walnut (*Juglans nigra*), and knockout rose (*Rosa* ‘Radrazz’) were obtained and transplanted in 10 to 20 L plastic pots. Plant species were selected based on their economic importance in Missouri. Apple, peach, walnut, pecan, grape, raspberry, and strawberry were transplanted as one-year-old bare root plants in early-April each year prior to treatment. The remaining species were two-year-old plants purchased from Forrest Keeling Nursery (Elsberry, MO). Potting soil was a custom blend, consisting of 59% pine bark, 9% sphagnum peat moss (BM1, Berger 121 1e Rang Saint-Modeste, QC G0L 3W0, Canada), 6% sand, 6% vermiculite (Vermiculite, Therm-O-Rock West, Inc, 6732 W. Willis Road #5014, Chandler, AZ 85226), and 20% perlite (Perlite, Therm-O-Rock West, Inc, 6732 W. Willis Road #5014, Chandler, AZ 85226). Media was supplemented with 1.17 liters (L) of granular slow release fertilizer with an N-P-K analysis of 38-0-0 (Nitroform, Koch Turf & Ornamental 4111 East 37th St. N Wichita, KS 67220), 0.83 L granular micronutrient fertilizer (Micromax, ICL Specialty Fertilizers PO Box 3310 Dublin, OH 43016) containing 17% Fe, 2.5% Mn, 0.10% B, 1% Cu, 0.05% Mo, and 1% Zn, and 1.5 L slow release fertilizer with an N-P-K analysis of 13-13-13 (Osmocote, ICL Specialty Fertilizers PO Box 3310 Dublin, OH 43016) was added to every 1.0 cubic meter batch of soil mix, respectively. In both years, all plants were maintained in at the University of Missouri Horticulture and Agroforestry Research Center located in New Franklin, Missouri (39.0161° N, 92.7534° W) in an outdoor nursery area where overhead irrigation was provided twice daily. The outdoor nursery area was equipped with a shade cloth on all sides to prevent wildlife interference. Furthermore, overhead shade cloths were installed for dogwood due to the sensitivity of this species to full sun. Prior to herbicide treatments both years, plants of each species were pruned to uniform height. Additionally, fertilizer (Osmocote Plus 15-9-12, ICL Specialty Fertilizers PO Box 3310

Dublin, OH 43016) was added to each pot to prevent nutrient deficiencies throughout the experiments.

The herbicide treatments evaluated in this research included 2,4-D choline (Enlist One, Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268), 2,4-D choline plus glyphosate (Enlist Duo, Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268), dicamba DGA plus Vapor Grip (Xtendimax plus Vapor Grip, Monsanto Company, 800 North Lindbergh Blvd, St. Louis, Missouri 63167), and dicamba DGA plus Vapor Grip plus glyphosate (Roundup Powermax, Monsanto Company, 800 North Lindbergh Blvd, St. Louis, Missouri 63167). Each of these herbicide treatments was applied at 1/2, 1/20th, and 1/200th of the manufacture's recommended use rates, or 1X rates. The 1X rates used for the calculation of the fractions of the use rate for 2,4-D choline, 2, 4-D choline plus glyphosate, dicamba and dicamba plus glyphosate were 1.09, 1.09 plus 1.10, 0.56, and 0.56 plus 1.10 kg ae ha^{-1}, respectively. A non-treated control of each species was included for comparison. On June 8 in 2017 and June 12 in 2018 all herbicide treatments were applied with a CO_2-pressurized backpack sprayer equipped with 8002 XR nozzles (TeeJet®, Spraying Systems Co., PO Box 7900, Wheaton, IL 60187) at 140 L ha^{-1} and 131 kPa by spraying with a 3 m boom approximately 45 cm directly over the top of each species. Flat fan nozzles were used to simulate an off-target movement occurrence of these herbicides. All species, except roses were vegetative at the time of treatment. Plants were kept separate after application and then returned to the outdoor nursery under overhead irrigation one day following herbicide treatment. The experimental design was a randomized complete block design in a split-plot arrangement with five single-plant replications of each treatment. Individual species were whole plots while subplots consisted of herbicide treatments.

Prior to herbicide treatments, three shoots per plant were tagged and initial shoot length measurements were recorded. Initial trunk diameter was also recorded prior to herbicide treatments by measuring 10 centimeters (cm) above the soil surface for dogwood, maple, oak, crabapple, elm, redbud, sweetgum, pecan, and walnut by using a digital caliper (Absolute, Mitutoya, 965 Corporate Blvd., Aurora, IL 60502). For apple and peach, initial trunk diameter measurements were recorded 10 cm above the graft union. Following treatments, shoot lengths were recorded again 28 and 112 DAT. Additionally, trunk diameter measurements were recorded again 112 DAT. Increase in tree trunk diameter was calculated by subtracting the initial measurement from the measurement 112 DAT. Overall visual injury evaluations were recorded 28 and 56 DAT. Visual injury included an assessment of epinasty/leaf malformation, chlorosis, necrosis, stem cracking/swelling, auxiliary branching, and reduced growth compared to the non-treated control, and occurred on a scale of 0 (no injury) to 100% (plant death). Additionally, three individual leaves were sampled from each plant 28 DAT to measure leaf length and width. Leaves were removed from each plant so close-up pictures could be taken with a reference scale. Following sampling, length and width measurements were recorded using ImageJ software (ImageJ, U.S. National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892). Leaf length:width ratios were determined for each leaf by dividing the leaf length by width to quantify leaf malformation, similar to Nath et al. (2003). Data were analyzed in SAS (SAS 9.4, SAS® Institute Inc. 100 SAS Campus Drive Cary, NC 27513) using the PROC GLIMMIX procedure. Means were separated using Fishers Protected Least Significant Difference (LSD) with $P \leq 0.05$. Herbicide, rate, and perennial species were considered fixed effects, while year and replication were considered random effects. Years were classified as random to provide conclusions about species or treatments over a wide range of environments (Blouin et al. 2011; Carmer et al. 1989).

Results and Discussion

Visual injury. Following treatment with either 2,4-D or dicamba, a variety of injury symptoms were observed 28 and 56 DAT. These included epinasty of stems and petioles, leaf cupping, strapping, and rolling, axillary branching, chlorosis, necrosis, leaf margin malformation, and upper and lower stem swelling and cracking. Figure 3.1 illustrates some of the unique differences between 2,4-D and dicamba injury on grape, sweetgum, maple, viburnum, oak, and walnut that was observed in 2017 and 2018. For example, dicamba injury to grapevines (Figure 3.1 A.) resulted in the newest leaves to become cupped and have significantly reduced size. In contrast, 2,4-D injury to grapevines (Figure 3.1 B.) resulted in leaf strapping to the newly emerging leafs. A similar difference in symptomology between 2,4-D and dicamba on grapes was noted by Mohseni-Moghadam et al. (2015). Viburnum injured by dicamba (Figure 3.1 G.) resulted in leaf rolling while 2,4-D treatments caused viburnum to have significant epinasty of stems, petioles, and leaves (Figure 3.1 H.). Walnut exhibited unique symptomology of 2,4-D and dicamba. Figure 3.1 K. illustrates leaf strapping and leaf margin malformation on walnut leaflets injured by dicamba. In contrast, 2,4-D injury caused epinasty, chlorosis, and necrosis of leaf tissue on walnut (Figure 3.1 L.). Feucht (1988), Al-Khatib et al. (1992), Samtani et al. (2008), and Mohseni-Moghadam et al. (2015) have reported similar symptoms in response to applications of 2,4-D and dicamba on other tree and grapevine species.

There was a rate by herbicide by perennial species interaction (*P<0.0001*) for visual injury 28 and 56 DAT. As rates increased across herbicide treatments and species, visual injury increased 28 and 56 DAT (Tables 3.1 and 3.2). When averaged across all species 2,4-D plus glyphosate at the 1/2X rate resulted in the greatest injury (60%) compared to dicamba plus glyphosate at the 1/2X rate (50%; data not shown). Across all species, greater visual injury occurred in response to 1/2X treatments of 2,4-D and dicamba in combination with glyphosate compared to 2,4-D or dicamba alone. For example, 2,4-D and dicamba applied to strawberry at the 1/2X rate caused 3 and 24% visual injury, respectively. However, when glyphosate was added to these treatments, visual injury increased to 61 and 70%, respectively (Table 3.1). McMurray et al. (1996) found that when 2,4-D was applied at 0.84 kg ae ha^{-1}, strawberry visual injury did not exceed 10%, which is consistent with the results from these experiments that strawberry foliage have a high tolerance to applications of 2,4-D in the spring. At the 1/20X rates, additive effects of 2,4-D or dicamba plus glyphosate were observed on dogwood, grape, maple, and walnut. Mohseni-Moghadam et al. (2015) also found that combinations of 2,4-D or dicamba with glyphosate caused greater visual injury on grape compared to either of these herbicides alone. Synergistic effects of 2,4-D or dicamba in combination with glyphosate have also been reported in terms of weed control (Flint and Barrett 1989; Johnson et al. 2010; Craigmyle et al. 2013). However, there was not greater injury to crabapple, elderberry, grape, and pecan in response to dicamba plus glyphosate compared to dicamba alone (Table 3.1).

The 1/20X rate of dicamba and dicamba plus glyphosate generally caused greater visual injury compared with 2,4-D or 2,4-D plus glyphosate (data not shown). With the exception of grape, all plant species exhibited less than 8% visual injury in response to the 1/200X rates of all treatments 28 and 56 DAT (Tables 3.1 and 3.2). Grape was the most sensitive species to the 1/200X rates of herbicide treatments and resulted in up to 19% visual injury (Table 3.1). Al-Khatib et al. (1993) found that grapes exhibited 21% visual injury from a 1/100X treatment of 2,4-D plus glyphosate, while Bhatti et al. (1996) reported that one-year-old grapevines expressed visible injury 30 DAT with 2,4-D applications of 1.2 g ha^{-1} (approximately 1/900X).

On average across all species, few differences in visual injury were observed between 28 and 56 DAT. For example, all rates of dicamba applied to oak caused only a 1 to 4% increase in visual injury from 28 to 56 DAT (Tables 3.1 and 3.2). Previous research has also shown that cotton expressed similar or slightly elevated injury up to 120 DAT in response to treatments of 2,4-D (Marple et al. 2007; Everitt and Keeling 2009). Grapes also expressed similar or greater levels of injury 90 DAT compared to 14 DAT with treatments of 2,4-D (Al-Khatib et al. 1993). These findings are in contrast to those found in annual species such as soybeans or vegetables, which begin to show signs of recovery by 28 DAT (Solomon and Bradley 2014; Mohseni-Moghadam and Doohan 2015). Therefore results from this study indicate that ornamental, fruit and nut plant species express visual injury symptoms from 2,4-D or dicamba for extended periods of time compared to annual plant species.

Some species exhibited a greater sensitivity towards 2,4-D while others were more sensitive to dicamba. Elm, grape, sweetgum, and walnut were more sensitive to treatments containing 2,4-D with or without glyphosate compared to dicamba (Tables 3.1 and 3.2). Mohseni-Moghadam et al. (2015) found that a wide range of grape cultivars were highly sensitive to low dose treatments of 2,4-D, and slightly less sensitive to dicamba. Alternatively, apple, maple, peach, and strawberry were found to be more sensitive to treatments containing dicamba compared with 2,4-D (Tables 3.1 and 3.2). For example, maple trees had 51 and 11% visual injury from 1/2X treatments of dicamba and 2,4-D, respectively (Table 3.2). Perry and Upchurch (1968), Sterrett (1968, 1969) also reported that maple trees were more susceptible to dicamba in comparison to 2,4-D. Some species, such as elderberry, hydrangea, redbud and raspberry, did not express a consistent difference in sensitivity between dicamba and 2,4-D with or without glyphosate. Hydrangea was found to be the least susceptible species in this study, exhibiting less than 18% injury from all treatments. In contrast, grape was the most sensitive species, with visual injury ranging from 4 to 19% across all herbicide treatments applied at the 1/200X rates (Table 3.1).

Shoot length. As herbicide rate increased, shoot length generally decreased (Table 3.3). There were no differences in shoot lengths between the herbicide treatments and the non-treated control for elm, hydrangea, oak, pecan, redbud, and sweetgum (data not shown). Dicamba plus glyphosate applied at the 1/2X rate caused reductions in shoot length on all species except dogwood, rose, and raspberry. Glyphosate plus 2,4-D applied at the 1/2X rate caused reductions in shoot length in all species except maple, rose, raspberry, and viburnum. Greater reductions in shoot length were observed with 1/2X treatments containing glyphosate in combination with either 2,4-D or dicamba in comparison to 2,4-D or dicamba alone (Table 3.3). For example, 2,4-D plus glyphosate at the 1/2X rate on strawberry resulted in a 28% shoot length reduction compared to 2,4-D alone. These results are similar to the visual injury assessments on strawberry (Table 3.1).

Herbicide treatments applied at the 1/20X rate reduced shoot length on crabapple and grape only. The 1/200X rates did not reduce shoot length, except for 2,4-D plus glyphosate applied to crabapple (Table 3.3). These results are in contrast to those found by Mohseni-Moghadam et al. (2015), who found that 2,4-D or 2,4-D plus glyphosate applied at the 1/300X rate resulted in significant shoot length loss on grapevines. Mohseni-Moghadam et al. (2015) may have observed greater reductions in shoot length with lower doses of 2,4-D because grapevines were grown in a greenhouse following treatment and were maintained as single shoot plants. Differences between these two studies could have also be observed due to the different formulations of 2,4-D and dicamba applied. Select treatments applied at the 1/200 or 1/20X rates resulted in greater shoot lengths on rose and raspberry. The 1/200 and 1/20X rates of 2,4-D and 2,4-D plus glyphosate resulted in greater shoot lengths (124%) compared to the non-treated control on rose (Table 3.3). This is most likely a result of hormesis, a phenomenon which states that at low does, inhibitors become stimulants (Mattson 2008). Hemphill and Montgomery (1981) also reported that 2.1 g ha^{-1} 2,4-D (approximately 1/500X) resulted in 136% pepper yield of the non-treated control and concluded this result was likely due to increased branching and flowering on pepper. Additionally, dicamba applied to raspberry at the 1/200X rate caused greater shoot length (150%) than the non-treated control (Table 3.3).

When shoot length was measured again 112 DAT, 1/2X rates of herbicides caused the greatest reductions in shoot length (Table 3.4). There were no differences in shoot lengths between the herbicide treatments and the non-treated control for apple, crabapple, hydrangea, redbud, and sweetgum (data not shown). Herbicide treatments did not cause reduced shoot length of elm 28 DAT, however by 112 DAT 1/2X rates of all herbicides reduced elm shoot length up to 15 cm compared to the non-treated control (Table 3.4). Dogwood shoot length was only reduced by the 1/2X treatment of 2,4-D plus glyphosate 28 DAT, however by 112 DAT all herbicides applied at the 1/2X rates reduced shoot lengths by as much as 14 cm (Tables 3.3 and 3.4). Pecan, raspberry, and rose also had greater shoot length reductions 112 DAT compared with 28 DAT. In contrast, by 112 DAT some species, such as grape, were able to recover from initial shoot length reductions that occurred to 1/20X rates of 2,4-D plus glyphosate and dicamba (Tables 3.3 and 3.4). Similar results from Mohseni-Moghadam et al. (2015) show that Riesling grapevines recovered shoot length from 1/30X treatments of 2,4-D or dicamba with or without glyphosate by 357 DAT. Apple and crabapple also had shoot length recovery by 112 DAT (Tables 3.3 and 3.4). Overall, these results indicate that some species may not experience reductions in shoot length until later in the growing season while others may experience shoot length reductions early in the season, but recover by 112 DAT.

Leaf length:width ratio. To quantify leaf malformation or curvature from herbicide treatments, leaf length and width was measured 28 DAT. Nath et al. (2003) also measured leaf length and width and determined the ratio of length to width in order to quantify leaf curvature in *Antirrhinum*. Ratios greater than the control indicate leaf width has decreased and/or leaf length has increased. Figure 3.2 shows a pin oak leaf that was treated with a 1/20X rate of dicamba which resulted in a greater ratio due to decreased leaf width compared to the non-treated control. Samtani et al. (2008) described similar symptomology on white oak seedlings that were injured with dicamba. Alternatively, ratios can be less than the control indicating an increase in leaf width and/or a decrease in leaf length. Figure 3.3 shows a grape leaf that was treated with a 1/20X rate of 2,4-D plus glyphosate which resulted in increased leaf width compared to the non-treated control and a corresponding decrease in the overall ratio. Similar symptomology effects were described on grapevines injured with 2,4-D by Mohseni-Moghadam et al. (2015).

There were no differences between any herbicide treatments and the non-treated control in the leaf length:width ratio for dogwood, hydrangea, maple, redbud, strawberry, and viburnum (data not shown). Overall, the 1/2X rates caused the greatest change in leaf length:width ratio across all remaining species. Glyphosate in combination with 2,4-D applied at the 1/2X rate caused reduced ratios on elderberry, elm, grape, oak, peach, and walnut (Table 3.5). These results suggest that 2,4-D plus glyphosate caused leaves to become expanded and/or shortened compared to the non-treated control. For raspberry and sweetgum 2,4-D plus glyphosate applied at the 1/2X rate caused increased ratios with elongated and / or narrow leaves (Table 3.5). Therefore, it is likely that 2,4-D plus glyphosate injury will result in elongated and/or narrow leaves in raspberry and sweetgum. The 1/2X rate of dicamba plus glyphosate caused fewer differences in the leaf length:width ratio compared to the 1/2X rate of 2,4-D plus glyphosate. However, dicamba plus glyphosate altered leaf shapes on apple, crabapple, elderberry, oak, and sweetgum (Table 3.5). In some cases, 2,4-D or dicamba caused similar leaf symptomology. For example, 1/2X rates of 2,4-D and dicamba increased leaf length:width ratios of 3.8 and 4.9 on pecan, respectively, as compared to with the control (2.7) (Table 3.5). These altered ratios for pecan correspond to elongated and narrow leaves. Grape and oak were the only species to show any difference in leaf ratios with 1/20X rates of certain 2,4-D and dicamba treatments compared to the non-treated control. No species exhibited significant differences from the non-treated control in response to 1/200X rates of the herbicide treatments (Table 3.5).

Trunk diameter. Several studies have quantified tree growth by measuring trunk diameter (Norton and Storey 1970; Daniell and Hardcastle 1972; Otta 1974; Putnam 1976; Comes et al. 1984; Patterson and Goff 1994). In this research, as herbicide rate increased, trunk diameter growth generally decreased for most species (Table 3.6). There were no differences in tree trunk diameter growth between any herbicide treatments and the non-treated control for pecan and sweetgum (data not shown). Across the nine remaining tree species, the 1/2X rate of dicamba plus glyphosate caused reductions in the trunk diameters for all species except crabapple and redbud, whereas 2,4-D plus glyphosate at the 1/2X rate caused reductions in trunk growth in all species except apple, maple, and peach. Dogwood and walnut had the least trunk diameter growth in response to 1/2X treatments of 2,4-D plus glyphosate, which was 0.3 and 0.0 mm, respectively.

No herbicide treatments at the 1/200X rates caused a reduction in tree trunk diameter. The 1/20X rates of dicamba and 2,4-D plus glyphosate did result in reduced trunk diameter growth on some species. Walnut trees exhibited 1.3 mm of growth as a result of 2,4-D plus glyphosate applied at the 1/20X rate, while non-treated walnut trees resulted in nearly 3 mm of trunk diameter growth by 112 DAT (Table 3.6). Dicamba applied at the 1/20X rate resulted in 32, 37, and 53% reduction in trunk diameter growth on maple, oak, and redbud tree species, respectively (Table 3.6). These results indicate that driftable fractions of dicamba adversely affects trunk growth of oak, maple, and redbud trees whereas walnut trunk diameter growth is reduced from a driftable fraction of 2,4-D plus glyphosate.

Based on a combination of measurements and visual evaluations, several species expressed a difference in sensitivity to 2,4-D or dicamba. For example, apple trunk diameter losses and visual injury evaluations were greater in response to 1/2X rates of dicamba compared with 2,4-D (Tables 3.1 and 3.6). Also, maple shoot and trunk diameter growth was reduced by the 1/20X rate of dicamba, while the 1/20X rate of 2,4-D resulted in similar growth as the non-treated control (Tables 3.3, 3.4, and 3.6). Peach tree shoot length and visual injury assessments also revealed that peach trees are more sensitive to dicamba compared with 2,4-D while visual injury, trunk diameter growth, and leaf length:width ratios indicate that oak trees were more sensitive to1/20X rates of dicamba compared to 2,4-D (Tables 3.1, 3.2, 3.5 and 3.6). Sterrett (1969) also reported that dicamba had greater efficacy on chestnut oak compared with 2,4-D. Collectively, these results demonstrate that apple, maple, oak, and peach trees expressed greater sensitivity to dicamba based on a combination of visual injury, trunk diameter, and shoot length measurements. However, some species exhibited a greater sensitivity towards 2,4-D. For example, elm leaf length:width ratios for 2,4-D and dicamba applied at the 1/2X rate were 0.9 and 2.4, respectively. Additionally, elm trees exhibited reduced trunk diameter growth in response to the 1/2X rate of 2,4-D while the 1/X rate of dicamba was not different from the non-treated control (Tables 3.5 and 3.6). Walnut trees exhibited 60 and 41% greater visual injury and greater reduced trunk growth, respectively, as a result of

1/2X treatments of 2,4-D compared with dicamba (Tables 3.1 and 3.6). Overall, grapevines, elm, and walnut trees expressed greater sensitivity to dicamba based on a combination of measurements assessed in this study.

The results from this research indicate that perennial plant species respond differently from one another to treatments containing 2,4-D or dicamba. As rates increased from 1/200 to 1/2X, visual injury and reductions in trunk diameter growth and reductions in shoot length increased. Across most plant species in this study, treatments containing glyphosate typically resulted in greater visual injury and reductions in shoot length compared to treatments containing 2,4-D or dicamba alone. Based on visual injury, grape was the most sensitive species to the lowest doses of all herbicide treatments. Following grape, dogwood, oak and elderberry were the next most sensitive species in this study. Leaf length:width ratios confirmed that grape and oak experienced the greatest symptoms from 1/200 and 1/20X rates of all treatments and were found to be the most sensitive species to the lowest doses (Tables 3.1, 3.2, and 3.5). Conversely, hydrangea was found to be the least sensitive species in this study. However, based on all of the combined measurements, we conclude that apple, peach, oak, and maple trees are more sensitive to dicamba compared to 2,4-D. In contrast, grape, walnut, and elm express a greater sensitivity towards 2,4-D compared to dicamba, while some species did not differ in sensitivity to 2,4-D or dicamba. For example, dogwood and elderberry were found to be highly sensitive to all herbicides while hydrangea, pecan, redbud, raspberry, and rose had similar levels of injury as a result of 2,4-D and dicamba treatments. This research also demonstrates that 2,4-D and dicamba can cause varying symptomology on the leaves of various species; causing leaves to become elongated and/or slender or to become shortened and/or expanded. Unique symptomology differences observed in these studies between 2,4-D and dicamba can be useful in determining if plants were injured from 2,4-D or dicamba, a concern in the future when dicamba and 2,4-D resistant crop technology coexists.

Results from this study also indicate that many species can experience visual injury symptoms in response to 1/200X rates of 2,4-D or dicamba with or without glyphosate. Egan et al. (2014) reported that driftable fractions corresponding to the 1/200X rate was likely to occur in field settings. However, in many instances in this research, these rates did not result in reductions in shoot length or trunk diameter growth. Compared to previous studies that examined the effects of 2,4-D and dicamba on annual species such as soybean or vegetable crops (Kruger et al. 2012; Solomon and Bradley 2014; Culpepper et al. 2018), most perennial species in this study were found to be less susceptible to driftable fractions of 2,4-D or dicamba. However, it appears that perennial species may experience injury for longer periods of time compared to annual plant species. Many woody species examined in this study are native or ornamental species that have a high monetary and aesthetic value to a wide range of audiences. Therefore, it is unlikely that visual injury resulting from 2,4-D or dicamba would be tolerated by homeowners or commercial growers of ornamental species. Off-target movement of these herbicides can also ruin edible fruit and nut crops also necessitates their disposal. Furthermore, if 2,4-D or dicamba drifts onto a certified organic crop, these producers must destroy their current crop and would also lose organic certification for three years according to the Organic Materials Review Institute (OMRI). Thus, applications of 2,4-D or dicamba with or without glyphosate should be made with extreme care near areas where these ornamental, fruit, and nut species may exist.

Literature Cited

Al-Khatib K, Parker R, Fuerst EP (1992) Sweet cherry (*Prunus avium*) response to simulated drift from selected herbicides. Weed Technol 6:975-979

Al-Khatib K, Parker R, Fuerst EP (1993) Wine grape (*Vitis vinifera L.)* response to simulated herbicide drift. Weed Technol 7:97-102

Alves GS, Kruger GR, da Cunha JPA, de Santana DG, Pinto LAT, Guimarães F, Zaric M (2017) Dicamba spray drift as influenced by wind speed and nozzle type. Weed Technol 31:724-731

Bajwa AA, Mahajan G, Chauhan BS (2015) Nonconventional weed management strategies for modern agriculture. Weed Sci 63:723-747

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 (1979) Dicamba volatility. Weed Sci 27:486-493

Bhatti MA, Al-Khatib K, Parker R (1996) Wine grape (*Vitis vinifera*) response to repeated exposure of selected sulfonylurea herbicides and 2, 4-D. Weed Technol 10:951-956

Bhatti MA, Al-Khatib K, Parker R (1997) Wine grape (*Vitis vinifera*) response to fall exposure of simulated drift from selected herbicides. Weed Technol 11:532-536

Blouin DC, Webster EP, Bond JA (2011) On the analysis of combined experiments. Weed Technol 25:165-169

Carmer S, Nyquist W, Walker W (1989) Least significant differences for combined analyses of experiments with two-or three-factor treatment designs. Agron J 81:665-672

Comes RD, Marquis LY, Kelley AD (1984) Response of Concord grapes (*Vitis labrusca*) to 2, 4-D in irrigation water. Weed Sci 32:455-459

Craigmyle, BD, JM Ellis, KW Bradley (2013) Influence of weed height and glufosinate plus 2,4-D combinations on weed control in soybean with resistance to 2,4-D. Weed Technol 27:271–280

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

Daniell JW, Hardcastle WS (1972) Response of peach trees to herbicide and mechanical weed control. Weed Sci 20:133-136

Egan JF, Barlow KM, Mortensen DA (2014) A meta-analysis on the effects of 2, 4-D and dicamba drift on soybean and cotton. Weed Sci 62:193-206

Egan JF, Mortensen DA (2012) Quantifying vapor drift of dicamba herbicides applied to soybean. Environ Toxicol Chem 31:1023-1031

Everitt JD, Keeling JW (2009) Cotton growth and yield response to simulated 2, 4-D and dicamba drift. Weed Technol 23:503-506

Feucht JR (1988) Herbicide injuries to trees-symptoms and solutions. J Arboric 14:215-219

Flint JL, Barrett M (1989) Effects of glyphosate combinations by 2, 4-D or dicamba on field bindweed (*Convolvulus arvensis*). Weed Sci 37:12-18

Hemphill DD, Montgomery ML (1981) Response of vegetable crops to sublethal application of 2, 4-D. Weed Sci 29:632-635

Holterman H, Van De Zande J, Porskamp H, Huijsmans J (1997) Modelling spray drift from boom sprayers. Comput Electron Agric 19:1-22

Johnson B, Young B, Matthews J, Marquardt P, Slack C, Bradley K, York A, Culpepper S, Hager A, Al-Khatib K (2010) Weed control in dicamba-resistant soybeans. Crop Manag 9:00-00

Kniss AR (2018) Soybean response to dicamba: A meta-analysis. Weed Technol 32:507-512

Kruger GR, Davis VM, Weller SC, Johnson WG (2010) Growth and seed production of horseweed (*Conyza canadensis*) populations after exposure to postemergence 2, 4-D. Weed Sci 58:413-419

Kruger GR, Johnson WG, Doohan DJ, Weller SC (2012) Dose response of glyphosate and dicamba on tomato (*Lycopersicon esculentum*) injury. Weed Technol 26:256-260

Marple ME, Al-Khatib K, Shoup D, Peterson DE, Claassen M (2007) Cotton response to simulated drift of seven hormonal-type herbicides. Weed Technol 21:987-992

Mattson MP (2008) Hormesis defined. Ageing Res Rev 7:1-7

McMurray G, Monks D, Leidy R (1996) Clopyralid use in strawberries (*Fragaria× ananassa* Duch.) grown on plastic mulch. Weed Sci 44:350-354

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

Mohseni-Moghadam M, Wolfe S, Dami I, Doohan D (2015) Response of wine grape cultivars to simulated drift rates of 2, 4-D, dicamba, and glyphosate, and 2, 4-D or dicamba plus glyphosate. Weed Technol 30:807-814

Nath U, Crawford BC, Carpenter R, Coen E (2003) Genetic control of surface curvature. Science 299:1404-1407

Nordby A, Skuterud R (1974) The effects of boom height, working pressure and wind speed on spray drift. Weed Res 14:385-395

Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR (2012) Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Sci 60:31-62

Norton JA, Storey JB (1970) Effect of herbicides on weed control and growth of pecan trees. Weed Sci 18:522-524

Ogg AG, Ahmedullah MA, Wright GM (1991) Influence of repeated applications of 2, 4-D on yield and juice quality of concord grapes (*Vitis labruscana*). Weed Sci 39:284-295

Otta J (1974) Effects of 2, 4-D herbicide on Siberian elm. Forest Sci 20:287-290

Palhano MG, Norsworthy JK, Barber T (2018) Cover crops suppression of Palmer amaranth (*Amaranthus palmeri*) in cotton. Weed Technol 32:60-65

Patterson MG, Goff WD (1994) Effects of weed control and irrigation on pecan (*Carya illinoinensis*) growth and yield. Weed Technol 8:717-719

Perry, PW, RP Upchurch (1968) Growth analysis of red maple and white ash seedlings treated with eight herbicides. Weed Sci 16:32–37

Putnam A (1976) Fate of glyphosate in deciduous fruit trees. Weed Sci 24:425-430

Robinson AP, Simpson DM, Johnson WG (2012) Summer annual weed control with 2, 4-D and glyphosate. Weed Technol 26:657-660

Samtani JB, Masiunas JB, Appleby JE (2008) Injury on white oak seedlings from herbicide exposure simulating drift. HortScience 43:2076-2080

Shergill LS, Bish MD, Biggs ME, Bradley KW (2017) Monitoring the changes in weed populations in a continuous glyphosate-and dicamba-resistant soybean system: A five-year field-scale investigation. Weed Technol 32:166–173

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

IL:315

Solomon CB, Bradley KW (2014) Influence of application timings and sublethal rates of synthetic auxin herbicides on soybean. Weed Technol 28:454-464

Sosnoskie LM, Culpepper AS, Braxton LB, Richburg JS (2015) Evaluating the volatility of three formulations of 2, 4-D when applied in the field. Weed Technol 29:177-184

Spaunhorst DJ, Siefert-Higgins S, Bradley KW (2014) Glyphosate-resistant giant ragweed (Ambrosia trifida) and waterhemp (Amaranthus rudis) management in dicamba-resistant soybean (*Glycine max*). Weed Technol 28:131-141

Sterrett JP (1968) Response of oak and red maple to herbicides applied with an injector. Weed Sci 16:159-160

Sterrett JP (1969) Injection of hardwoods with dicamba, picloram, and 2, 4-D. J For 67:820-821

[USDA-APHIS] US Department of Agriculture-Animal and Plant Health Inspection Service. (2014) Determination of nonregulated status for Dow AgroSciences DAS-68416-4 soybean. Washington, DC: US Department of Agriculture. https://www. aphis.usda.gov/brs/aphisdocs/11_23401p_det.pdf Accessed February 7, 2018

[USDA-APHIS] US Department of Agriculture-Animal and Plant Health Inspection Service. (2015) Determination of nonregulated status for Monsanto Company MON 88708 soybean. Washington, DC: US Department of Agriculture. https://www.aphis.usda.gov/brs/aphisdocs/10_18801p_det.pdf. Accessed February 7, 2018

Van Wychen L (2017) WSSA survey ranks most common and most troublesome weeds in broadleaf crops, fruits and vegetables. http://wssa.net/2017/05/wssa-survey-ranks-most-common-and-most-troublesome-weeds-in-broadleaf-crops-fruits-and-vegetables/ Accessed February 5, 2019

Wang M, Rautmann D (2008) A simple probabilistic estimation of spray drift—factors determining spray drift and development of a model. Environ Toxicol Chem 27:2617-2626

Wolf TM, Grover R, Wallace K, Shewchuk SR, Maybank J (1993) Effect of protective shields on drift and deposition characteristics of field sprayers. Can J Plant Sci 73:1261-1273

Wright TR, Shan G, Walsh TA, Lira JM, Cui C, Song P, Zhuang M, Arnold NL, Lin G, Yau K (2010) Robust crop resistance to broadleaf and grass herbicides provided by aryloxyalkanoate dioxygenase transgenes. Proceedings of the National Academy of Sciences 107:20240-20245

Table 3.1. Visual injury of ornamental, fruit, and nut plant species in response to driftable fractions of 2,4-D and dicamba 28 days after treatment.

	Fraction of 1X Use Rate											
	2,4-D			2,4-D + glyphosate			Dicamba			Dicamba + glyphosate		
Species	1/200	1/20	1/2	1/200	1/20	1/2	1/200	1/20	1/2	1/200	1/20	1/2
	---------- Visual Injury (%) ----------											
Apple	1	2	7	1	6	26	1	7	26	2	12	36
Crabapple	1	2	16	0	6	54	0	3	10	0	1	13
Dogwood	2	8	68	1	25	93	0	17	69	4	28	82
Elderberry	0	13	65	1	8	81	6	25	73	6	27	77
Elm	2	11	37	2	15	71	0	9	29	1	10	40
Grape	6	31	91	19	66	100	4	29	75	7	32	78
Hydrangea	0	0	3	0	2	12	0	1	6	0	3	18
Maple	1	4	14	0	7	25	3	21	40	3	30	48
Oak	4	13	52	2	14	78	4	21	39	5	28	53
Peach	0	1	26	0	1	49	2	19	64	2	23	78
Pecan	0	4	32	1	10	53	0	4	28	0	5	28
Redbud	0	12	32	2	17	59	3	30	35	4	20	54
Rose	0	2	24	0	9	71	0	0	26	1	5	50
Raspberry	1	1	6	0	8	46	0	2	15	0	0	46
Strawberry	0	0	3	0	1	61	0	5	24	1	7	70
Sweetgum	0	2	32	0	4	54	0	3	23	0	6	40
Viburnum	1	4	52	5	10	72	0	23	40	3	17	66
Walnut	1	9	80	0	18	95	0	1	20	0	1	38
[a] LSD $_{(0.05)}$							8					

[a] species x rate x herbicide LSD $_{(0.05)}$.

Table 3.2. Visual injury of ornamental, fruit, and nut plant species in response to driftable fractions of 2,4-D and dicamba 56 days after treatment.

	Fraction of 1X Use Rate											
	2,4-D			2,4-D + glyphosate			Dicamba			Dicamba + glyphosate		
Species	1/200	1/20	1/2	1/200	1/20	1/2	1/200	1/20	1/2	1/200	1/20	1/2
	------------------- Visual Injury (%) -------------------											
Apple	1	2	12	0	4	25	1	4	27	2	8	40
Crabapple	0	3	17	0	5	75	0	4	11	1	4	16
Dogwood	1	9	75	2	41	91	1	19	65	6	38	97
Elderberry	1	12	58	2	9	81	4	20	63	2	26	62
Elm	0	6	35	0	11	68	0	6	33	0	9	41
Grape	7	26	86	12	59	100	3	25	61	5	30	69
Hydrangea	0	1	5	1	1	24	0	1	4	0	1	25
Maple	1	4	11	0	3	26	7	19	51	3	29	57
Oak	4	14	38	3	15	80	5	22	43	5	24	56
Peach	0	1	23	0	3	40	2	15	57	2	25	70
Pecan	2	11	36	6	16	60	1	16	35	3	13	46
Redbud	0	11	23	4	21	56	0	20	31	3	25	53
Rose	0	0	1	0	7	60	0	1	25	0	2	37
Raspberry	0	0	7	0	1	28	0	0	5	0	2	29
Strawberry	0	0	3	0	0	52	0	2	14	0	2	49
Sweetgum	0	1	23	0	2	56	0	1	14	0	4	41
Viburnum	2	4	44	2	12	67	4	22	43	1	11	58
Walnut	0	7	94	0	17	99	0	2	25	0	3	49
[a] LSD $_{(0.05)}$	8											

[a] species x rate x herbicide LSD $_{(0.05)}$.

Table 3.3. Shoot length of ornamental, fruit, and nut plant species in response to driftable fractions of 2,4-D and dicamba 28 days after treatment.

	Fraction of 1X Use Rate													
	Non-treated Control	2,4-D			2,4-D + glyphosate			Dicamba			Dicamba + glyphosate			LSD $_{(0.05)}$[a]
Species	--	1/200	1/20	1/2	1/200	1/20	1/2	1/200	1/20	1/2	1/200	1/20	1/2	
	---------- Shoot Length (cm) ----------													
Apple	37	32	37	29	36	38	26	37	36	30	35	38	22	7
Crabapple	33	27	26	29	23	25	23	28	26	26	29	27	24	7
Dogwood	26	27	27	20	29	25	16	28	27	22	28	23	22	8
Elderberry	40	35	37	29	36	30	28	37	33	25	38	35	30	8
Grape	77	65	71	18	70	39	10	74	58	28	63	67	32	18
Maple	34	39	41	36	33	29	27	36	28	24	32	31	23	9
Peach	68	64	69	58	71	74	48	63	70	51	66	66	47	6
Rose	21	25	25	22	25	25	20	22	25	19	23	22	22	4
Raspberry	40	42	47	48	40	49	34	60	50	48	56	51	29	17
Strawberry	53	52	54	53	61	56	38	51	62	48	56	55	34	15
Viburnum	46	38	41	36	42	36	47	39	39	39	46	33	33	10
Walnut	10	10	8	6	8	8	4	9	10	10	8	11	5	4

[a] species were analyzed separately.

Table 3.4 Shoot length of ornamental, fruit, and nut plant species in response to driftable fractions of 2,4-D and dicamba 112 days after treatment.

	Fraction of 1X Use Rate													
	Non-treated Control	2,4-D			2,4-D + glyphosate			Dicamba			Dicamba + glyphosate			LSD $_{(0.05)}$[a]
Species	--	1/200	1/20	1/2	1/200	1/20	1/2	1/200	1/20	1/2	1/200	1/20	1/2	
	---------- Shoot Length (cm) ----------													
Dogwood	35	35	40	22	40	28	21	39	34	22	40	33	21	11
Elderberry	45	35	38	31	35	46	31	42	39	25	40	34	28	12
Elm	54	42	48	39	54	48	40	54	47	42	46	47	43	11
Grape	86	98	116	19	93	61	7	99	89	47	92	81	39	26
Maple	48	60	56	50	45	47	35	50	34	29	43	37	29	11
Oak	22	23	22	20	26	27	15	25	30	22	23	23	22	7
Peach	71	68	75	61	76	79	55	67	75	47	72	66	43	7
Pecan	6	6	5	4	6	7	2	7	5	7	6	6	6	3
Rose	30	33	31	28	29	30	23	27	31	26	29	29	26	5
Raspberry	108	97	89	96	87	90	86	95	90	98	110	103	80	25
Strawberry	55	49	49	59	48	38	34	57	51	46	57	54	31	21
Viburnum	49	39	45	36	47	38	45	43	38	45	52	38	36	10
Walnut	12	14	12	5	12	10	5	11	11	14	9	12	7	4

[a] species were analyzed separately.

Table 3.5. Leaf length:width ratio of ornamental, fruit, and nut plant species in response to driftable fractions of 2,4-D and dicamba 28 days after treatment.

	Fraction of 1X Use Rate													
	Non-treated Control	2,4-D			2,4-D + glyphosate			Dicamba			Dicamba + glyphosate			LSD $_{(0.05)}$[a]
Species	--	1/200	1/20	1/2	1/200	1/20	1/2	1/200	1/20	1/2	1/200	1/20	1/2	
	---- Leaf length:width ratio (cm) ----													
Apple	3.3	3.5	3.4	3.5	3.2	3.5	3.7	3.2	3.2	3.8	3.7	3.1	5.3	1.0
Crabapple	1.4	1.4	1.7	1.4	1.4	1.7	1.5	1.4	1.6	1.6	1.6	1.6	1.9	0.5
Elderberry	4.8	4.6	4.3	2.6	4.0	4.3	2.1	4.3	3.4	3.4	4.0	4.2	0.7	2.0
Elm	2.4	2.2	2.4	0.9	2.6	2.3	0.8	2.0	2.5	2.4	2.5	2.3	2.1	0.8
Grape	1.3	1.4	1.6	0.2	1.3	0.9	0.2	1.2	1.2	0.7	1.4	1.3	1.0	0.3
Oak	1.8	2.2	2.4	1.7	2.0	2.1	0.2	2.1	2.9	4.1	2.2	2.8	2.0	0.9
Peach	9.0	9.2	9.1	10.7	9.0	8.6	3.9	8.4	8.6	5.0	8.6	10.3	0.8	2.1
Pecan	2.7	2.9	2.9	3.8	3.1	3.0	3.3	3.2	3.1	4.9	3.2	3.0	3.6	0.9
Rose	2.5	2.2	2.1	4.1	2.3	2.2	1.2	2.4	2.1	3.0	2.1	2.2	2.2	1.5
Raspberry	2.2	2.0	2.0	2.0	2.3	2.3	4.7	2.1	2.2	2.1	2.1	2.2	3.3	1.4
Sweetgum	0.9	0.8	0.8	1.1	0.8	0.8	1.3	0.8	0.9	0.9	0.8	0.8	1.1	0.2
Walnut	2.9	2.7	2.5	2.0	2.6	2.8	1.5	2.6	2.4	3.6	2.4	2.7	2.5	0.8

[a] species were analyzed separately.

Table 3.6. Tree trunk diameter growth of ornamental, fruit, and nut tree species in response to driftable fractions of 2,4-D and dicamba 112 days after treatment.

	Fraction of 1X Use Rate													
	Non-treated Control	2,4-D			2,4-D + glyphosate			Dicamba			Dicamba + glyphosate			LSD $_{(0.05)}$[a]
Species	--	1/200	1/20	1/2	1/200	1/20	1/2	1/200	1/20	1/2	1/200	1/20	1/2	
	Trunk Diameter Growth (mm)													
Apple	4.9	3.8	4.3	4.0	4.0	4.0	4.0	4.0	3.8	3.0	4.3	4.8	2.2	1.2
Crabapple	5.3	4.6	5.3	4.6	4.8	4.0	1.3	4.8	5.3	4.3	4.8	4.1	3.9	1.8
Dogwood	4.3	4.2	5.0	2.8	5.0	3.2	0.3	4.5	3.3	1.5	4.5	3.4	0.5	1.9
Elm	5.1	5.6	4.7	3.4	5.0	5.2	2.5	5.1	4.9	4.6	4.9	4.5	3.6	1.4
Maple	6.3	5.7	5.8	5.7	5.2	6.1	6.2	5.7	4.3	4.3	6.7	4.8	4.5	1.8
Oak	6.7	5.8	6.4	5.4	7.6	5.7	1.9	5.2	4.5	4.8	5.3	5.3	4.4	1.6
Peach	6.1	5.2	5.3	4.0	5.4	5.4	5.4	5.3	5.4	3.2	5.3	5.0	3.6	1.4
Redbud	3.0	3.0	2.3	3.0	3.2	2.9	1.6	2.7	1.6	2.2	2.0	1.7	2.0	1.3
Walnut	2.9	1.8	1.5	0.9	2.1	1.3	0.0	2.9	3.1	2.1	2.3	2.6	0.8	1.4

[a] species were analyzed separately

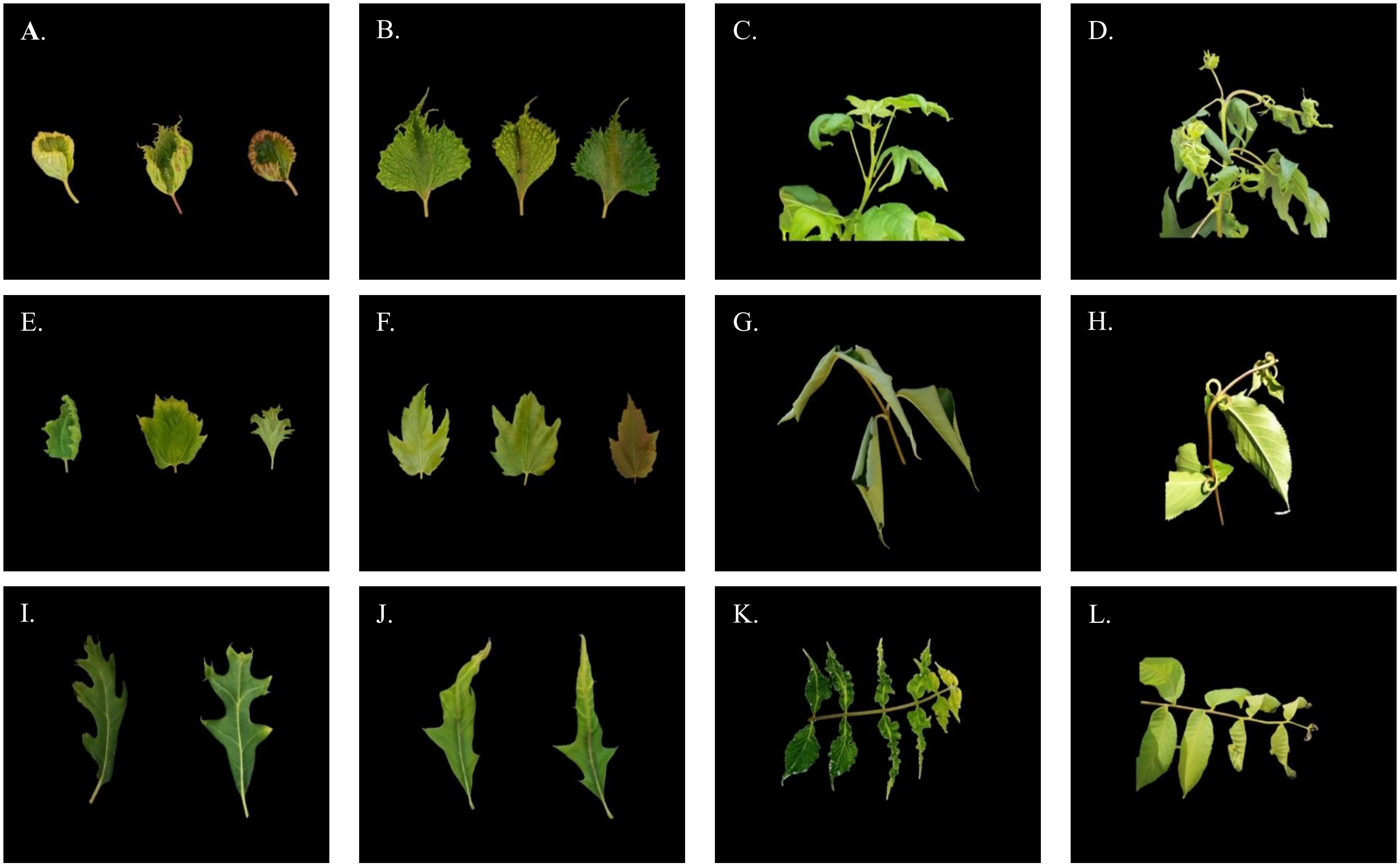

Figure 3.1. Symptomology differences between dicamba (A,C,E,G,I, and K) and 2,4-D (B,D,F,H,J, and L) on various ornamental, fruit, and nut tree species. The response to 1/20X rates of dicamba and 2,4-D is shown for grape (A and B), maple (E and F), and pin oak (I and J), while the response to 1/2X rates of dicamba and 2,4-D is shown for sweetgum (C and D), viburnum (G and H), and walnut (K and L).

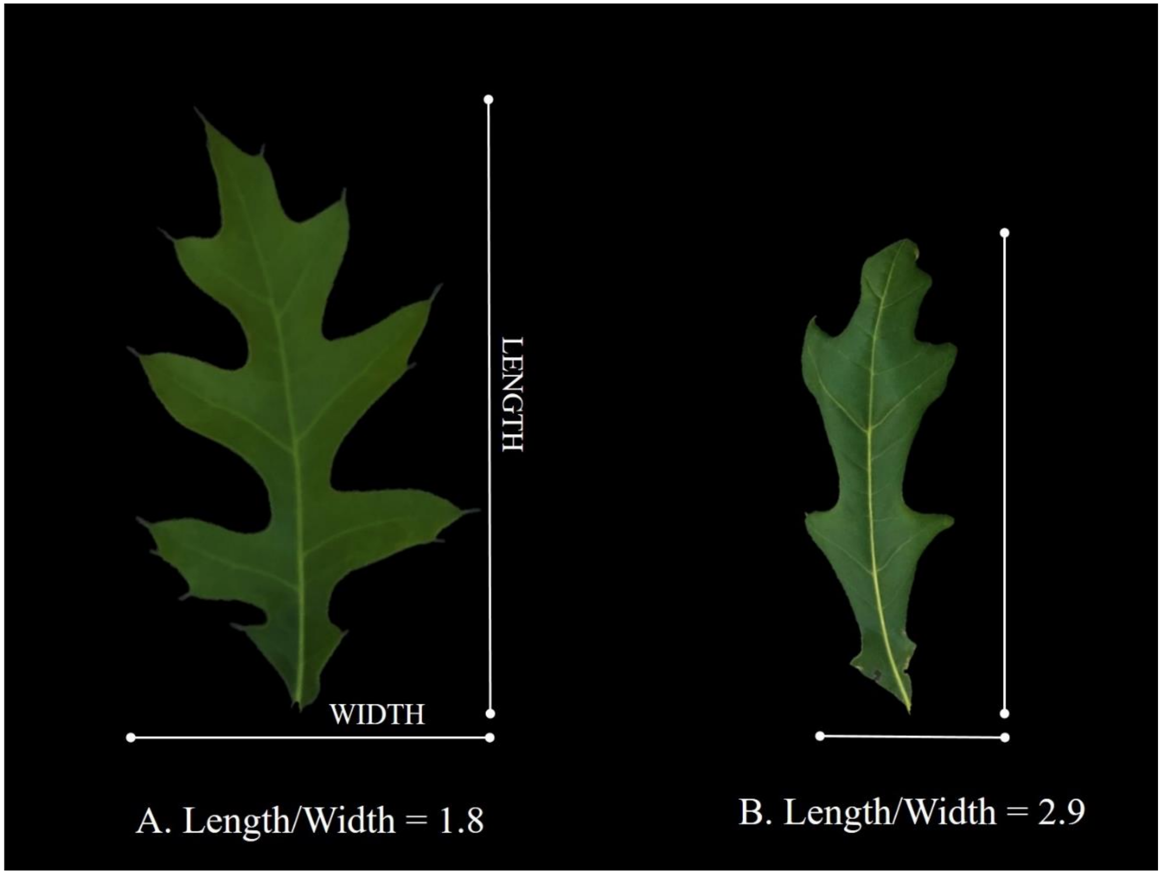

Figure 3.2. Increased leaf length:width ratio in response to 1/20X treatment of dicamba on pin oak. The length:width ratio is determined by dividing overall leaf length by width. **(A.)** Non-treated control. **(B.)** 1/20X dicamba.

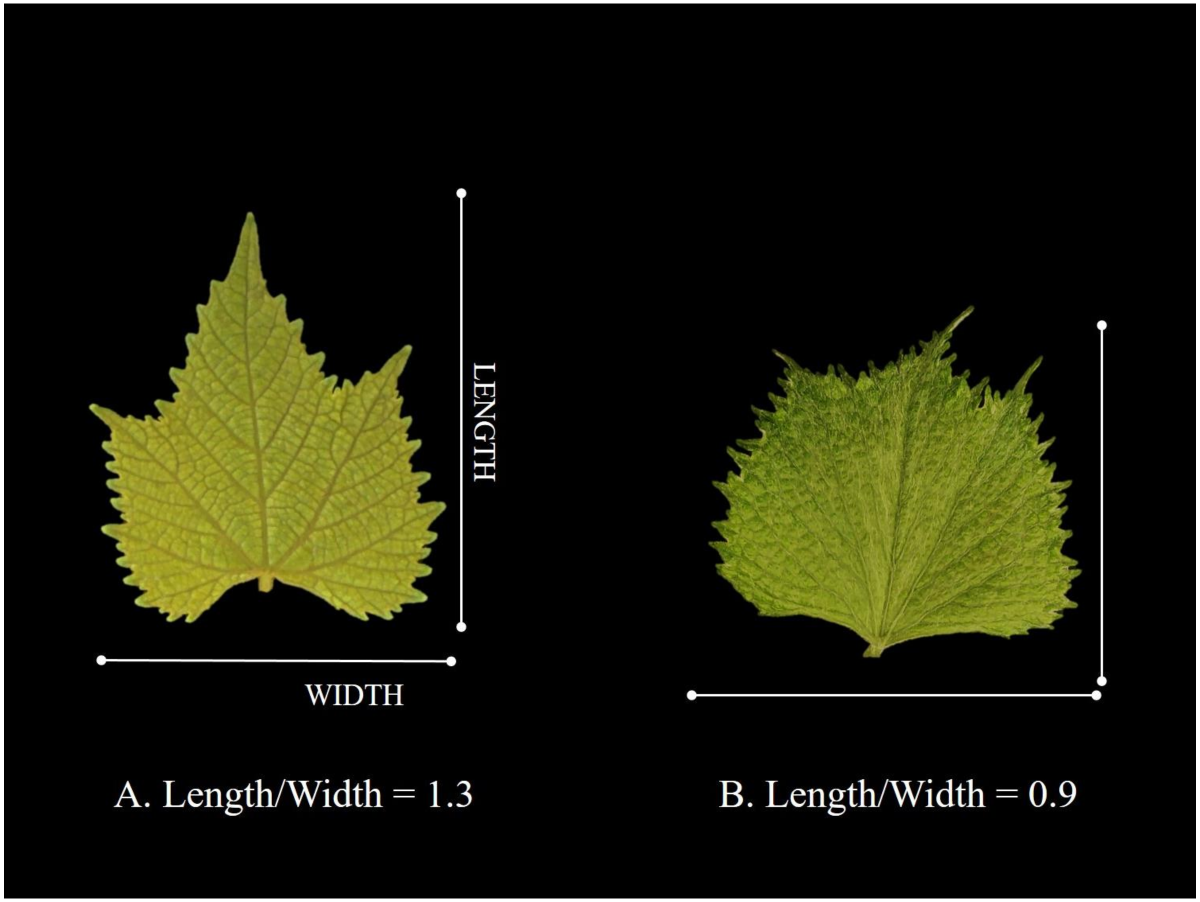

Figure 3.3. Decreased leaf length:width ratio in response to 1/20X treatment of 2,4-D plus glyphosate on grape. The length:width ratio is determined by dividing overall leaf length by width. **(A.)** Non-treated control, **(B.)** 1/20X 2,4-D plus glyphosate.