# EFFECTS OF TANK-CONTAMINATION WITH DICAMBA AND 2,4-D ON SUGARBEET

By

Michael Anthony Probst

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## ABSTRACT

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The use of dicamba- and 2,4-D-resistant soybean will lead to increased use of both dicamba and 2,4-D in Michigan cropping systems. In turn, the potential for sensitive crop exposure to these herbicides by tank-contamination will be greater, which is of great concern to sugarbeet growers. Field experiments were conducted at two locations in Michigan in 2016 and 2017 to determine how tank-contamination with dicamba and 2,4-D impacts sugarbeet growth, yield, and quality when applied at multiple growth stages. Five rates of dicamba and 2,4-D were applied at the 2-, 6-, and 14-leaf growth stages. Herbicide injury to sugarbeet was detected at all application timings with dicamba and 2,4-D rates ranging from 2.8-22.4 g ae $ha^{-1}$. At a rate of 22.4 g $ha^{-1}$, 2,4-D reduced yield and recoverable white sucrose $ha^{-1}$ (RWSH) at both locations, while the same rate of dicamba reduced yield and RWSH at one location. No reductions in yield or RWSH were found at any other rate of either herbicide. Applications of 2,4-D at the 14-leaf growth stage resulted in lower yields compared with applications at the 2- and 6-leaf growth stages. In addition, herbicide residues of 2,4-D and dicamba were found to decline following exposure. By harvest, dicamba and 2,4-D residues were below 10 ppb. Unfortunately, there is no maximum residue limit established for either herbicide in sugarbeet, meaning that any level of residues found is unacceptable. The rates necessary to cause yield and RWSH reductions in sugarbeet would typically be associated with improper spray-system cleanout. This further stresses the importance for proper cleanout, as sugarbeet exposure to low rates of dicamba and 2,4-D do not always equate to yield or quality loss.

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# REVIEW OF LITERATURE

## Introduction

Sugarbeet (*Beta vulgaris* L.) is an important commercial crop grown in Michigan as well as other regions throughout the United States. Today's sugarbeet can be traced back to beets that were domesticated in the Mediterranean region in the second and third century B.C.; however, it was not discovered that beets could be used for the production of sucrose until the mid-1800s (Winner 1993). Sugarbeet is a biennial plant that is grown as an annual crop. In Michigan, sugarbeet is typically planted in April, and the roots are harvested in the fall (September – October) following the mechanical removal of the aboveground tissue. Once harvested, the roots are transported to a processing facility, where sucrose is extracted from the roots, and products such as beet pulp and pure crystalline sugar are produced. Of the 130 million metric tons of sucrose produced globally, 35% comes from sugarbeet, and in the United States, sugarbeet accounts for 50-55% of the 8.4 million metric tons of sucrose produced (Panella et al. 2009). In 2017, sugarbeet was planted on approximately 457,000 ha in the United States. Of that, 58,000 ha were planted in Michigan, ranking fourth behind Minnesota, North Dakota, and Idaho (NASS 2018). Total sugarbeet production in the United States in 2016 was valued at 1.3 billion dollars, while Michigan sugarbeet production that same year was valued 135 million dollars (NASS 2018).

## Weed Control in Sugarbeet

Successful weed control is a critical component of sugarbeet production. Weeds must be controlled in a timely and effective manner, or serious yield reductions can occur. Scott et al. (1979) found that uncontrolled weeds resulted in yield reductions as high as 96%. They also found that delaying weed control longer than 4 weeks after crop emergence resulted in unrecoverable yield loss. Length of weed control to prevent yield loss was variable, but as long as weed dry weights did not exceed 120 g $m^{-2}$, sugar yields were unaffected (Scott et al. 1979). Alternatively, Kemp et al. (2009) reported 66 and 67% yield loss to glyphosate- and glufosinate-resistant sugarbeet, respectively, when weeds were not controlled. The critical time of weed removal ranged from 8 to greater than 11 weeks after planting, while the critical weed-free period ranged from 1.5 to 6.5 weeks after planting, assuming a 10% acceptable level of yield loss (Kemp et al. 2009). Spangler et al. (2014) found that maximum sugarbeet yields were more likely when weeds were controlled prior to reaching 2 cm, while 30 cm tall weeds reduced sugarbeet yield by as much as 21%. They also determined that early-season weed control was crucial, as weeds were more competitive for nitrogen early in the growing season (Spangler et al. 2014). Armstrong and Sprague (2010) came to similar conclusions, as they determined sugarbeet yield was 7-21% greater when controlling weeds with glyphosate prior to reaching 2 cm compared with weeds controlled at 15 cm.

Weed type and density also influences the level of sugarbeet yield loss. A broadleaf weed mixture containing redroot pigweed (*Amaranthus retroflexus* L.), kochia (*Kochia scoparia* L.), and common lambsquarters (*Chenopodium album* L.) was capable of reducing sugarbeet yield 13% at a density of 6 plants per 30 meters of row, whereas a density of 24 plants per 30 meters of row reduced yield 39% (Schweizer 1981). Mesbah et al. (1994) found that 1.5 kochia plants

per meter of row proved to be more competitive than 6 green foxtail (*Setaria viridis* L.) plants per meter of row, reducing yield up to 67 and 37%, respectively. However, the combination of these two weeds at the same densities reduced sugarbeet yield up to 83%. Common lambsquarters and Powell amaranth (*Amaranthus powellii* S. Wats.), both at a density of 24 plants per 30 meters of row, reduced sugar beet root yield by 48 and 24%, respectively (Schweizer 1983;Schweizer and Lauridson 1985). With such a wide variety of weed types capable of causing serious yield reductions, effective weed control in sugarbeet is essential.

Prior to the release of glyphosate-resistant (GR) sugarbeet, weed control in sugarbeet production consisted of diverse programs including multiple applications of both PRE and POST herbicides along with in-crop cultivations (Dale et al. 2006). PRE herbicides used in sugarbeet included cycloate, pyrazon, ethofumesate, and EPTC, while POST herbicides included desmedipham, phenmedipham, triflusulfuron, and clopyralid (Dale et al. 2006; Dexter 1998; Wilson et al. 2005). Unfortunately, several problems with these systems arose: high clay content and high soil organic matter could cause PRE herbicides to be less effective, while initial labeled rates of POST herbicides like desmedipham (1.1-1.4 kg ai $ha^{-1}$) and phenmedipham (1.1-1.7 kg ai $ha^{-1}$) often caused severe injury to sugarbeet (Dale et al. 2006; Dexter 1994). Because of this, weed management practices shifted towards the use of reduced rates of multiple POST herbicides applied several times throughout the season. According to Dexter (1994), a split application of desmedipham at half the labeled rate (0.56 kg $ha^{-1}$) resulted in 83% weed control and 39% herbicide injury to sugarbeet compared with 66% weed control using a single application of the full rate. A split application of the quarter rate (0.28 kg $ha^{-1}$) of desmedipham reduced sugarbeet injury from 49% using the single full rate to 8% without sacrificing weed control. Split applications of desmedipham plus phenmedaphim at half the labeled rate provided

87% control of redroot pigweed while only injuring sugarbeet 5%, and the three-quarter rate split application provided 98% redroot pigweed control and injured sugarbeet 15% (Dexter 1994). Another study found that the addition of a methylated seed oil to POST split-applications of reduced-rate herbicide tank-mixtures tended to increase weed control, but sugarbeet injury increased along with it (Wilson et al. 2005). With the increased adoption of POST split applications, the use of PRE herbicides sharply declined in Minnesota and North Dakota. By the mid-1980s, less than 5% of the sugarbeet hectares in these states received PRE herbicides (Dexter and Luecke 2003). Michigan was slow to follow suit, as the issues associated with PRE herbicides in North Dakota and Minnesota were not present in Michigan. That is, however, until the introduction of the micro-rate herbicide program in 1998. The micro-rate consisted of desmedipham plus phenmedipham at 0.045 + 0.045 kg $ha^{-1}$ plus triflusulfuron at 0.004 kg ai $ha^{-1}$ plus clopyralid at 0.023 kg ai $ha^{-1}$ plus methylated seed oil at 1.5% v $v^{-1}$ applied multiple times throughout the growing season (Dale et al. 2006). Michigan sugarbeet growers were quick to adopt the micro-rate program, as 60% of sugarbeet hectares received micro-rate applications in 2002; alternatively, the use of PRE herbicides on sugarbeet in Michigan declined from greater than 90% before 2000 to 75% in 2001 and 60% in 2002 (Dale et al. 2006). Dale et al. (2006) compared weed control and sugarbeet injury of the micro-rate herbicide program (consisting of 4 applications) against standard split-applications (consisting of 2 applications), both with and without ethofumesate PRE in Michigan. They found that in dry conditions (2001), sugarbeet injury was less than 6% for all treatments, but injury ranged from 29 to 43% in wetter conditions (2002). Both micro-rate programs, along with the standard split-application including ethofumesate, resulted in similar injury of at least 38%. No differences in weed control were found in 2001, but the addition of ethofumesate to both the standard-split and micro-rate

programs increased common lambsquarters control in 2002 (Dale et al. 2006). Neither Dexter (1994) testing split applications nor Dale et al. (2006) testing both split applications and micro-rates were able to achieve 100% weed control consistently, meaning that surviving weeds would be capable of producing seed unless removed through alternative means. Bollman and Sprague (2009) found that the use of a PRE herbicide followed by four POST micro-rate applications increased control of several weed species, but 100% weed control was still not achieved. Field studies in Michigan tested the use of the soil residual herbicides *s*-metolachlor and dimethenamid-p in combination with micro-rate applications and found that weed control increased compared with the standard micro-rate program. However, sugarbeet injury also had the tendency to increase with the inclusion of the soil residual herbicides, even at reduced rates split throughout the season (Bollman and Sprague 2007). Despite differences in weed control and sugarbeet injury, there have been few differences in recoverable white sucrose between herbicide programs (Bollman and Sprague 2007; Bollman and Sprague 2009; Dale et al. 2006). Another common theme among these weed control programs is the requirement of multiple herbicide applications throughout a single growing season.

With the commercial release of glyphosate-resistant (GR) sugarbeet in 2007, the approach to weed control in sugarbeet shifted drastically. Glyphosate is a non-selective herbicide capable of controlling a broad spectrum of weed species, including perennial weed species (Franz et al. 1997; Baylis 2000). The implementation of GR crops, including sugarbeet, allowed glyphosate to be applied to naturally susceptible crops to control weeds without the concern of seriously damaging the crop (Dill 2005). Madsen and Jensen (1995) found that glyphosate proved to be the more potent and effective method of weed control when compared with a more conventional mixture of phenmedipham, ethofumesate, and metamitron. This is in agreement with the findings

of Kemp et al. (2009), who found that glyphosate provided better weed control of *Amaranthus* sp. and common lambsquarters along with greater root yield compared with a conventional POST herbicide program. They also found that weed control only increased in certain instances when a PRE was used followed by glyphosate, and there were no differences in yield (Kemp et al. 2009). Field studies conducted in Oregon found that single applications of glyphosate to 2-4 leaf sugarbeet could not provide consistent weed control, and yields did not consistently match those of a standard herbicide program; however, multiple applications of glyphosate throughout the season provided excellent weed control while consistently achieving similar or greater yields than the standard program (Guza et al. 2002). Guza et al. (2002) also found that a single application of glyphosate in combination with dimethenamid-P at the 2-4 leaf stage achieved similar weed control and yields as the standard program that required four herbicide applications, while treatments in which glyphosate was combined with ethofumesate could cause sugarbeet injury and reduce yield. Contrary to Bollman and Sprague's (2007) findings, Guza et al. (2002) did not see any significant injury to sugarbeet with the inclusion of dimethenamid-P applied POST. These discrepancies could be attributed to several different factors between Oregon and Michigan, including soil type and higher humidity in Michigan. Wilson et al. (2002) came to more definitive conclusions in their field studies in Nebraska, where they found that a single application of glyphosate did not sufficiently control weeds and resulted in yield reductions. The addition of a second glyphosate application decreased overall weed density and increased yield, but a third application of glyphosate did not improve upon this. Glyphosate programs with two applications achieved similar weed control and 15% greater sucrose yields when compared with three passes of desmedipham plus phenmedipham, triflusulfuron, and clopyralid (Wilson et al. 2002). Kniss et al. (2004) reached similar conclusions, finding that two applications of

glyphosate could achieve 99% weed control, while conventional programs achieved a maximum of 95% weed control.

Along with the potential for reduced applications and decreased risk of injury, there are also economic benefits associated with a GR sugarbeet system. While conventional weed control systems in sugarbeet averaged \$336 $ha^{-1}$ in 2001, two applications of glyphosate, along with the technology fee for GR seed, costs \$188 $ha^{-1}$, which would have saved growers \$148 $ha^{-1}$ in 2001 (Gianessi 2005). Kniss et al. (2004) determined that switching from a conventional or micro-rate program to glyphosate-resistant sugarbeet could result in \$435 $ha^{-1}$ greater net return, attributing this drastic increase to reduced input costs and increased sucrose content associated with the glyphosate weed control system. Dillen et al. (2013) stated that the adoption of glyphosate-resistant sugarbeet saves growers an average of \$257 $ha^{-1}$.

## Glyphosate-Resistant Weeds and Control with Dicamba and 2,4-D

Following their introduction in 1996 through 2001, over 185 million hectares of GR crops have been planted in the United States, and the use of glyphosate has increased from 1.8 million kg in 1990 to 45 million kg in 2005 (Gianessi 2005; Gianessi 2008). These values continued to increase, as 125 million kg of glyphosate was used in 2014, with 90% of that being agricultural use (Benbrook 2016). The effectiveness and simplicity of GR crops allowed growers to completely rely on glyphosate for weed control, but this heavy reliance on a single herbicide resulted in strong selection pressure for weeds possessing GR traits (Powles 2008; Young 2006; Owen 2008). There are currently 37 weed species worldwide that have developed resistance to glyphosate, with 17 of those species present in the United States (Heap 2018). Several of these species, including horseweed (*Conyza canadensis* L.), waterhemp (*Amaranthus tuberculatus* var.

*rudis* L.), Palmer amaranth (*Amaranthus palmeri* S. Watson), are very problematic weeds in a range of cropping systems (Powles 2008). In response to these GR weeds, new herbicide resistance technologies have been developed: specifically, dicamba and 2,4-D resistance in soybean (*Glycine max* L.) (Powles 2008; Behrens et al. 2007; Wright et al. 2010).

Both dicamba and 2,4-D are auxin mimics that selectively control a range of broadleaf species (Grossman 2003). The ability to use these herbicides in soybean, a crop that is naturally sensitive to both, would allow for increased control of several GR weeds (Behrens et al. 2007). Soybean resistance to dicamba and 2,4-D was achieved through the insertion of resistance genes: dicamba monooxygenase for dicamba and the aryloxyalkanoate dioxygenase enzyme AAD-12 for 2,4-D (Behrens et al. 2007; Wright et al. 2010). Dicamba resistance in soybean has been stacked with glyphosate resistance, while 2,4-D resistance has been stacked with both glyphosate and glufosinate resistance. Dicamba-resistant soybean were first commercialized in 2016 with PRE and over the top dicamba applications allowed in 2017, while 2,4-D-resistant soybean have yet to be commercially released.

Dicamba and 2,4-D have been shown to be effective against waterhemp and Palmer amaranth. The addition of 2,4-D to a POST application of glufosinate increased waterhemp control by 11-25% compared with glufosinate applied alone to soybean resistant to both herbicides (Craigmyle et al. 2013), while 2,4-D alone provided 94% control of waterhemp at a rate of 1,120 g ae $ha^{-1}$ (Robinson et al. 2012). Merchant et al. (2013) noted similar results, finding that combinations of glufosinate plus 2,4-D or glufosinate plus dicamba increased Palmer amaranth control compared with each of those herbicides applied alone. Field studies conducted by Joseph et al. (2017) found that two POST applications of glyphosate plus 2,4-D provided 98 to 100% control of GR Palmer amaranth, while greenhouse studies conducted by Norsworthy et

al. (2008) found that both 2,4-D and dicamba provided at least 97% control of GR Palmer amaranth. In addition, both single and sequential applications of dicamba decreased Palmer amaranth density and increased cotton (*Gossypium hirsutum*) yield compared with a glyphosate-only system and a non-dicamba system (Cahoon et al. 2015).

Much like with waterhemp and Palmer amaranth, combining dicamba or 2,4-D with other herbicides can result in greater control of GR horseweed. Combinations of glyphosate plus 2,4-D and glyphosate plus dicamba provided 99% control of GR horseweed, while combining glufosinate with either dicamba or 2,4-D provided greater than 90% control of GR horseweed (Chahal and Johnson 2012; Steckel et al. 2006). Additionally, Kruger et al. (2010) found that both 2,4-D and dicamba were capable of providing 90% or greater control GR horseweed. Greenhouse studies by Flessner et al. (2015) determined that the presence or absence of glyphosate did not influence the level of GR horseweed control with dicamba, indicating that dicamba was the only effective active ingredient in a dicamba-glyphosate tank-mixture. Contrary to most studies, Byker et al. (2013) found that control of GR horseweed with a glyphosate-dicamba mixture ranged from 65 to 97%, indicating that there is some variability in the control of horseweed using POST applications of dicamba. Certain populations of horseweed have also been shown to have a heightened tolerance to 2,4-D and can produce seed after exposure to labeled rates (Kruger et al. 2010). Despite these contradictions, it appears that dicamba and 2,4-D are still effective options for control of GR horseweed.

As GR weeds continue to become more of an issue, technologies such as dicamba and 2,4-D resistant soybean will become influential tools for diversifying the approach to weed management (Powles 2008). However, as the use of this technology expands, dicamba and 2,4-D

use will increase as well and be implemented in entirely new ways. While this is exciting from a weed management standpoint, it poses serious concerns as well.

## Effects of Dicamba and 2,4-D on Sensitive Crops

Dicamba and 2,4-D are two of the oldest chemical herbicides: 2,4-D was discovered and patented in the mid-1940s, and dicamba was later discovered in 1958 (Peterson 1967; Shaner 2014). Despite their age, these herbicides still maintain significance as effective herbicides for achieving selective control of broadleaf weeds, especially with the recent development of transgenic crops expressing resistance to dicamba and 2,4-D (Behrens et al. 2007; Peterson et al. 2016). The fact that both dicamba and 2,4-D degrade quickly in the soil, have limited mobility, and have relatively low health risks make them very desirable herbicides, and although they are classified in different chemical families, dicamba and 2,4-D are both auxin mimicking herbicides, meaning they have the same mode of action for killing sensitive plants (Peterson et al. 2016; Shaner 2014). Auxin mimics affect sensitive plants by overloading their auxin receptors, leading to several different physiological and morphological reactions. The high concentration of auxins activates the expression of genes that promote ethylene biosynthesis, which causes abnormal tissue growth such as epinasty, swelling, and curling (Grossman 2010). The enzymes involved in the cell elongation process are also overproduced and growth inhibition occurs in both the root and shoot, while reduced transpiration from stomatal closure, carbon assimilation, and the over-production of reactive oxygen species lead to the phytotoxic effects that eventually cause plant death (Grossman 2010). While these herbicides are effective for controlling sensitive weed species, they can be detrimental when exposed to sensitive non-target species.

There are multiple avenues through which sugarbeet can be exposed to dicamba and 2,4-D including volatilization, particle drift, and spray system contamination (Behrens and Lueschen 1979; Boerboom 2004; Cundiff et al. 2017). Volatilization is the conversion of the herbicide into a gaseous state that is released into the atmosphere and can then move to another crop. Dicamba has been shown to degrade to the free acid form following application and, at a vapor pressure of 200000 mm Hg at 25 C, volatilize into a gaseous form (Behrens and Lueschen 1979). In addition, butyl ester formulations of 2,4-D have been shown to be extremely volatile, while the dimethylamine and choline salt formulations are much less volatile (Grover et al. 1972; Sosnoskie et al. 2015). Particle drift is the movement of herbicide particles by wind. There are many different factors that can contribute to the extent of particle drift, including wind speed, boom height, speed of the sprayer, and spray particle size. Spray system contamination is when a herbicide or herbicides from a previous application remain in the spray system and are then unintentionally applied to a non-target crop. This can result from the lack of or improper cleanout of the spray tank and sprayer booms, as well as tanks, hoses, and lines involved in the mixing and transportation of the spray solution. Boerboom (2004) detected 0.021-0.63% of the field use rate of dicamba in a sprayer following proper cleanout procedures that consisted of draining and washing the system and flushing with an ammonia-water solution. Another study that simulated an in-field tank cleanout following application with 0.56 kg $ha^{-1}$ of dicamba found that approximately 0.3% of the field use rate remained within the hoses (Cundiff et al. 2017). This same study also found that PVC polyurethane hoses and synthetic rubber hoses had greater retention of dicamba than polyethylene hoses, and these differences were attributed to imperfections found within the PVC polyurethane and synthetic rubber hoses that were not found within the polyethylene hoses. Steckel (2010) stated that dicamba and 2,4-D have a greater

likelihood of adhering to plastic and rubber sprayer parts, making cleanout more difficult. Higher rates than what Boerboom (2004) and Cundiff et al (2017) found are feasible if proper cleanout is not used, or if herbicides get left in sections of the boom that can lead to spikes in the concentration of these herbicides. These low rates, as well as the low rates of herbicides typically associated with volatility and particle drift, could be enough to impact sensitive crops, including sugarbeet.

**Soybean**. Numerous studies have been conducted to evaluate the negative effects of dicamba and 2,4-D on non-dicamba or non-2,4-D resistant soybean. Based on regression analysis, the rate of 2,4-D that caused 20% soybean injury was 29 and 109 g ae $ha^{-1}$ at the V5 and R2 growth stages, respectively (Robinson et al. 2013a). In addition, Andersen et al. (2004) found that a 2,4-D rate as low as 56 g $ha^{-1}$ was enough to cause 25% injury to soybean. In another study, 28 g $ha^{-1}$ of 2,4-D only caused 3% injury applied at the V3 soybean growth stage and did not impact yield at any stage (Solomon and Bradley 2014). Robinson et al. (2013a) determined that a 10% soybean yield reduction was caused by 149 and 202 g $ha^{-1}$ of 2,4-D at the V5 and R2 growth stages, respectively. The requirement of relatively high rates of 2,4-D to cause significant yield reductions support the findings of Egan et al. (2014), who found that soybean was relatively tolerant to vapor and particle drift of 2,4-D at all growth stages, and that there were rarely yield reductions except in the case of serious exposure ($\geq$56.1 g $ha^{-1}$).

Depending on the growth stage, 0.75 g ae $ha^{-1}$ of dicamba caused 13-23% soybean injury (Soltani et al. 2015). This same study found that yield was reduced 10% by dicamba rates of 11.8 and 2.0 g $ha^{-1}$ applied to V2/V3 and R1 soybean, respectively. Dicamba vapor drift at 0.56 g $ha^{-1}$ reduced soybean yield 1%, while particle drift at 5.6 g $ha^{-1}$ reduced yield 8.7%, with a greater

likelihood for yield loss when soybean was exposed in the reproductive stages (Egan et al. 2014). All dicamba rates caused greater injury in the vegetative growth stages, with the lowest rate of 0.028 g $ha^{-1}$causing 21% injury. At the same time, dicamba applied at a rate of 2.8 g $ha^{-1}$ reduced yield 3.4% and 13.8% at the V3 and R2 growth stage, respectively (Solomon and Bradley 2014). Robinson et al. (2013b) found that yield was impacted at lower rates of dicamba, with 10% yield reduction occurring at rates of 1.1 and 0.53 g $ha^{-1}$ applied at the V5 and R2 growth stages, respectively. Griffin et al. (2013) found that dicamba rates of 4.4 and 17.5 g $ha^{-1}$ caused 2.5 times more yield reduction when applied to soybeans in the R1 growth stage than to soybeans in the V3/V4 growth stage.

Researchers have often found that when comparing injury differences between application timings, dicamba and 2,4-D injured soybean in vegetative growth stages more than soybean in reproductive growth stages (Griffin et al. 2013; Robinson et al. 2013a; Solomon and Bradley 2014; Soltani et al. 2015). Contrary to this trend, other studies found that greater 2,4-D injury occurred during the reproductive stages (Kelley et al. 2005) or that there were no differences in application timing (Auch and Arnold 1978). Although more injury generally occurred in the vegetative stages, greater yield loss occurred to soybean when exposed to dicamba in the reproductive stages (Solomon and Bradley 2014; Robinson et al. 2013b; Griffin et al. 2013; Soltani et al. 2015). Egan et al. (2014) determined through a meta-analysis that sub-lethal rates of dicamba caused greater yield reductions when applied to soybean in the reproductive stages, while soybean were generally tolerant to sub-lethal rates of 2,4-D at all stages.

While soybean yield is more heavily impacted by dicamba in the reproductive stages, some rates that caused yield loss in the vegetative stages are within the aforementioned range of rates

following sprayer cleanout. Based on these studies, however, rates of 2,4-D necessary to reduce yield were typically outside of this range.

**Sugarbeet**. Although not as sensitive to dicamba as soybean, sugarbeet have shown sensitivity to both 2,4-D and dicamba. 2,4-D at rates ranging from 17-280 g $ha^{-1}$ has been shown to cause injury to sugarbeet at all growth stages, with 2,4-D exposure to sugarbeet at earlier stages resulting in stand loss and exposure at later stages causing a reduction in extractable sucrose from the harvested crop (Byford and Prince 1976; Holksvig 1950; Schroeder et al. 1983). Schroeder et al. (1983), concerned with 2,4-D and dicamba drift onto sugarbeet from applications to cereal crops, found that 280 g $ha^{-1}$ of 2,4-D reduced sugarbeet yield by 27% when averaged across multiple application timings. They also found that dicamba ranging from 17-140 g $ha^{-1}$ did not significantly reduce sugarbeet yield. Schweizer (1978) found that sugarbeet top growth was reduced 55% and yield was reduced 10% from 70 g $ha^{-1}$ of 2,4-D applied at the 8-leaf stage. A study in England determine that sugarbeet root yield was reduced by 12% following an application of 70 g $ha^{-1}$ of 2,4-D (Byford and Prince 1976). While these studies did note injury symptoms from both herbicides along with yield reductions from 2,4-D, the relatively high rates necessary for significant effects indicates that sugarbeet have a certain level of tolerance to these herbicides. Unlike soybean, sugarbeet seem to be more sensitive to 2,4-D and have a greater tolerance to dicamba; however, they are similar in that late-stage exposure has a greater impact on yield.

**Other crops**. Along with soybean and sugarbeet, other important broadleaf crops can be impacted due to their sensitivity to dicamba and 2,4-D. Cotton can be injured at low doses of

these herbicides, with potential yield loss. Johnson et al. (2012) found that cotton seed yield was reduced at 20 g $ha^{-1}$ of 2,4-D according to regression analysis. Depending on the growth stage, 2.8 g $ha^{-1}$ of 2,4-D was capable of injuring cotton more than 20% and reducing yield 16-68%, while 28 g $ha^{-1}$ caused 35-40% injury and reduced yield 61-97% (Everitt and Keeling 2009). These results suggest that cotton is much more sensitive to 2,4-D than soybean. Dicamba also has negative effects, as 2-leaf cotton was injured as much as 42% by 28 g $ha^{-1}$ of dicamba, while yield was reduced 10% by rates as high as 14 g $ha^{-1}$ (Everitt and Keeling 2009). Johnson et al. (2012), using regression analysis, found that seed cotton yield was reduced by 41 g $ha^{-1}$ of dicamba. Hamilton and Arle (1979) determined that dicamba rates of 16-64 g $ha^{-1}$ did not reduce cotton boll weight when applied prebloom, but 8 g $ha^{-1}$ reduced boll weight by 9% when applied at bloom. Egan et al. (2014) found that vapor drift exposures to 2,4-D could reduce yield 9-19%, while particle drift exposure could reduce yield 32-33%. They also determined that 56 g $ha^{-1}$ of dicamba would reduce cotton yield by 10% across all growth stages. All studies indicate that cotton, unlike soybean, is more sensitive to 2,4-D than dicamba, but both herbicides are capable of causing severe injury and reducing yield at very low doses.

Like the crops already discussed, other broadleaf crops have the potential to be affected by dicamba and 2,4-D. Tomato plants exposed to 13.44 g $ha^{-1}$ of 2,4-D experienced a 93% yield reduction (Fagliari et al. 2005). Johnson et al. (2012) found that peanut was more tolerant of 2,4-D than dicamba, as 41 g $ha^{-1}$ of dicamba and 269 g $ha^{-1}$ of 2,4-D were the lowest rates that reduced yield. Prostko et al. (2011) determined that 40 g $ha^{-1}$ of dicamba reduced peanut yield anywhere from 2-29% and 560 g $ha^{-1}$ reduced yield from 23-100%, depending upon the location. Dicamba at a rate of 2.8 g $ha^{-1}$ was capable of injuring potatoes up to 30%, while 22.2 g $ha^{-1}$ reduced tuber yields by 40% and marketable yields by 74% (Wall 1994). It is clear that both

dicamba and 2,4-D can negatively impact sensitive crops by causing both injury and yield reductions. While sugarbeet may differ in the level of sensitivity to these herbicides, it is safe to say that growers should be concerned about their sugarbeet crops being exposed to dicamba and 2,4-D.

## Dicamba and 2,4-D Residues in Susceptible Crops

Aside from potential injury and yield reduction, there is another level of concern related to tank-contamination with dicamba and 2,4-D on sugarbeet. Because sugarbeet is a food crop, the level of herbicide residue within the harvested crop is extremely important. Sugarbeet have shown the ability to translocate herbicides to the root. Multiple foliar applications of labeled herbicides resulted in the detection of herbicide residues in the harvested root, although these levels did not exceed the maximum limit (Kucharski 2009). Reduced rates of these herbicides caused reductions in the residue level of harvested beets, but the residues were still detectable (Kucharski et al. 2008). Contrary to these findings, when ethofumesate was both soil- and foliar-applied, the herbicide only moved through the xylem, resulting in no translocation to the roots (Eshelb 1978).

Multiple studies have been conducted to determine the persistence of dicamba and 2,4-D residues in soybean. Dicamba and 2,4-D herbicide residues were still detectable in soybean foliage 48 days after treatment (DAT) when applied at rates of 5.6-56 g $ha^{-1}$ and 11.2-112 g $ha^{-1}$, respectively (Andersen et al. 2004). Auch and Arnold (1978) found dicamba residues at approximately 900 parts per billion seven days after an application of 56 g $ha^{-1}$, but this level was down to zero 11 days later. At the same time, Boerboom (2004) was not able to detect any dicamba residue in soybean foliage three days after simulating drift. While there is a lack of

information regarding the persistence of dicamba and 2,4-D residues in sugarbeet, the activity of these herbicides gives cause for concern. Both dicamba and 2,4-D move within the phloem of plants, meaning that translocation to sugarbeet roots is likely to occur. This could pose a serious issue to sugarbeet growers, as there is currently no maximum residue limit (MRL) set for either dicamba or 2,4-D in sugarbeet. Because of this, any detectable level of either herbicide in the harvested crop would result in rejection. Several crops have established MRLs for dicamba and 2,4-D. The soybean seed MRLs for dicamba and 2,4-D are 10 and 0.02 parts per million (ppm), respectively, while molasses from sugarcane has MRLs for dicamba and 2,4-D of 5.0 and 0.2 ppm, respectively (Cornell Law School 2016; Cornell Law School 2017). In addition, the MRL for 2,4-D in root vegetables (excluding potato) is 0.1 ppm, while potato itself is 0.4 ppm (Cornell Law School 2017). Additional MRLs for other crops can be found in Table 1.1. The establishment of MRLs for dicamba and 2,4-D in sugarbeet would be extremely beneficial to growers, as exposure to the herbicides would not necessarily indicate that residues will still be present above the MRL at harvest.

While the release of dicamba- and 2,4-D-resistant soybean will provide growers with increased options to selectively control GR weeds in soybean, the increased use in dicamba and 2,4-D is cause for concern to growers of sensitive crops, including sugarbeet. The risk of exposure to these herbicides will increase with their expanded use, leaving sugarbeet growers vulnerable to injured crops as well as potential loss of yield. Along with this, exposure to dicamba and 2,4-D could result in the presence of unacceptable herbicide residues in the harvested crop, resulting in rejection and the loss of profits. Because of this, it is essential to gain a better understanding of the effects that 2,4-D and dicamba have on sugarbeet growth, yield, and

quality. It is also important to determine how long the residues of dicamba and 2,4-D persist in sugarbeet, with the hopes of possibly establishing MRLs for both herbicides in sugarbeet.

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# CHAPTER 2

# EFFECTS OF TANK-CONTAMINATION WITH DICAMBA AND 2,4-D ON SUGARBEET

## Abstract

The recent registrations of soybean varieties resistant to dicamba and 2,4-D will lead to an increased risk of sensitive crop exposure to these herbicides through tank-contamination. This is concerning to sugarbeet growers, as they typically require multiple herbicide applications for effective weed control each year. Field experiments were conducted in 2016 and 2017 in Richville and East Lansing, Michigan to evaluate the effects of dicamba and 2,4-D tank-contamination on sugarbeet growth, yield, and quality. In addition, sugarbeet tissue samples were analyzed to quantify dicamba and 2,4-D residues shortly after exposure and at harvest. Dicamba and 2,4-D rates of 1.4, 2.8, 5.6, 11.2, and 22.4 g ae $ha^{-1}$ were applied to sugarbeet at either the 2-, 6-, or 14-leaf growth stages. These rates were equivalent to 0.125, 0.25, 0.5, 1, and 2% of the field use rate of 1.12 kg $ha^{-1}$ for each herbicide. Herbicide injury to sugarbeet was detected from dicamba and 2,4-D at rates ranging from 2.8 to 22.4 g $ha^{-1}$ at all application timings. Symptomology was typical of plant growth regulator herbicides, consisting of leaf crinkling and malformation and twisted, elongated petioles. At 14 DAT, the effective dose for 20% injury ($ED_{20}$) for 2,4-D ranged from 0.79-1.01% at Richville and 0.64-0.78% at East Lansing, averaged across all application timings. The $ED_{20}$ values for dicamba 14 DAT ranged from 0.86-0.89% at Richville and 0.45-0.78% at East Lansing. While results from field experiments did not provide a clear answer as to which herbicide caused greater injury to sugarbeet, results from greenhouse experiments indicate that 2,4-D caused greater injury to sugarbeet than dicamba. Canopy closure was slowed by the 1% rate of dicamba and 2,4-D at the 2-leaf stage and by the 2% rate of both herbicides applied at the 6- and 14-leaf stages. The interaction between herbicide rate and

application timing was not significant for sugarbeet yield and recoverable white sucrose $ha^{-1}$ (RWSH). Yield was reduced 28% at Richville and 20% at East Lansing by 22.4 g $ha^{-1}$ of 2,4-D. Similarly, 22.4 g $ha^{-1}$ of dicamba reduced yield 17% at Richville. No yield reductions were detected at any other rates. Similar to sugarbeet yield, RWSH was only reduced by 22.4 g $ha^{-1}$ of both herbicides. Herbicide residues declined quickly in sugarbeet following application. Following an application of 2.8 g $ha^{-1}$, 2,4-D residues 0 DAT were found as high as 0.30 and 0.14 ppm at East Lansing and Richville, respectively, while dicamba residues ranged from 0.08-0.17 ppm. By 21 DAT, 2,4-D and dicamba residues were below 0.02 ppm. At 14 DAT, 2,4-D residues at both locations were highest from 22.4 g $ha^{-1}$ applied to 14-leaf sugarbeet, although concentrations were below 0.20 ppm. At rates ranging from 5.6 to 22.4 g $ha^{-1}$, 2,4-D residues were highest in sugarbeet treated at the 14-leaf timing. Dicamba residues 14 DAT were below 0.15 ppm at all growth stages and rates applied. At harvest, sugarbeet treated with higher rates of 2,4-D at the 14-leaf growth stage tended to have higher 2,4-D residue concentrations. However, concentrations at all combinations of rate and timing were less than 10 ppb in the shoot and root tissue across both locations. Dicamba residue levels were also low at harvest, as residues in combined root and shoot tissue never exceeded 10 ppb at either location. Despite these low residue concentrations at harvest, improper spray system cleanout could result in rates of dicamba or 2,4-D high enough to reduce sugarbeet yield or RWSH. This further stresses the importance for proper cleanout in order to make sure exposure to dicamba and 2,4-D does not result in sugarbeet yield or RWSH reductions.

**Nomenclature:** Dicamba; glyphosate; 2,4-D; soybean, *Glycine max* (L.); sugarbeet, *Beta vulgaris* L.

**Key words:** Exposure, herbicide residues, spray system contamination, sugarbeet injury

## Introduction

The rapid spread of glyphosate- and multiple-resistant weeds in soybean cropping systems has led to the development of both dicamba- and 2,4-D-resistant soybean. Dicamba and 2,4-D resistance in soybean was achieved through the insertion of the resistance genes, dicamba monooxygenase and the aryloxyalkanoate dioxygenase enzyme AAD-12, respectively (Behrens et al. 2007; Wright et al. 2010). Currently, dicamba resistant soybean are stacked with glyphosate resistance, while 2,4-D resistant soybean are stacked with both glyphosate and glufosinate resistance. The use of dicamba and 2,4-D in soybean will allow for a more diversified approach to weed management (Powles 2008), as these herbicides have been shown to be effective against some of the most problematic glyphosate-resistant weeds, including horseweed (*Conyza canadensis* L.), waterhemp (*Amaranthus tuberculatus* var. *rudis* L.), and Palmer amaranth (*Amaranthus palmeri* S. Watson) (Chahal and Johnson 2012; Kruger et al. 2010; Norsworthy et al. 2008; Robinson et al. 2012). However, the expected increase in use of dicamba and 2,4-D is likely to result in issues with sensitive crops.

Several important broadleaf crops have shown sensitivity to dicamba and 2,4-D, including sugarbeet (Everitt and Keeling 2009; Schroeder et al. 1983; Solomon and Bradley 2014). Sugarbeet is an important crop in the United States, accounting for 50-55% of U.S. sucrose produced (Panella et al. 2009), and a 1.3 billion dollar production value (NASS 2018). Of the 457,000 ha of sugarbeet planted in the United States, Michigan ranks fourth with 58,000 ha planted in 2017 and a 135 million dollar production value in 2016 (NASS 2018). Sugarbeet can be exposed to dicamba or 2,4-D as a result of volatility, herbicide particle drift, or spray system contamination (Behrens and Lueschen 1979; Boerboom 2004; Cundiff et al. 2017). Growers using the same sprayer for pesticide applications in both dicamba or 2,4-D resistant soybean and

sugarbeet have an even greater risk of spray tank-contamination, especially since sugarbeet requires multiple applications of glyphosate throughout the season for effective weed control (Guza et al. 2002; Wilson et al. 2002).

Several studies have shown that sugarbeet are negatively impacted by relatively low rates of dicamba and 2,4-D. 2,4-D causes injury to sugarbeet at all growth stages, with injury at earlier stages resulting in stand loss and injury at later stages causing reductions in extractable sucrose (Byford and Prince 1976; Holksvig 1950; Schroeder et al. 1983). Schweizer (1978) found that top growth was reduced 55% and root yield was reduced 10% when sugarbeet was exposed to 70 g ae $ha^{-1}$ of 2,4-D at the 8-leaf stage. In a similar study, Byford and Prince (1976) determined that sugarbeet root yield was reduced by 12% following an application of 70 g $ha^{-1}$ of 2,4-D. Schroeder et al. (1983), concerned with 2,4-D and dicamba drift onto sugarbeet from applications to cereal crops, found that 280 g $ha^{-1}$ of 2,4-D reduced sugarbeet yield by 27% when averaged across multiple application timings, while 140 g $ha^{-1}$ did not decrease yield significantly. Alternatively, rates of dicamba ranging from 17-140 g ae $ha^{-1}$ did not significantly reduce yield or sucrose content. From this study, sugarbeet appeared to be more sensitive to 2,4-D and have a greater tolerance to dicamba. Depending on the field use rate, as well as the sugarbeet growth stage at the time of exposure, it is possible that dicamba and 2,4-D levels from tank-contamination could impact sugarbeet growth and yield. Boerboom (2004) and Cundiff et al. (2017) found that as much as 0.63% of the field use rate of 560 g $ha^{-1}$ dicamba remained in the spray system when following proper cleanout procedures. It is likely that improper tank cleanout procedures would result in herbicide levels much higher than this, increasing the potential for herbicide injury to sugarbeet and yield loss.

Along with the potential for injury and the loss of yield and sucrose content, there is another negative aspect of dicamba and 2,4-D exposure to sugarbeet. The presence of dicamba and 2,4-D residues in the harvested roots of sugarbeet could pose a serious issue for sugarbeet growers. Currently there are no maximum residue limits (MRLs) set for either dicamba and 2,4-D in sugarbeet (Cornell Law School 2016; Cornell Law School 2017). Because of this, any detectable level of either herbicide in the harvested crop would result in rejection. There has been no research conducted concerning dicamba and 2,4-D residues in sugarbeet. However, in soybean, Auch and Arnold (1978) found dicamba residues at approximately 900 parts per billion seven days after an application of 56 g $ha^{-1}$, but this level was down to zero 11 days later. Andersen et al. (2004) detected dicamba residues at 0.03 ppm and 2,4-D residues ranging from 0.073-0.80 in soybean foliage 48 days after treatment with rates as low as 5.6 g $ha^{-1}$ of dicamba and 11.2 g $ha^{-1}$ of 2,4-D. On the other hand, Boerboom (2004) was not able to detect any dicamba residue in soybean foliage three days after simulating drift. Considering that both dicamba and 2,4-D are systemic herbicides that translocate to the growing points of plants, it would be expected that these herbicides would translocate to sugarbeet roots (Grossman 2010; Shaner 2014).

The release of dicamba and 2,4-D resistant soybean will lead to an increase in use of dicamba and 2,4-D, leaving sugarbeet growers vulnerable to injured crops as well as potential loss of yield. Along with this, exposure to dicamba and 2,4-D could result in the presence of unacceptable herbicide residues in the harvested crop, resulting in rejection and the loss of profits. Because of this, it is essential to gain a better understanding of the effects that 2,4-D and dicamba have on sugarbeet growth, yield, and quality. It is also important to determine how long the residues of dicamba and 2,4-D persist in sugarbeet, with the hopes of possibly establishing MRLs for both herbicides in sugarbeet. Therefore, the objectives of this research were to: (1)

evaluate the effects of a simulated tank-contamination on sugarbeet using multiple rates of dicamba and 2,4-D at three growth stages; (2) determine if glyphosate or AMS influence the effects of dicamba and 2,4-D on sugarbeet; and (3) quantify the residue levels of dicamba and 2,4-D in sugarbeet following exposure.

## Materials and Methods

**Field Experiments.** Field experiments were conducted in 2016 and 2017 at two locations: the Michigan State University (MSU) Saginaw Valley Research and Extension Center near Richville, MI (43.4°N, 83.7°W) and the MSU Agronomy Farm in East Lansing, MI (42.71°N, 84.47°W). The soil type in Richville was a Tappan-Londo loam (fine-loamy, mixed, active, calcareous, mesic Typic Epiaquolls), with pH 7.8 and 2.5% organic matter in 2016, and pH 7.7 and 2.4% organic matter in 2017. The soil type in East Lansing was a Capac loam (fine-loamy, mixed, active, mesic Aquic Glossudalfs), with pH 7.3 and 2.5% organic matter in 2016 and pH 5.9 and 3.1% organic matter in 2017 for the 2,4-D experiment, and pH 6.1 and 2.6% organic matter for the dicamba experiment. Soil preparation at both locations consisted of fall chisel plow followed by two passes of a soil finisher in the spring prior to planting. Fertilizer applications were standard for sugarbeet production in Michigan. Glyphosate-resistant sugarbeet varieties were planted at Richville on April 16, 2016 (Hilleshög 9616, Syngenta Seeds Inc, Longmont, CO) and April 18, 2017 (Crystal G515, American Crystal Sugar Company, Moorhead, MN) at populations of 118,500 and 122,000 seeds $ha^{-1}$, respectively. Sugarbeet was planted at East Lansing on April 18, 2016 (Crystal 059) and May 17, 2017 (Crystal G515) at populations of 118,500 seeds $ha^{-1}$ for both years.

Separate field experiments were conducted at each location for dicamba and 2,4-D. Experiments were set up as a split-plot design with four replications. The main plot factor was sugarbeet growth stage at herbicide application timing, and the sub-plot factor was herbicide application rate. Plots were four rows wide and 12.2 m long, with a row spacing of 76 cm. Herbicides were applied to sugarbeet at either the 2-, 6-, or 14-leaf growth stages. The 6-leaf application timing was omitted from East Lansing, due to space constraints. Herbicide application rates were 0, 1.4, 2.8, 5.6, 11.2, and 22.4 g ae $ha^{-1}$ for both the diglycolamine salt of dicamba (Clarity®, BASF, Florham Park, NJ) and the choline salt of 2,4-D (Enlist One®, Dow AgroSciences, Indianapolis, IN). These rates are equivalent to 0, 0.125, 0.25, 0.5, 1, and 2% of the field use rates of these herbicides, assuming a field use rate of 1.12 kg ae $ha^{-1}$. Each herbicide application included a full rate of glyphosate at 0.84 kg ae $ha^{-1}$ plus ammonium sulfate (AMS) at 2% w $w^{-1}$. Research plots were kept weed-free with additional applications of glyphosate plus AMS. Herbicides were applied using a $CO_2$-pressurized backpack sprayer calibrated to deliver 177 L $ha^{-1}$ at a pressure of 207 kPa through 11003 AIXR flat-fan nozzles (TeeJet® Spraying Systems, Wheaton, IL).

Herbicide injury to sugarbeet was evaluated 7, 14, and 21 days after treatment (DAT) and at harvest. Injury was evaluated on a scale of 0-100%, with 0% representing no injury and 100% indicating complete plant death. Starting in mid-June, approximately 8 weeks after planting, canopy closure measurements were taken weekly or bi-weekly at the Richville location in 2016 and 2017 until aboveground leaf growth ceased using a SunScan Canopy Analysis System with a 1 m by 13 mm wand (Dynamax Inc, Houston, TX). Three light measurements were taken simultaneously in each plot beneath and above the plant canopy to calculate percent canopy closure using Equation 1:

$$Canopy\ closure\ (\%) = \left(\frac{Light\ above\ canopy - Light\ below\ canopy}{Light\ above\ canopy}\right) x\ 100 \qquad [1]$$

Samples for residue analysis were taken from field plots 14 DAT and at harvest. Following applications at the 2-leaf stage, 20-30 plants were sampled for sufficient tissue. Following applications at the 6- and 14-leaf stages, six plants were sampled. Samples taken at harvest were split into above and belowground tissue, while samples taken 14 DAT were kept intact. Additional samples were taken 0, 7, 14, and 21 DAT of sugarbeet sprayed with the 0.25% rate of both dicamba and 2,4-D at each application timing to measure the decline of herbicide residues over time. Samples were stored at -20 C until processed.

Sugarbeet was mechanically harvested and weighed from two rows of each plot in Richville in late September, while only a single row was harvested in East Lansing in late September to early October (Table 2.1). The dicamba study at East Lansing was not harvested in 2017 due to unfavorable growing conditions resulting in a poor stand. A sub-sample of sugarbeet roots was taken from each plot and analyzed for quality by the Michigan Sugar Company laboratory (Michigan Sugar Company, Bay City, MI). Sugarbeet quality is expressed as kg of recoverable white sucrose per Mg of root (RSWMg). Total sugar production is expressed as kg of recoverable white sucrose $ha^{-1}$(RWSH).

**Herbicide Residue Quantification.** *Sample preparation.* Stored frozen sugarbeet samples were ground using a Ninja® Blender (SharkNinja, Needham, MA). Blenders were cleaned with a water/detergent solution between each sample to avoid cross-contamination. Samples weighing

5±0.05 and 10-10.99 g for 2,4-D and dicamba residue analysis, respectively, were placed in 50 mL centrifuge tubes and stored at -20 C until sample extraction.

*2,4-D residue analysis.* Sugarbeet samples collected from the 2,4-D experiments were analyzed at Dow AgroSciences headquarters (Dow AgroSciences, Indianapolis, IN). Residues of 2,4-D were extracted from tissue by adding 25 mL of 90:10 methanol:1.0 N NaOH extraction solution to the 5±0.05 g samples. Samples were homogenized for 60 seconds, shaken for 30 minutes, and centrifuged. The supernatant was then decanted. Another 25 mL of extraction solution was added to the original tube, vortexed, shaken, and centrifugation was repeated. The supernatant was again decanted. The combined supernatant was mixed, and an aliquot of either 2 mL or 200 µL was taken, depending upon the dilution factor. The 2 mL and 200 µL aliquots were acidified with 2 mL or100 µL of 0.2 N HCl, respectively. The 2 mL aliquots were then taken through solid phase extraction, and cartridges were eluted with 90:10 methanol:water plus 0.5% acetic acid. All samples then had an internal standard and keeper solution added before being dried with $N_2$. Samples were then reconstituted to a final volume of 1.0 mL with 50:50 methanol:water + 0.1% acetic acid and passed through a polytetrofluoroethylene (PTFE) syringe filter to further clean the sample prior to injection into the negative-ion liquid chromatograph coupled with a tandem mass spectrometer. Residues of 2,4-D were determined using an Agilent 1290 HPLC (Agilent Technologies, Santa Clara, CA) coupled with a Sciex API5000 triple quad mass spectrometer (Sciex LLC, Framingham, MA) using multiple reaction monitoring (MRM) acquisition mode, monitoring *m/z* 219/161 for quantitation and *m/z* 221/163 for confirmation. Injection volumes were 10 µL. Analytical separation was achieved using an Agilent Zorbax SB-C8 3.5 µm, 4.6 x 75 mm column. The limit of quantitation (LOQ) was 1.0 ppb, and the limit of detection (LOD)

was defined as 30% of the LOQ, 0.3 ppb. The method is selective for the determination of 2,4-D by virtue of the chromatographic separation and MS/MS detection. The validity of the analytical method was demonstrated by including procedural recovery samples with each sample set. Precision and accuracy were demonstrated by achieving average recoveries within 90-100% at each fortification level.

*Dicamba residue analysis.* Sugarbeet samples collected from the dicamba experiments were prepared for detection in the Michigan State University Weed Science lab, and analysis was conducted at the MSU Environmental Chemistry and Pesticide Research Lab (East Lansing, MI). Samples were prepared for analysis using the QuEChERS method as outlined by Koesukwiwat et al. (2008) modified for the detection of dicamba. Twenty mL of 47% NaOH was added to each sample. Samples were hand-shaken vigorously for one minute to and allowed to incubate for approximately 12 h. Following incubation, 5 mL of 9 M $H_2SO_4$ was added slowly (approximately 1 mL $minute^{-1}$) to avoid excessive reactions between the reagents. Ten mL of acetonitrile was added, followed by 1 g of NaCl and 4 g $MgSO_4$. Samples were hand-shaken for one minute, then centrifuged for 5 minutes at 2500 rpm. A 2 mL aliquot was taken from the upper phase extract containing the dicamba analyte. Residues of dicamba were determined using a Waters 2695 Separations Module HPLC (Waters Corporation, Milford, MA) coupled with a Waters Aquity SQ single quadrupole mass spectrometer detector using multiple acquisition mode, monitoring *m/z* 173/203/205 for quantitation and *m/z* 203 and *m/z* 173/203/205 for confirmation. Injection volumes were 10 µL. Analytical separation was achieved using a Waters X-Bridge $C_{18}$ 3.5µm phase, 3.0 X 50 mm column. The LOQ was 1.0 ppb, and the LOD was 30% of the LOQ, 0.3 ppb. This method is selective for the determination of dicamba by virtue of the

chromatographic separation of HPLC/MS detection. The validity of the analytical method was demonstrated by including procedural recovery samples with each sample set. Precision and accuracy were demonstrated by achieving average recoveries within 50-150% at each fortification level.

**Greenhouse Experiments.** *General plant culture and herbicide application.* Sugarbeet were planted 2.54 cm deep in 10 by 10 cm pots filled with potting media (Suremix™ Perlite, Michigan Gower Products, Galesburg, MI). One week after emergence, sugarbeet were thinned to two plants per pot, and thinned again to one plant per pot a week later. Plants were grown in the greenhouse at a temperature of 25±5 C with a 16 h photoperiod of natural sunlight, with supplemental lighting at 1,000 μmol $m^{-2}$ $s^{-1}$ photosynthetic photon flux. Plants were watered daily and fertilized two weeks after emergence with 25 mL of 20:20:20 ($N:P_2O_5:K_2O$) solution at a concentration of 6.61 g $L^{-1}$. Starting three weeks after emergence, plants were fertilized weekly with 50 mL of the same fertilizer solution. All herbicide applications were made using a track sprayer with a single 8001E TeeJet®flat-fan nozzle (TeeJet®Technologies, Wheaton, IL) calibrated to deliver 187 L $ha^{-1}$ at 193 kPa of pressure.

*Experiment 1: Sugarbeet response to dicamba and 2,4-D.* Sugarbeet were grown in the greenhouse using methods previously described. The experiment was arranged as a three factor completely randomized design with four replications and repeated in time. Factors included sugarbeet growth stage, herbicide active ingredient, and herbicide rate. When sugarbeet were at the 2- and 6-leaf growth stages, 12 rates of dicamba and 2,4-D were applied. Dicamba and 2,4-D rates were 0, 1.4, 2.8, 5.6, 11.2, 22.4, 56, 112, 280, 560, 840, and 1120 g ae $ha^{-1}$. These rates

were equivalent to 0, 0.125, 0.25, 0.5, 1, 2, 10, 25, 50, 75 and 100% of the field use rates of these herbicides, assuming a field use rate of 1.12 kg ae $ha^{-1}$. All applications of 2,4-D and dicamba included glyphosate at 0.84 kg ae $ha^{-1}$ plus ammonium sulfate at 2% w $w^{-1}$. Sugarbeet injury was evaluated 7 and 14 DAT. Injury was evaluated on a scale of 0-100%, with 0% representing no injury and 100% indicating complete plant death. Above and belowground biomass was harvested 14 DAT, air dried for 7 d, and dry weights were recorded

*Experiment 2: Influence of glyphosate.* Sugarbeet at the 2- and 6-leaf growth stages were sprayed with dicamba and 2,4-D at rates of 0, 1.4, 2.8, 5.6, 11.2, and 22.4 g ae $ha^{-1}$ for each herbicide. Each herbicide-rate combination was sprayed twice, with and without the full rate of glyphosate (0.84 kg ae $ha^{-1}$). All treatments included ammonium sulfate at 2% w $w^{-1}$. Each experiment had four replications and was repeated in time. Sugarbeet injury was evaluated 14 DAT, above and belowground biomass was harvested, and dry weights were recorded.

*Experiment 3: Influence of ammonium sulfate.* Sugarbeet at the 6-leaf stage were treated with dicamba and 2,4-D at rates of 0, 2.8, 5.6, 11.2, and 22.4 g ae $ha^{-1}$. All treatments included glyphosate at 0.84 kg ae $ha^{-1}$. Each herbicide-rate combination was sprayed twice, with and without ammonium sulfate at 2% w $w^{-1}$. Each experiment had four replications and was repeated in time. Sugarbeet injury was evaluated 14 DAT, above and belowground biomass was harvested, and dry weights were recorded.

**Statistical Analysis.** Herbicide injury in field experiments was combined over years and analyzed separately by location. Effective doses for 20% injury ($ED_{20}$) are calculated as

percentages of a field use rate of 1.12 kg ae $ha^{-1}$ for both dicamba and 2,4-D. The $ED_{20}$ at each application timing was calculated and analyzed using the drc package in R (R version 3.0.0, The R Foundation for Statistical Computing), and curves were fitted to the data using the four-parameter log-logistic model (Equation 2) with an upper limit of 100 as selected by the drc modelFit function.

$$y = c + \frac{100 - c}{1 + \exp[b(\log(x) - \log(e))]} \quad [2]$$

The variable *y* is herbicide injury, *x* is the herbicide rate, *c* is the lower limit, *b* is the slope around *e*, and *e* is the $ED_{50}$. $ED_{20}$ values for the different application timings were compared using a paired t-test at $p \leq 0.05$.

Canopy closure data was combined over years and analyzed separately for each herbicide application timing. Curves for the untreated sugarbeet, the 1% rate at the 2-leaf timing, and the 2% rate at the 6- and 14-leaf timings were fitted to data using SigmaPlot 11.0 (Systat Software Inc., San Jose, CA), and a three-parameter log-logistic model (Equation 3) was used for the 2- and 6-leaf applications, while a linear equation (Equation 4) was used for the 14-leaf application.

$$y = \frac{d}{1 + \exp[b(\log(x) - \log(e))]} \quad [3]$$

$$y = ax + b \quad [4]$$

The variables in Equation 3 are the same as the variables in Equation 2, with the exception that *y* is the percentage of canopy closure and *x* is days after planting. For Equation 4, *y* is the

percentage of canopy closure, *x* is days after planting, *a* is the slope, and *b* is the *y*-value when *x* equals 0. Lines for the untreated and treated sugarbeet were compared using the extra sum of squares principle for nonlinear regression (Lindquist et al. 1996).

Yield, RWSH, and herbicide residues at harvest were analyzed using SAS® 9.4 (SAS Institute, Cary, NC). Assumptions of normality and homogeneity of variances was confirmed using PROC UNIVARIATE for yield and RWSH, and herbicide residues were log-transformed to meet these assumptions. Analysis of variance was conducted using PROC MIXED. The statistical model included herbicide rate and application timing, as well as their interaction. Random effects included replication and year, as well as their interaction. Herbicide residues at harvest were analyzed separately by tissue type. When interactions between main effects were not significant, data were combined over main effects. Means were separated using Tukey's HSD at $p \leq 0.05$.

Decline sampling of the herbicide residues over time at the three application timings was plotted in SigmaPlot 11.0 using the two-parameter exponential decay equation (Equation 5), while herbicide residue concentrations 14 DAT for the different application timings were plotted using the two-parameter exponential growth equation (Equation 6).

$$y = a * \exp(-bx) \quad [5]$$

$$y = a * \exp(bx) \quad [6]$$

For Equation 5, *y* is herbicide residue concentration, *x* is weeks after treatment, *a* is the initial residue amount, and *b* is rate of decline. For Equation 6, *y* is herbicide residue concentration, *x* is herbicide rate, *a* is the initial residue amount, and *b* is the rate of growth. Data were combined

over year when there were no significant interactions between years. Comparisons between application timings were made using standard error comparisons.

The linear correlation between herbicide injury to sugarbeet and herbicide residues at harvest was analyzed using PROC REG in SAS® 9.4. Graphs were plotted using SigmaPlot 11.0.

$ED_{20}$ values for herbicide injury for the greenhouse glyphosate influence experiment as well as $ED_{50}$ values for biomass (rate required to reduce biomass by 50%) for the greenhouse dose response experiment were plotted using the drc package in R, and lines were fit using the three-parameter Weibull model (Equation 7).

$$y = d * \exp\{-\exp[b(\log(x) - e)]\} \quad [7]$$

*Y* is herbicide injury for the glyphosate comparison and biomass for the dose response experiment, *x* is herbicide rate, *d* is the upper limit, *b* is the slope about *e*, and *e* is the $ED_{50}$. For the greenhouse experiment, the upper limit was set at 100. For the glyphosate comparison, herbicides were analyzed separately. Data were combined over repetition in time, and comparisons between effective doses were made using a paired t-test at $p \leq 0.05$.

Herbicide injury for the dose response experiment and the ammonium sulfate influence experiment were plotted using the drc package in R, and lines were fitted using Equation 3. $ED_{20}$ values were obtained for injury for the ammonium sulfate comparison as well as $ED_{50}$ values for injury for the dose response experiment. Data were combined over repetitions in time. Effective doses were compared using a paired t-test at $p \leq 0.05$.

Biomass data for the glyphosate influence and ammonium sulfate influence experiments were compared using SAS® 9.4. Assumptions of normality and homogeneity of variances was confirmed using PROC UNIVARIATE, and analysis of variance was conducted using PROC

MIXED. The statistical model for the glyphosate comparison included the main effects of glyphosate, herbicide rate, and application timing, while the main effects for the ammonium sulfate comparison were ammonium sulfate and herbicide rate. The interactions between all main effects were included as well, and data was combined over main effects when interactions were not significant. Data were combined over repetition in time, and replication was treated as a random effect. Means were separated using Tukey's HSD at $p \leq 0.05$.

## Results and Discussion

**Field Experiments.** *Sugarbeet injury.* Herbicide injury to sugarbeet was detected at all application timings of dicamba and 2,4-D rates ranging from 2.8-22.4 g $ha^{-1}$ (Figure 2.1, Figure 2.2). Symptoms were consistent with typical plant growth regulator injury, consisting of leaf epinasty and crinkling, twisted and elongated petioles, and general shoot tissue malformation. Higher rates (11.2-22.4 g $ha^{-1}$) tended to restrict the upward growth of leaves, instead causing leaves to grow outward, often resulting in sugarbeet having a "flattened" appearance. Similar symptoms in sugarbeet were reported by Schweizer (1978) and Byford and Prince (1976). Injury following herbicide application at the 2-leaf growth stage often resulted in a restricted, upright growth pattern, as opposed to more outward growth in untreated plants. Dicamba tended to cause more severe crinkling along leaf margins than 2,4-D, while 2,4-D often resulted in a "goose-neck" growth pattern that caused leaf petioles to grow downward and then back up. These differences in symptoms were more apparent at higher herbicide rates, and may be helpful for determining the herbicide responsible for sugarbeet injury from an unknown source. Although injury symptoms were typically apparent within three days of treatment, the effective dose for

20% injury ($ED_{20}$) was often lowest at 14 DAT for both herbicides, indicating a peak in injury response at 14 DAT (Table 2.2, Table 2.3). Similar trends have also been noted in soybean, as injury from dicamba and 2,4-D tends to be highest at 14-28 DAT (Robinson et al. 2013a; Robinson et al. 2013b; Soltani et al. 2015). Injury to sugarbeet, as expected, was most severe at the 22.4 g $ha^{-1}$ rate of dicamba and 2,4-D.

Herbicide injury to sugarbeet was combined over years and analyzed separately by location. At Richville, sugarbeet injury across all application timings was minimal from 1.4 g $ha^{-1}$ of 2,4-D, ranging from 3-5% 14 DAT, while injury from 22.4 g $ha^{-1}$ ranged from 31-40% 14 DAT (Figure 2.1a). There were no differences between $ED_{20}$ values of 2,4-D amongst the three application timings at Richville 14 DAT, with values ranging from 0.79-1.01% of the field use rate (8.8-11.3 g $ha^{-1}$) (Table 2.2). At 7 and 21 DAT, $ED_{20}$ values for the 6- and 14-leaf timings were lower than the $ED_{20}$ value for the 2-leaf timing, indicating greater injury at the later growth stages at these evaluation timings. At 21 DAT, $ED_{20}$ values ranged from 1.01-1.53% of the field use rate (11.3-17.1 g $ha^{-1}$), indicating that sugarbeet may have recovered from 2,4-D injury, although the $ED_{20}$ value for the 6-leaf timing did not change (Table 2.2). At the 6-leaf timing, the $ED_{20}$ increased from 0.90% at 7 DAT to 1.01% at 14 DAT. As this is generally a period of rapid growth for sugarbeet, it is likely that recovery occurred quicker at this stage as new tissue was produced (Trebbi and McGrath 2009).

Results were similar following 2,4-D treatments at East Lansing as sugarbeet injury peaked at 36% 14 DAT at the 22.4 g $ha^{-1}$ rate of 2,4-D (Figure 2.1b). At 14 DAT, there were no differences in $ED_{20}$ values between application timings, with values of 0.78 and 0.64% of the field use rate (8.7 and 7.2 g $ha^{-1}$) for the 2- and 14-leaf timing, respectively (Table 2.2). By 21 DAT, these values increased to 1.33 and 1.02% (14.9 and 11.4 g $ha^{-1}$) for the 2- and 14-leaf

timing, respectively. Schweizer (1978) found that sugarbeet foliar growth continued to decrease from 2 to 8 WAT when 2,4-D was applied to 4-leaf sugarbeet, but at a rate of 70 g $ha^{-1}$, which is 3X higher than the highest rate used in the present study. Robinson et al. (2013a) found that $ED_{20}$ values for 2,4-D in soybean were no less than 29 and 109 g $ha^{-1}$ at 14 and 28 DAT, respectively. Solomon and Bradley (2014) noted even less injury in soybean, as they found that 28 g $ha^{-1}$ of 2,4-D only caused 3% injury 2 WAT. Based on these results, sugarbeet appear to be more sensitive to injury from 2,4-D than soybean.

Similar to 2,4-D, injury from 1.4 g $ha^{-1}$ of dicamba at Richville was minimal (<5%) across all timings 14 DAT (Figure 2.2a). Increasing the rate to 22.4 g $ha^{-1}$ increased injury to 32-37%. The $ED_{20}$ values for the three application timings of dicamba were similar 14 DAT. Values ranged from 0.86-0.95% of the field use rate (9.6-10.6 g $ha^{-1}$) (Table 2.3). This range increased to 0.90-1.46% (10.1-16.4 g $ha^{-1}$) at 21 DAT, with the 6- and 14-leaf timings displaying greater injury than the 2-leaf timing. Injury was greatest at the 6-leaf timing 7 DAT, with $ED_{20}$ values of 0.70, 0.91, and 1.56% of the field use rate for the 6-, 14-, and 2-leaf timings, respectively. Like 2,4-D, the $ED_{20}$ for the 6-leaf timing increased from 7 to 14 DAT. The rapid growth at the 6-leaf stage may have allowed for sugarbeet to recover from dicamba injury. Similar to Richville, injury from dicamba 14 DAT at East Lansing ranged from 2-5% at 1.4 g $ha^{-1}$ to 37-40% at 22.4 g $ha^{-1}$ (Figure 2.2b). Injury was greater at the 2-leaf timing than the 14-leaf timing at all three evaluation timings. The lowest $ED_{20}$ values for both timings were at 14 DAT, with values of 0.45 and 0.78% of the field use rate for the 2- and 14-leaf timings, respectively (Table 2.3). These values increased from 14 to 21 DAT, ranging from 0.78-1.19% of the field use rate. Robinson et al. (2013b) determined that the $ED_{20}$ of dicamba for soybean 14 DAT ranged from 0.676-0.937 g $ha^{-1}$, while Solomon and Bradley (2014) detected 21% injury 2 WAT from 0.028 g $ha^{-1}$ of

dicamba applied to V3 soybean. The results of these studies indicate that 20% injury in soybean is reached at lower rates of dicamba compared with sugarbeet. Additionally, at 8 WAT, Soltani et al. (2015) detected 20 and 40% injury to soybean from 6 g $ha^{-1}$ of dicamba applied to V2-3 and R1 soybean, respectively. These results suggest that sugarbeet may have a greater tolerance to dicamba compared with soybean.

Based on these results, it is not clear whether 2,4-D or dicamba is more injurious to sugarbeet. While injury symptoms have been discussed in past research, no statistical comparisons between dicamba and 2,4-D injury have been made in sugarbeet. Sugarbeet exposed to dicamba and 2,4-D at the 2- and 6-leaf growth stages displayed the ability to recover from injury throughout the growing season. Across both locations, injury at harvest from all rates of 2,4-D applied at the 2- and 6-leaf timings was less than 17% (data not shown). Similarly, sugarbeet injury at harvest from all rates of dicamba applied at the same timings across both locations was less than 14%. This recovery is likely due to the extended amount time between treatment and harvest, as applications to 2-leaf and 6-leaf sugarbeet occurred 14-18 weeks before harvest. Sugarbeet exposed to dicamba and 2,4-D at the 14-leaf timing showed little to no recovery prior to harvest as injury was as high as 34 and 39% across both locations at the 22.4 g $ha^{-1}$ rate of dicamba and 2,4-D, respectively. This lack of recovery does not come as a surprise, considering that they had less time to recover (10-12 weeks) and the majority of foliar growth had already occurred.

According to the findings of Boerboom (2004) and Cundiff (2017), the amount of herbicide remaining in the spray system following proper cleanout did not exceed 0.63% of the field use rate. Based on these results, the rates of dicamba and 2,4-D necessary to cause 20% injury to sugarbeet is greater than this amount at all application timings, with the exception of

dicamba applied to 2-leaf sugarbeet at East Lansing. This is promising, as following proper cleanout procedures should be sufficient in preventing severe injury to sugarbeet. However, improper cleanout that results in higher rates remaining in the system could have a much more severe impact. Results such as this further stress the importance of adhering to proper spray system cleanout protocols.

*Canopy closure.* Sugarbeet treated with the 1% rate of 2,4-D at the 2-leaf stage and the 2% rate of 2,4-D at the 6- and 14-leaf stages had a significantly slower rate of canopy closure than untreated sugarbeet (Figure 2.3). The same results were found for sugarbeet treated with the same rates of dicamba (Figure 2.4). These rates caused significant injury to sugarbeet at all growth stages, likely leading to these differences in canopy closure. Sugarbeet treated with the 1% rate of dicamba and 2,4-D at the 2-leaf stage had slightly slower canopy closure than untreated sugarbeet, but never reached an equal level of row closure at the end of the season (Figure 2.3a, Figure 2.4a). At the 6-leaf stage, the 2% rate of both herbicides caused a significant delay in canopy development. However, sugarbeet treated with the 2% rate had nearly reached the same level of canopy closure as untreated sugarbeet (Figure 2.3b, Figure 2.4b). This indicates a similar trend to herbicide injury, as sugarbeet injured at the 6-leaf growth stage recovered throughout the growing season. The same was not true for sugarbeet treated at the 14-leaf growth stage. Canopy closure was delayed by both 2,4-D and dicamba shortly after treatment, and this difference between treated and untreated sugarbeet persisted through the growing season (Figure 2.3c, Figure 2.4c). These results are likely related to herbicide injury to sugarbeet, as injury symptoms appeared within 2-3 days after dicamba and 2,4-D application to 14-leaf sugarbeet, and recovery from injury appeared to be minimal. As with injury, this is likely because of the

majority of sugarbeet shoot growth had occurred prior to herbicide applications at the 14-leaf growth stage. Schweizer (1978) observed a similar response to 2,4-D in sugarbeet, as 2,4-D applied to 8-12 leaf sugarbeet suppressed foliar growth more than when applied at earlier stages, and that foliar suppression was still apparent at harvest when 70 g $ha^{-1}$ of 2,4-D was applied to 8-leaf sugarbeet. The potential delay in canopy closure can have effects that extend beyond impacts on sugarbeet yield, as the delay in canopy closure could have an effect on weed control. Both Armstrong and Sprague (2010) and Alford et al. (2004) noted that weed biomass was less when sugarbeet was planted in 38-51 cm rows compared with 76 cm rows and attributed this difference to more rapid canopy closure in narrower rows. Delays in canopy closure from exposure to dicamba and 2,4-D could similarly lead to increased weed biomass and competition later in the season and possibly require an additional herbicide application to control weeds.

*Sugarbeet yield and quality.* There was not an interaction between herbicide exposure rate and timing for 2,4-D or dicamba at either location. Only the highest rate of both herbicides reduced yield and RWSH. Yield and RWSH were both reduced 28% by 2,4-D at 22.4 g $ha^{-1}$ at Richville (Table 2.4). The 2% rate of 2,4-D reduced yield by 20% and RWSH by 23% at East Lansing. Previous studies also found that 2,4-D was capable of reducing sugarbeet yield, although Schroeder et al. (1983) did not detect a significant yield reduction at rates lower than 280 g $ha^{-1}$, which reduced yield by 27%. Other studies found that sugarbeet yield was reduced 10-12% by 2,4-D at 70 g $ha^{-1}$ (Schweizer 1978; Byford and Prince 1976). These discrepancies could likely be attributed to the fact that sugarbeet in the previous studies were thinned to approximately half the population of the present study, meaning sugarbeet had less intraspecific competition as they attempted to recover from injury. Schroeder et al. (1983) also found that RWSH was reduced at

170 g $ha^{-1}$ of 2,4-D, while Schweizer (1978) found a RWSH reduction at 35 g $ha^{-1}$. These results further support the idea that sugarbeet may be more sensitive to 2,4-D than soybean but less sensitive than cotton, as Robinson et al. (2013a) determined that 149 g $ha^{-1}$ was needed to reduce soybean yield, while cotton yield was reduced by 2,4-D at 2.8 g $ha^{-1}$ (Everitt and Keeling 2009).

Sugarbeet growth stage at the time of 2,4-D application also influenced sugarbeet yield and RWSH. When compared with applications at the 2- and 6-leaf growth stages, applications of 2,4-D at the 14-leaf growth stage reduced yield at Richville and East Lansing and reduced RWSH at Richville only (Table 2.5), despite similar injury severity being detected at all three timings. This is likely associated with the lack of recovery from injury following 14-leaf applications of 2,4-D. The restriction of canopy closure may have influence yield as well, as sugarbeet treated at the 14-leaf stage were more heavily impacted than at the 2- and 6-leaf stage. Also, sugarbeet at this stage had likely begun allocating more resources to increasing root biomass, which would increase the potential impacts that herbicide injury could have on yield and RWSH. Other studies have also found that sucrose content was decreased by later applications of 2,4-D, although they often found that these same applications tended to increase root yield, although not significantly (Byford and Prince 1976; Holksvig 1950; Schroeder et al. 1983). There were no differences in yield or RWSH between the 2- and 6-leaf application timings, despite injury occurring at all three growth stages. As with herbicide injury, this is most likely due to the extended recovery time following exposure to the herbicide. This indicates that sugarbeet are capable of recovering from significant injury without associated yield loss if exposure occurs early.

Sugarbeet yield and RWSH were only impacted by dicamba at 22.4 g $ha^{-1}$ at Richville. Yield was reduced 17% while RWSH was reduced 19% averaged across the three application timings (Table 2.6). These results contradict the findings by Schroeder et al. (1983), as they were not

able to detect significant yield loss in sugarbeet from dicamba rates as high as 140 g $ha^{-1}$. Timing of dicamba application did not impact sugarbeet yield, although significant injury and slowed canopy closure occurred at all three timings. This lack of yield loss is likely due to the combination of ample recovery time prior to harvest as well as sugarbeet possessing a greater tolerance for dicamba compared with 2,4-D (Schroeder et al. 1983). Neither rate nor timing influenced yield or RWSH at East Lansing in 2016, despite noting similar injury symptoms to the dicamba study at Richville (Table 2.6). Greater foliar disease pressure at East Lansing led to more variation in yields that may explain why yield loss did not occur. These results suggest that sugarbeet may be less sensitive to dicamba than soybean, as the rates applied in the present study have been shown to significantly reduce soybean yield (Egan et al. 2014; Robinson et al. 2013b; Solomon and Bradley 2014; Soltani et al. 2015).

No rate of dicamba or 2,4-D impacted RWSMg at any timing or location (data not shown). This is further reflected in the similar patterns in yield and RWSH reductions for both herbicides, as RWSH is a function of both yield and percent sucrose. Contrary to this, Schroeder et al. (1983) found that 140 g $ha^{-1}$ of 2,4-D reduced percent sucrose. Schweizer (1978) detected a 0.3 and 0.4% reduction from 35 and 70 g $ha^{-1}$ of 2,4-D, respectively. However, these rates are much greater than the rates of dicamba and 2,4-D applied in the present study, making them difficult to compare.

According to these results, greater than 11.2 g $ha^{-1}$ (1% of the field use rate) of both dicamba and 2,4-D is necessary to significantly reduce both yield and RWSH. These rates are outside the 0.63% maximum found by Boerboom (2004) and Cundiff (2017) that remains in a spray system following proper cleanout, indicating that proper cleanout should prevent yield or RWSH reductions resulting from spray-system contamination. This is promising to growers who are at a

higher risk of tank-contamination with dicamba and 2,4-D, but at the same time further stresses the importance of following proper cleanout protocol. Improper cleanout could easily result in dicamba or 2,4-D rates sufficient to reduce sugarbeet yield and RWSH. Because sugarbeet yield and RWSH were reduced by 2,4-D applications at the 14-leaf growth stage, it would be wise to avoid applying 2,4-D to other crops this late in the season or to use a different sprayer for pesticide applications in sugarbeet.

**Herbicide Residue Quantification.** *2,4-D residues.* Sugarbeet treated with 2.8 g $ha^{-1}$ (0.25% rate) of 2,4-D were sampled to measure the decline of herbicide residues over time following exposure. 2,4-D residue levels as high as 0.14 and 0.30 ppm at Richville and East Lansing, respectively, were found in sugarbeet an hour after treatment (0 days after treatment - DAT) (Figure 2.5, Figure 2.6). At Richville in 2017, residue concentrations in both 2- and 6-leaf sugarbeet were higher than residues in 14-leaf sugarbeet 0 DAT. However, residues in 2-leaf sugarbeet were higher in 2017 than in 2016 (Figure 2.5). Across both years at East Lansing, 2,4-D residues in 2-leaf sugarbeet were also higher than 14-leaf sugarbeet 0 DAT (Figure 2.6). Residues in 14-leaf sugarbeet were consistent at 0.05 ppm across both year and location. While the larger plants intercepted a greater amount of herbicide at application, the low residue levels are likely due to the low rate of 2.8 g $ha^{-1}$ being diluted within a relatively high amount of plant biomass. 2,4-D residues at all application timings declined within 1 week after treatment (WAT). At Richville in 2017, 2,4-D residues levels declined below 0.04 ppm 1 WAT from all three application timings. The decline in residues was not as rapid at Richville in 2016, although levels in 6-leaf sugarbeet dropped from approximately 0.14 ppm at 0 DAT to 0.07 ppm at 1 WAT. Residues declined similarly at East Lansing, as residues in 2-leaf sugarbeet decreased from 0.30

ppm at 0 DAT to less than 0.05 ppm at 1 WAT, resulting in comparable residue levels between 2-leaf and 14-leaf sugarbeet. Similar declines in 2,4-D residues over time have been noted in other species as well. Andersen et al. (2004) found that 2,4-D applied at 11.2 g $ha^{-1}$ to V3 soybean decreased from 5.4 μg $g^{-1}$ at 0 DAT to 0.6 μg $g^{-1}$ at 6 days after treatment in 2001 and from 4.8 μg $g^{-1}$ to 1.0 μg $g^{-1}$ in 2002 . In wheat, Cessna (1980) found that 2,4-D residues decreased from 155.4 ppm at 0 DAT to 0.89 ppm at 21 DAT at a rate of 560 g $ha^{-1}$. 2,4-D residues in sugarbeet continued to decline to levels below 0.04 ppm 2 WAT and below 0.02 ppm 3 WAT at all timings, locations, and years.

2,4-D residues 14 DAT and at harvest were combined across years at both locations. Timing of exposure and herbicide rate both influenced 2,4-D residues in sugarbeet 14 DAT, although timing appeared to be the more influential factor. As herbicide rate increased, 2,4-D residues increased more rapidly in 6- and 14-leaf sugarbeet as compared with 2-leaf sugarbeet at both locations (Figure 2.7). Across all rates at both locations, residue levels never surpassed 0.05 ppm when applied at the 2-leaf stage. The smaller plants at this stage would have had less herbicide interception compared with sugarbeet at the 6- or 14-leaf stages. At the highest rate (22.4 g $ha^{-1}$), residue concentrations were different between all three timings at Richville 14 DAT (Figure 2.7a). At this rate, residue levels were the highest in 14-leaf sugarbeet at 0.16 ppm, while concentrations were 0.09 and 0.007 ppm in 6-leaf and 2-leaf sugarbeet, respectively. Residue levels were below 0.10 ppm for all timings at 11.2 g $ha^{-1}$ and below 0.05 ppm at 5.6 g $ha^{-1}$, although significant differences remained between the three timings at these rates. At East Lansing, 2,4-D residue levels peaked at 0.10 ppm from 22.4 g $ha^{-1}$ applied to 14-leaf sugarbeet (Figure 2.7b). This was significantly higher than the 0.03 ppm found in 2-leaf sugarbeet from the same rate of 2,4-D. Residues in 14-leaf sugarbeet were 0.05 and 0.02 ppm at 2,4-D rates of 11.2

and 5.6 g $ha^{-1}$, respectively. These results seem to contradict the findings of Andersen et al. (2004), as they were unable to detect a difference at 12 DAT in 2,4-D residues in soybean between rates of 11.2 and 56 g $ha^{-1}$. However, they did determine that 2,4-D residues at this time from a rate of 112 g $ha^{-1}$ were higher than residues from the previously mentioned rates. This would suggest that, at lower herbicide rates, differences in rate are more influential in sugarbeet than soybean, as the decrease in residues as rates decreased was more apparent in sugarbeet, despite a greater disparity in exposure rates in the soybean study.

Residues of 2,4-D in sugarbeet tissue continued to decline through harvest. Because of the extremely low levels, residues are expressed in parts per billion (ppb) for the remainder of this section. Only the exposure rate of 22.4 g $ha^{-1}$ of 2,4-D applied to 14-leaf sugarbeet resulted in residues above 10 ppb in combined root and shoot tissue at harvest. Despite these low levels, significant differences were still detected between treatments. At Richville, 2,4-D residues in root tissue were higher from the 22.4 g $ha^{-1}$ rate applied to 14-leaf sugarbeet than the 5.6 or 2.8 g $ha^{-1}$ rate (Table 2.7). The 11.2 g $ha^{-1}$ rate also resulted in higher residues than the 2.8 g $ha^{-1}$ rate but was not different than the 5.6 g $ha^{-1}$ rate. This same pattern was reflected in sugarbeet shoot tissue, with the exception that the 11.2 g $ha^{-1}$ rate was not different than the 2.8 or 5.6 g $ha^{-1}$ rate. Exposure rate did not influence 2,4-D residues in either root or shoot tissue at the other application timings. Andersen et al. (2004) found similar results, as they could not detect a difference in 2,4-D residue concentration in soybean 48 DAT between rates of 11.2, 56, and 112 g $ha^{-1}$. These rates were applied to V3 soybean, which would be more comparable with 2- and 6-leaf sugarbeet than 14-leaf sugarbeet. Cessna (1980) also found that 2,4-D residues in wheat drastically decreased throughout the season, as 2,4-D applied at 560 g $ha^{-1}$ in June resulted in residue levels less than 0.05 ppm in September. However, this may not be a suitable comparison

considering that wheat is much more tolerant to 2,4-D than sugarbeet. At the 2,4-D rate of 22.4 g $ha^{-1}$, residues in root tissue from the 14-leaf application timing were higher at 2.3 ppb than residues of 0.5 ppb and 0.6 ppb from 2- and 6-leaf application timings, respectively (Table 2.7). This was also reflected at the 5.6 g $ha^{-1}$ rate. All three application timings had different concentrations in root tissue at the 11.2 g $ha^{-1}$ rate, with a later application time equating to higher concentrations. Similarly, the concentration of 2.2 ppb in the shoot tissue from the 14-leaf application was higher than the concentrations of 1.0 ppb and 0.7 ppb from the 2- and 6-leaf applications, respectively, when 2,4-D was applied at a rate of 22.4 g $ha^{-1}$. At the next lowest rate, residues in shoot tissue from the 14-leaf application were higher at 1.4 ppb than residues from the 2-leaf application at 0.8 ppb, although residues from the 6-leaf application were similar to both. The influence of application timing on residues is likely because applications to 14-leaf sugarbeet occurred 10-12 weeks prior to harvest while the earlier applications occurred 14-18 weeks prior to harvest (Table 2.1). In addition, results also suggest that the decline in residues is likely due to dilution within plant biomass, meaning that later applications would have less additional growth to dilute residue concentrations. Total residues were below 5 ppb for all combinations of 2,4-D rate and application timing.

At East Lansing, herbicide application timing appeared to be even more influential towards residue levels, as there were no differences in residue concentrations between rates within each application timing (Table 2.8). Only the main factor of application timing was significant in shoot tissue residues at harvest, as residues from the 14-leaf application were 2.1 ppb, compared with 0.8 ppb from the 2-leaf application, averaged over the rates applied. 2,4-D residues were higher in root tissue from applications to 14-leaf sugarbeet than applications to 2-leaf sugarbeet at both 11.2 and 22.4 g $ha^{-1}$. The highest residue level at harvest in the combined root and shoot

tissue was 12.0 ppb from applications of 2,4-D to 14-leaf sugarbeet at 22.4 g $ha^{-1}$, while all other combinations of rate and timing had residues less than 10 ppb.

Rates of 2,4-D at 22.4 g $ha^{-1}$ or less applied to sugarbeet resulted in surprisingly low residue levels both shortly after treatment as well as at harvest. Residues declined rapidly following exposure, especially when exposure occurred to sugarbeet earlier in the season. This appeared to be due to rapid foliar growth that diluted the residues within the plant biomass. The decline in residues continued through to harvest, although residues at harvest are consistently higher from applications of higher 2,4-D rates to 14-leaf sugarbeet. Despite this, residues at all combinations of rate and application timing are low. Total residue concentration at harvest only surpassed 10 ppb from the highest rate of 2,4-D applied at the latest timing, and no concentrations within root tissue were higher than 10 ppb at harvest. Because of this, it would be extremely beneficial to sugarbeet growers if a maximum residue level (MRL) were established for 2,4-D in harvested sugarbeet roots. The levels at harvest fall well below the MRLs of comparable crops, such as 0.4 ppm for potato and 0.1 ppm for other root crops (Table 1.1). If a similar limit was established for sugarbeet, the negative impacts of tank-contamination with 2,4-D would be diminished. Unless a more extreme level of exposure occurred, the results of this study suggest that growers would still be able to harvest and profit from sugarbeet exposed to 2,4-D through tank-contamination.

*Dicamba residues.* Sugarbeet treated with 2.8 g $ha^{-1}$ (0.25% rate) of dicamba were sampled to measure the decline of herbicide residues over time following exposure. At one hour after treatment (0 days after treatment - DAT), dicamba residues were only as high as 0.09 and 0.16 ppm at Richville and East Lansing, respectively (Figure 2.8). Dicamba residues were higher in 2-leaf sugarbeet than in 14-leaf sugarbeet 0 DAT at East Lansing, however there was little

difference between dicamba residues in sugarbeet treated at the 2-, 6-, and 14-leaf stages 0 DAT at Richville. This is contrary to 2,4-D residue levels following exposure, as sugarbeet growth stage at the time of 2,4-D exposure had more influence. At both locations, residues began to decline following application. Residues in 2-leaf sugarbeet declined more rapidly in the first week following application than residues in 14-leaf sugarbeet at East Lansing, resulting in similar dicamba residue levels 1 WAT. At Richville, the decline in dicamba residues was similar for all three growth stages. This continued through 3 WAT, where dicamba residues were all below 0.02 ppm. At East Lansing, residues in both 2- and 14-leaf sugarbeet followed a similar pattern of decline from 1 to 3 WAT. Similarly to Richville, dicamba residues were all below 0.02 ppm 3 WAT. Andersen et al. (2004) found that dicamba residues in soybean foliage also decreased more rapidly in the first week after exposure to 5.6 g $ha^{-1}$ of dicamba, from 3.0 to 0.6 $\mu g\ g^{-1}$ in 2001 and from 2.9 to 0.6 $\mu g\ g^{-1}$ in 2002. Like dicamba residues in sugarbeet, residues in soybean foliage continued to decline at a slower rate from 6 to 24 DAT (Andersen et al. 2004). Auch and Arnold (1978) found similar results in soybean foliage following exposure to 11 g $ha^{-1}$ of dicamba, as dicamba residues decreased from 1.2 to 0.3 ppm within the first week after exposure, and then continued to decrease through 18 DAT.

Dicamba residues 14 DAT and at harvest were combined across years at both locations. Contrary to 2,4-D, application timing of dicamba to sugarbeet seemed to have little influence on dicamba residue levels 14 DAT at both Richville and East Lansing when rates were below 22.4 g $ha^{-1}$ (Figure 2.9). However, growth stage at application did have greater influence when dicamba was applied at 22.4 g $ha^{-1}$. At Richville, dicamba residue levels in 6-leaf sugarbeet were 0.08 ppm when dicamba was applied at 22.4 g $ha^{-1}$, while residue levels in 2- and 14-leaf sugarbeet were between 0.01 and 0.05 ppm, respectively (Figure 2.9a). A difference at this rate was also

noted in East Lansing, where dicamba residue levels 14 DAT were 0.01 and 0.1 ppm in 2- and 14-leaf sugarbeet, respectively (Figure 2.9b). Dicamba rates ranging from 0.125-11.2 g $ha^{-1}$ resulted in dicamba residue levels below 0.05 ppm at all three growth stages at both Richville and East Lansing. Andersen et al. (2004) detected higher levels of dicamba residues in soybean foliage compared to dicamba residue levels found in sugarbeet at comparable rates, but still found that dicamba residues were higher with an increased exposure rate. In 2001, at 12 DAT, dicamba rates of 5.6 and 11.2 g $ha^{-1}$ resulted in residue levels of 0.2 and 0.3 ppm, respectively, while a rate of 56 g $ha^{-1}$ resulted in a residue level of 2.2 ppm. Andersen et al. (2004) reported similar dicamba residue levels in soybean foliage for these same rates when the trial was repeated in 2002. (Andersen et al. 2004).

Dicamba rate continued to influence dicamba residue levels in sugarbeet tissue at harvest. Because of the low levels at harvest, residues will now be reported in parts per billion (ppb). At Richville, dicamba residue levels in sugarbeet shoot tissue from the 22.4 and 5.6 g $ha^{-1}$ rates were higher than residue levels from the and 11.2 g $ha^{-1}$ rate, while there was no difference between the 22.4 and 5.6 g $ha^{-1}$ rates (Table 2.9). Residues in shoot tissue were below 5 ppb at all three rates. Unlike in sugarbeet shoot tissue, dicamba rate did not influence residue levels in root tissue, as residues ranged from 0.2 to 1.1 ppb. However, sugarbeet growth stage at the time of application did influence dicamba residue levels in root tissue. Averaged across rates, dicamba residues were higher from an application to 14-leaf sugarbeet compared to 2- and 6-leaf sugarbeet, while there was no difference between the two earlier growth stages. Dicamba residue levels in combined root and shoot tissue at Richville ranged from 1.1 to 4.7 ppb, with the 5.6 and 22.4 g $ha^{-1}$ rates resulting in higher residue levels than the 11.2 g $ha^{-1}$ rate. These results contradict the findings of Andersen et al. (2004), as they could not detect a difference in dicamba

residues in soybean foliage 48 days after being treated with a broader range of dicamba rates from 0-56 g $ha^{-1}$. However, both dicamba and 2,4-D residue levels were low at harvest, as 2,4-D residues never exceeded 12.0 ppb in combined root and shoot tissue. Dicamba rate had a similar effect on harvest residue levels at East Lansing. Similar to Richville, the 22.4 g $ha^{-1}$ rate of dicamba resulted in higher residue levels in shoot tissue than the 11.2 g $ha^{-1}$ rate, while the 5.6 g $ha^{-1}$ rate did not differ from the other two rates (Table 2.10). Residue levels in shoot tissue ranged from 1.7 to 6.2 ppb. Dicamba rate again had no influence on residue levels in root tissue at East Lansing, as residues levels ranged from 0.3 to 1.7 ppb. While dicamba residue levels were slightly higher at East Lansing than Richville, residues in combined root and shoot tissue were not higher than 8.0 ppb across all dicamba rates. Unlike at Richville, the main effect of sugarbeet growth stage at the time of dicamba application did not influence dicamba residue levels in either root or shoot tissue at East Lansing.

Like 2,4-D, dicamba residues showed that they can quickly decline following exposure to sugarbeet, while all dicamba rates resulted in residue levels below 0.15 ppm 14 DAT. Much like 2,4-D, dicamba residue levels at harvest were low, as residues in combined root and shoot tissue never exceeded 10 ppb at both Richville and East Lansing. Boerboom (2003) found a maximum of 0.63% of the field use rate of dicamba following a proper three-rinse cleanout, which falls within the range of dicamba rates tested in this study. These findings indicate that a tank-contamination event at this rate would result in dicamba residue levels less than 10 ppb in harvested sugarbeet, while a more extreme event may result in higher residue levels. This again stresses the importance of both proper tank cleanout to avoid high exposure rates as well as establishing an MRL for dicamba in sugarbeet. Without an MRL, these low levels of dicamba residues at harvest are still considered unacceptable, despite being lower than the MRL for

dicamba in several other crops, such as 0.10 ppm in field corn and 0.30 ppm in sugarcane (Table 1.1). However, if a similar MRL was established for dicamba in sugarbeet, the potential risks of exposure to dicamba would be reduced. These results suggest that dicamba residues at harvest would be low enough that farmers could still sell their product despite exposure.

**Greenhouse Experiments.** *Response of sugarbeet to dicamba and 2,4-D.* Sugarbeet growth stage at the time of herbicide application did not significantly influence the $ED_{50}$ values for herbicide injury to sugarbeet 7 DAT for either dicamba or 2,4-D. The $ED_{50}$ for 2,4-D applied to 2-leaf sugarbeet was 7.9%, which was less than the $ED_{50}$ for dicamba, 13.9%, applied at the same stage (Table 2.11). The same was found for herbicides applied to 6-leaf sugarbeet, as the $ED_{50}$ values for 2,4-D and dicamba were 5.9 and 13.7%, respectively. Similar to injury noted in field experiments, $ED_{50}$ values were lower 7 DAT compared with 14 DAT, indicating an increase in sugarbeet injury over time. Similar to injury at 7 DAT, sugarbeet growth stage at application did not result in significantly different $ED_{50}$ values 14 DAT for either dicamba or 2,4-D. However, the differences in herbicides persisted. $ED_{50}$ values 14 DAT were different for applications to 2-leaf sugarbeet, with values of 4.8 and 6.8% for 2,4-D and dicamba, respectively. For herbicides applied to 6-leaf sugarbeet, $ED_{50}$ values were 4.1 and 7.8% for 2,4-D and dicamba, respectively.

Only application timing influenced the $ED_{50}$ of sugarbeet shoot biomass, as herbicides did not differ within either application timing. The $ED_{50}$ values for 2,4-D and dicamba applied to 6-leaf sugarbeet were 13.6 and 12.2%, respectively, while the values for 2-leaf sugarbeet were 2.1 and 3.8 for 2,4-D and dicamba, respectively (Table 2.11). Comparisons were similar for $ED_{50}$ values of total sugarbeet biomass, as only application timing influenced these values. While

Schweizer (1978) found that sugarbeet foliage following 2,4-D application to 4- and 8-leaf sugarbeet was suppressed 55% by 70 g $ha^{-1}$ 2 WAT, the results of the present study indicate that the rate for this level of growth suppression is approximately 23 g $ha^{-1}$. However, the numbers reported by Schweizer (1978) were based on visual estimates. In sugarbeet root biomass, the $ED_{50}$ value for dicamba applied to 6-leaf sugarbeet was significantly higher at 15.4% than all other herbicide and timing combinations. The $ED_{50}$ of 5.0% for both dicamba and 2,4-D applied to 2-leaf sugarbeet and 9.1% for 2,4-D applied to 6-leaf sugarbeet were not significantly different. These results show that herbicide injury with dicamba and 2,4-D does not necessarily equate to biomass reductions. This is somewhat characteristic of plant growth regulator herbicides such as dicamba and 2,4-D, as they cause plant malformation but not necessarily tissue reduction or necrosis.

*Influence of glyphosate.* The presence of glyphosate in the spray solution only influenced herbicide injury to sugarbeet when dicamba was applied to 6-leaf sugarbeet. Glyphosate increased the herbicidal activity of dicamba at this stage, as the $ED_{20}$ value was reduced from 0.9% for dicamba alone to 0.6% for dicamba plus glyphosate (Table 2.12). $ED_{20}$ values with and without glyphosate were not different for 2,4-D or dicamba applied to 2-leaf sugarbeet or 2,4-D applied to 6-leaf sugarbeet. This is in agreement with Flessner et al. (2015), who determined that the addition of glyphosate to the spray solution did not increase the control of glyphosate-resistant horseweed compared with dicamba alone. Similarly, Kelley et al. (2005) found that glyphosate did not increase the herbicidal effects of dicamba on soybean injury and yield.

When dry weights were measured, there was no interaction between glyphosate and herbicide rate or application timing for 2,4-D or dicamba, so the main effect of glyphosate was

compared. The addition of glyphosate did not influence the effects of either dicamba or 2,4-D on sugarbeet shoot biomass (Table 2.13). However, the addition glyphosate did reduce sugarbeet root biomass. Root biomass was 0.1 g lower when glyphosate was added to dicamba and 2,4-D (Table 2.13). Similarly, differences were noted in total sugarbeet biomass, as total biomass was lower when glyphosate was added to both herbicides. These results raise some concerns, as glyphosate is the primary herbicide for weed control in sugarbeet. Therefore, it is most likely that, if sugarbeet are exposed to these low rates of dicamba and 2,4-D, it will be in conjunction with a full rate of glyphosate. This combination appears to have the potential to affect sugarbeet with slightly more severity than if sugarbeet were exposed to dicamba or 2,4-D alone. Based on these results, it cannot be determined if these effects would persist past 14 DAT, if the same response would occur to sugarbeet in later growth stages, or if they would impact final yield. More extensive research would be needed to further evaluate these possibilities.

*Influence of ammonium sulfate.* The addition of ammonium sulfate (AMS) to dicamba and 2,4-D did not result in a significant difference in the $ED_{20}$ values for either herbicide (Table 2.14). The addition of AMS to dicamba or 2,4-D did not have a significant interaction with herbicide rate for sugarbeet biomass. When combined over rates, the addition of AMS to 2,4-D decreased shoot biomass by 0.2 g. However, the same pattern was not reflected in root biomass or total biomass. The addition of AMS to dicamba did not impact sugarbeet shoot, root, or total biomass. These results contradict the findings of Roskamp et al. (2013), as they found that AMS increased the herbicidal activity of both dicamba and 2,4-D on several different weed species in the presence of a range of cationic solutions as well as deionized water. However, they also found that the addition of AMS did not influence injury to common lambsquarters from 2,4-D when mixed in

deionized water. The fact that sugarbeet and common lambsquarters are both members of the *Chenopodiaceae* family could be why these results are similar. Considering that the present study was also conducted using deionized water, these results are similar. Nalewaja and Matysiak (1993) also found that AMS increased the herbicidal activity of several different formulations of dicamba and 2,4-D against kochia. The discrepancies in results could be due to the large differences in herbicide rates applied, as Nalewaja and Matysiak (1993) applied dicamba and 2,4-D at 70 and 220 g $ha^{-1}$, respectively, while Roskamp et al. (2013) applied dicamba and 2,4-D at 280 and 266 g $ha^{-1}$, respectively. These rates are much higher than the rates applied to sugarbeet, which could magnify the effects that AMS has on the activity of both herbicides. Considering that the primary method of weed control in sugarbeet is glyphosate with the addition of AMS, these results are promising to growers who are concerned about tank-contamination with dicamba and 2,4-D. It appears that the presence of AMS does not seriously amplify the effects of these herbicides on sugarbeet at the low rates expected from tank-contamination.

Based on our research, both dicamba and 2,4-D have the ability to significantly injure sugarbeet at rates that would be typically found in a tank-contamination event. However, this injury does not always equate to losses in yield or recoverable white sucrose, which is encouraging to sugarbeet growers who are at a high risk for tank-contamination. The rates of dicamba and 2,4-D that could remain in a spray system following proper cleanout, as outlined by Boerboom (2004) and Cundiff et al. (2017), were not high enough to reduced sugarbeet yield or recoverable white sucrose. This stresses the importance of following the proper protocol for spray system cleanout, especially considering that improper cleanout could result in high enough rates remaining in the tank to negatively impact sugarbeet. Sugarbeet exposed to sub-lethal rates

of 2,4-D resulted at the 14-leaf growth stage resulted in reduced yields compared with sugarbeet exposed earlier in the season. Because of this, growers should avoid late-season applications of 2,4-D to other crops, especially if being applied in close proximity to sugarbeet or if the same sprayer is used for pesticide applications in sugarbeet.

Following exposure, residues of 2,4-D and dicamba declined in sugarbeet. Residue concentrations of both herbicides at harvest were extremely low. 2,4-D residues in both root and shoot tissue were well below the maximum residue limits of 2,4-D in comparable crops, while dicamba residue levels at harvest were far below maximum residue limits of dicamba established in other crops. Unfortunately, there are currently no maximum residue limits established for either dicamba or 2,4-D in sugarbeet, making even these low concentrations unacceptable. The establishment of MRLs for these herbicides in sugarbeet would be extremely beneficial to growers, as exposure to rates expected from tank-contamination would likely result in harvest residue levels being below this limit. This would allow growers to harvest and profit from sugarbeet despite exposure to dicamba and 2,4-D through tank-contamination.

# APPENDICES

# APPENDIX A

Tables and Figures

*Table 1.1*. Maximum residue levels (MRLs) for 2,4-D and dicamba in several different commodities (Cornell Law School 2016; Cornell Law School 2017).

| Commodity | 2,4-D MRL | Dicamba MRL |
|---|---|---|
| | ppm | |
| Corn, field, grain | 0.05 | 0.10 |
| Corn, sweet, kernel + cob | 0.05 | 0.04 |
| Potato | 0.40 | -- |
| Rye, grain | 2.00 | 2.00 |
| Sorghum, grain, grain | 0.20 | 4.00 |
| Soybean, seed | 0.02 | 10.00 |
| Sugarcane, cane | 0.05 | 0.30 |
| Vegetable, root & tuber, except potato | 0.10 | -- |
| Vegetable, cucurbit, group 9 | 0.05 | -- |
| Wheat, grain | 2.00 | 2.00 |

*Table 2.1*. Planting, herbicide application, and harvest dates for 2,4-D and dicamba tank-contamination experiments conducted in Richville and East Lansing, MI, in 2016 and 2017.

| | 2016 | | 2017 | |
|---|---|---|---|---|
| | Richville | East Lansing | Richville | East Lansing |
| Planting | April 16 | April 18 | April 18 | May 17 |
| Application timing | | | | |
| 2-leaf sugarbeet | May 20 | May 20 | May 16 | June 9 |
| 6-leaf sugarbeet[a] | June 2 | -- | June 2 | -- |
| 14-leaf sugarbeet | June 22 | June 22 | July 5 | August 2 |
| Harvest | September 20 | September 22 | September 20 | October 10[b] |

[a]The 6-leaf application timing was not included in East Lansing.

[b]Sugarbeets from the dicamba experiment were not harvested at the East Lansing location in 2017.

*Table 2.2*. The effective dose of 2,4-D to cause 20% injury ($ED_{20}$) 7, 14, and 21 days after treatment (DAT) when applied to 2-, 6-, and 14-leaf sugarbeet in Richville and East Lansing, MI.[a]

| Application timing | Richville | | | East Lansing | | |
|---|---|---|---|---|---|---|
| | 7 DAT | 14 DAT | 21 DAT | 7 DAT | 14 DAT | 21 DAT |
| | ——— % of field use rate[b] ——— | | | ——— % of field use rate ——— | | |
| 2-leaf | 1.46 a[c] | 0.82 a | 1.53 a | 1.11 a | 0.78 a | 1.33 a |
| 6-leaf | 0.90 b | 1.01 a | 1.01 b | -- | -- | -- |
| 14-leaf | 1.04 b | 0.79 a | 1.03 b | 0.79 b | 0.64 a | 1.02 a |

[a]Data was combined over 2016 and 2017.

[b]The 1X field use rate of 2,4-D was 1.12 kg $ha^{-1}$.

[c]Values followed by the same letter within the same column are not significantly different at $\alpha = 0.05$.

*Table 2.3*. The effective dose of dicamba to cause 20% injury ($ED_{20}$) 7, 14, and 21 days after treatment (DAT) when applied to 2-, 6-, and 14-leaf sugarbeet in Richville and East Lansing, MI.[a]

| Application timing | Richville | | | East Lansing | | |
|---|---|---|---|---|---|---|
| | 7 DAT | 14 DAT | 21 DAT | 7 DAT | 14 DAT | 21 DAT |
| | ——— % of field use rate[b] ——— | | | ——— % of field use rate ——— | | |
| 2-leaf | 1.56 a[c] | 0.89 a | 1.46 a | 0.48 b | 0.45 b | 0.78 b |
| 6-leaf | 0.70 c | 0.86 a | 0.90 b | -- | -- | -- |
| 14-leaf | 0.91 b | 0.95 a | 1.04 b | 0.90 a | 0.78 a | 1.19 a |

[a]Data was combined over 2016 and 2017.

[b]The 1X field use rate of dicamba was 1.12 kg $ha^{-1}$.

[c]Values followed by the same letter within the same column are not significantly different at $\alpha = 0.05$.

*Table 2.4.* The main effect of 2,4-D rate on sugarbeet yield and recoverable white sucrose $ha^{-1}$ (RWSH) following exposure in Richville and East Lansing, MI.[a]

| | | Richville | | East Lansing | |
|---|---|---|---|---|---|
| 2,4-D rate | | Yield | RWSH | Yield | RWSH |
| g ae $ha^{-1}$ | (%)[b] | — Mg $ha^{-1}$ — | — kg $ha^{-1}$ — | — Mg $ha^{-1}$ — | — kg $ha^{-1}$ — |
| 0 | 0 | 52.2 a[c] | 6840 a | 69.2 a | 7902 a |
| 1.4 | 0.125 | 54.7 a | 7226 a | 64.8 ab | 7386 ab |
| 2.8 | 0.25 | 54.9 a | 7450 a | 64.3 ab | 7403 ab |
| 5.6 | 0.5 | 53.8 a | 7199 a | 64.7 ab | 7433 ab |
| 11.2 | 1 | 49.7 a | 6614 a | 58.2 ab | 6563 ab |
| 22.4 | 2 | 37.8 b | 4936 b | 55.6 b | 6102 b |

[a]Data was combined over years and application timings since there was not a significant interaction between herbicide rate and sugarbeet growth stage.

[b]Percentages are based on the 1X field use rate of 2,4-D at 1.12 kg $ha^{-1}$.

[c]Means within the same column followed by the same letter are not significantly different at $\alpha = 0.05$.

*Table 2.5*. The main effect sugarbeet growth stage at the time of 2,4-D exposure on yield and recoverable white sucrose $ha^{-1}$ (RWSH) at Richville and East Lansing, MI.[a]

| Sugarbeet growth stage | Richville | | East Lansing | |
|---|---|---|---|---|
| | Yield | RWSH | Yield | RWSH |
| | — Mg $ha^{-1}$ — | — kg $ha^{-1}$ — | — Mg $ha^{-1}$ — | — kg $ha^{-1}$ — |
| 2-leaf | 54.6 a[b] | 7257 a | 65.3 a | 7415 |
| 6-leaf | 51.9 a | 6916 a | --[c] | -- |
| 14-leaf | 45.1 b | 5961 b | 60.3 b | 6848 |

[a]Data was combined over years and 2,4-D rates since there was not a significant interaction between 2,4-D rate and sugarbeet growth stage.

[b]Means within the same column followed by the same letter are not significantly different at $\alpha = 0.05$.

[c]The 6-leaf timing was not included at East Lansing.

*Table 2.6.* The main effect of dicamba rate on sugarbeet yield and recoverable white sucrose $ha^{-1}$ (RWSH) following exposure in Richville and East Lansing, MI.[a]

| | | Richville | | East Lansing[b] | |
|---|---|---|---|---|---|
| Dicamba rate | | Yield | RWSH | Yield | RWSH |
| g ae $ha^{-1}$ | (%)[c] | — Mg $ha^{-1}$ — | — kg $ha^{-1}$ — | — Mg $ha^{-1}$ — | — kg $ha^{-1}$ — |
| 0 | 0 | 65.0 a[d] | 8593 a | 69.2 | 6622 |
| 1.4 | 0.125 | 65.1 a | 8632 a | 71.6 | 6533 |
| 2.8 | 0.25 | 63.4 a | 8286 a | 68.9 | 6454 |
| 5.6 | 0.5 | 64.0 a | 8370 a | 68.4 | 6419 |
| 11.2 | 1 | 61.0 a | 8003 a | 67.2 | 6310 |
| 22.4 | 2 | 54.2 b | 6970 b | 65.8 | 6042 |

[a]Data was combined over years and application timings since there was not a significant interaction between herbicide rate and sugarbeet growth stage.

[b]Data is only presented from the 2016 experiment in East Lansing, since sugarbeet at this location were not harvested in 2017.

[c]Percentages are based on the 1X field use rate of dicamba at 1.12 kg $ha^{-1}$.

[d]Means within the same column followed by the same letter are not significantly different at $\alpha = 0.05$.

*Table 2.7*. Concentrations of 2,4-D residues in sugarbeet tissue at harvest following applications of multiple rates of 2,4-D at three sugarbeet growth stages in Richville, MI.[a]

| | Tissue source | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Shoot | | | Root | | | Combined tissue | | |
| 2,4-D rate | 2-leaf | 6-leaf | 14-leaf | 2-leaf | 6-leaf | 14-leaf | 2-leaf | 6-leaf | 14-leaf |
| g ae $ha^{-1}$ | ppb | | | | | | | | |
| 2.8 | 1.0 bc[a] | 0.8 bc | 1.0 bc | 0.6 d-f | 0.5 d-f | 0.9 cd | 1.6 cd | 1.3 cd | 1.9 b-d |
| 5.6 | 0.9 bc | 0.9 bc | 1.2 b | 0.4 ef | 0.6 d-f | 1.1 bc | 1.3 cd | 1.4 cd | 2.3 bc |
| 11.2 | 0.8 c | 0.8 bc | 1.4 ab | 0.4 f | 1.1 c-e | 1.8 ab | 1.1 d | 1.9 b-d | 3.2 ab |
| 22.4 | 1.0 bc | 0.7 bc | 2.2 a | 0.5 d-f | 0.6 d-f | 2.3 a | 1.5 cd | 1.4 cd | 4.6 a |

[a]Data was combined over both years of this research.

[b]Means followed by the same letter within tissue type are not significantly different at $\alpha = 0.05$.

*Table 2.8*. Concentrations of 2,4-D in sugarbeet tissue at harvest following applications of multiple rates of 2,4-D at two sugarbeet growth stages in East Lansing, MI.[a]

| | Tissue source | | | | | |
|---|---|---|---|---|---|---|
| | Shoot[b] | | Root | | Combined tissue | |
| 2,4-D rate | 2-leaf | 14-leaf | 2-leaf | 14-leaf | 2-leaf | 14-leaf |
| g ae $ha^{-1}$ | ppb | | | | | |
| 2.8 | 0.9 | 1.3 | 0.9 b-d[c] | 2.1 a-c | 1.8 c-e | 3.3 a-d |
| 5.6 | 0.8 | 1.8 | 1.5 b-d | 3.0 ab | 2.3 b-e | 4.8 a-c |
| 11.2 | 0.9 | 2.1 | 0.8 cd | 6.1 a | 1.7 de | 8.2 ab |
| 22.4 | 0.7 | 2.4 | 0.5 d | 8.6 a | 1.1 e | 12.0 a |

[a]Data was combined over both years of this research.

[b]The interaction between 2,4-D rate and sugarbeet growth stage at application timing was not significant for residue concentrations within shoot tissue. However, the main factor of application timing was significant at $\alpha \leq 0.05$., as residue levels from 14-leaf sugarbeet were 2.1 ppb compared with 0.8 ppb from the 2-leaf application timing.

[c]Means followed by the same letter within tissue type are not significantly different at $\alpha = 0.05$.

*Table 2.9*. Concentrations of dicamba residues in sugarbeet tissue at harvest following applications of multiple rates of dicamba at three sugarbeet growth stages in Richville, MI.[a]

| | Tissue source | | |
|---|---|---|---|
| Dicamba rate | Shoot | Root | Combined tissue |
| g ae $ha^{-1}$ | ppb | | |
| 5.6 | 4.1 a[a] | 0.6 | 4.7 a |
| 11.2 | 0.9 b | 0.2 | 1.1 b |
| 22.4 | 4.1 a | 1.1 | 4.5 a |

[a]Data was combined over both years of this research and over sugarbeet growth stage.

[b]The main effect of dicamba rate was not significant for residue concentrations within root tissue. However, the main effect of sugarbeet growth stage at application was significant. Dicamba residues from the 14-leaf application timing (1.0 ppb) were significantly greater than residues from the 2-leaf (0.2 ppb) and 6-leaf (0.4 ppb) application timings. Dicamba residues from the 2- and 6-leaf application timings were not significantly different.

[b]Means followed by the same letter within tissue type are not significantly different at $\alpha = 0.05$.

*Table 2.10*. Concentrations of dicamba in sugarbeet tissue at harvest following applications of multiple rates of dicamba at two sugarbeet growth stages in East Lansing, MI.[a]

| | Tissue source | | |
|---|---|---|---|
| Dicamba rate | Shoot | Root | Combined tissue |
| g ae $ha^{-1}$ | ppb | | |
| 5.6 | 3.9 ab[b] | 0.9 | 4.8 a |
| 11.2 | 1.7 b | 0.3 | 2.2 b |
| 22.4 | 6.2 a | 1.7 | 8.0 a |

[a]Data was combined over both years of this research and over sugarbeet growth stage.

[b]Means followed by the same letter within tissue type are not significantly different at $\alpha = 0.05$.

*Table 2.11*. The effective dose to cause 50% injury to sugarbeet and reduce biomass by 50% ($ED_{50}$) in sugarbeet exposed to dicamba and at the 2- and 6-leaf growth stages of sugarbeet in the greenhouse.

| | | $ED_{50}$ | | | | |
|---|---|---|---|---|---|---|
| | | Injury | | Biomass | | |
| Herbicide[a] | Timing | 7 DAT | 14 DAT | Shoot | Root | Total |
| | | % of field use rate[b] | | | | |
| 2,4-D | 2-leaf | 7.9 b[c] | 4.8 b | 2.1 b | 5.0 b | 2.1 b |
| | 6-leaf | 5.9 b | 4.1 b | 13.6 a | 9.1 b | 12.4 a |
| Dicamba | 2-leaf | 13.9 a | 6.8 a | 3.8 b | 5.0 b | 3.8 b |
| | 6-leaf | 13.7 a | 7.8 a | 12.2 a | 15.4 a | 12.8 a |

[a]All herbicide applications included glyphosate at 0.84 kg ae $ha^{-1}$ and ammonium sulfate at 2% w $w^{-1}$.

[b]Percentages are based on the 1X field use rate of 2,4-D and dicamba at 1.12 kg $ha^{-1}$.

[c]Values within the same column followed by the same letter are not significantly different at $\alpha = 0.05$.

*Table 2.12*. The effective dose to cause 20% injury ($ED_{20}$) to sugarbeet when 2,4-D and dicamba were applied with and without glyphosate at two sugarbeet growth stages in the greenhouse.

| Herbicide[a] | Timing | $ED_{20}$ Alone | $ED_{20}$ w/ GLY[b] | p-value[d] |
|---|---|---|---|---|
| | | ——— % of field use rate[c] ——— | | |
| 2,4-D | 2-leaf | 0.9 | 1.0 | 0.4811 |
| | 6-leaf | 0.6 | 0.6 | 0.9392 |
| Dicamba | 2-leaf | 1.12 | 1.0 | 0.6049 |
| | 6-leaf | 0.9 | 0.6 | 0.0064 |

[a]All herbicide applications included ammonium sulfate at 2% w $w^{-1}$.

[b]Glyphosate was included at a rate of 0.84 kg ae $ha^{-1}$.

[c]Percentages are based on the 1X field use rate of 2,4-D and dicamba at 1.12 kg $ha^{-1}$.

[d]P-values were determined using a paired t-test comparison. $ED_{20}$ values are significantly different at $\alpha = 0.05$.

*Table 2.13*. Influence of glyphosate on sugarbeet biomass following treatment with multiple rates of dicamba and 2,4-D at two sugarbeet growth stages.

| Herbicide[a] | Glyphosate[b] | Shoot Biomass | Root Biomass | Total Biomass |
|---|---|---|---|---|
| | | grams | | |
| Dicamba | Yes | 3.1 a[c] | 0.4 b | 3.5 b |
| | No | 3.2 a | 0.5 a | 3.7 a |
| 2,4-D | Yes | 2.9 a | 0.5 b | 3.4 b |
| | No | 3.0 a | 0.6 a | 3.6 a |

[a]Herbicides were analyzed separately, and data are combined across herbicide rate and sugarbeet growth stage since the presence of glyphosate did not have a significant interaction with either factor. All herbicide treatments included ammonium sulfate at 2% w $w^{-1}$.

[b]Glyphosate rate was 0.84 kg ae $ha^{-1}$.

[c]Means followed by the same letter within the same column and within herbicide are not significantly different at $\alpha = 0.05$.

*Table 2.14*. Influence of ammonium sulfate (AMS) on herbicide injury and sugarbeet biomass following application of multiple rates of dicamba and 2,4-D.

| Herbicide[a] | AMS[b] | Injury $ED_{20}$[c] | Shoot Biomass | Root Biomass | Total Biomass |
|---|---|---|---|---|---|
| | | — % — | — grams — | — grams — | — grams — |
| Dicamba | Yes | 0.4 a[d] | 2.9 a | 0.6 a | 3.5 a |
| | No | 0.5 a | 3.1 a | 0.6 a | 3.6 a |
| 2,4-D | Yes | 0.8 a | 2.7 b | 0.6 a | 3.4 a |
| | No | 1.0 a | 2.9 a | 0.7 a | 3.6 a |

[a]Herbicides were analyzed separately, and data are combined across herbicide rate since there was no interaction with the presence of AMS. All herbicide treatments included glyphosate at 0.84 kg ae $ha^{-1}$.

[b]AMS rate was 2% w $w^{-1}$.

[c]Values are expressed as the percentage of the 1X field use rate of 2,4-D and dicamba at 1.12 kg ae $ha^{-1}$.

[d]Means followed by the same letter within the same column and within herbicide are not significantly different at $\alpha = 0.05$.

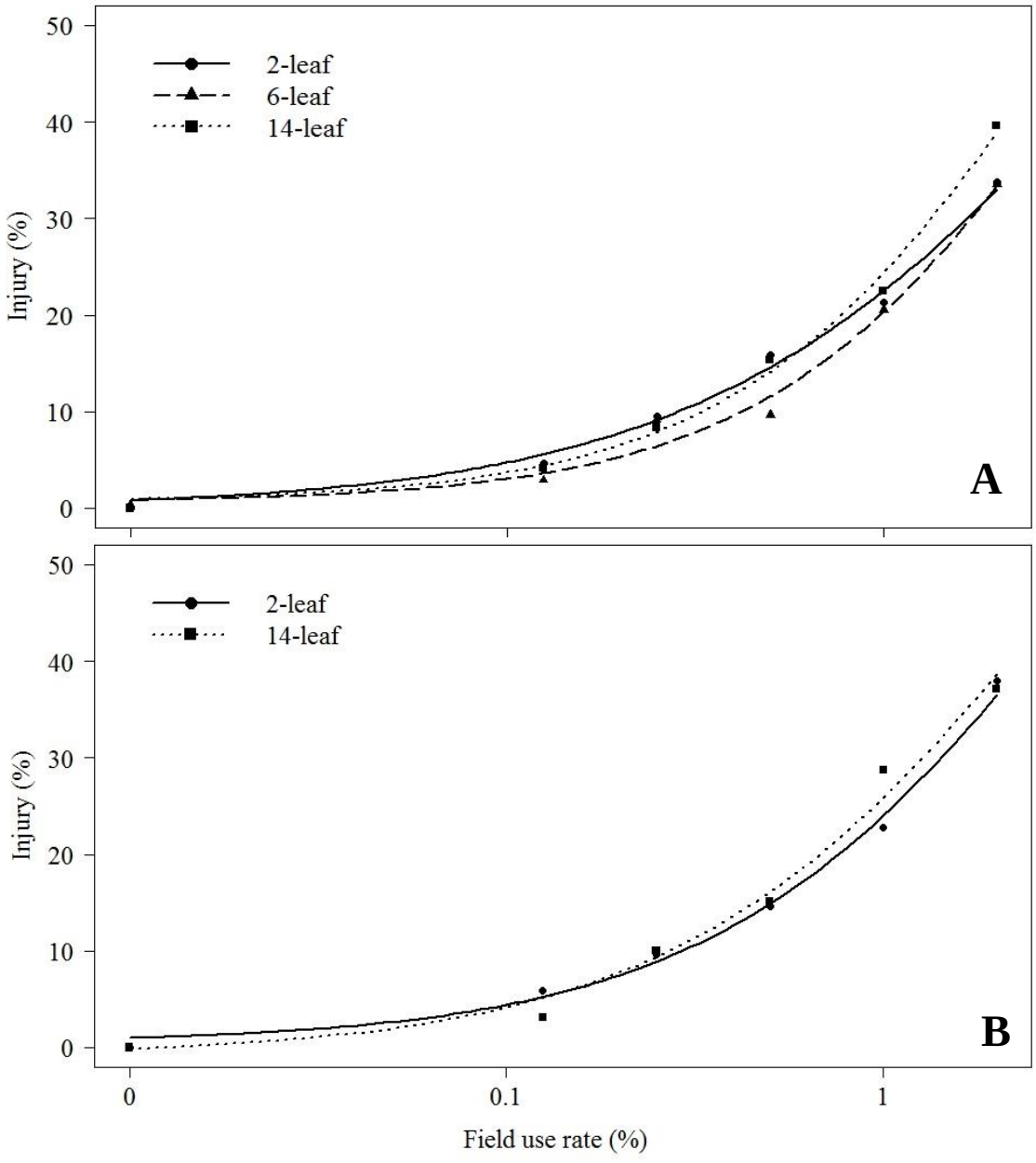



*Figure 2.1.* Herbicide injury to sugarbeet 14 DAT from multiple rates of 2,4-D applied at the 2-, 6-, and 14-leaf growth stages in Richville (A) and East Lansing (B), MI. Data was combined over the two years of this study.

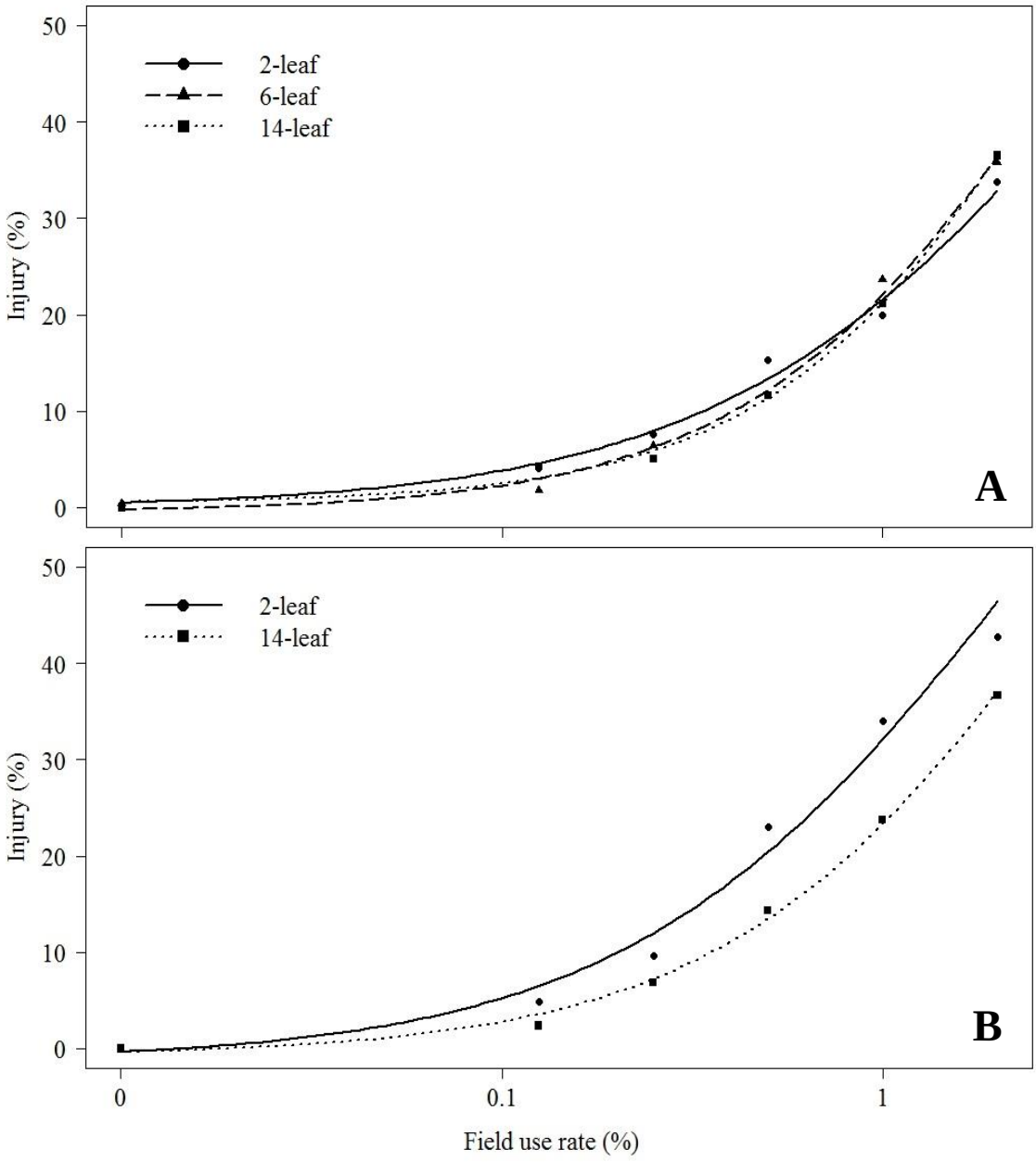



*Figure 2.2*. Herbicide injury to sugarbeet 14 DAT from multiple rates of dicamba applied at the 2-, 6-, and 14-leaf growth stages in Richville (A) and East Lansing (B), MI. Data was combined over the two years of the study.

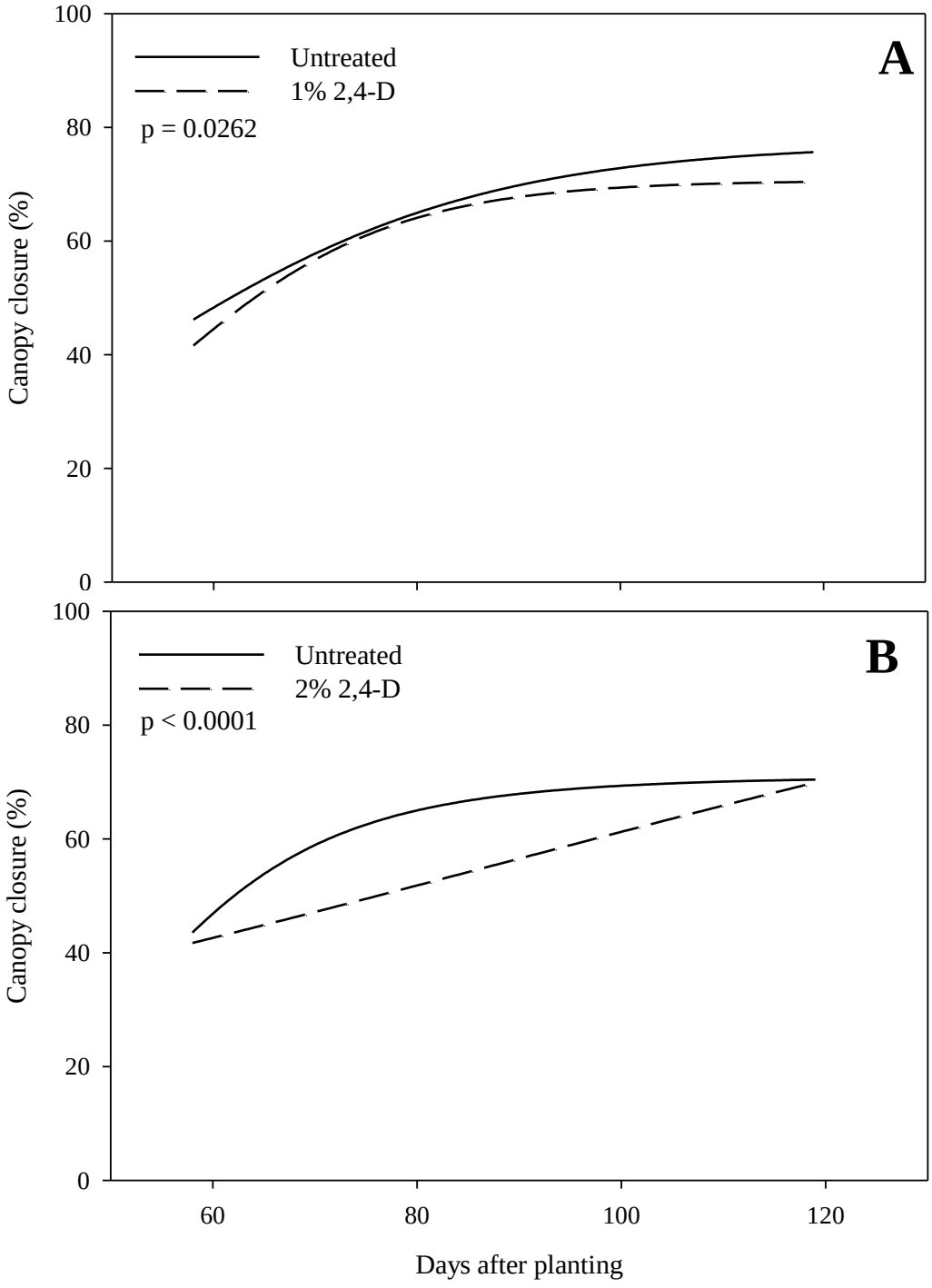
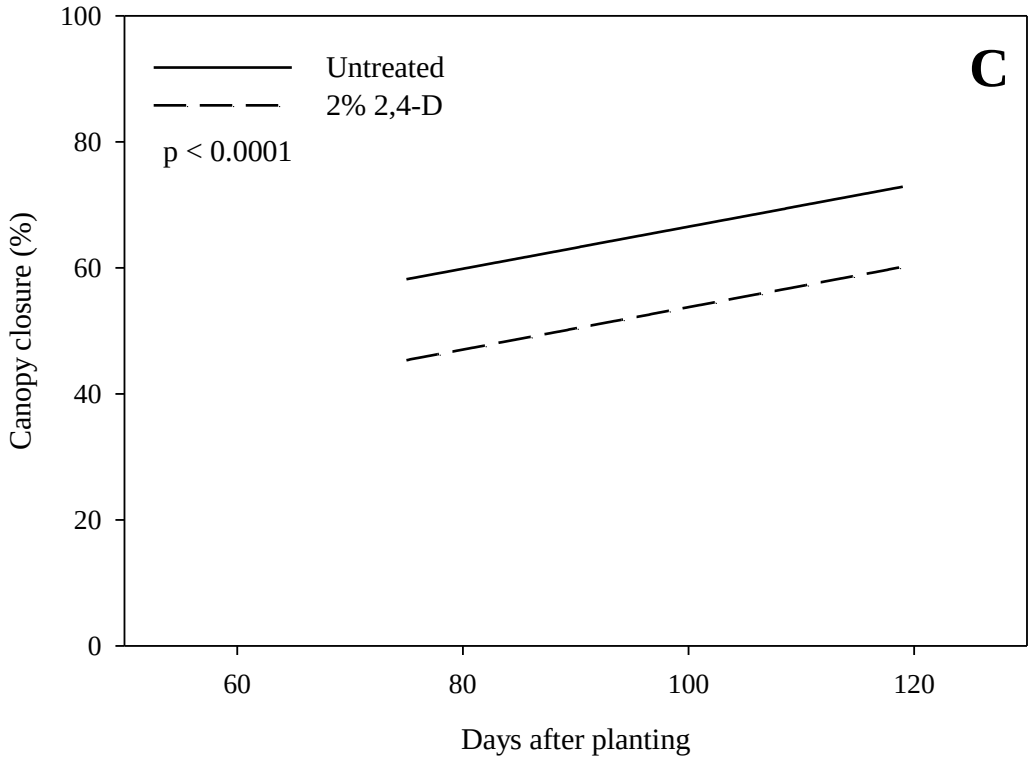



*Figure 2.3*. Canopy closure of sugarbeet in Richville, MI, exposed to the 1% (11.2 g ae $ha^{-1}$) rate of 2,4-D at the 2-leaf stage (A), and the 2% (22.4 g ae $ha^{-1}$) rate of 2,4-D at the 6- (B), and 14-leaf (C) growth stages compared with sugarbeet not exposed to 2,4-D.

*Figure 2.3* (cont'd).




Measurements started approximately 30, 14, and 7 DAT for the 2-, 6-, and 14-leaf stages, respectively. Data was combined over years, and lines were compared using the extra sum of squares principle for nonlinear regression at $\alpha = 0.05$. Canopy closure of sugarbeet exposed to 2,4-D was significantly slower at all growth stages.

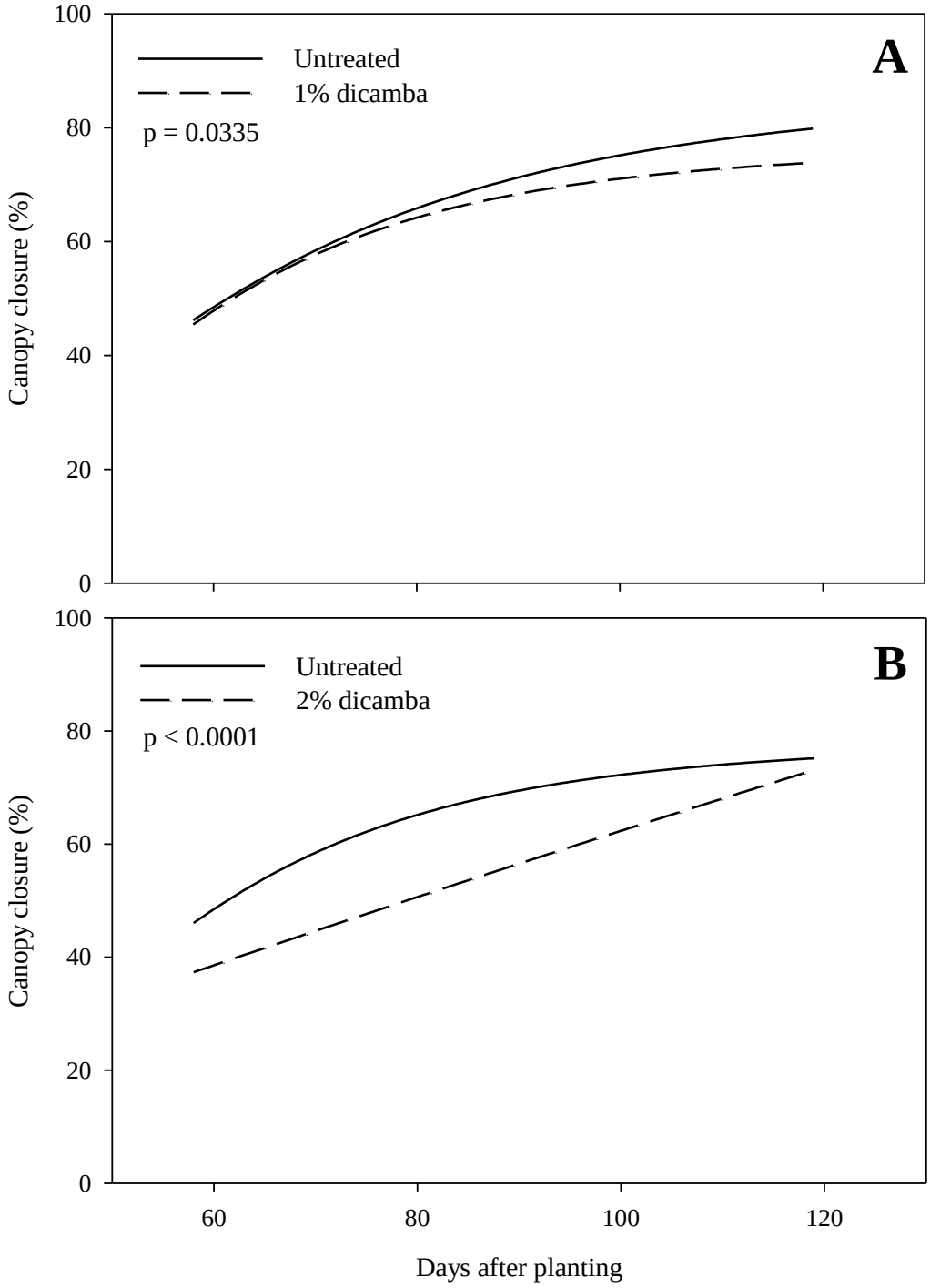
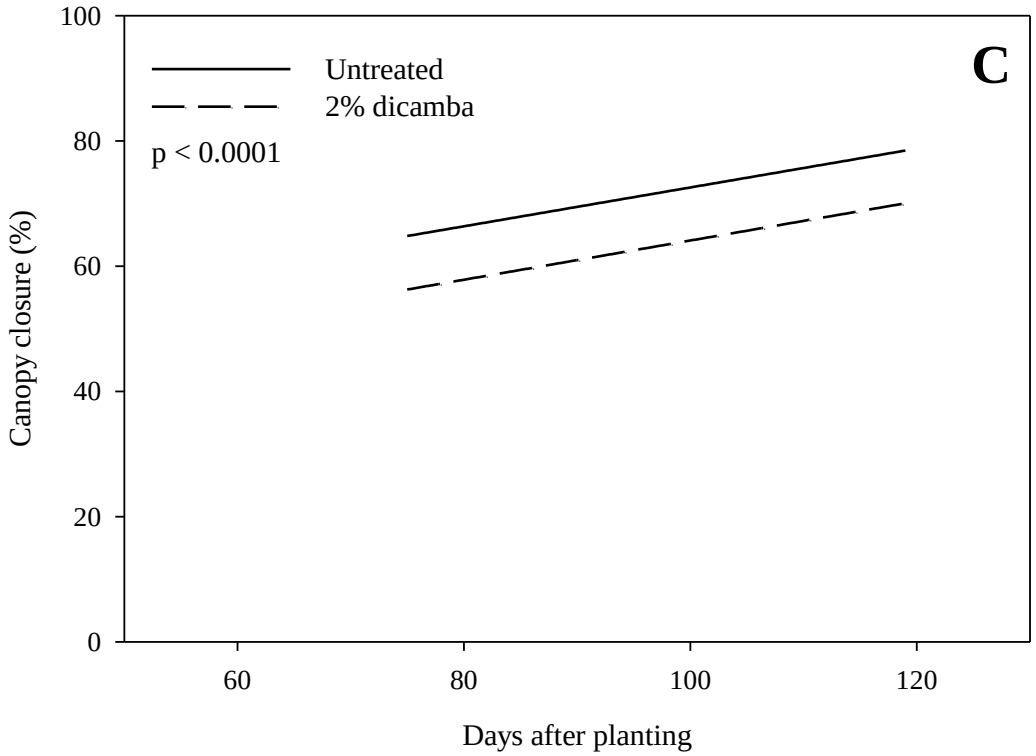



*Figure 2.4*. Canopy closure of sugarbeet in Richville, MI, exposed to the 1% (11.2 g ae $ha^{-1}$) rate of dicamba at the 2-leaf stage (A), and the 2% (22.4 g ae $ha^{-1}$) rate of dicamba at the 6- (B), and 14-leaf (C) growth stages compared with sugarbeet not exposed to dicamba.

*Figure 2.4* (cont'd).




Measurements started approximately 30, 14, and 7 DAT for the 2-, 6-, and 14-leaf stages, respectively. Data was combined over years, and lines were compared using the extra sum of squares principle for nonlinear regression at $\alpha = 0.05$. Canopy closure of sugarbeet exposed to dicamba was significantly slower at all growth stages.

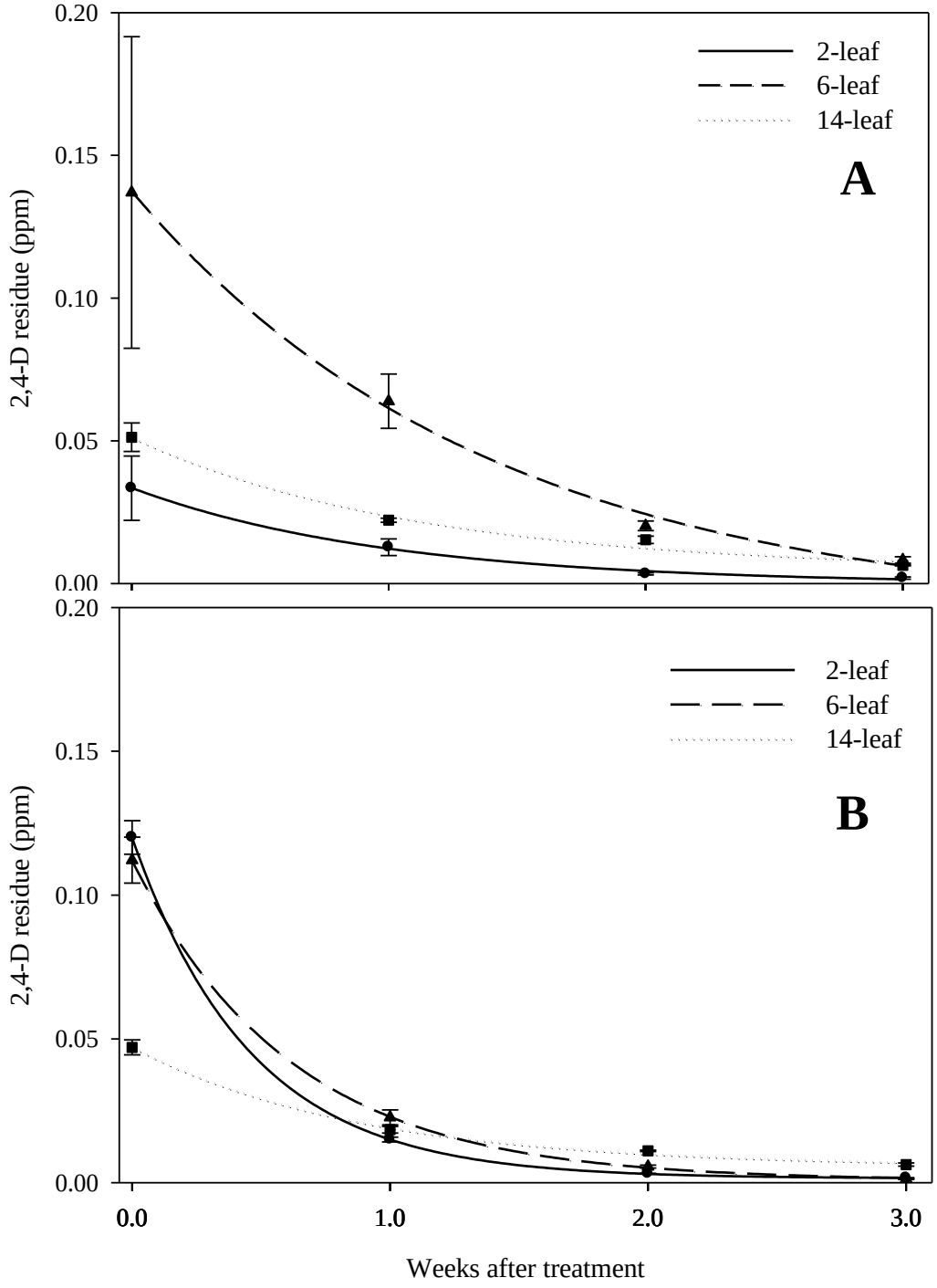



*Figure 2.5*. 2,4-D residues for the three weeks following exposure at the 2- (●), 6- (▲), and 14-leaf (■) growth stages in 2016 (A) and 2017 (B) in Richville. Data was combined over years. Vertical bars represent standard errors of the mean.

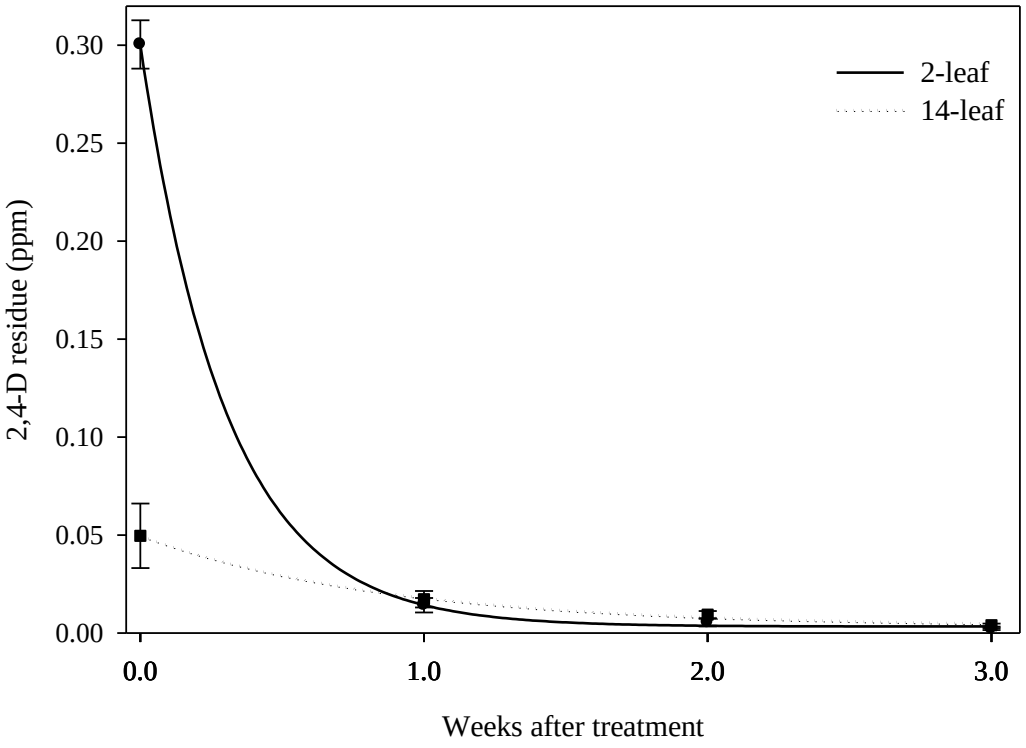



*Figure 2.6.* 2,4-D residues for the three weeks following exposure at the 2- (●) and 14-leaf (■) growth stages in East Lansing, MI. Data was combined over years. Vertical bars represent standard errors of the mean.

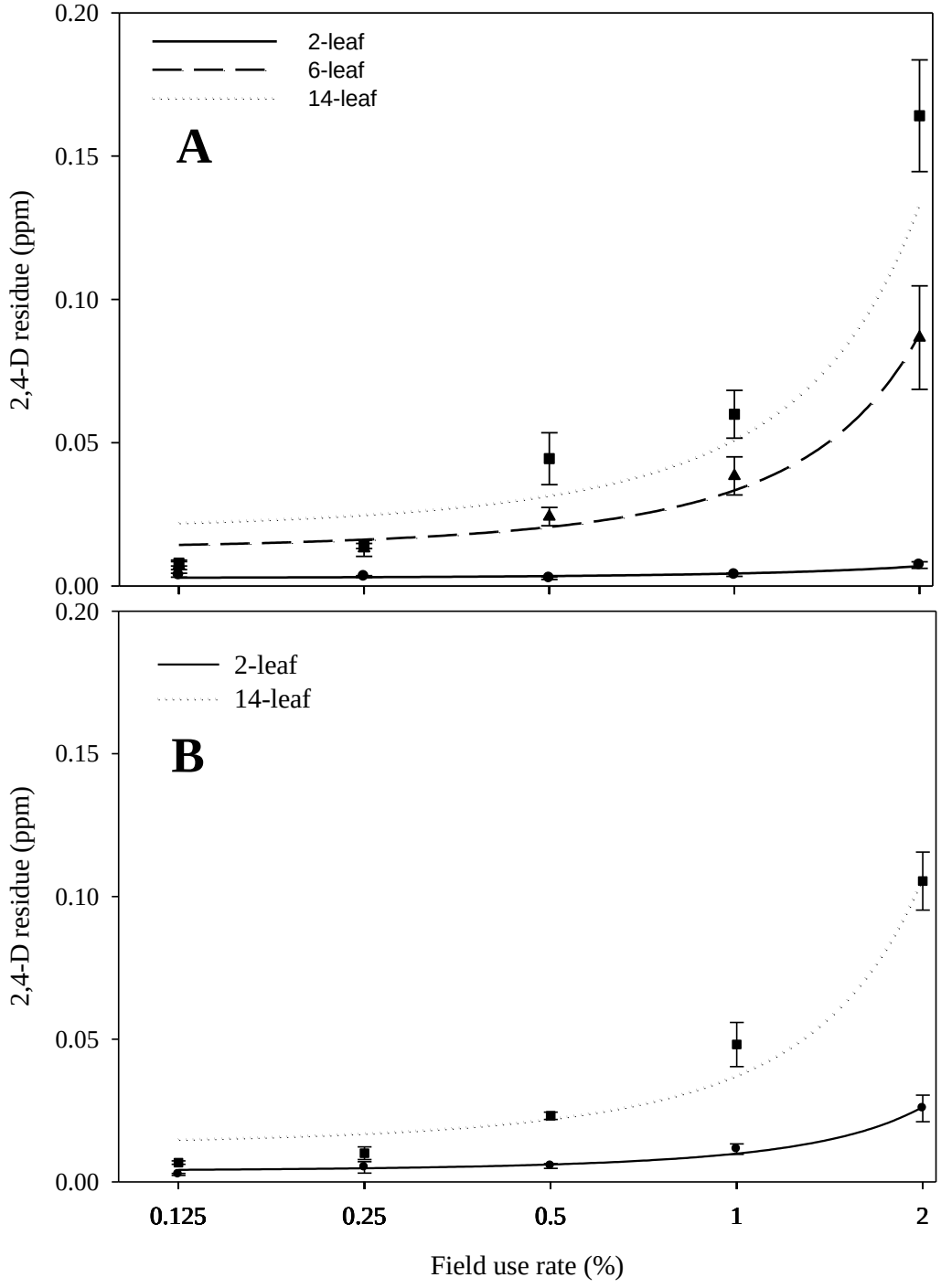



*Figure 2.7.* 2,4-D residues 14 DAT with multiple rates at the 2- (●), 6- (▲), and 14-leaf (■) growth stages in Richville (A) and East Lansing (B), MI. Data was combined over years. Vertical bars represent standard errors of the mean.

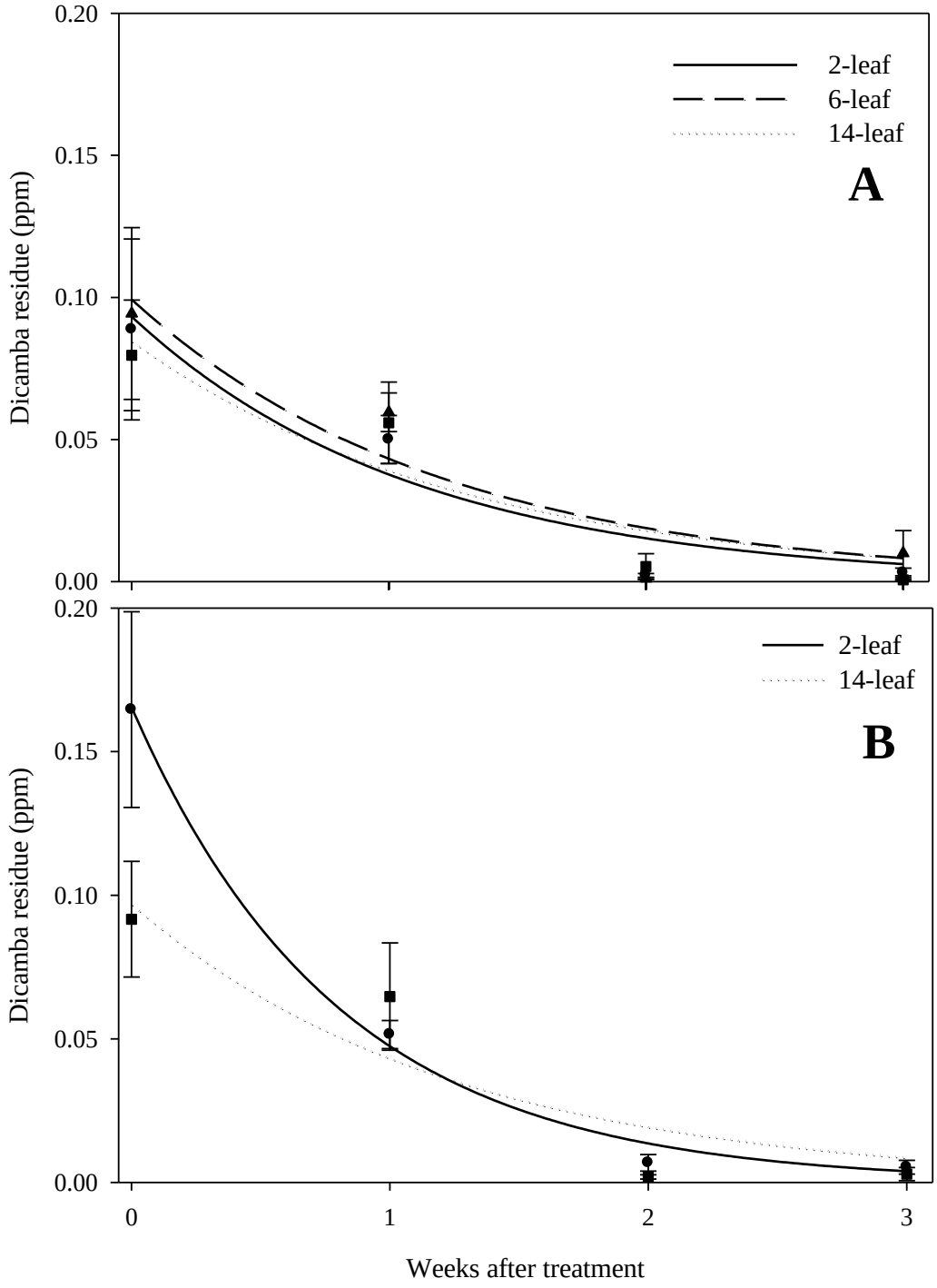



*Figure 2.8*. Dicamba residues for the three weeks following applications at the 2- (●), 6- (▲), and 14-leaf (■) growth stages at Richville (A) and East Lansing (B). Data was combined over years. Vertical bars represent standard errors of the mean.

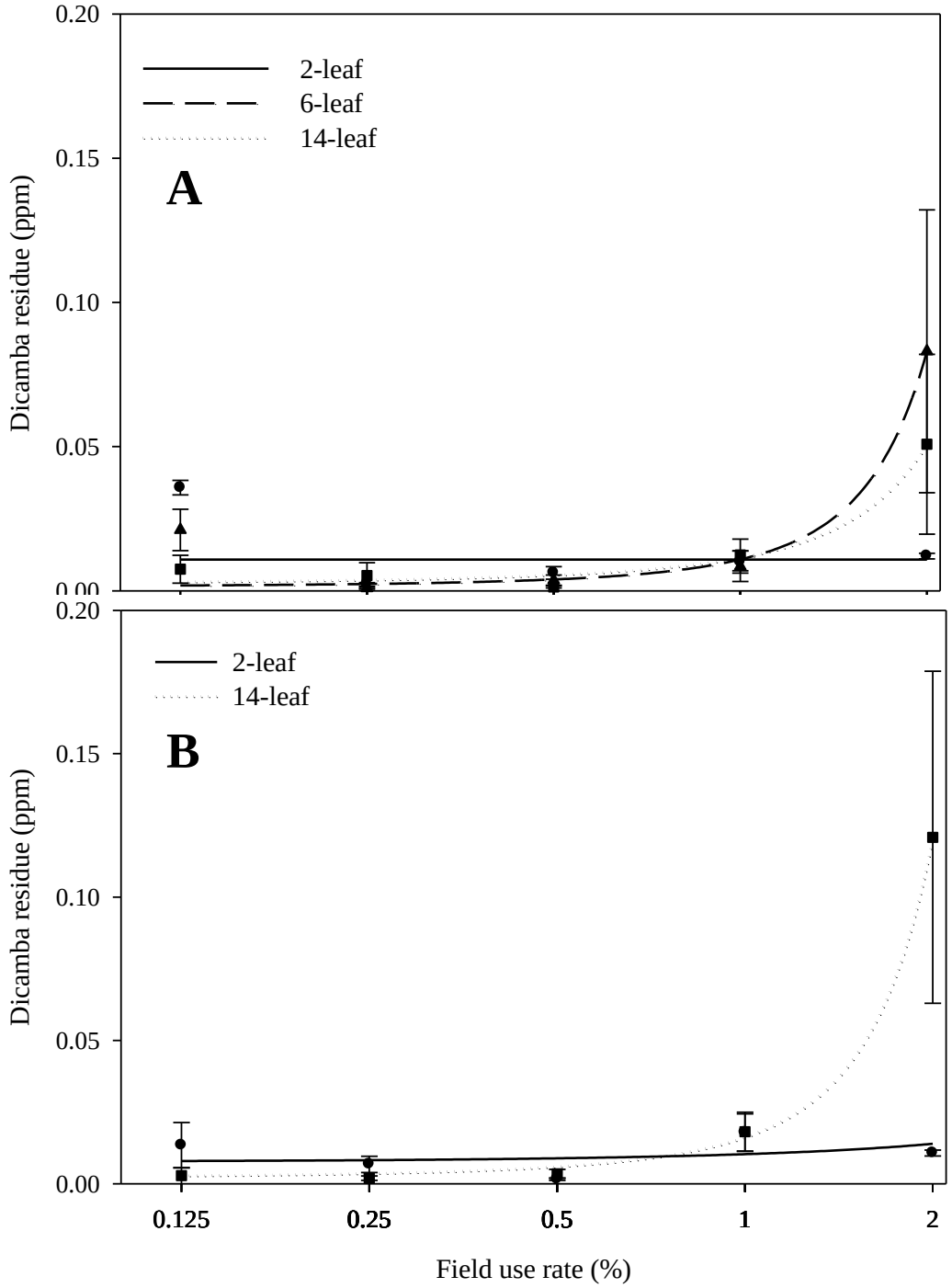



*Figure 2.9.* Dicamba residues 14 DAT with multiple rates at the 2- (●), 6- (▲), and 14-leaf (■) growth stages in Richville (A) and East Lansing (B), MI. Data was combined over years. Vertical bars represent standard errors of the mean.

# APPENDIX B

## Supplemental Tables and Figures

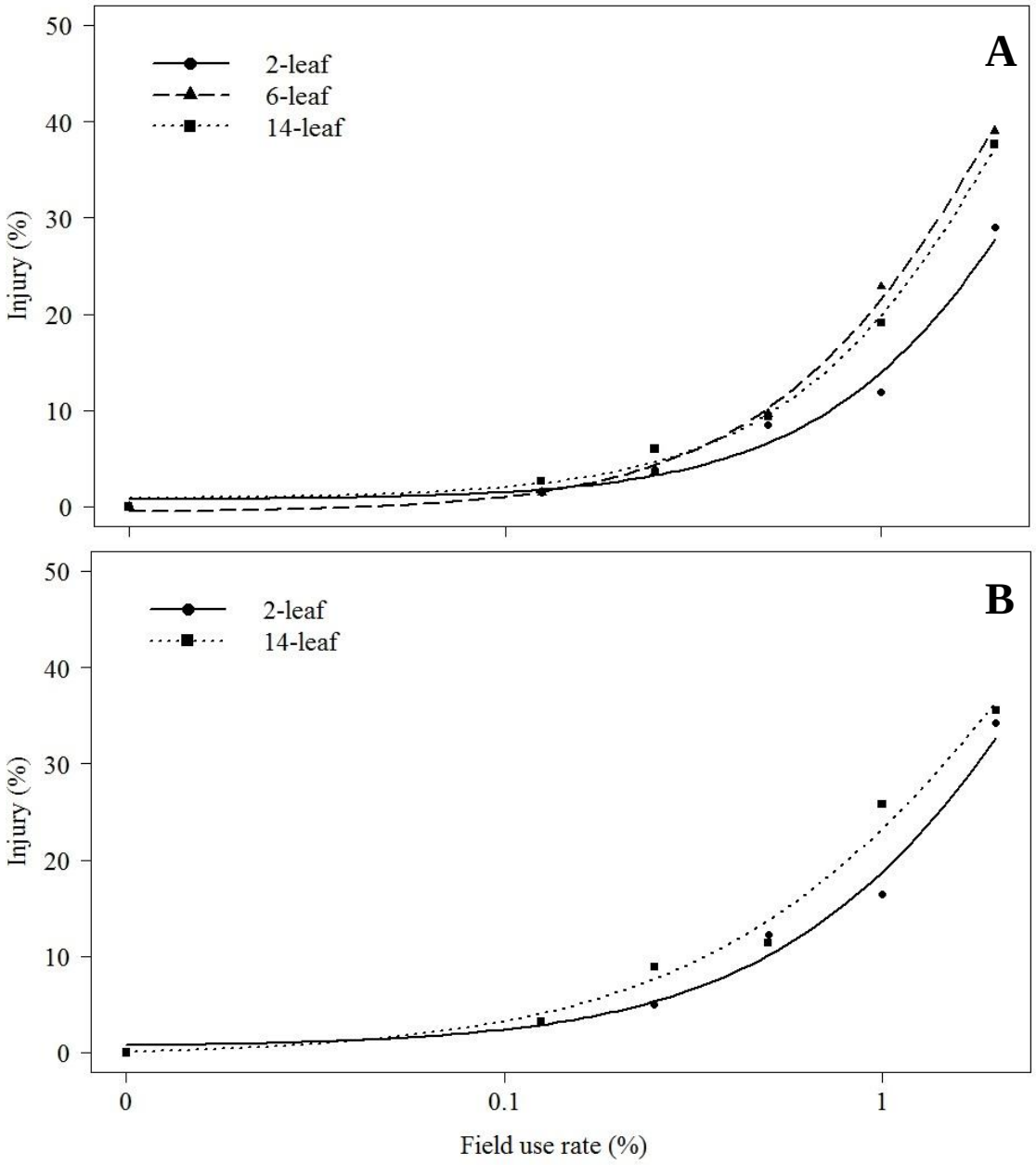



*Figure 2.10*. Herbicide injury to sugarbeet 7 DAT from multiple rates of 2,4-D applied at the 2-, 6-, and 14-leaf growth stages at Richville (A) and East Lansing (B). Data was combined over years.

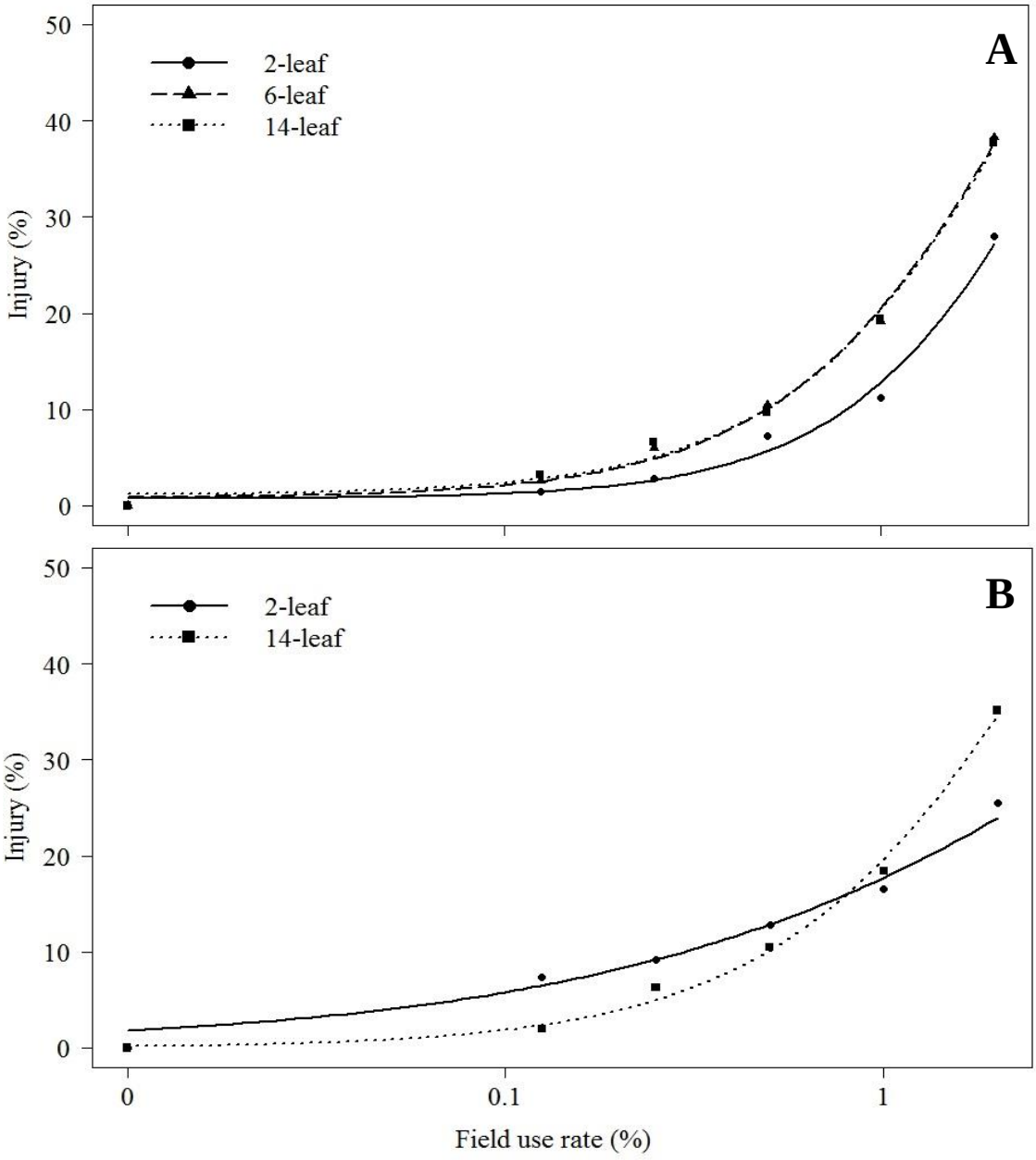



*Figure 2.11.* Herbicide injury to sugarbeet 21 DAT from multiple rates of 2,4-D applied at the 2-, 6-, and 14-leaf growth stages at Richville (A) and East Lansing (B). Data was combined over years.

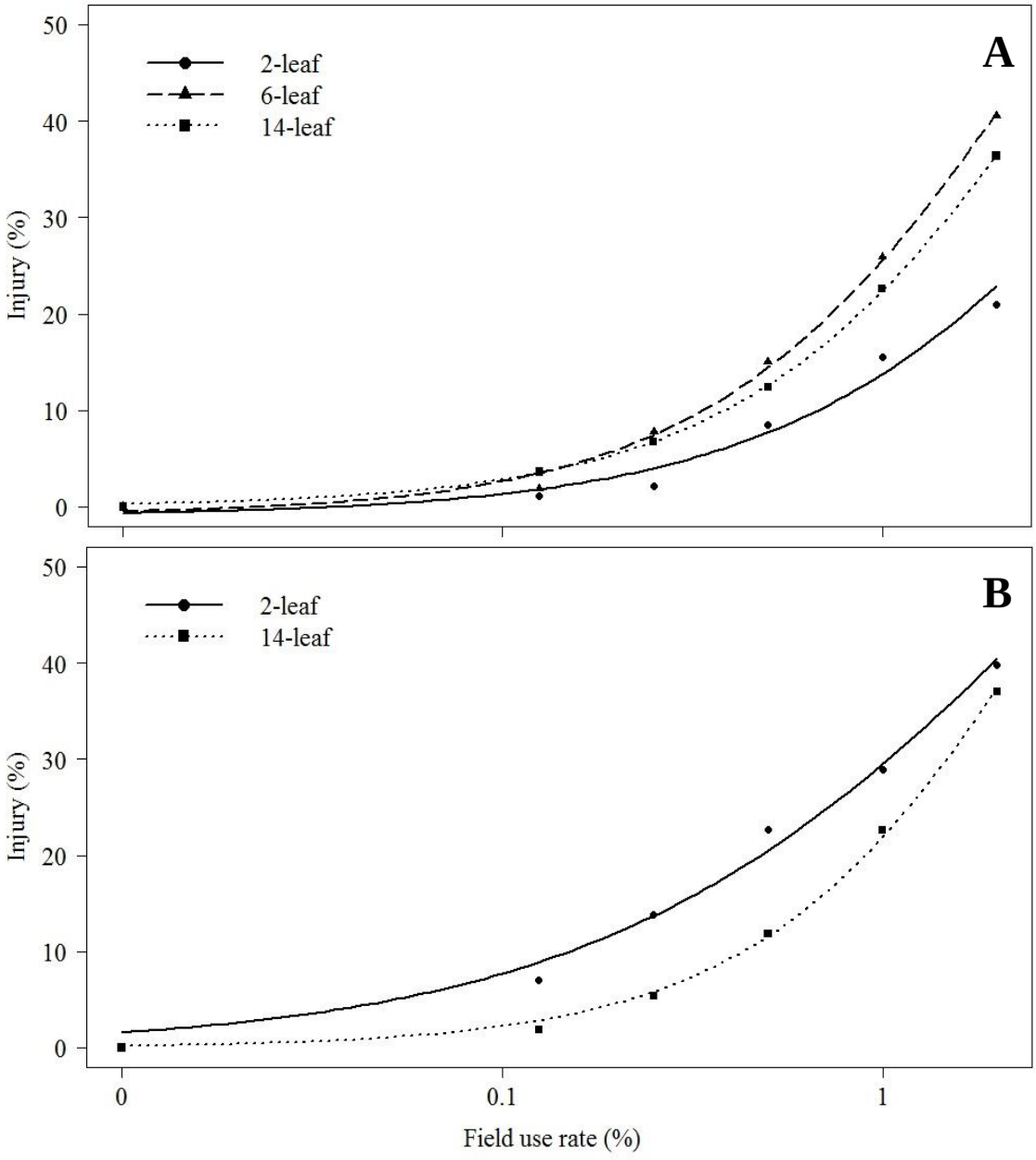



*Figure 2.12.* Herbicide injury to sugarbeet 7 DAT from multiple rates of dicamba applied at the 2-, 6-, and 14-leaf growth stages at Richville (A) and East Lansing (B). Data was combined over years.

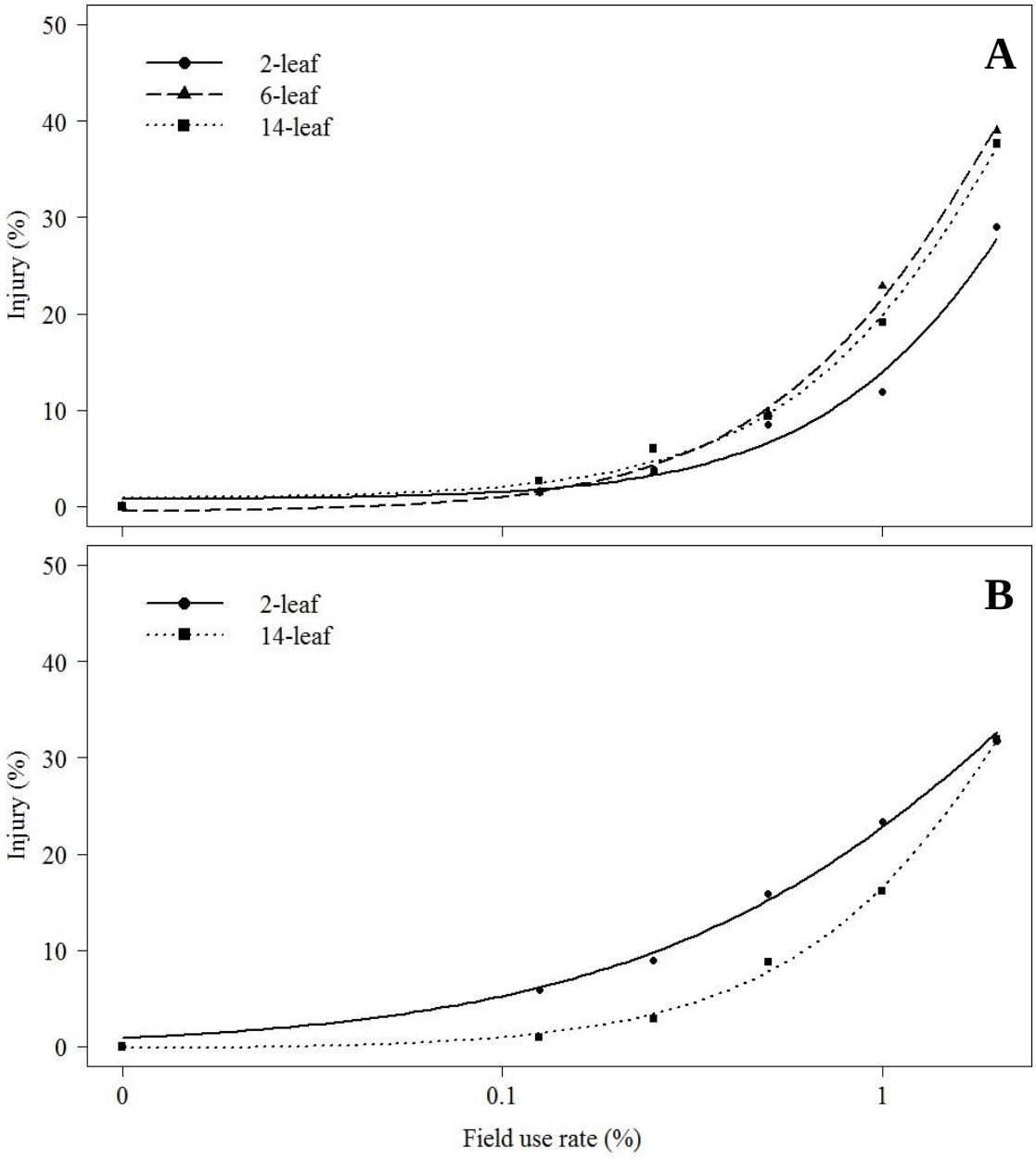



*Figure 2.13*. Herbicide injury to sugarbeet 21 DAT from multiple rates of dicamba applied at the 2-, 6-, and 14- growth stages at Richville (A) and East Lansing (B). Data was combined over years.

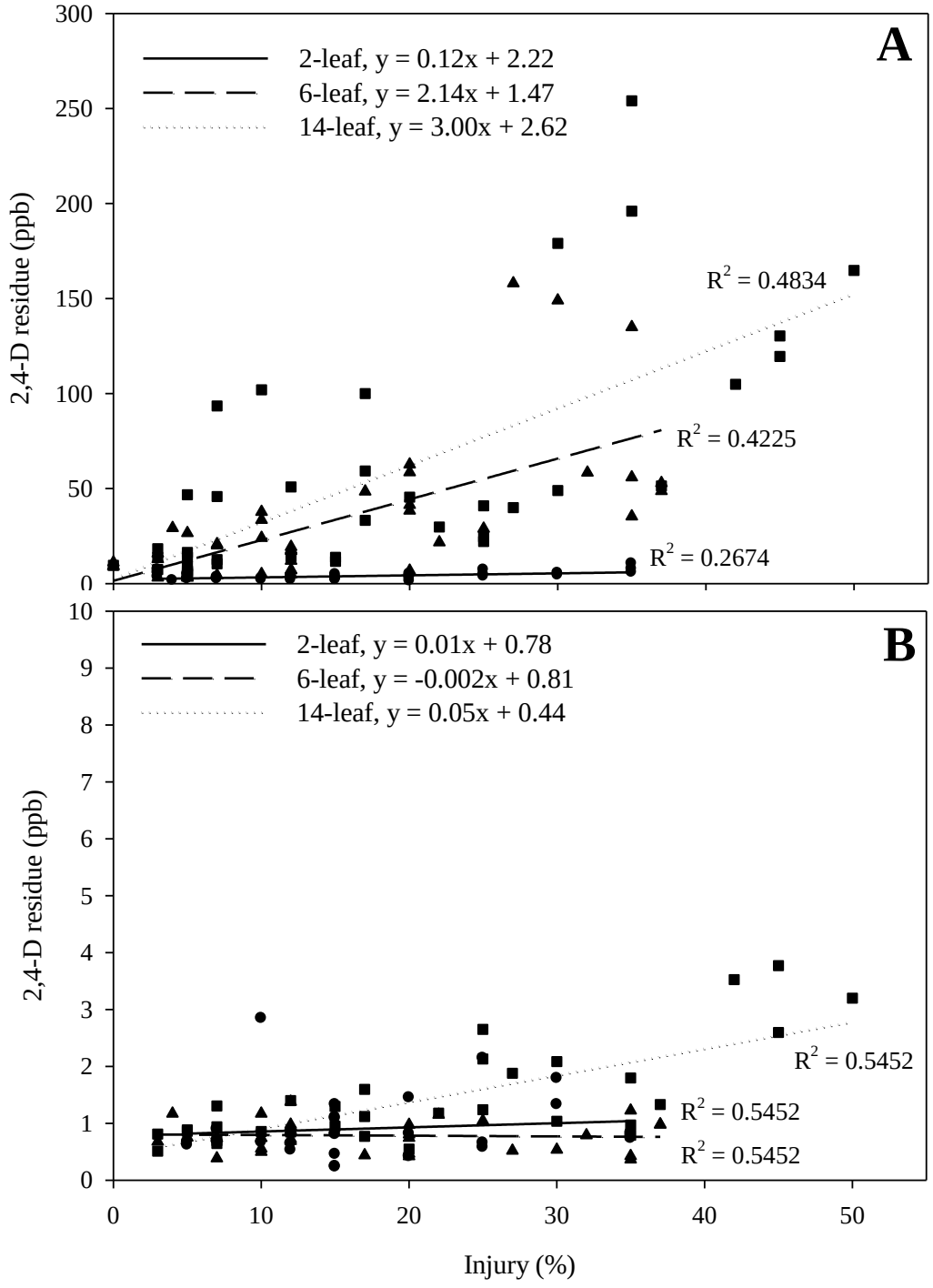
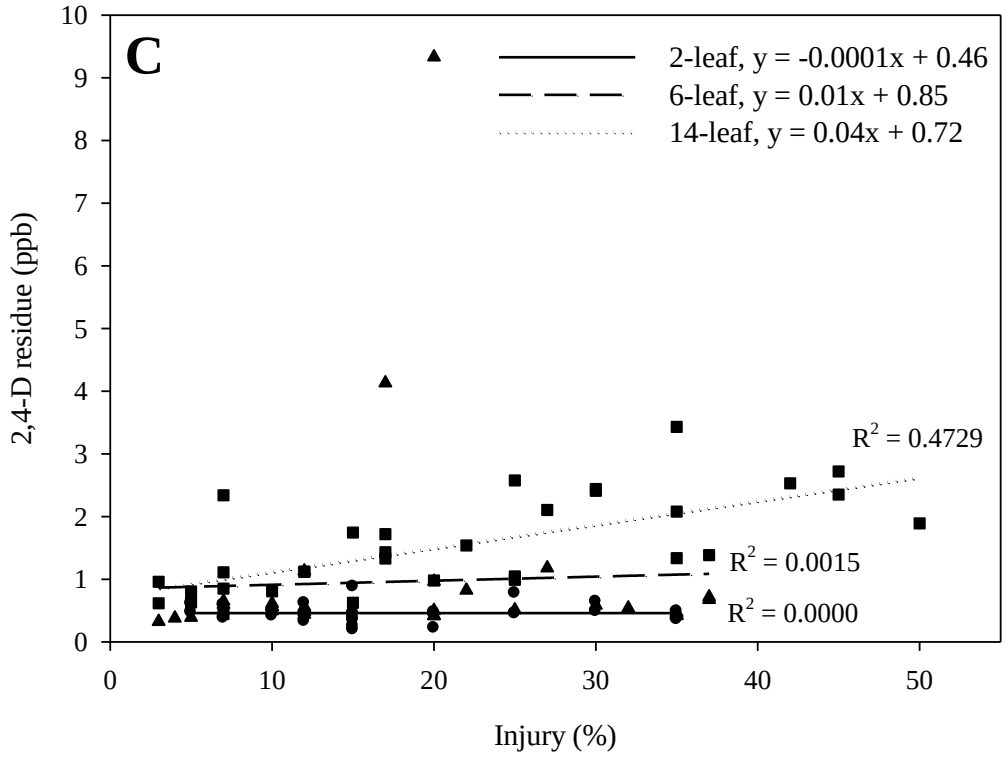



*Figure 2.14*. Linear relationship between sugarbeet injury caused by 2,4-D applied at the 2- (●), 6- (▲), and 14-leaf (■) growth stages and 2,4-D residues 14 days after treatment (DAT) (A) and in the shoot tissue (B) and root tissue (C) at harvest.

*Figure 2.14* (cont'd).




Data was combined over years and 2,4-D rates, and only data from the Richville location was used. There was a significant linear correlation between injury at the 14-leaf growth stage and 2,4-D residues 14 DAT and in the shoot and root tissue. At 14 DAT, there was also a significant linear correlation between injury at all three growth stages and 2,4-D residues.

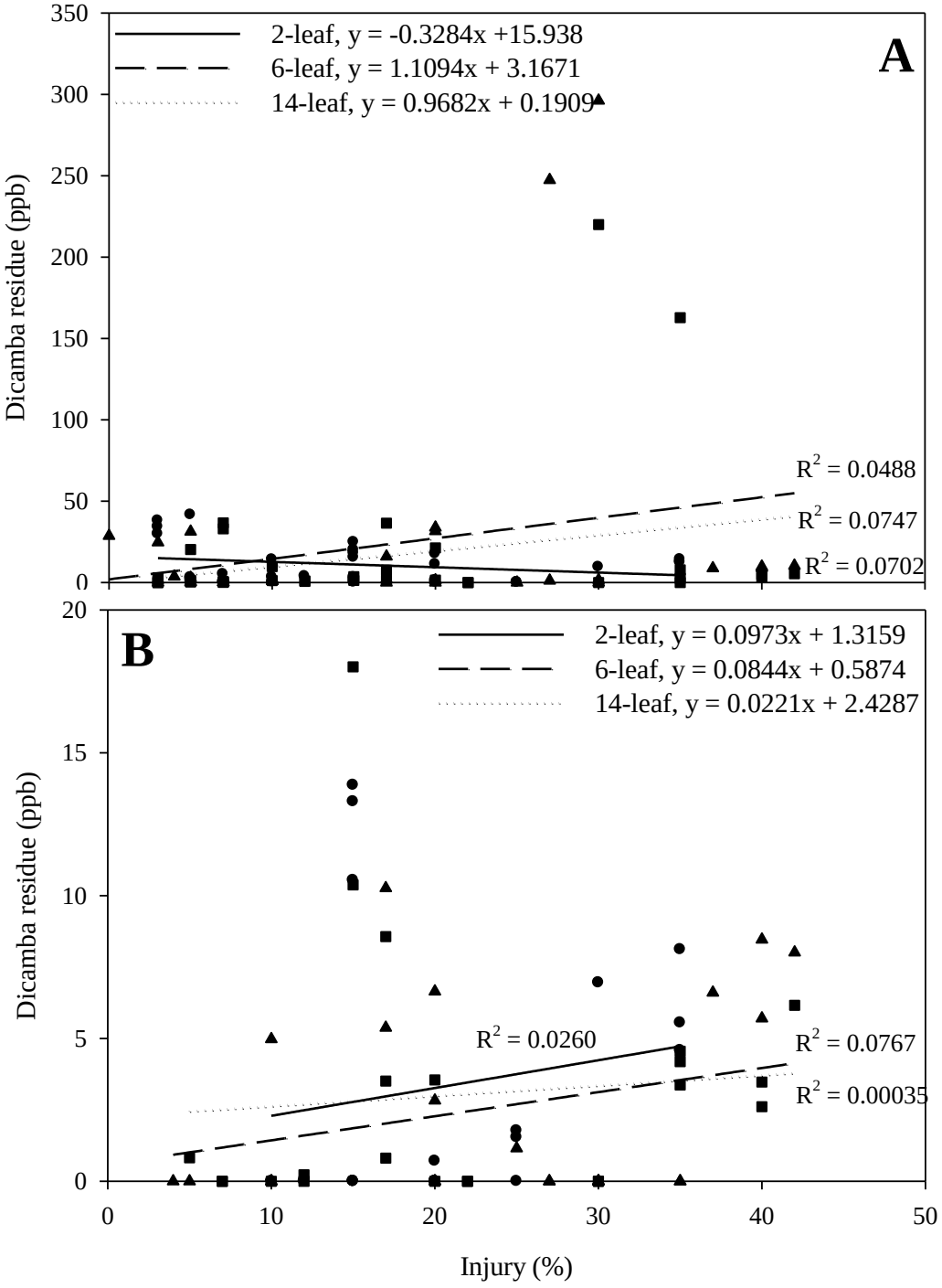
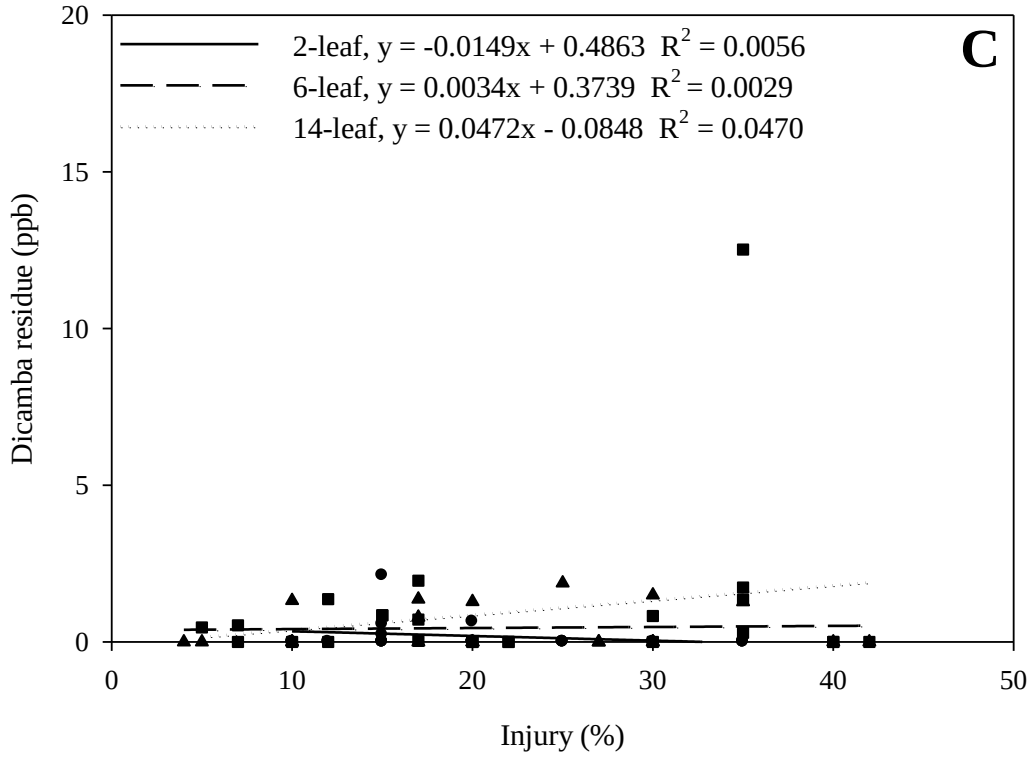



*Figure 2.15*. Linear relationship between sugarbeet injury caused by dicamba applied at the 2- (●), 6- (▲), and 14-leaf (■) growth stages and dicamba residues 14 days after treatment (DAT) (A) and in the shoot tissue (B) and root tissue (C) at harvest.

*Figure 2.15* (cont'd).




Data was combined over years and dicamba rates, and only data from the Richville location was used. There was no significant linear correlation between injury and dicamba residues for any combination of growth stage and sugarbeet tissue.

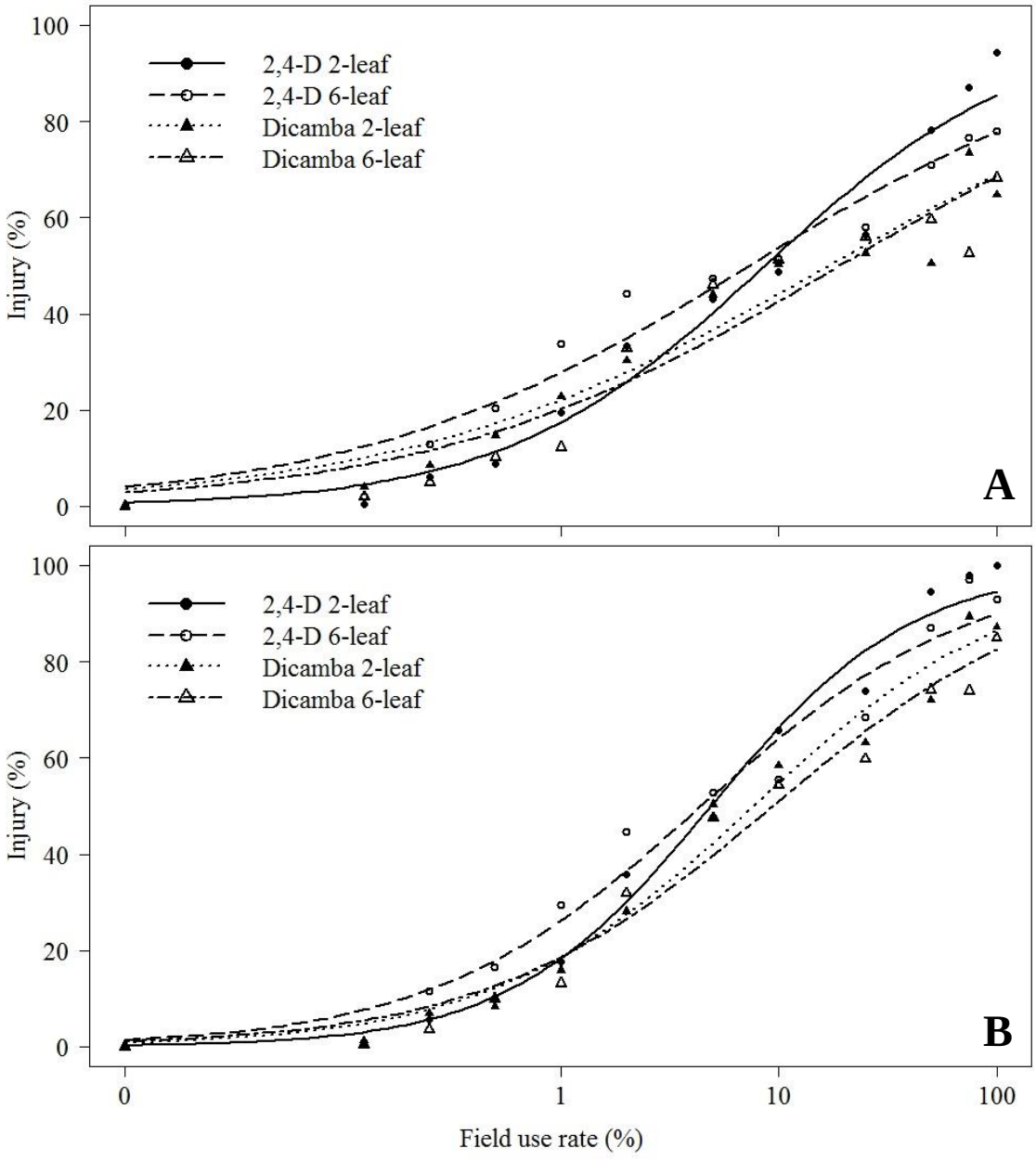



*Figure 2.16.* Sugarbeet injury 7 (A) and 14 (B) days after treatment (DAT) in a greenhouse with rates of dicamba and 2,4-D ranging from 0-100% of the field use rate (assuming a field use rate of 1120 g ae $ha^{-1}$ for both herbicides) at the 2-leaf and 6-leaf growth stages. Data were combined over repetitions in time.

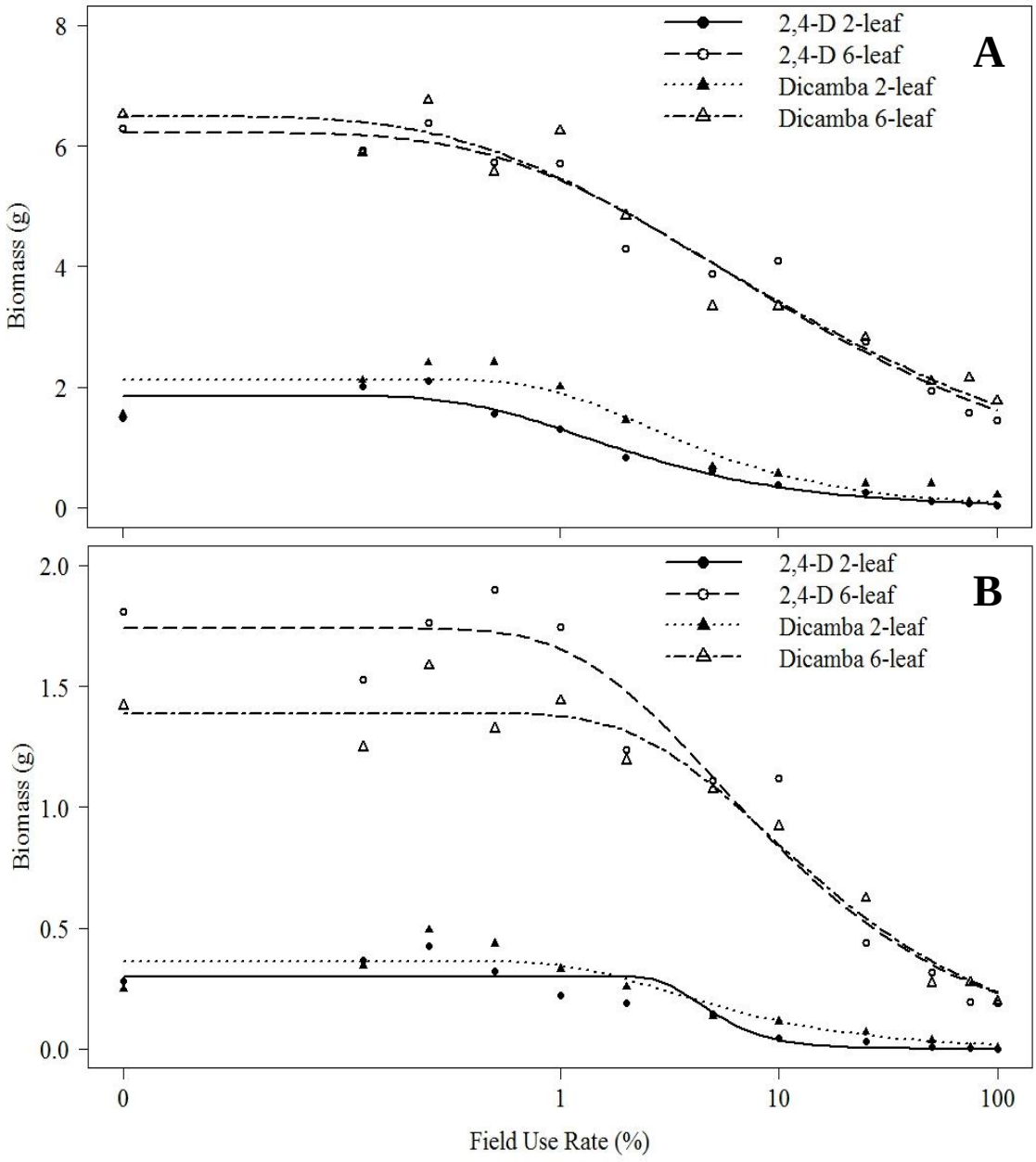
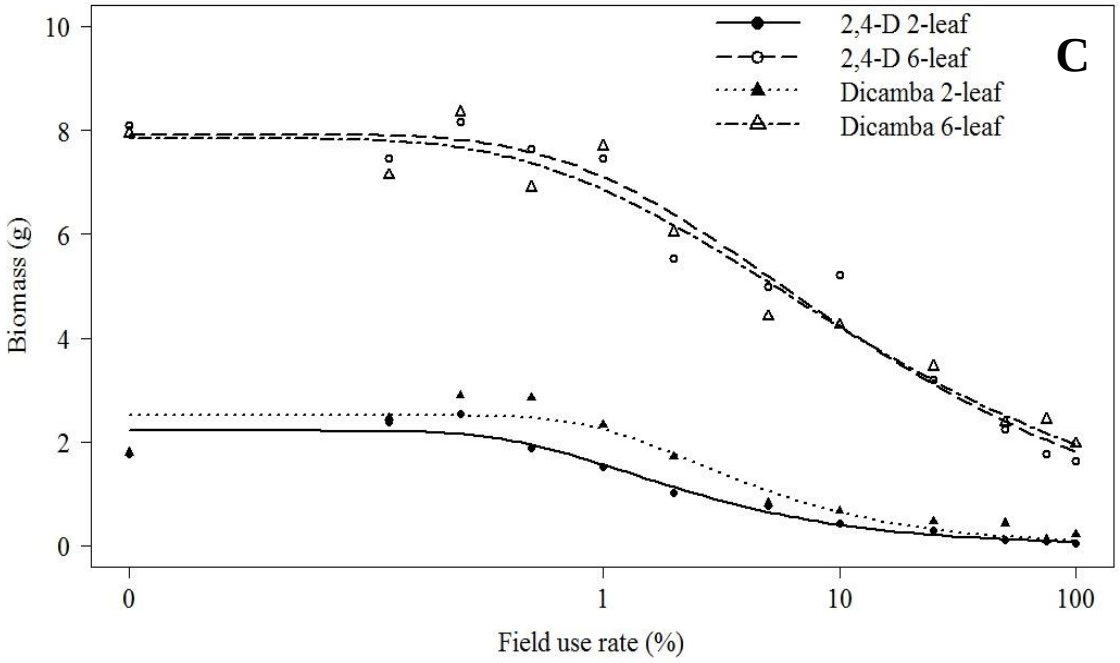



*Figure 2.17*. Sugarbeet shoot (A), root (B), and total (C) biomass harvested 14 days after treatment (DAT) in a greenhouse with rates of dicamba and 2,4-D ranging from 0-100% of the field use rate (assuming a field use rate of 1120 g ae $ha^{-1}$ for both herbicides) at the 2- and 6-leaf growth stages. Data were combined over repetitions in time.

*Figure 2.17* (cont'd).



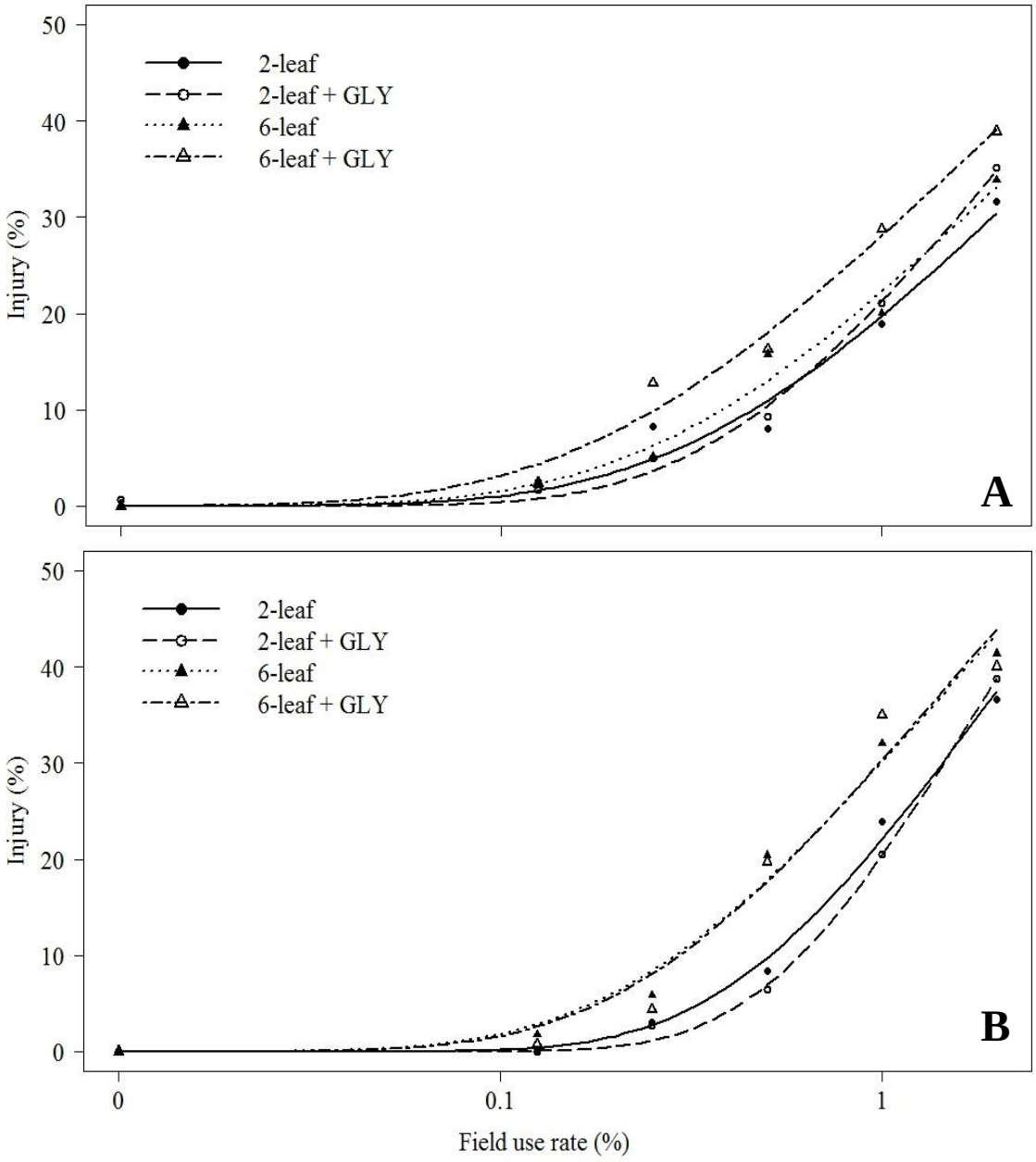



*Figure 2.18*. Herbicide injury 14 DAT to sugarbeet following applications of multiple rates of dicamba (A) and 2,4-D (B) in a greenhouse both with and without glyphosate at the 2- and 6-leaf growth stages. Data were combined over repetitions in time.

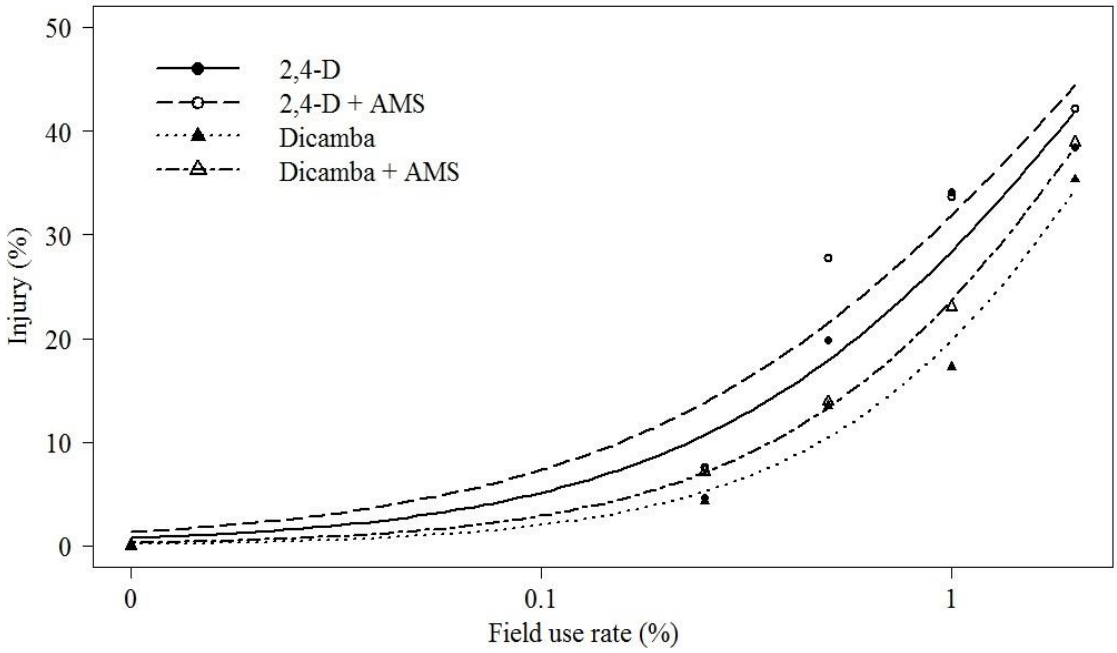



*Figure 2.19*. Herbicide injury to sugarbeet following application of multiple rates of dicamba and 2,4-D in a greenhouse both with and without ammonium sulfate at the 6-leaf growth stage. Data were combined over repetitions in time.

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