DRY EDIBLE BEAN SENSITIVITY TO DICAMBA AND 2,4-D

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**ABSTRACT**

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The recent registrations of dicamba- and 2,4-D-resistant soybeans will likely increase the amounts of dicamba and 2,4-D used in Michigan. Off-target movement to sensitive crops, such as dry bean, will be of greater concern with increased use. In 2017 and 2018, field experiments were conducted at two locations in Michigan to examine the effects of low rates of dicamba and 2,4-D on dry bean. In the first experiment, three rates of dicamba and 2,4-D were applied to V2 and V8 black and navy beans. Dicamba and 2,4-D rates that caused 20% injury to dry beans 14 DAT were 4.5 and 107.5 g ae $ha^{-1}$, respectively. Delays to 50% maturity ranged between 8-16 d for 56 g ae $ha^{-1}$ of dicamba, and 2-9 d for 112 g ae $ha^{-1}$ of 2,4-D. Over four site-years, the rate of dicamba that caused a 10% reduction in yield was 9.8 g ae $ha^{-1}$, which was 1.7% of the field use rate. 2,4-D rates up to 112 g ae $ha^{-1}$ did not reduce dry bean yield. The second experiment examined the effects of the interaction between low rates of dicamba (5.6 g ae $ha^{-1}$) or 2,4-D (11.2 g ae $ha^{-1}$) alone and with glyphosate (8.4 g ae $ha^{-1}$) as tank-contaminants with dry bean herbicides. Results indicated that when dicamba tank-contaminations occurred with a dry bean herbicide there was a synergistic effect. Over two site-years, dry bean yields were between 6-10% lower when dry bean herbicide was applied with dicamba or dicamba plus glyphosate, compared to dicamba and dicamba plus glyphosate alone. Tank-contamination of 2,4-D (11.2 g ae $ha^{-1}$) had little effect on dry bean with or without dry bean herbicides. This work further stresses the need for caution when using dicamba or 2,4-D herbicides near sensitive crops.

Dedicated to my best friend
Alycia Marie Burch

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# LITERATURE REVIEW

## Introduction

Dry edible bean, taxonomically classified as *Phaseolus vulgaris* L., is within the Leguminosae family. *Phaseolus vulgaris* L. is divided into two groups; snap beans that are harvested for the immature pods and dry beans that are harvested for mature seed. Dry bean is a short-seasoned, annual crop that is grown around the world. Dry beans are divided into 13 different market classes and within each class there are several varieties or cultivars (USDA 2014). Dry beans typically mature in 85 to 100 days, depending on variety and growing conditions. As a result, dry beans are often grown in the northern regions of the United States or at higher elevations in the intramountainous regions (Kelly and Cichy 2013). In the United States 14 states currently grow dry beans. Among these states, Michigan ranks second in total production behind North Dakota. Seventy nine percent of dry beans grown in Michigan are in the East Central District, consisting of Bay, Huron, Sanilac, Saginaw and Tuscola counties (USDA-NASS 2018). Michigan leads the nation in the production of three classes of dry bean: black beans, cranberry beans, and small red beans. However, several other market classes are also grown in Michigan and the top five classes harvested 2017 were 48,500 ha of black, 29,900 ha of navy, 2,400 ha of light red kidney, 2,100 ha of small red, and 1,500 ha of cranberry beans (USDA-NASS 2018). In 2017, the total number of dry bean hectares harvested in Michigan was 88,500 with an average yield of 2,250 kg $ha^{-1}$, equating to a production value of $129,184,000 (USDA-NASS 2018). Not only are dry beans important economically, they are also a critical staple of the human diet. Dry beans are recognized as a nutrient-dense food rich in protein, dietary fiber, folate and minerals (Kelly and Cichy 2013).

There are two basic growth habits of dry beans; determinant and indeterminant. A determinate growth habit ceases vegetative growth when reproductive development begins, while an indeterminate growth habit continues vegetative growth even after reproductive growth begins. Determinant dry beans are usually considered a bush type bean, while indeterminate beans have typically been classified as vining or trailing types (Kelly 2001). However, within the indeterminate growth habit dry bean types differ in vine extension. Currently, there are four different types classified within the two different growth habits of beans (Kelly 2001; Urwin et al. 1996). These types include:

- Type I -determinate bush;
- Type II -indeterminate, upright short vine;
- Type III -indeterminate, prostrate vine;
- Type IV -indeterminate with climbing tendencies

In Michigan the majority of dry beans grown are classified as Type II beans. These beans are typically seeded in the month of June. Because dry bean are considered a short-seasoned crop, the delayed planting provides optimum conditions for seed germination and emergence. Dry bean seed requires soil temperatures of 13° C at a 5 cm depth for optimum germination (Kelly and Cichy 2013). Average annual precipitation in Michigan's dry bean growing regions is 84 cm, with approximately 43 cm received during a typical dry bean growing season (26-year average for Caro, MI State Climatologist Office 2018). Dry bean seeding rates are dependent on market class and row widths. Typical Michigan seeding rate recommendations for black and navy beans are 259,000 seeds ha$^{-1}$ in 76 cm rows and 296,000 seeds ha$^{-1}$ in 50-56 cm rows (G. Varner, personal communication, Michigan Bean Commission 2018). However, Holmes (2012) found that seeding rates from 196,500 to 327,000 seeds ha$^{-1}$ did not affect dry bean yield, regardless of row widths. A fluctuation in the number of pods per plant and seeds per pod were determined to be the source of yield compensation at varying plant populations.

Based on growth habit, seed size, and weather conditions, two different harvest methods are used widely across the United States. The first method, a traditional multistep system, begins with undercutting or pulling the plants from the soil at physiological maturity and windrowing them to further dry. This can be done using a knife- or a rod-puller, with the main difference between the two methods being the mechanism that removes the beans from the soil. After the beans have sufficiently dried in windrows, they are thrashed using a pickup header on a commercial combine. The traditional method of harvest works well in semi-arid regions where beans can be allowed to dry for 7-10 days before thrashing. In the Midwest, where rains can be more erratic, beans are often pulled and windrowed early in the morning while the dew dampens the pods to reduce shattering, and thrashed later that same day (Kelly and Cichy 2013). In traditional harvest systems, yield losses often occurred during the drying phase. Dry beans were left exposed on the soil surface and subject to changes in moisture levels due to environmental conditions as well as the possibility of insect feeding (Boudreaux and Griffin 2001; Cook 2004; Wilson and Smith 2002). However, breeding efforts began in the 1970s to develop Type II varieties. These varieties resisted lodging and were better suited for a second method of harvest, direct harvest (Adams 1996; Kelly 2001; Kelly and Cichy 2013).

Type II dry bean varieties have a similar growth type as soybean (*Glycine max* L.) and make direct harvest an efficient option (Kelly 1994; 2001; 2010; Welacky and Park 1987). Direct harvest systems were an attractive option for dry bean producers because they allow dry beans to be harvested in a one-step operation, versus the two- or three- step process of traditional harvest. The consolidation of this process allows for a reduction in time, equipment and labor needed during harvest. Growers transitioning from a traditional harvest system to a direct harvest system can produce dry beans on 25-30% more hectares with the same amount of time allocated to harvest (Kelly and Cichy 2013). In direct harvest systems, a traditional grain combine cuts, gathers, and thrashes dry beans all in one operation. This eliminates the need for specialized equipment used to pull and thrash dry beans. The direct harvest system also reduces the amount of soil that passes through harvest machinery, extending equipment longevity (Schwartz et al. 2004; Harrigan et al. 1992). However, with the implementation of direct harvest systems with combines that were originally designed for soybean harvest, yield losses of 20-40% were often observed (Schwartz et al. 2004). Improvements that have been made to direct harvest equipment to mitigate harvest losses include the use of a flexible floating cutter bar with a narrow pitch, as well as the use of vine lifters to raise lodged plants and pods over the cutter bar (Schwartz et al. 2004; Harrigan et al. 1992). Other advancements in harvest technology include the replacement of the traditional combine reel with an air reel, which uses air to raise plants and pods above the cutter bar, reducing loss. The combination of these advancements reduced harvest lost in direct harvest systems down to 3-7% (Schwartz et al. 2004). Other changes in production that have occurred with the adoption of direct harvest systems include the transition from planting dry beans at wider row widths (70-75 cm) to narrow rows (35-55 cm) (Kelly and Cichy 2013). In narrower rows, the improved plant architecture of Type II varieties creates a denser plant canopy that results in fewer pods that touch the soil surface. This added benefit results in decreased disease pressure in narrower rows (Blackshaw et al. 2000; Kelly and Adams 1987; Saindon et al. 1993).

### Weed control in dry bean

Weed management is a critical component of dry bean production. Weeds compete with dry bean for water, light and nutrients, ultimately leading to reduced crop yields and economic returns (Blackshaw 1991; Blackshaw and Esau 1991; Burnside et al. 1993, 1994; Dawson 1964; Wilson et al. 1980; Woolley et al. 1993; Zollinger and Kells 1993). Uncontrolled weeds can reduce dry bean yield by up to 80% (Blackshaw and Esau 1991; Blackshaw et al. 2000; Burnside et al. 1998; Chikoye et al. 1995; Homes 2012; Malik et al. 1993; Soltani et al. 2018; Wall 1995; Wooley et al. 1993).

Dry bean yield loss due to weed competition is affected by multiple factors including, timing of weed control practices, timing of weed emergence, species, density, and biomass. To avoid yield loss, weeds must be controlled by the V2 growth stage of dry bean and continue until dry beans reach the R1 stage (Woolley et al. 1993). Defined slightly differently, Burnside et al. (1993) found that weed control measures should begin no later than three weeks after planting, and continue to 5 or 6 weeks after planting, to keep weeds from limiting dry bean yield. The most sensitive agronomic component of dry bean yield is the number of pods per plant, whereas the weight per 100 seeds and the number of seeds per pod were relatively stable under competition (Wooley et al. 1993). Chikoye et al. (1995) found that common ragweed (*Ambrosia artemisiifolia* L.) reduced navy bean seed yield by 10-22% when 1.5 ragweed seedlings $m^{-1}$ of row emerged at the same time as dry bean (VE). Alternatively, yield reductions were only 4-9% when common ragweed emergence was delayed until V3. Blackshaw (1991) observed similar yield losses from interference with hairy nightshade (*Solanum sarrachoides* L.); yield losses of 13% occurred with as few as 2 hairy nightshade seedlings per meter of row. Interference of nightshade species (*S. sarrachoides* L. and *S. ptychantum* L.) also reduces harvest quality and market value of dry bean cultivars that are light in color, as the purple liquid in the fruit can stain the bean seed (Brouwer et al. 2015).

In Michigan, there are certain key weed species that pose a challenge to control in dry beans. Annual broadleaves that are challenging to control include: common lambsquarters (*Chenopodium album* L.), pigweed species (*Amaranthus powellii* L. and *A. Retroflexus* L.), common ragweed, eastern black nightshade, horseweed (*Conyza canadensis* L.) and velvetleaf (*Abutilion theophrasti* L.) (Hill 2014). Chemical control becomes more challenging as Group 2 (acetolactate synthase or ALS) herbicide resistance has been documented in all of these difficult to control species in Michigan's dry bean production regions (Hill 2018). Group 14 (protoporphyrinogen oxidase or PPO) resistance has also occurred in Michigan populations of common ragweed. Multiple resistance within common ragweed limits the number of effective herbicide options for weed control in dry bean. Annual grasses are also prevalent across the state of Michigan and require control in dry bean production systems, common grasses include foxtail species (*Setaria faberi* L., *S. pumila* (Pior.), and *S. viridis* L.) (Hill 2014).

Historically, weed management in dry bean has relied heavily on mechanical and cultural control methods (Arnold et al. 1993; Blackshaw et al. 2000; Burnside et al. 1998). However, advancements in dry bean herbicides, coupled with the increasing movement to limit in-season soil and crop disturbance, has led to an increased reliance on chemical weed control. Current herbicide recommendations for weed control in dry bean include the use of soil-applied herbicides, either preplant incorporated (PPI) or preemergence (PRE) followed by a postemergence (POST) herbicide application to control the remaining weeds (Blackshaw et al. 2000; Burnside et al. 1994; Holmes and Sprague 2013).

Limited POST herbicide options has driven research in the direction of identifying additional options for weed control. Multiple researchers examined the possibility of safening dry beans from POST injury from imidazolinone herbicides by adding bentazon, as imidazolinone herbicides applied alone caused plant injury (Bauer et al. 1995a). This research began when Bauer et al. (1995b) examined imazethapyr tank mixtures to control problematic weeds in dry bean. They found that when imazethapyr was mixed with bentazon and crop oil concentrate, some antagonism occurred. This combination resulted in reduced herbicide adsorption and translocation. While discouraging for control of certain weeds, these findings helped identify the use of bentazon as a safener for imadizolinone herbicides to improve crop safety.

Later on, Hekmat et al. (2008) examined this strategy to safen applications on dry beans with a different imidazolinone herbicide, imazamox. They found that when imazamox was tank-mixed with bentazon at rates of 25 g ai ha$^{-1}$ plus 600 g ai ha$^{-1}$, respectively, crop safety could be improved. This work confirmed the safety of application on multiple market classes of dry bean including black, cranberry, kidney, Otebo, pinto, white and yellow eye beans. The effectiveness of the combination for weed control was not reported. However, recently Wilson et al. (2014) confirmed the effectiveness of imazamox plus bentazon. Across three years, imazamox plus bentazon effectively reduced weed density, as well as, or better than any other POST herbicide program tested, proving that this tank mixture remains an effective herbicide option for dry bean producers.

Other POST options for chemical weed control in dry bean include fomesafen and fomesafen tank mixtures. Fomesafen is registered for use in the United States for both PRE and POST applications in dry bean. While crop injury can occur, overall crop safety with fomesafen has been confirmed on multiple dry bean classes (Soltani et al. 2006). Fomesafen is used to help control, pigweed species, common ragweed, wild mustard (*Sinapis arvensis* L.), ladysthumb (*Polygonum persicaria* L.) and eastern black nightshade (Shaner 2014). Fomesafen can cause transient crop injury that includes stunting, leaf crinkling, and brown spotting on the leaves 7 days after treatment (DAT) (Wilson 2005). However, by 21 DAT dry bean plants generally show full recovery from this injury. When used at a use rate of 210 g ai $ha^{-1}$ redroot pigweed, kochia (*Bassia scoparia* L.), and common purslane (*Portulaca oleracea* L.) were controlled at a level of 90% or greater (Wilson 2005). When the rate was increased to 280 g ai $ha^{-1}$ common lambsquarters and hairy nightshade control was 71% and 92%, respectively. Applying fomesafen to unifoliate dry bean caused the least amount of injury, while providing the best weed control (Wilson 2005).

To date, the combination of imazamox and bentazon is the most common POST application in Michigan (Michigan Bean Commission 2018). The use of fomesafen POST is also very common for pigweed species, eastern black nightshade and ALS-resistant common ragweed control. Herbicide resistance in weed species has been considered a ‘wicked’ problem by many and will continue to be a growing challenge for crop producers. Herbicide selection and rotation will be more important than ever to help overcome weed resistance issues in the future.

## New options for glyphosate-resistant weed control in soybean

Since the introduction of glyphosate-resistant (GR) crops in 1996, over 185 million hectares have been planted in the United States. This has led to an increase in the use of glyphosate (Gianessi 2005; Benbrook 2016). Ninety percent of the glyphosate used worldwide is in agricultural applications. The amount of glyphosate used has increased from 635,000 kg of active ingredient in 1974 to 125 million kg in 2014 (Benbrook 2016). Glyphosate-resistant crops offer simple and convenient solutions for broad-spectrum broadleaf and grass weed control. In some instances, this has led to the abandonment of best agronomic practices for avoiding the development of herbicide resistance including: crop rotation, the use of herbicides with multiple effective sites of action, and mechanical weed control (Wright et al. 2010). The heavy reliance on glyphosate as the sole weed control method increased selection pressure for the development of glyphosate-resistant (GR) weed species. Currently, 37 different weed species have evolved resistance to glyphosate worldwide (Heap 2018). In the United States alone, 17 different GR weeds have been documented. Several of these GR weeds including: giant ragweed (*Ambrosia trifida* L.), horseweed, waterhemp (*Amaranthus tuberculatus* L.), Palmer amaranth (*Amaranthus palmeri* L.), and common ragweed are problematic for Michigan farmers. The emergence of glyphosate and multiple-resistant weeds has led to the development of new herbicide-resistant traits in broadleaf crops, soybean specifically.

The insertion of the dicamba monooxygenase and aryloxyalkanoate dioxygenase enzyme AAD-12 into soybean confers resistance to dicamba and 2,4-D, respectively (Behrens et al. 2007; Wright et al. 2010). The dicamba resistant Roundup Ready 2 Xtend® (Bayer Crop Science, Research Triangle Park, NC) soybeans are also resistant to glyphosate. Low volatility dicamba formulations were developed for use with these soybeans and are marketed with trade names of XtendiMax ® (Bayer Crop Science, Research Triangle Park, NC), FeXapan ® (Dupont, Wilmington, DE), and Engenia ® (BASF, Research Triangle Park, NC). Soybeans that are resistant to 2,4-D are sold as Enlist E3 ® (Dow AgroSciences, Indianapolis, IN) soybeans. These soybeans are engineered to be resistant to three different herbicides; 2,4-D, glufosinate and glyphosate. Enlist E3 soybeans are labeled for both PRE and POST applications of 2,4-D choline, a lower volatility formulation of 2,4-D than 2,4-D amine or ester formulations (Sosnoskie et al. 2015). The 2,4-D choline formulation is sold under the trade name Enlist One ® (Dow AgroSciences, Indianapolis, IN). It is also marketed and sold in a premixture with glyphosate as Enlist Duo ® (Dow AgroSciences, Indianapolis, IN).

Dicamba and 2,4-D provide excellent control of many problematic glyphosate-resistant weeds. Byker et al. (2013) examined the effectiveness of dicamba as a POST treatment in dicamba-resistant soybean for the control of GR horseweed. Prior to the development of dicamba and 2,4-D resistant soybean, there were very few effective herbicide options for multiple-resistant horseweed (ALS and glyphosate) control. Preplant dicamba applications of 600 g ae $ha^{-1}$ provided 90 to 100% horseweed control, and POST applications of dicamba at 300 g ae $ha^{-1}$ provided good control (>88%), 8 weeks after application (Byker et al. 2013). Treatments that utilized an effective soil-applied herbicide followed by a POST application of glyphosate plus dicamba, provided the best overall control of GR horseweed. Kruger et al. (2010) determined that GR populations of horseweed could be controlled POST with both dicamba and 2,4-D. Dicamba provided 90% and 2,4-D provided 81% control of horseweed plants that were 30 cm tall at application. When plants were less than 30 cm tall, both herbicides provided over 90% control. In addition to improved control of horseweed, Norsworthy et al. (2008) documented control of multiple herbicide resistant populations of Palmer amaranth with both dicamba and 2,4-D POST. Greater than 93% Palmer amaranth control was recorded for both dicamba and 2,4-D.

Soybeans that are resistant to 2,4-D are also resistant to glufosinate POST. The combination of 2,4-D and glufosinate is beneficial for the improved control of weeds across multiple populations and species. Problematic species controlled with this combination include

GR horseweed and GR Palmer amaranth (Merchant et al. 2013; Steckel et al. 2006). Craigmyle et al. (2013) compared the use of 2,4-D (1120 or 840 g ae $ha^{-1}$) plus glufosinate (450 g ai $ha^{-1}$) to glufosinate alone (450 g ai $ha^{-1}$) at different application timings. All applications of 2,4-D plus glufosinate provided similar common waterhemp control to two POST applications of glufosinate. However, when only one herbicide application was made POST, the addition of 2,4-D to glufosinate increased control from common waterhemp control from 74 to >93%.

Dicamba and 2,4-D are valuable herbicides to control many difficult to control weeds species. New herbicide resistance traits in soybean to dicamba and 2,4-D will give producers additional weed management options for the control of problematic GR weed species.

## Characteristics of dicamba and 2,4-D

Dicamba and 2,4-D are classified as plant growth regulator (PGR) or synthetic auxin herbicides. These herbicides are part of the Group 4 site of action group (Mallory-Smith and Retzinger 2003). As the first commercially available herbicide, 2,4-D was patented in 1942 and became commercially available in 1945 (Peterson 1967). 2,4-D is in the phenoxy chemical family and has multiple uses (Shaner 2014). At low rates (10-24 ppm) 2,4-D serves as a fruit-drop-prevention aid in citrus crops. At higher rates (>280 g ae $ha^{-1}$) 2,4-D is commonly used both PRE and POST for selective broadleaf weed control in most grass crops and now varieties of 2,4-D resistant soybean. In the acid form, 2,4-D has a Log $K_{ow}$ of 2.81 and is more lipophilic than dicamba (Shaner 2014). The 2,4-D acid is often formulated as ester derivatives or amine salts, which are readily dissolvable in organic solvents (Shaner 2014). More recently a low volatility formulation, 2,4-D choline has been introduced. This formulation does not have a reported $K_{ow}$ since it dissociates rapidly to the acid form (Shaner 2014). The vapor pressure measurements of 2,4-D salts are often not meaningful, since they typically reflect the vapor pressure of the acid (1.2 X $10^{-7}$ mm hg at 25 C) after the salt dissociates from the counterion (Peterson et al. 2016). However, salts that have a lower dissociation and more stable counterions will have less vapor loss after an application than formulations that do not (Hillger et al. 2012). 2,4-D choline has greater stability of counterions after dissociation than other salts, thus providing less vapor loss than other formulations (Eytcheson et al. 2012; Hillger et al. 2012; Sosnoskie et al. 2015).

Patented 16 years after 2,4-D, dicamba became commercially available in 1967 (Shaner 2014). Dicamba is in the benzoic acid chemical family and has been primarily used for POST control broadleaf weeds in corn, small grains, and grasses. Dicamba can also be used PRE at rates of 560 g ae $ha^{-1}$ for weed control prior to corn or dicamba-resistant soybean. Dicamba is a weak acid with a pKa value of 1.87 and a $K_{ow}$ 0.29 (Shaner 2014). These characteristics, along with Burnside and Lavy (1966) findings of little to no adsorption of dicamba to soil, indicate that dicamba is prone to leaching through the soil profile. However, dicamba is also rapidly degraded (half-life 4.4 days), which effectively lowers the leaching potential (Burnside and Lavy 1966). The free acid of dicamba has a solubility of 4500 mg $L^{-1}$ (Shaner 2014). Like 2,4-D, when dicamba is sold as a herbicide, it is formulated as a salt. Current salt formulations of dicamba include: diglycolamine (DGA), dimethylammonium, Na salt and N, N-Bis-(aminopropyl) methylamine (BAPMA) salt. The salt formulations of dicamba have higher solubilities in water than the acid. The solubility of the dimethylamine salt of dicamba is 720,000 mg $L^{-1}$ (Shaner 2014). The dimethylamine salt was sold commercially as the product Banvel ® (Arysta LifeScience, Cary, NC). While this formulation is very soluble, which is conducive for plant uptake and mixing, it is also the most volatile commercial formulation of dicamba. This raises concerns for the off-target movement of dicamba vapors. In response, less volatile formulations such as the DGA and BAPMA salt have been developed. These low volatility formulations are the only labeled dicamba formulations for applications on dicamba-resistant soybean.

Dicamba and 2,4-D are systemic herbicides and are able to move in both the xylem and the phloem (Change et al. 1971). Characteristic symptoms of susceptible plants affected by dicamba and 2,4-D include: stunting, stem twisting or epinasty, leaf cupping, and the death of growing points (Grossman 2010). Currently the mechanism of action of these two herbicides is not fully understood. However, when these herbicides are applied to a susceptible plant, auxin receptors are overloaded, causing a hyperaccumulation of ethylene, abscisic acid, and other reactive oxygen species (Grossman 2010). Effects of these herbicides are first observed in areas of high metabolic activity, i.e., new growth (Change et al. 1971).

## Off-target movement

Sensitive plants can be exposed unintentionally to dicamba and 2,4-D by various means, including movement by vapor drift, particle drift, and spray system contamination (Behrens and Lueschen 1979; Boerboom 2004; Cundiff et al. 2017; Dexter 1993). Vapor drift occurs when molecules of an herbicide's active ingredient are converted from a liquid to a gas, and then move to an unintended location by air currents (Peterson et al. 2016). The vaporization potential of the herbicide is a function of both the herbicides physical properties and the environmental conditions at the time of and after application (Behrens and Lueschen 1979; Burgoyne and Hites 1993).

Dicamba and 2,4-D are subject to vapor drift when salt formulations dissociate into the acid forms, and the instability of the molecule allows for vaporization (Behrens and Lueschen

1979; Hillger et al. 2012). For example, dicamba can degrade from the dicamba salt to the free acid in water; the free acid has a vapor pressure of 20 x $10^{-4}$ mm Hg at 25 C. This high vapor pressure can allow for the volatilization of dicamba acid into a gaseous form (Behrens and Lueschen 1979).

While new low volatility formulations of dicamba have been developed and implemented into soybean systems, significant levels of vapor drift have been still reported. Jones et al. (2019) compared the vapor drift potential of the DGA and BAPMA salts under field conditions. Over two years it was recorded that the DGA salt was able to cause plant injury on sensitive soybean at a maximum distance of 180 m from the application location. The BAPMA salt was recorded to have moved a maximum distance of 108 m after application.

2,4-D also has the ability to volatize. Sosnoskie et al. (2015) compared the relative volatility of three formulations of 2,4-D, the ester, amine, and choline salt under field conditions. It was found that 2,4-D ester had the greatest volatility followed by 2,4-D amine, and 2,4-D choline had the lowest volatility (Sosnoskie et al. 2015). Results are consistent with findings of the greater stability of 2,4-D choline after salt dissociation (Hillger et al. 2012)

Another method of off-target movement can be from particle drift. Particle drift is the physical movement of the herbicide solution by wind. Factors that can affect particle drift and need consideration prior to application include, boom height, droplet size, and distance from susceptible vegetation (Maybank et al. 1978; Nordby and Skuterud 1975; Thistle 2004; Wolf et al. 1993). Particle drift has been an issue in agriculture as long as pesticides have been applied. Under optimal environmental conditions and sprayer specifications (boom height and pressure), up to 6% of the total spray solution can drift, meaning it does not reach its intended target (Nordby 1974). When boom height and pressure was raised total drift increased up to 37% of the total solution (Nordby 1974). Not only does equipment affect drift but environmental conditions as well. Holterman et al. (1997) modeled drift from a boom sprayer and concluded that wind speed had a significant effect on the severity of particle drift. The farther the distance from the sprayer the more of an effect wind speed had on the amount of spray solution deposited off-target or downwind. At wind speeds of 5 m $s^{-1}$ and 0.8 m $s^{-1}$ the amount of spray solution that drifted 1 m from the spray nozzle were not affected by windspeed. However, 5 m from the nozzle windspeeds of 5 m $s^{-1}$ and 0.8 m $s^{-1}$ caused drift of 1 and 0.1% of the total spray solution (Holterman et al. 1997). Nozzle type can also have a significant effect on spray particle drift and be an effective drift mitigation technique. Alves et al. (2017) modeled drift of dicamba and dicamba plus glyphosate comparing standard flat fan to air-induction nozzles. Overall air-induction nozzles produced coarser spray droplets and a more uniform spray pattern with dicamba alone compared with dicamba plus glyphosate. However, with standard flat fan nozzles dicamba alone had a greater drift potential. Regardless of herbicide, drift 5 m away from the nozzle at a wind speed of 2.2 m $s^{-1}$ on average was 14 and 0.75% for flat fan an air-induction nozzles, respectively.

Using sensitive soybeans as a bioindicator, Jones et al. (2019) determined that particle drift from the DGA salt of dicamba could travel up to 152 m from the application boarder under field conditions. While injury was present at this great distance, yield reductions of 5% only occurred up to 42.8 m from the application border.

A third way that sensitive plants can be exposed to dicamba and 2,4-D is by contamination of application equipment. Contamination of spray equipment can occur from an improper, inadequate, or lack of clean out following an application of a PGR herbicide. This can lead to the unintentional application of the remaining herbicide or herbicide residues on the next application. Spray equipment can be difficult to clean completely as there are many components that require attention. Spray equipment parts that can come into contact with the herbicide include: the spray tank, sprayer boom, hoses, connectors and chemical inductors. Even after a sprayer had been drained and flushed with an ammonia-water solution Boerboom (2004) was able to detect dicamba residues in the rinse water at 822- 24800 ppb. Based on a dicamba use rate of 560 g ae $ha^{-1}$, these detections ranged from 0.021-0.63% of the recommended field use rate.

Cundiff et al. (2017) compared the retention of dicamba in several different sprayer hose types: synthetic rubbers, plastic polymers, and polyethylene blends. When the sprayer was loaded with 560 g ae $ha^{-1}$ of dicamba, the synthetic rubber hoses retained 16 parts per million by volume (ppmv) after rinsing, which is equivalent to 0.39% field use rate. Whereas low-density polyethylene hoses retained less than 1 ppmv after rinsing. After analysis it was determined that the difference in dicamba retention was related to interior imperfections from the manufacturing process. Imperfections in the lining, such as pockets and cracks had the ability to retain dicamba during the rinse. The polyethylene hose was free of these imperfections, thus explaining the low level of dicamba retention.

On a broader scale Osborn et al. (2015) conducted a survey of 46 commercial applicators and analyzed dicamba and 2,4-D residues in sampled rinse water from spray equipment. Samples were taken from: (1) the herbicide solution from the sprayer tank, (2) the rinse water used in the first rinse of the tank, (3) the rinse water from the second rinse, and (4) the water from the third and final rinse. Detectable herbicide concentrations were found at all sampling times. By the third water rinse dicamba detection averaged 0.41 mg $L^{-1}$ across all samples, this was 0.16% of the initial herbicide concentration (245 mg $L^{-1}$). 2,4-D residue detections were higher than dicamba in all three rinse water samplings. By the third rinse 3.3 mg $L^{-1}$ of 2,4-D was still detectable, or 1% of the initial concentration (312 mg $L^{-1}$).

In combination these findings confirm that herbicide resides from dicamba and 2,4-D can be left in spray equipment at detectable concentrations even after rinsing with water or ammonia tank cleaners (Boerboom 2004; Cundiff et al. 2017; Osborn et al. 2015). However, it is possible that higher rates can be retained if proper procedures are not followed. Sensitive crop exposures to these rates of PGR herbicides can have negative effects. Through this research it is evident that caution needs to be taken when using dicamba or 2,4-D as the potential for off-target movement or unintended applications of significant levels of these herbicides are possible.

**Sensitive crop exposure**

As dicamba and 2,4-D have been in use for many years numerous studies have been conducted examining the implications of exposure to sensitive crops. Soybean is extremely susceptible to very low levels of exposure to dicamba and 2,4-D. Kniss (2018) determined through a meta-analysis that dicamba rates as low as 0.03 g ae $ha^{-1}$ could elicit a response from soybean. Early research conducted by Auch and Arnold (1978) demonstrated that dicamba rates ranging from 1 to 56 g ae $ha^{-1}$ applied to V1 to R3 soybean resulted in a foliar response. Soybean height was also reduced when dicamba was applied after the V3 growth stage. However, soybean yield only reduced when exposures occurred during the reproductive stages of growth. Yield was reduced 63% when dicamba was applied at 56 g ae $ha^{-1}$ at the early-pod stage (R3). Germination of harvested seed was also reduced when dicamba at 56 g ae $ha^{-1}$ was applied to R3 soybean. Applications to R3 soybean reduced germination by 60%. Auch and Arnold (1978) also screened five soybean varieties for differences in sensitivity to dicamba. None of the soybean varieties examined were more tolerant to dicamba than others.

More recently, Soltani et al. (2015) examined soybean sensitivity to dicamba at rates ranging from 0 to 60 g ae $ha^{-1}$. When V2 soybean were exposed to dicamba the estimated rates to reduce yield 20 and 50% were 25.2 and >60 g ae $ha^{-1}$, respectively. However, when exposure occurred at R1 estimates dropped to 4.3 and 11.5 g ae $ha^{-1}$ for 20 and 50% yield loss, respectively. Griffin et al. (2013) also found that soybean were more sensitive at early reproductive stage (R1) than a vegetative growth stages, as yield was 2.5 times lower when dicamba was applied to soybean in the reproductive stage of growth at 4.4 and 17.5 g ae $ha^{-1}$.

Soybean are not only sensitive to dicamba, but also to 2,4-D. However, several researchers have found when comparing the two herbicides soybean was always more sensitive to dicamba than 2,4-D at equivalent rates (Anderson et al. 2004; Egan et al 2014; Kelley et al. 2005; Wax et al. 1969). Anderson et al. (2004) reported 40% more injury when soybean were treated with 0.0112 g ae $ha^{-1}$ of dicamba than 2,4-D, 24 DAT. This confirmed findings by Wax et al. (1969) who also reported greater soybean injury from dicamba than 2,4-D. Soybean yield was also reduced by all rates of dicamba tested from 5.6 to 56 g ae $ha^{-1}$. Whereas 2,4-D only reduced yield at the highest rate tested, 112 g ae $ha^{-1}$, even though soybean injury was as high as 30% with lower 2,4-D rates. Weidenhamer et al. (1989) also found that soybean could to tolerate considerable amounts of early-season foliar injury from 2,4-D without reducing yield. Basing yield losses off of soybean injury from herbicides can overestimate yield losses, since soybeans have the ability to tolerate high levels of injury without resulting in equivalent levels of yield loss (Al-Katib and Peterson 1999). Egan et al. (2014) also confirmed that soybeans are more sensitive to dicamba than 2,4-D by conducting a meta-analysis of 11 separate studies. Dose-response curves generated by this analysis documented that soybean was relatively more tolerant to 2,4-D than dicamba during both vegetative and reproductive growth stages. Soybean yield reductions ranged from 1.5 to 3% from 2,4-D at 56 g ae $ha^{-1}$.

*POST herbicide/adjuvants effects on soybean response from PGR herbicides.* Because there are multiple ways that dicamba and 2,4-D can be exposed to sensitive crops, it is important to also understand what effects other herbicides and/or adjuvants may have on their response to exposure. Kelley et al. (2005) examined the effects that postemergence soybean herbicides had on soybean injury from exposure to a low rate of dicamba, 5.6 g ae $ha^{-1}$ (1% of the labeled field rate). They found a synergistic response when dicamba was applied with imazamox, imazethapyr and fomesafen. This response resulted in increased soybean injury and further reduced yield in some circumstances. However, there was not a synergistic response when glyphosate was added, since glyphosate-resistant soybean was the indicator species. Responses from dicamba also included: reduced seed weight and the number of seeds per pod. In addition, reductions were greater when the applications were made to V7 versus V3 soybean. Results indicate that the synergistic responses exist between dicamba and herbicides that have metabolism-based selectivity in soybean (imidazolinones and fomesafen), and not with an insensitive target site (glyphosate).

Brown et al. (2009) examined simulated dicamba plus diflufenzopyr (0-40 g ai $ha^{-1}$) drift to soybean, followed three days later by applications of imazethapyr (100 g ai $ha^{-1}$), bentazon (1080 g ai $ha^{-1}$), or chlorimuron-ethyl (9 g ai $ha^{-1}$). Synergistic responses were not as consistent as Kelley et al. (2005). In some environments, soybean injury was considered synergistic when the other herbicides followed dicamba plus diflufenzopyr drift, 14 DAT. In environments where injury was caused by the soybean herbicides, all three soybean herbicides caused a synergistic response. Regardless of environment, there was no synergistic responses for dry biomass reduction or plant height. However, when chlorimuron-ethyl was applied following dicamba plus diflufenzopyr drift, soybean yield was decreased by 4-7% more than expected, indicating a synergistic response.

Other research has been conducted focusing on the effects of glyphosate in combination with dicamba on sensitive crops. Jones et al. (2018) found on non-glyphosate or dicamba-resistant soybean, that the addition of glyphosate to dicamba, both at 1.5 and 0.4% of the field use rates elicited a synergistic response. The addition of glyphosate to dicamba increased leaf malformation by 6% at R1 and pod malformation by 10% at R3. However, this combination did not affect the number of plants injured from grow outs of harvested seed.

Adjuvants can also effect sensitive crop injury from PGR herbicides. Anderson et al. (2004) reported increased soybean injury and yield loss when crop oil concentrate (COC) at 1% v $v^{-1}$ was added to low rates of dicamba compared with dicamba alone. When applied to sensitive soybean the addition of COC increased soybean injury by 5-10%, and increased yield loss by 9-10% in comparison to dicamba applied alone (Anderson et al. 2004).

*Other crops.* In addition to soybean, several other broadleaf crops are susceptible to dicamba and 2,4-D. In the Southern United States, cotton and peanuts can also be affected by unintended applications of dicamba and 2,4-D. Unlike soybean, where there is a greater tolerance for 2,4-D, cotton is much more sensitive to 2,4-D than dicamba (Everitt et al. 2009; Marple et al. 2008). Exposure to 280 g ae $ha^{-1}$ of 2,4-D at pinhead square growth stage injured cotton 77% and reduced lint yield by 96% (Everitt et al. 2009). At the same timing, dicamba at 280 g ae $ha^{-1}$

injured cotton 46% and reduced lint yield by 63%. The rates examined were equal fractions of each herbicides labeled use rates. Cotton is also more susceptible to dicamba and 2,4-D when exposure happens at 3-4 leaf and 8-18 leaf growth stages (Marple et al. 2008). Injury often intensified over time until 28 DAT and by 56 DAT cotton plants began to recover (Marple et al. 2008). Similar to soybean, peanut is more tolerant to 2,4-D than dicamba (Johnson et al. 2012). At applications rates of 280 ae g $ha^{-1}$ of dicamba and 540 g ae $ha^{-1}$ of 2,4-D, peanut had a maximum injury of 55 to 80% from dicamba and 30 to 40% from 2,4-D. (Johnson et al. 2012)

Across the U.S. a multitude of other specialty crops are grown in unique areas. In Southern Illinois one of these unique niche crops is horseradish (*Armoracia rusticana* L.). Wiedau et al. (2019) determined that horseradish is more tolerant to dicamba than 2,4-D. Horseradish root yield was reduced by exposure to 2,4-D at rates as low as 1.06 g ae $ha^{-1}$. However, these reductions did not occur with dicamba until rates of 560 g ae $ha^{-1}$ were applied. Even though 2,4-D reduced horseradish root yield, there were no detections of 2,4-D residues in the roots at harvest. Only the 1120 g ae $ha^{-1}$ rate of dicamba resulted in a significant residue detection of 32 parts per billion (ppb) in the harvested root. This level was well below the Environmental Protection Agencies established maximum residue limits (MRLs) of 100 ppb for root crops.

Other specialty crops that are relevant to Michigan production systems have shown sensitivity to dicamba and 2,4-D as well, including sugarbeet (*Beta vulgaris* L.). Sugarbeet is less sensitive to dicamba than soybean, but more sensitive to 2,4-D similar to peanut and horseradish (Johnson et al. 2012; Kniss 2018; Probst 2018, Wiedau et al. 2019). 2,4-D rates ranging from 2.8 to 280 g $ha^{-1}$ have been shown to cause injury across various sugarbeet growth stages (Byford and Prince 1976; Holksvig 1950; Probst 2019; Schroeder et al. 1983). Yield reductions of 27%

have also been documented from 2,4-D at 280 g ae $ha^{-1}$, where dicamba did not reduce root yield at rates from 17 to 140 g ae $ha^{-1}$ (Schroeder et al. 1983). At rates of 22.4 g ae $ha^{-1}$ for both herbicides, Probst (2018) documented yield reductions of 17 and 28% from dicamba and 2,4-D, respectively.

*Dry bean sensitivity to dicamba and 2,4-D*. Previous research is limited on the response of dry edible beans to exposure of dicamba and 2,4-D. In the mid-1980s, when dicamba and 2,4-D were commonly used for weed control in corn and wheat, Lyon and Wilson (1986) examined the sensitivity of the Great Northern market class of dry bean to both dicamba and 2,4-D amine at three rates (1.1, 11.2 and 112.5 g ae $ha^{-1}$) and four different growth stages (PRE, V2, R1 and R3). They reported the response of Great Northern beans to dicamba and 2,4-D exposure paralleled others work on the sensitivity of soybean to dicamba and 2,4-D. They concluded that dry bean sensitivity to dicamba and 2,4-D increased with herbicide rate and plant stage and that dry beans would be less likely to be damaged by 2,4-D than dicamba. Dicamba reduced yield starting at 11.2 and 112.5 g ae $ha^{-1}$ at R1 and V2 dry bean, respectively. However, 2,4-D did not reduce yield until a 112.5 g ae $ha^{-1}$ was applied at R1. The 112.5 g ae $ha^{-1}$ rate of dicamba and 2,4-D reduced yield 96 and 65%, respectively, at R3. In addition to yield reductions, Lyon and Wilson (1986) also reported reduced seed quality, including lower test weights and reduced germination of progeny seed. Test weight was only reduced by dicamba applications of 11.2 and 112.5 g ae $ha^{-1}$, but not by 2,4-D. Reductions in test weight can serve as an indication of reduced plant maturity at the time of harvest. Often treatments that had a high percentage in yield loss also had higher reductions in test weight. Test weight was reduced by as much as 63% with 112.5 g ae $ha^{-1}$ of dicamba at R3. Germination of progeny seed was reduced most often when R1 dry beans were exposed to at least 11.2 g ae $ha^{-1}$ of dicamba and 2,4-D. Lyon and Wilson (1986) hypothesized that these herbicides were translocating to the rapidly forming pods and seed at this time and contributed to the reduced germination of seed harvested from these plants.

More recently with the commercialization of dicamba-resistant soybean, Hatterman-Valenti et al. (2017) examined pinto and navy beans sensitivity to dicamba, glyphosate, and dicamba plus glyphosate at the R1 stage of growth. Dicamba rates of 4.5 g ae $ha^{-1}$ or greater caused significant plant injury to both classes of beans. Dry beans were also more sensitive to low rates of dicamba than glyphosate. Pinto bean injury did not occur at rates of glyphosate lower than 10 g ae $ha^{-1}$, and navy beans were not injured by any rate of glyphosate tested up to 100 g ae $ha^{-1}$. The addition of glyphosate to dicamba did not significantly affect plant injury or yield on either class of dry bean when compared with dicamba alone. Effects of dicamba on dry bean yield were variable. Delays in seed set, ultimately led to pinto beans not being harvested when dicamba at 4.48 g ae $ha^{-1}$ or higher was applied. However, in navy beans yield reductions started at 18 g ae $ha^{-1}$ or higher rate of dicamba. Navy bean yield was reduced 56% at this time. Data from this study was only collected in one location per dry bean class, suggesting that yield may also have been affected by environmental conditions. Although not measured researchers noted that exposures and subsequent plant injury from dicamba or dicamba plus glyphosate slowed the plants natural maturation process and could have had a greater effect on dry bean yield in the event of an early frost prior to harvest.

As we build our knowledge base on the response of dry bean to plant growth regulator herbicides, we have research questions that remain to be answered with confidence. Research in soybean has been beneficial in elucidating what research questions are important to ask. The investigation of these questions in dry bean will be beneficial to Michigan dry bean producers in the understanding of plant response to an off-target exposure of a PGR herbicide on dry beans.

Questions that remain to be answered:

1. Do modern cultivars of dry beans have similar sensitives to PGR herbicides?
2. Are results comparable over multiple classes of dry bean?
3. During vegetative growth stages is timing significant?
4. Does the addition of glyphosate and/or tank-mixtures with dry bean herbicides effect dry bean response?

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# CHAPTER II

# SENSITIVITY OF DRY EDIBLE BEAN TO DICAMBA AND 2,4-D

## Abstract

Dicamba and 2,4-D exposure to sensitive crops, such as dry beans, is of great concern with the recent registrations of dicamba and 2,4-D resistant soybeans. In 2017 and 2018, field experiments were conducted at two Michigan locations to understand how multiple factors, including dry bean market class, herbicide rate, and application timing influence dry bean response to dicamba and 2,4-D. Dicamba and 2,4-D at rates of 0.1, 1 and 10% of the field use rate for dicamba and 2,4-D choline were applied to V2 and V8 black and navy beans. Field use rates for dicamba and 2,4-D choline were 560 and 1120 g ae ha$^{-1}$, respectively. There were very few differences between market classes or application timings when dry beans were exposed to dicamba or 2,4-D. Rates that caused 20% dry bean injury 14 DAT were 4.5 and 107.5 g ae ha$^{-1}$ for dicamba and 2,4-D, respectively. When dicamba was applied at 56 g ae ha$^{-1}$ canopy closure was reduced up to 51% and maturity was delayed up to 16 days. Even though both herbicides caused high levels of injury to dry beans, yield reductions were not consistently observed. At four site-years, 2,4-D did not reduce dry bean yield or seed weight with any rate tested. However, when averaged over site-years dicamba rates of 3.7, 9.8 and 17.9 g ae ha$^{-1}$ caused 5, 10 and 15% yield loss, respectively. Dicamba also reduced seed weight by 10% when 56 g ae ha$^{-1}$ was applied. However, the germination rate of progeny seed was not affected by dicamba or 2,4-D. While dry bean yield and quality is not always directly impacted by dicamba or 2,4-D herbicides, long delays in maturity from injury can have negative impacts on yield and quality due to harvestability issues. This work further stresses the need for caution when using dicamba or 2,4-D herbicides near sensitive crops.

## Introduction

Glyphosate and multiple resistant weeds are a management challenge for U.S. soybean (*Glycine max* L.) farmers. New soybean varieties developed to be resistant to dicamba or 2,4-D provide alternative options for control of glyphosate resistant weeds (Kruger et al. 2010). For example, postemergence (POST) applications of dicamba in dicamba resistant soybean have provided 88-100% control of glyphosate resistant weeds, including horseweed (*Conyza canadensis* L.), Palmer amaranth (*Amaranthus palmeri* L.), common waterhemp (*Amaranthus tuberculatus* L.), and giant ragweed (*Ambrosia trifida* L.) (Byker et al. 2013; Kruger et al. 2010; Norsworthy et al. 2008; Spaunhorst et al. 2014). In 2,4-D resistant soybean, the use of 2,4-D alone or in combination with glufosinate has also provided greater than 93% control of glyphosate resistant populations of both horseweed and Palmer amaranth (Craigmyle et al 2013; Norsworthy et al. 2008).

Dicamba and 2,4-D are classified as plant growth regulator (PGR) or synthetic auxin herbicides and are in the Group 4 herbicide site of action group (Mallory-Smith and Retzinger 2003). Historically, these herbicides have been used for selective control of broadleaf (dicots) plants in monocot crops including: turf, small grains, corn and pastures (Shaner 2014). In fact, almost all broadleaf plants show some response to applications of these herbicides. Selectivity between dicots and monocots with these herbicides is a function of both plant metabolism and target-site sensitivity (Sterling and Hall 1997; Grossman 2003). In dicamba resistant soybean, the insertion of the dicamba monooxygenase gene catalyzes dicamba metabolism to non-toxic forms allowing for the safe use of soil-applied and postemergence dicamba (Behrens et al. 2007). In 2,4-D resistant soybean, the aryloxyalkanoate dioxygenase enzyme AAD-12 was inserted to increase the degradation of 2,4-D into the non-herbicidal metabolite dichlorophenol (Wright et al. 2010). Prior to the insertion of these resistance genes, soybean was extremely sensitive to dicamba and 2,4-D and could be injured if exposed unintentionally (Anderson et al. 2004; Wax et al 1969).

Sensitive plants can be exposed unintentionally to dicamba and 2,4-D by various means including: off-target movement by vapor drift, particle drift, and spray system contamination (Behrens and Lueschen 1979; Boerboom 2004; Cundiff et al. 2017; Dexter 1993). Vapor drift occurs when molecules of a herbicide active ingredient are converted from a liquid to a gaseous form and are moved to an unintended location by air currents (Peterson et al. 2014). The vaporization potential of the herbicide is a function of both the herbicides physical properties and the environmental conditions at the time of and after application (Behrens and Lueschen 1979; Burgoyne and Hites 1993).

Dicamba and 2,4-D are subject to vapor drift when salt formulations dissociate into the acid forms, thus increasing the vapor pressures of these herbicides (Behrens and Lueschen 1979; Hillger et al. 2012). Particle drift is another way sensitive plants can be exposed to low doses of dicamba and 2,4-D. Particle drift is the physical movement of the spray solution by wind. Factors that affect particle drift include: application speed, droplet size, nozzle type, boom height, and wind speed (Alves et al. 2017; Maybank et al. 1978; Nordby and Skuterud 1975; Thistle 2004; Wolf et al. 1993). Steps can be taken to help reduce particle drift, such as nozzle selection. However, Alves et al. (2017) found that even when drift reduction nozzles were used, 0.6% rates of dicamba could drift up to 12 m with a wind speed of 4.9 m $s^{-1}$.

Sensitive plants can also be exposed to low doses of dicamba and 2,4-D through spray system contamination. Improper sprayer clean out or herbicide residues remaining in spray tanks, hoses, or nozzles even after proper clean out can lead to unintended injury on sensitive crops during the next herbicide application. Boerboom (2004) documented that as much as 0.63% of the original use rate of dicamba (0.56 kg ae $ha^{-1}$) was detected in discharge water from the boom even after the spray system had been flushed with an ammonia water solution. These findings were also supported by Osborn et al. (2015) who found that in the water from the third rinse of a spray tank, rates of dicamba and 2,4-D of 0.16 and 1% were still detected, respectively. These findings confirm the possibility of dicamba and 2,4-D residues being deposited on sensitive crop during the applicators next application.

Several research studies have been conducted examining the effects of dicamba and 2,4-D exposure on sensitive crops. Soybean has been the focus of the majority of these trials, as they are extremely sensitive and are grown on a large number of hectares in the United States. Previous research has shown that soybeans are more sensitive to dicamba than 2,4-D (Anderson et al. 2004; Wax et al. 1969). Through a meta-analysis of 11 studies, Kniss (2018) determined that rates as low as 0.03 g ae $ha^{-1}$ of dicamba can injure soybean. However, yield losses of 5% did not occur until 0.9 g ae $ha^{-1}$ of dicamba or higher were applied at the flowing stage of soybean (R1 to R2). Soybean injury from 2,4-D can also occur at relatively low rates, with 11.2 g ae $ha^{-1}$ causing 5% plant injury (Anderson et al. 2004). However, even with plant injury up to 30% yield reductions did not always occur (Wax et al. 1969; Weidenhamer et al. 1989). While soybean is an economically important crop to the United States and Michigan, there are a multitude of other sensitive broadleaf crops grown in areas that are at risk of unintended dicamba or 2,4-D exposure.

Dry edible bean (*Phaseolus vulgaris* L.) is another sensitive broadleaf crop that is at risk of unintended exposure to dicamba and 2,4-D. This unique high value crop, is a short-seasoned legume that typically matures in 85-100 days (Kelly and Cichy 2013). In the United States, 14 states produce 13 different market classes of dry beans (USDA-NASS 2018). Currently, Michigan leads the nation in production of three of these classes: black, cranberry, and small red beans (USDA-NASS 2018). While Michigan is not the number one producer of navy beans, this class is the second largest class grown in Michigan. On average, Michigan harvests 106,000 ha of dry beans with an average yield of 2,300 kg ha$^{-1}$ (USDA-NASS 2018). Yearly this equates to a farm-gate value of $140 million. Michigan dry beans are typically planted starting the first week of June and continue throughout the month (Kelly and Cichy 2013). This later planting date historically kept dry beans at a lower risk from exposure to dicamba and 2,4-D applications when these herbicides were used in corn and small grains, since applications were often made before dry beans emerged. However, with the use of postemergence applications of dicamba and 2,4-D to soybean later in the season, dry beans are at an increased risk of exposure.

Limited research has been conducted examining the effects of dicamba and 2,4-D exposure on dry edible beans. Lyon and Wilson (1986) published research documenting that great northern beans were more sensitive to dicamba than 2,4-D and that dicamba at 112.5 g ae ha$^{-1}$ reduced yield up to 96%. They also reported that exposure to dicamba and 2,4-D delayed bean maturity, and reduced test weight and germination of harvested seed. More recently, Hatterman-Valenti et al. (2017) reported that dicamba exposure at flowering (R1) at a rate of 18 g ae ha$^{-1}$ injured navy and pinto beans up to 53%, delayed maturity, and reduced yield 56-100%.

With the recent commercialization of soybean varieties resistant to dicamba and 2,4-D, we expect to see an increased use of these herbicides in Michigan. This change will put Michigan dry beans at an elevated risk for exposure from dicamba and 2,4-D. Previous research indicates that low doses of these herbicides has the potential to impact dry bean yield and quality (Lyon and Wilson 1986; Hatterman-Valenti et al. 2017). As we build our knowledge base on the response of dry beans to dicamba and 2,4-D, we have several research questions that remain to be answered with confidence on modern dry bean cultivars and market classes that are economically important to Michigan. Therefore, the objectives of this research were to understand how multiple factors, including dry bean market class, herbicide rate, and application timing influence dry bean response to dicamba and 2,4-D.

## Materials and methods

Field experiments were conducted in 2017 and 2018 at the Michigan State University (MSU) Agronomy Farm in East Lansing, Michigan (42.71 N, -84.47 W) and at the MSU Saginaw Valley Research and Extension Center near Richville, Michigan (43.399 N, -83.697 W). The soil types at East Lansing were a Colwood-Brookston loam (fine-loamy, mixed, mesic typic haplaquolls) with pH 7.0 and 2.7% organic matter in 2017, and a pH 6.0 and 3.4% organic matter in 2018. Soils at the Richville location were a Tappan-Londo clay loam (fine-loamy, mixed, active, calcareous, mesic typic epiaquolls) with pH 7.0 and 2.7% organic matter in 2017, and a pH 7.7 and 2.5% organic matter in 2018. Dry beans were planted into conventionally tilled soils. Soil preparation consisted of either fall chisel or moldboard plowing followed by two passes of a soil finisher in the spring prior to planting. Fertilizer applications were standard for dry bean production in Michigan. In East Lansing, 19-19-19 (N-P-K) at 112 kg $ha^{-1}$ was applied with the planter 5 cm down and 5 cm over from the seed. At Richville, 336 kg $ha^{-1}$ of 17-8-15 (N-P-K) fertilizer containing 1.5% manganese and 1.5% zinc was broadcast applied prior to spring tillage.

Two Type II (upright indeterminate vine) varieties, ‘Zenith’ black beans (Michigan Crop Improvement Association, Okemos, MI) and ‘Merlin’ navy beans (ProVita Seeds, Pigeon, MI) were planted in 76 cm rows at 269,000 seeds $ha^{-1}$ at all locations. Black and navy beans were selected, since they are the top two market classes grown in Michigan (NASS-USDA 2018). Dry beans were planted on June 6, 2017 and June 5, 2018 in East Lansing, and on June 8, 2017 and June 19, 2018 in Richville. Plots were four rows wide by 9.1 m long.

The diglycolamine salt of dicamba (XtendiMax ® Bayer Crop Science, Research Triangle Park, NC) and the choline salt of 2,4-D (Enlist One ® Dow AgroSciences LLC, Indianapolis, IN) were applied at 0.1, 1.0, and 10% of their standard rates of 560 and 1120 g ae $ha^{-1}$ used in dicamba resistant and 2,4-D resistant soybean, respectively. Each herbicide treatment was applied at two different stages of growth, V2 (two-trifoliate) and V8 (pre-bloom) dry bean. Herbicides were applied using a tractor-mounted, compressed air sprayer calibrated to deliver 177 L $ha^{-1}$ at 206 kPa using AIXR 11003 nozzles (TeeJet Technologies, Wheaton, IL).

Experiments were arranged in a split-plot design with three (East Lansing 2018) or four replications. The main plot factor was application timing and subplot factors within in the main plots were arranged in a two-way factorial between dry bean class and herbicide treatment. All field experiments were maintained weed free through mechanical cultivation and supplemented by hand weeding.

Precipitation data were obtained throughout the growing season from the Michigan Automated Weather Network (http://www.agweather.geo.msu.edu/mawn/, Michigan State University, East Lansing, MI). The Michigan Automated Weather Network maintains weather stations within one kilometer of the experiment locations in both East Lansing and Richville, MI (Table 2.1).

Dry bean injury was evaluated 7, 14 and 28 days after treatment (DAT) on a scale from 0-100%, with 0 equivalent to no plant response and 100 representing complete plant death. Dry bean canopy closure was measured 55 days after planting (DAP) using a SunScan Canopy Analysis System (Dynamax Inc, Houston, TX), consisting of a 1 m by 13 mm wand containing 64 light sensors, connected to a hand-held computer. The wand was placed perpendicular to the center two rows above and below the plant canopy. Three measurements were taken per plot sequentially. Percent canopy closure was calculated by subtracting the amount of light below the canopy from the amount of light measured above the canopy, then dividing that by the amount of light above the canopy and multiplying by 100. Measurements were taken around solar noon in full or nearly full sun. Dry bean maturity was evaluated weekly for 6 weeks prior to harvest. A 0-100% scale was used, with a 0 indicating all green tissue and 100 indicating complete plant maturity (no green tissue).

Dry bean plots were mechanically harvested after natural plant senescence. When possible, plots were direct harvested using a small-plot research combine (Massey-Ferguson 8XP, AGCO, Duluth, GA) with a 1.5 m header. Treatments that had severe delays in maturity were hand pulled and dry bean seed was separated from pods, stems, and leaves by using a stationary thrasher (Almaco LPR, Almaco, Nevada, IA). Excess rainfall near harvest at East Lansing in 2018 lead to dry bean regrowth requiring the use of a chemical desiccant prior to harvest. Dry beans were desiccated with saflufenacil (Sharpen® BASF Corporation, Research Triangle Park, NC) at 50 g ai $ha^{-1}$ plus 1% v $v^{-1}$ methylated seed oil 14 d prior to harvest. Dry bean yield was adjusted to 18% moisture.

Samples of harvested dry bean seed were taken from each plot during harvest. Seed samples were sorted for mechanical damage and weight per 100 seeds was recorded on undamaged seeds. Seeds were tested for germination by placing 25 seeds per sample in a germination chamber for 5 d at 20 C. At the conclusion of the 5 d the number of germinated seeds were counted, divided by the total, and multiplied by 100 to calculate percent germination.

**Statistical analysis.** Data analysis was conducted using PROC MIXED procedure in SAS ® 9.4 (SAS institute, Cary, NC). The statistical model consisted of herbicide treatment, application timing, and dry bean class as fixed effects and site-year (individual years and locations), replication nested within site-year and the interaction between application timing and replication nested within site-year as random effects. Replications were used as an error term for testing the effects of site-year, when an interaction of site-year by one of the fixed effects was significant, data was analyzed separately. Normality assumptions were checked by examining histogram and normal probability plots of the residuals. Unequal variance assumption was assessed by visual inspection of the side-by-side box plot of the residuals followed by the Levene’s test for unequal variances.

Further analysis was preformed through the use of nonlinear regression to predict the effects of multiple rates of dicamba and 2,4-D using the drc package in R (R, Version 3.0) (Knezevic et al. 2007). Model fit was confirmed using the ModelFit function in R. Nonlinear regression was used to predict the herbicide rate that caused 20% dry bean injury ($I_{20}$), and yield reductions of 5, 10, and 15% ($YR_{5-15}$). Nonlinear regression was also used to predict the number of days from planting to 50% dry bean maturity ($MR_{50}$). The non-linear models used were the three-parameter log-logistic model [1] and the three-parameter Weibull model [2].

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

The log-logistic model was used for the estimate $I_{20}$ and $MR_{50}$ values. For $I_{20}$ values *y* represented dry bean injury, *x* is herbicide rate, *c* is the lower limit, *b* is the slope of the line around $e$ and $e$ is the $I_{20}$. $I_{20}$ values were compared using a paired t-test at $p \leq 0.05$. For $MR_{50}$ variables in equation 1 are the same as for $I_{20}$, with the exception that $y$ is dry bean maturity and $x$ represents days after planting. $MR_{50}$ values were analyzed using the PROC MIXED procedure as previously described.

$$y = d(\exp\{-\exp[b(\log(x - e)]\}) \quad [2]$$

The Weibull model used for estimating yield reductions from dicamba and 2,4-D in comparison to the control where $y$ represents dry bean yield as a percentage of the control, $b$ the relative slope of the curve, $d$ is the upper limit, $e$ is the $YR_{5\text{-}15}$, and $x$ the herbicide rate. $YR_{5\text{-}15}$ values were compared using a paired t-test at $p \leq 0.05$.

## Results and discussion

**Dry bean injury.** Dry bean injury from dicamba and 2,4-D was rate dependent and varied by dry bean class or application time, depending on the time of evaluation (DAT) (Figures 2.1a-2.1c). At 7 DAT, there was a herbicide treatment by dry bean class interaction ($P = 0.01$). Black beans were slightly more sensitive to dicamba than navy beans and both classes of dry beans were more tolerant to 2,4-D compared with dicamba (Table 2.2). Injury from the 0.1% (0.56 g ae $ha^{-1}$) rate of dicamba and the 1% (11.2 g ae $ha^{-1}$) rate of 2,4-D was relatively low (3-4%) (Figures 2.1a) and symptoms consisted of leaf crinkling and cupping. At higher rates, dry bean injury was as high as 39% and additional symptoms included outward growth that resulted in a more "flattened" appearance of the plants.

Differences in injury between dry bean classes were not apparent by 14 DAT. However, there was a herbicide treatment by application timing interaction for dry bean injury at 14 and 28 DAT ($P < 0.0001$). Dry beans were more sensitive to dicamba at the V2 compared with the V8 application timing, 14 DAT (Table 2.2). Injury from dicamba continued to progress and was as high as 46 and 38% from the 10% (56 g ae $ha^{-1}$) rate for the V2 and V8 application timings, respectively (Figure 2.1b). Additional injury symptoms included stem twisting or epinasty and were consistent with symptoms described by Lyon and Wilson (1986) and Hatterman-Valenti et al. (2017). However, by 28 DAT injury was highest from dicamba applications to V8 compared with V2 dry beans. The $I_{20}$ values were 0.8 and 1.5% of the dicamba field use rate for the V2 and V8 application timings, respectively 14 DAT and were 3.75 and 2.0%, 28 DAT (Table 2.2).

The higher injury observed from dicamba applications to V8 dry beans 28 DAT may be due to slower growth, as dry beans transition from vegetative to reproductive stages (Fageria and Santos 2008). While the $I_{20}$ values for dicamba increased from 14 to 28 DAT, there was little to no signs of recovery from dicamba injury at the 10% rate (56 g ae $ha^{-1}$) (Figure 2.1c). At this rate, dry bean plants exhibited death of axial growing points as well as callous formation on stems. Hatterman-Valenti et al. (2017) also noted these symptoms 20 DAT when pinto and navy beans were treated at R1 with 44 g ae $ha^{-1}$ of dicamba.

Unlike injury from dicamba, when dry beans were exposed to 2,4-D at rates ranging from 0.1-10% (1.12 to 112 g ae $ha^{-1}$) there were no differences in injury between dry bean class or application timing (Table 2.2). $I_{20}$ values for 2,4-D injury to dry beans averaged 4.5, 9.7, and >10% of the field use rate at 7, 14, and 28 DAT, respectively. The higher $I_{20}$ values at the later evaluation timings demonstrates dry bean’s ability to recover from 2,4-D injury over time. This has also been demonstrated with 2,4-D injury to soybean. Robinson et al. (2013) reported that $I_{20}$ values for 2,4-D injury to soybean increased from 6.8 to 9.7%, 14 and 28 DAT, respectively.

**Canopy closure.** Overall dry bean canopy closure was different between site-years. Average canopy closure 55 DAP for dry beans not exposed to dicamba or 2,4-D was 64, 99, and 86% for Richville 2017, Richville 2018 and East Lansing 2018, respectively (Table 2.3). Canopy closure was not measured in East Lansing 2017. Differences in canopy closure between site-years is most likely a reflection of greater canopy development resulting from precipitation received within the 30 days prior to the canopy closure measurement. At Richville 2018 where canopy closure was nearly complete (99%) 13.7 cm of rainfall occurred in the 30 days prior to measurement (Table 2.1). Rainfall during this period was 2.8 and 4 cm for Richville 2017 and East Lansing 2018, respectively.

Dicamba reduced canopy closure in all three site-years. The high rate of dicamba (56 g ae $ha^{-1}$) had the greatest impact, reducing canopy closure 8 to 51% compared with the untreated control (Table 2.3). In most cases, greater reductions in canopy closure from dicamba were recorded for the V8 compared with V2 application timing. Differences in canopy closure were also observed with the 1% (5.6 g ae $ha^{-1}$) rate of dicamba in two site-years. Dry beans exposed to dicamba from the V2 application timing had 7 days longer to recover from herbicide injury compared with the V8 application timing, since all measurements were taken 55 DAP. One exception was at East Lansing 2018, where a greater reduction in canopy closure occurred when the 10% rate of dicamba was applied to V2 dry beans. The high rate of 2,4-D (112 g ae $ha^{-1}$) only reduced canopy closure for the V2 application timing at East Lansing 2018 (Table 2.3). Canopy closure was reduced by 11% compared with the untreated control.

In general, there were no differences in canopy closure between dry bean classes. However, at two locations black beans had greater canopy closure than navy beans when exposed to 10% (56 g ae $ha^{-1}$) rates of dicamba for two of the application timings. In East

Lansing 2018 at the V2 application, there was a 27% difference in canopy closure between the dry bean classes ($P < 0.0001$). This resulted in 24 and 51% canopy closure for navy beans and black beans, respectively. The same interaction occurred at Richville 2018 when the 10% (56 g ae $ha^{-1}$) rate of dicamba was applied at V8. Canopy closure for navy beans was 68% and black beans was 76% ($P = 0.01$).

Increased canopy closure in dry beans has advantages for both weed control and yield. Greater canopy development is essential for capturing higher levels of photosynthetically active radiation (PAR) that has been associated with increased yield potential (Blackshaw et al 2000). Several studies have shown that PAR can be maximized through the use of narrower row widths (Blackshaw et al. 2000; Holmes and Sprague 2013). Blackshaw et al. (2000) reported that dry beans captured 25% more PAR when planted in 23 compared with 69 cm rows. This increase in PAR led to an improvement in weed control and dry bean yield. In our study, we found that dicamba injury to dry beans can reduce canopy closure by twice that amount, 51%, that would likely affect dry bean yield and increase difficulties for weed control.

**Dry bean maturity**. Dry bean maturity was similar between black and navy beans, regardless of herbicide treatment, application timing, or site-year. At Richville, maturity was influenced by the interaction of herbicide treatment and application timing ($P<0.0001$). There was an 8 to 9 day delay to 50% maturity compared to the control when dicamba was applied at the 10% (56 g ae $ha^{-1}$) rate (Table 2.4). Maturity was also delayed 10 days when the 1% (5.6 g ae $ha^{-1}$) rate of dicamba was applied to V8 dry beans. Applications of 2,4-D had less of an impact on dry bean maturity. Maturity to 50% was delayed by 6 days when the 10% (112 g ae $ha^{-1}$) rate of 2,4-D was applied to V8 dry beans. Lower rates of 2,4-D did not affect dry bean maturity (Table 2.4).

Dry bean maturity was not affected by application timing at East Lansing. In general, longer delays in maturity were documented at East Lansing than Richville. The longest delay in maturity was 16 days when dicamba was applied at the 10% (56 g $ha^{-1}$) rate (Table 2.4). Lower rates of dicamba also delayed maturity. The 1% (5.6 g ae $ha^{-1}$) rate of dicamba delayed dry bean maturity 8 days. Only the highest rate of 2,4-D (112 g ae $ha^{-1}$) delayed dry bean maturity (9 days) (Table 2.4).

At both locations dry bean maturity was affected more by dicamba than 2,4-D. Our data supports findings by Lyon and Wilson (1986) that reported dicamba caused longer delays in maturity than 2,4-D when applied at equal rates. Delays in maturity and complete crop loss have been reported in dry bean by Hatterman-Valenti et al. (2017). Dicamba at a rate of 4.4 g ae $ha^{-1}$ applied to R1 pinto beans resulted in abortion of initial flowers and new flower formation did not develop until the end of the season, resulting in complete yield loss. Delays in navy bean maturity were also recorded from dicamba rates up to 44 g ae $ha^{-1}$, however beans were able to be harvested after chemical desiccation (Hatterman-Valenti et al. 2017).

**Dry bean yield and quality.** Few differences in dry bean yield existed between herbicide treatments (Table 2.5). Yield was only reduced by the highest rate of dicamba and was 26 and 33% lower than the untreated control in 2017 and 2018, respectively. Yield was not affected by any rate of 2,4-D. While dry bean classes did not differ in how they responded to herbicide exposure, black beans did yield 8% more than navy beans in 2017 ($P= 0.001$).

To help explain the impact of different dicamba and 2,4-D rates on dry bean yield, the effective dose required to reduce yields 5, 10 and 15% were calculated (Figure 2.2). Regardless of dry bean class, application timing, location or year the effective dose to reduce dry bean yield up to 15% was always greater than the highest rate of 2,4-D tested (>112 g ae $ha^{-1}$) (Table 2.6). While previous dry bean studies have not reported yield loss predictions from various rates of 2,4-D, Robinson et al. (2013) estimated that 87 g ae $ha^{-1}$ (8% of the field use rate) would reduce soybean yield 5% when exposure occurred during the vegetative stage of growth. Unlike 2,4-D, effective doses of dicamba to reduce yield 5, 10, and 15% were in the rate range tested. Dicamba rates estimated to reduce dry bean yield 5, 10, and 15% were 0.6, 1.8 and 3.6% of a field use rate (3.3, 10.1, 20.0 g ae $ha^{-1}$), respectively, in 2017 (Table 2.6). While dry bean yields where generally higher in 2018, dicamba rate estimates for yield reductions were similar between the two years. Estimated dicamba rates were 0.75, 1.7 and 2.8% (4.2, 9.5, 15.6 g ae $ha^{-1}$) for 5, 10 and 15% yield reductions. Similar to studies with 2,4-D, currently no dry bean studies have reported dicamba rate estimates for yield reductions. However, Kniss (2018) conducted a meta-analysis of published research on soybean yield losses from dicamba. Reported yield loss from dicamba exposure in soybean at the vegetative stage were variable and estimated rates to impact yield 5% ranged from 1.6 to 97 g ae $ha^{-1}$ (0.28-17% of the field use rate).

The higher level of sensitivity of dry bean has to dicamba than 2,4-D supports previous research by Lyon and Wilson (1986). When dicamba and 2,4-D were sprayed at V2, with rates up to 112 g ae $ha^{-1}$ for both herbicides, only that highest rate of dicamba (112 g ae $ha^{-1}$) reduced yield by 70%. However, dry beans did show more sensitivity to both dicamba and 2,4-D when exposed in the reproductive growth stages (Lyon and Wilson 1986). This was also confirmed by Hatterman-Valenti et al. (2017), where yield losses in navy bean were 56% from 17.9 g ae $ha^{-1}$ applied at R1.

*Seed weight and germination.* Interactions with years, locations or application timings did not exist for dry bean seed weight. Only the main effects of herbicide treatment and dry bean class were significant. Dicamba at the 10% (56 g ae $ha^{-1}$) rate was the only herbicide treatment that affected dry bean seed weight (Table 2.5). This was also the only treatment that reduced dry bean yield. Seed weight of dry beans that were exposed to the 10% (56 g ae $ha^{-1}$) rate of dicamba was reduced 10% compared with the untreated control. Regardless of herbicide treatment, seed weight of black beans (22.2 g 100 $seeds^{-1}$) was greater than navy beans (19.4 g 100 $seeds^{-1}$) (data not shown). This difference is likely more associated with differences in the cultivars examined than the actual dry bean class. The black bean cultivar 'Zenith' used in this study tends to have a naturally higher seed weight than other black bean or navy bean cultivars (Kelley et al. 2014).

Germination was tested for the black beans harvested from this research. Neither application timing nor herbicide treatment affected black bean germination in either year, even though overall germination rates were higher from seed harvested in 2018 compared with 2017 (Table 2.5). Other researchers have found that dicamba applied to dry beans at V2 can reduce harvested seed germination (Lyon and Wilson 1986). However, this was only observed when dicamba was applied at 112.5 g ae $ha^{-1}$, which was twice the amount of dicamba that we tested. They also found that when dicamba applications were made to dry beans after flowering (R1), larger reductions in seed viability were observed. Our findings were more similar to Auch and Arnold (1978), where soybean seed germination was not affected when dicamba at rates up to 56 g ae $ha^{-1}$ was applied in the vegetative stage of growth.

Overall both classes of dry bean responded similarly to dicamba and 2,4-D. The timing of application often did not affect dry beans response when applied during vegetative growth stages. Previous research however does indicate that dry beans are more sensitive to dicamba and

2,4-D once reproductive growth begins (Lyon and Wilson 1986). When applied at equal percentages of their field use rate and compared across all response variables dry beans were more sensitive to dicamba than 2,4-D. Dicamba at rates as low as 0.6% (3.36 g ae $ha^{-1}$) reduced dry bean yield. This rate is similar to what has been reported as possible dicamba residue levels found in a sprayer even after following recommended sprayer clean out procedures (Boerboom 2004). This is concerning for dry bean producers as direct yield loss is possible even when clean out procedures are followed. In the event that a sprayer is not cleaned out properly, dry beans could be exposed to even higher rates of dicamba and thus higher yield loss. While no rate of 2,4-D tested caused direct yield loss, significant delays in maturity did occur. Indirect yield losses could result from harvest difficulties influenced by delayed maturity and erratic precipitation patterns in Michigan during the harvest season. Furthermore, dry beans currently do not have an established maximum residue limits (MRLs) for dicamba or 2,4-D residues that may be present in the harvested seed following exposure. Future research could investigate the possibility of these herbicide residues in harvested dry bean seed.

APPENDICES

# APPENDIX A
## Tables and figures

Table 2.1. Monthly and 5-yr average precipitation at East Lansing and Richville, MI in 2017 and 2018.[a]

| | East Lansing | | | Richville | | |
|---|---|---|---|---|---|---|
| Month | 2017 | 2018 | 5-yr ave. | 2017 | 2018 | 5-yr ave. |
| | | cm | | | cm | |
| June | 8.4 (8.2)[b] | 3.7 (3.0) | 7.6 | 12.2 (11.5) | 4.0 (2.9) | 7.0 |
| July | 6.7 | 2.7 | 5.8 | 2.8 | 5.0 | 7.0 |
| August | 3.5 | 11.7 | 9.3 | 5.7 | 20.0 | 12.6 |
| September | 3.3 | 10.3 | 7.8 | 4.0 | 4.9 | 6.1 |
| October | 17.4 | 9.3 | 8.9 | 8.7 | 5.0 | 6.5 |
| November | 3.5[c] | - | 8.0 | - | - | 4.5 |
| Total | 37.6[d] | 37.0 | 47.4 | 32.7 | 37.8 | 43.7 |

[a] Michigan Automated Weather Network, http://www.agweather.geo.msu.edu/mawn/, Michigan State University, East Lansing, MI

[b] Precipitation data in parenthesis is from the time of planting.

[c] The harvest month does not include rainfall after harvest.

[d] Total precipitation is a total of rainfall from planting until harvest.

Table 2.2. The effective dose of dicamba and 2,4-D to cause 20% injury ($I_{20}$) 7, 14 and 28 DAT when applied to V2 and V8 black and navy beans[a].

| | 7 DAT[b] | | 14 DAT | | 28 DAT | |
|---|---|---|---|---|---|---|
| Herbicide | Black | Navy | V2 | V8 | V2 | V8 |
| | —%— | | —%— | | —%— | |
| Dicamba | 1.2 a[c] | 1.6 b | 0.8 a | 1.5 b | 3.75 b | 2.0 a |
| 2,4-D | 4.4 c | 4.6 c | 9.6 c | 9.7 c | >10 c | >10 c |

[a]Data was combined over site-years and presented by the significant interactions between main effects.

[b]Effective use rates are presented as a percentage of the field use rate of dicamba (560 g ae ha$^{-1}$) and 2,4-D choline (1120 g ae ha$^{-1}$).

[c]Values followed by the same letter within evaluation timing are not significantly different ($\alpha \leq 0.05$).

Table 2.3. Canopy closure of dry beans 55 days after planting (DAP) at Richville 2017 and 2018, and East Lansing 2018, MI when V2 and V8 dry beans were exposed to dicamba and 2,4-D.[a,b]

| | | | Richville | | | | East Lansing | |
|---|---|---|---|---|---|---|---|---|
| | | | 2017 | | 2018 | | 2018 | |
| Herbicide | Rate | | V2[c] | V8 | V2 | V8 | V2[e] | V8 |
| | g ae ha$^{-1}$ | (%) | % | | % | | % | |
| Dicamba | 0.56 | 0.1 | 61 c-f[c] | 60 d-f | 99 a | 98 a | 88 ab | 83 c-e |
| | 5.6 | 1.0 | 61 c-f | 58 fg | 99 a | 93 b | 83 de | 76 f |
| | 56 | 10 | 54 g | 30 h | 89 c | 72 d[d] | 37 h[e] | 68 g |
| 2,4-D | 1.12 | 0.1 | 64 b-d | 63 b-e | 99 a | 99 a | 86 b-d | 87 a-c |
| | 11.2 | 1.0 | 63 b-e | 66 ab | 99 a | 99 a | 90 a | 81 e |
| | 112.0 | 10 | 69 a | 59 ef | 99 a | 98 a | 77 f | 82 de |
| None | - | - | 62 b-f | 65 a-c | 99 a | 99 a | 88 ab | 84 b-e |

[a]Data is presented as the herbicide treatment by application timing interaction and were combined over dry bean class, unless otherwise noted.

[b]Canopy closure was measured 55 DAP, which was 27 and 20 DAT for the V2 and V8 application timings, respectively.

[c]Means followed by the same letter within a site-year are not significantly different $\alpha \leq 0.05$.

[d]There was a significant interaction including dry bean class for the 10% rate of dicamba at the V8 timing. Canopy closure was greater for black beans (76%) than navy beans (68%).

[e]There was a significant interaction including dry bean class for the 10% rate of dicamba at the V2 timing. Canopy closure was greater for black beans (51%) than navy beans (24%).

Table 2.4. Delay in dry bean maturity at Richville and East Lansing, MI in 2017 and 2018 when V2 and V8 dry beans were exposed to dicamba and 2,4-D.[a,b]

| Herbicide | Rate | | Richville | | East Lansing |
|---|---|---|---|---|---|
| | | | V2 | V8 | |
| | g ae $ha^{-1}$ | (%) | days | | days |
| Dicamba | 0.56 | 0.1 | 0 a[c] | 0 a | 2 a |
| | 5.6 | 1.0 | 2 a | 10 c | 8 b |
| | 56 | 10 | 8 bc | 9 c | 16 c |
| 2,4-D | 1.12 | 0.1 | 0 a | 0 a | 0 a |
| | 11.2 | 1.0 | 0 a | 1 a | 1 a |
| | 112 | 10 | 2 a | 6 b | 9 b |
| None | - | - | 0 a | 0 a | 0 a |

[a]Data was combined over dry bean class and application timings when interactions were not significant.

[b]Values are estimates of days delayed in comparison to the untreated control to 50% maturity ($MR_{50}$), generated by a three-parameter log-logistic model.

[c]Means followed by the same letter within location are not significantly different $\alpha \leq 0.05$.

Table 2.5. Main effect of herbicide treatment on dry bean yield, seed weight and black bean germination after harvest when exposed to multiple rates of dicamba and 2,4-D at V2 and V8 growth stages in Richville and East Lansing, MI.

| Herbicide | Rate | | Yield[b] 2017 | Yield[b] 2018 | Seed weight[c] | Germination 2017 | Germination 2018 |
|---|---|---|---|---|---|---|---|
| | g ae ha$^{-1}$ | (%)[a] | — kg ha$^{-1}$ — | — kg ha$^{-1}$ — | g 100 seeds$^{-1}$ | (%) | (%) |
| Dicamba | 0.56 | 0.1 | 2350 a | 3604 ab | 21.4 a | 86 a | 98 a |
| | 5.6 | 1.0 | 2168 a | 3342 b | 20.7 a | 89 a | 96 a |
| | 56 | 10 | 1714 b | 2370 c | 19.4 b | 82 a | 93 a |
| 2,4-D | 1.12 | 0.1 | 2297 a | 3575 ab | 21.0 a | 88 a | 96 a |
| | 11.2 | 1.0 | 2234 a | 3689 a | 21.2 a | 88 a | 95 a |
| | 112 | 10 | 2256 a | 3439 ab | 20.8 a | 85 a | 93 a |
| None | 0 | - | 2325 a | 3562 ab | 21.4 a | 86 a | 96 a |

[a]Rates based off standard rates of 0.56 and 1.1 kg ha$^{-1}$ for dicamba and 2,4-D, respectively.

[a]Means within the same column followed by the same letter are not significantly different $\alpha \leq 0.05$.

[b]Data were combined over locations and dry bean class.

[c]Data were combined over dry bean class, location and years.

Table 2.6. Estimates of dicamba and 2,4-D doses needed to reduced dry bean yield 5, 10 and 15%.[a]

| Herbicide | 2017 | | | 2018 | | |
|---|---|---|---|---|---|---|
| | $YR_5$[b] | $YR_{10}$ | $YR_{15}$ | $YR_5$ | $YR_{10}$ | $YR_{15}$ |
| | — %[c] — | — % — | — % — | — % — | — % — | — % — |
| Dicamba | 0.6 a[d] | 1.8 a | 3.6 a | 0.75 a | 1.7 a | 2.8 a |
| 2,4-D | >10 b | >10 b | >10 b | >10 b | >10 b | >10 b |

[a]Data was combined over locations, dry bean classes, and application timings.

[b]$YR_5$ = 5% yield reduction; $YR_{10}$ = 10% yield reduction; $YR_{15}$ = 15% yield reduction

[c]Estimates are presented as a % of the field use rate of dicamba at 560 g ae $ha^{-1}$ and 2,4-D chloine at 1120 g ae $ha^{-1}$.

[d]Means within the same column followed by the same letter are not significantly different $\alpha \leq 0.05$)

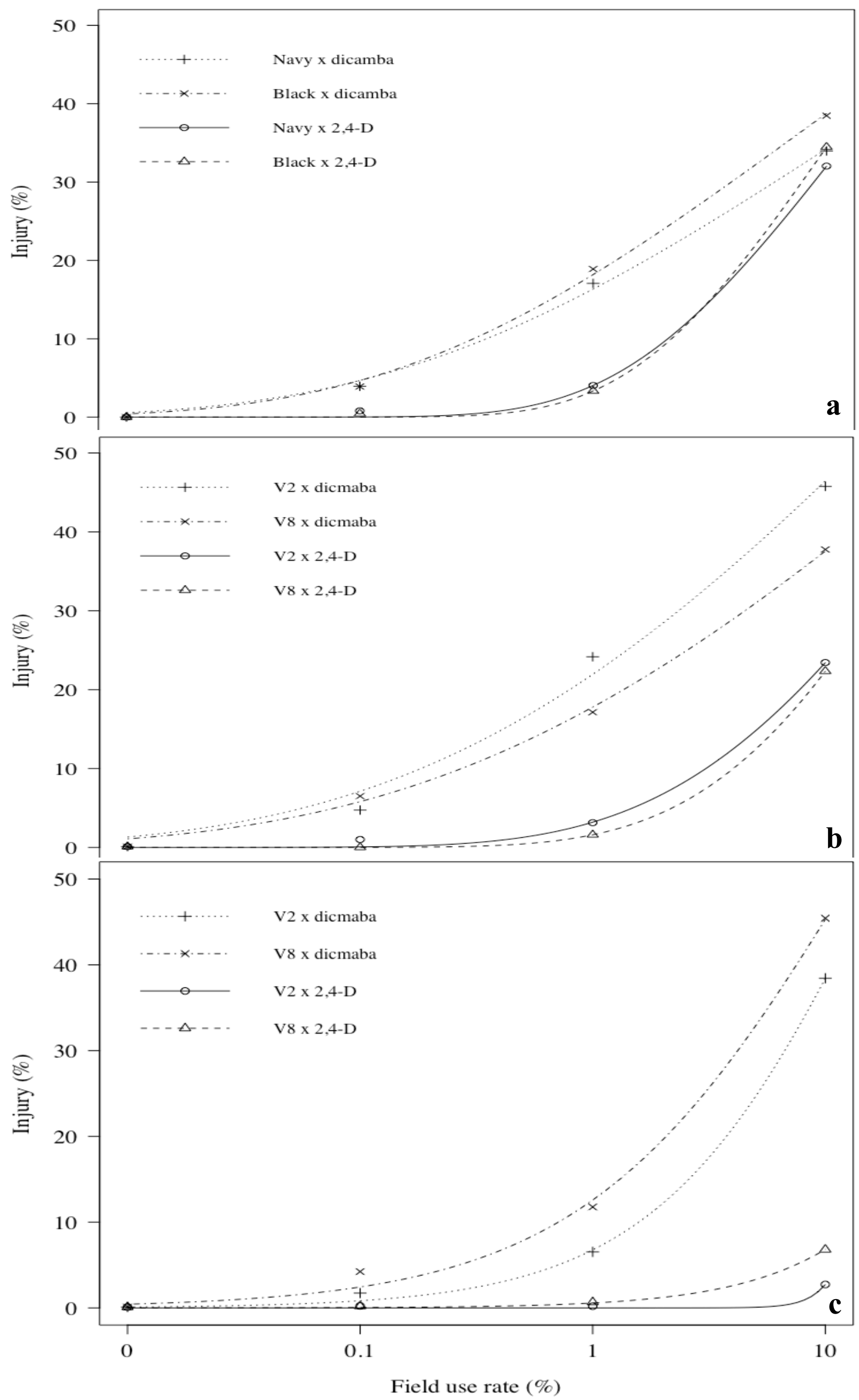


Figure 2.1. Herbicide injury to dry bean at 7 (a) 14 (b) and 28 DAT (c) from multiple rates of dicamba and 2,4-D applied at V2 and V8 in Richville and East Lansing, MI. Data was combined over locations and presented by the significant interaction between main effects. Interactions include herbicide treatment by dry bean class (a) (P=0.01) and herbicide treatment by application timing (b) (c) (P<0.0001)

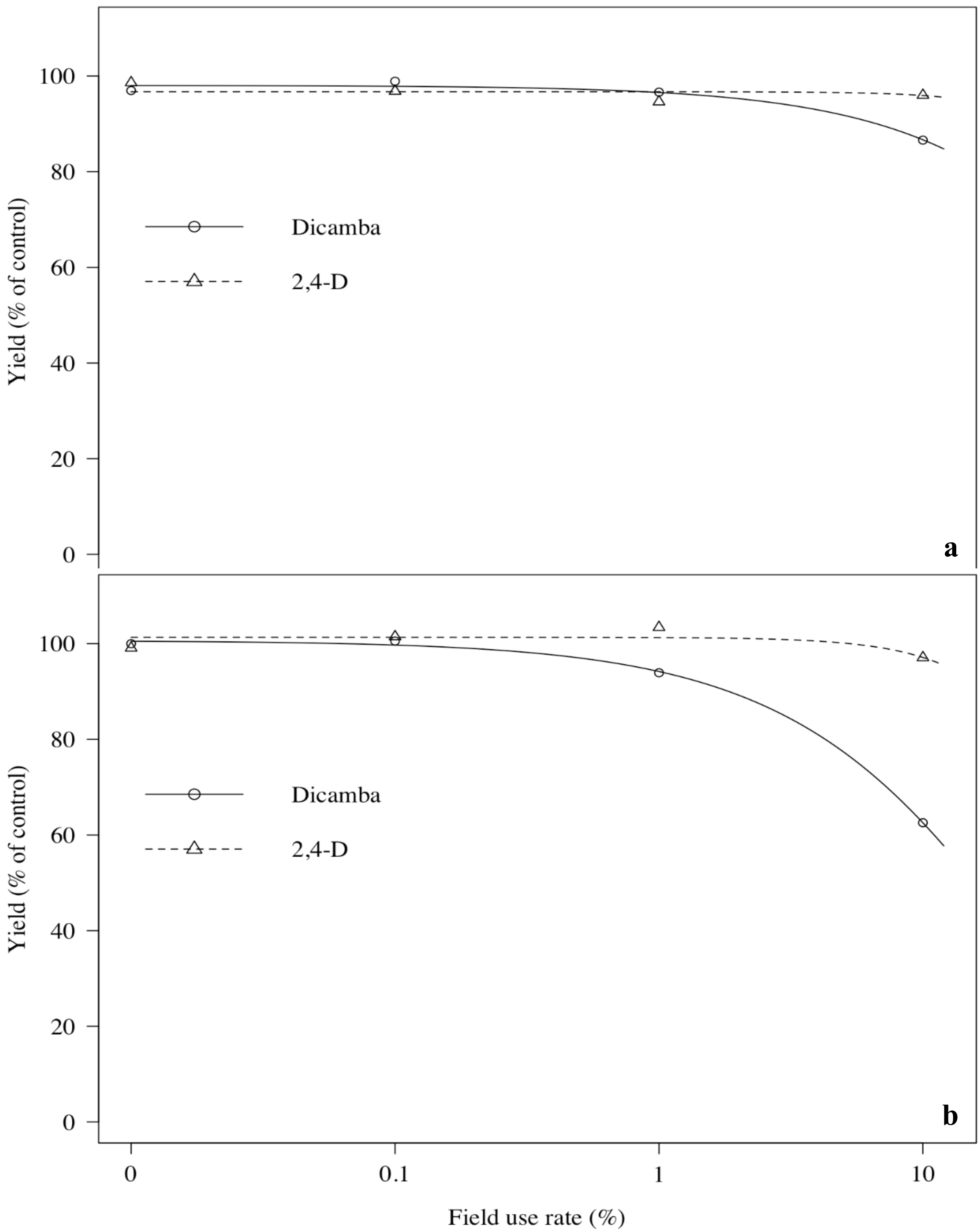


Figure 2.2. Dry bean yield as a percentage of the untreated when exposed to multiple rates of dicamba and 2,4-D at V2 and V8 growth stage in 2017 (a) and 2018 (b). Data were combined over dry bean class and Richville and East Lansing, MI location.

APPENDIX B

Supplemental figures

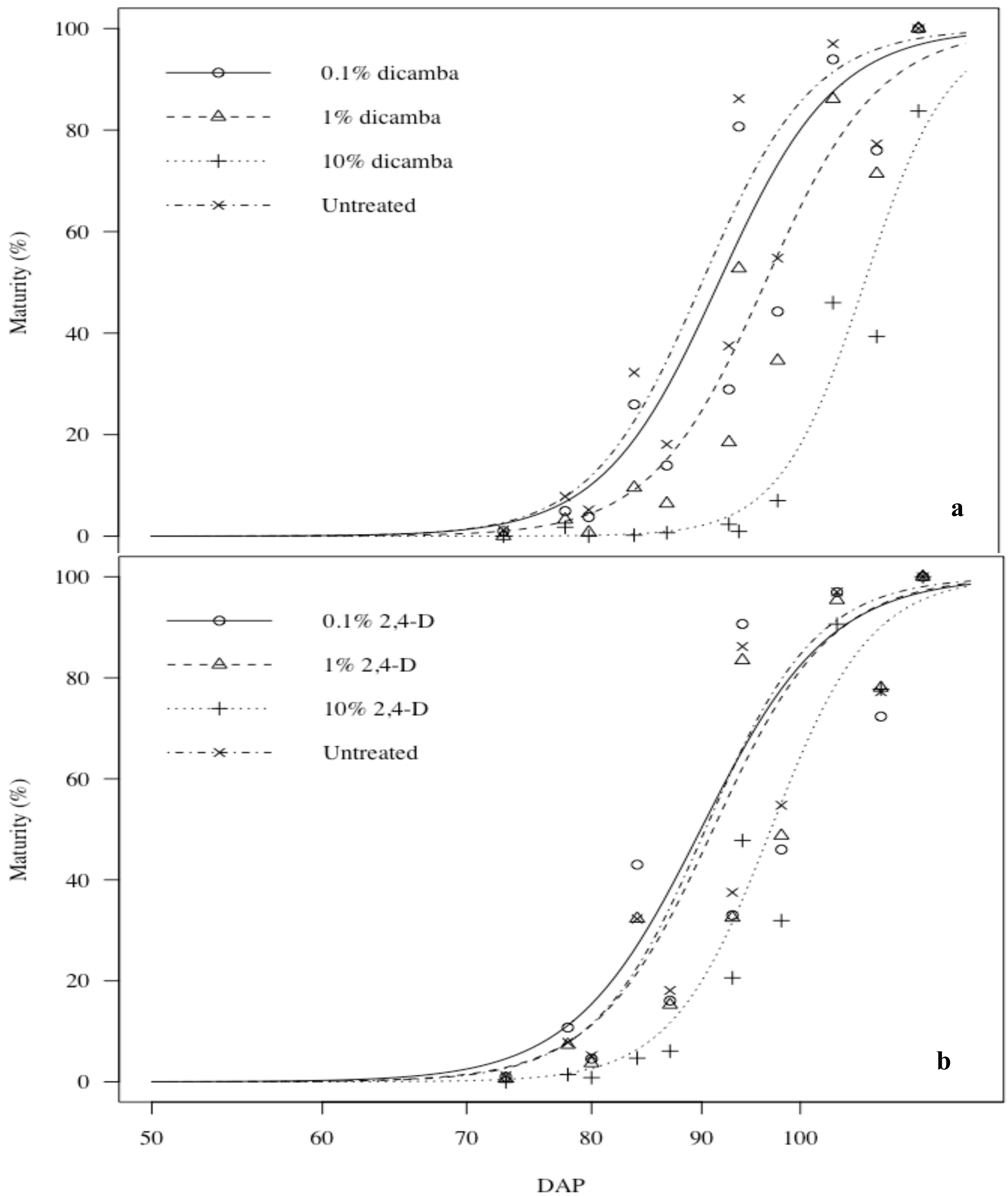


Figure 2.3. Dry bean maturity for signifigant main effects in East Lansing from applications of multiple rates of dicamba (a) and 2,4-D (b) when data were combined over dry bean class, application timing and years.

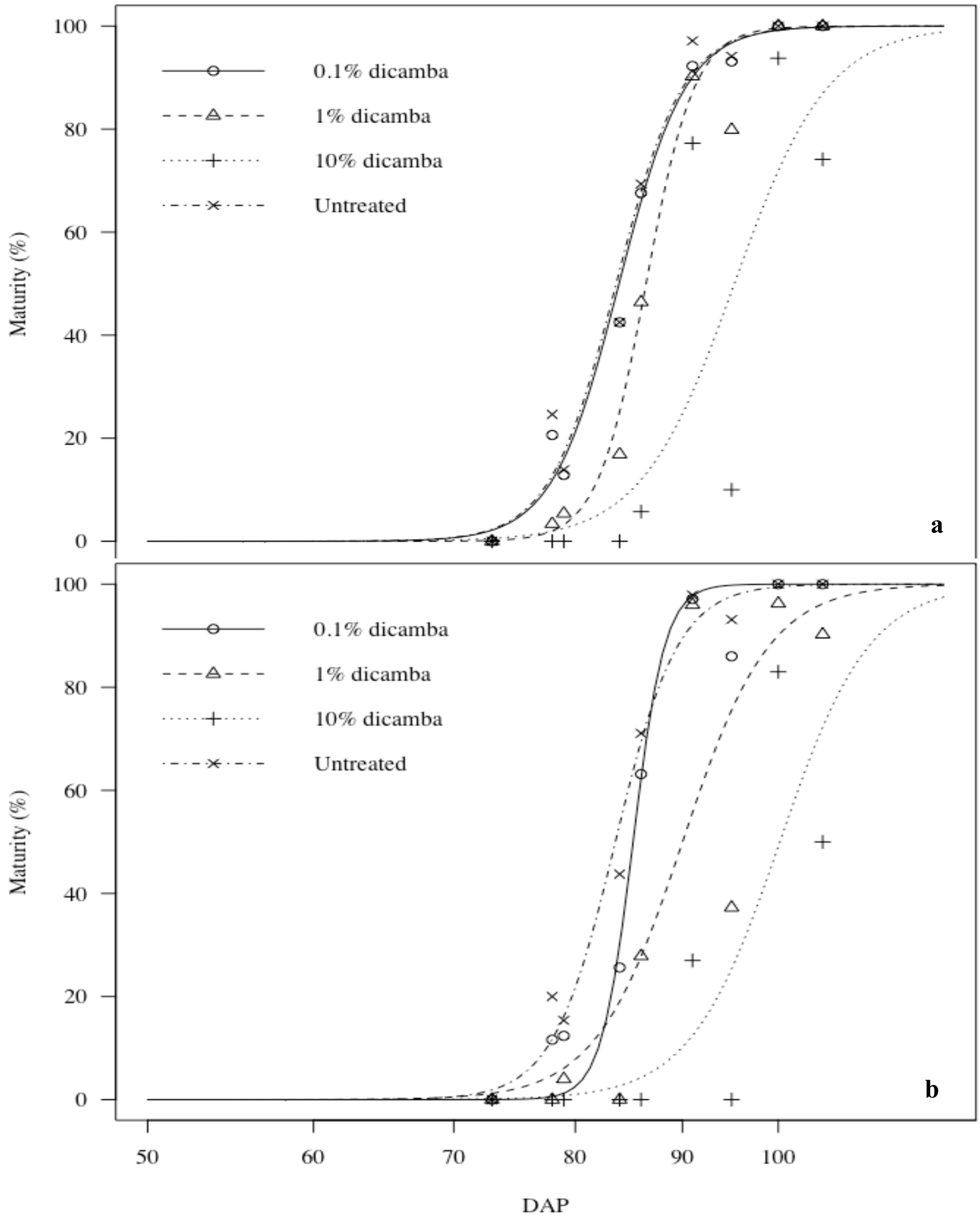


Figure 2.4. Dry bean maturity for the interaction between herbicide treatment and application timing in Richville from applications of multiple rates of dicamba at V2 (a) and V8 (b) when data were combined over dry bean class and years.

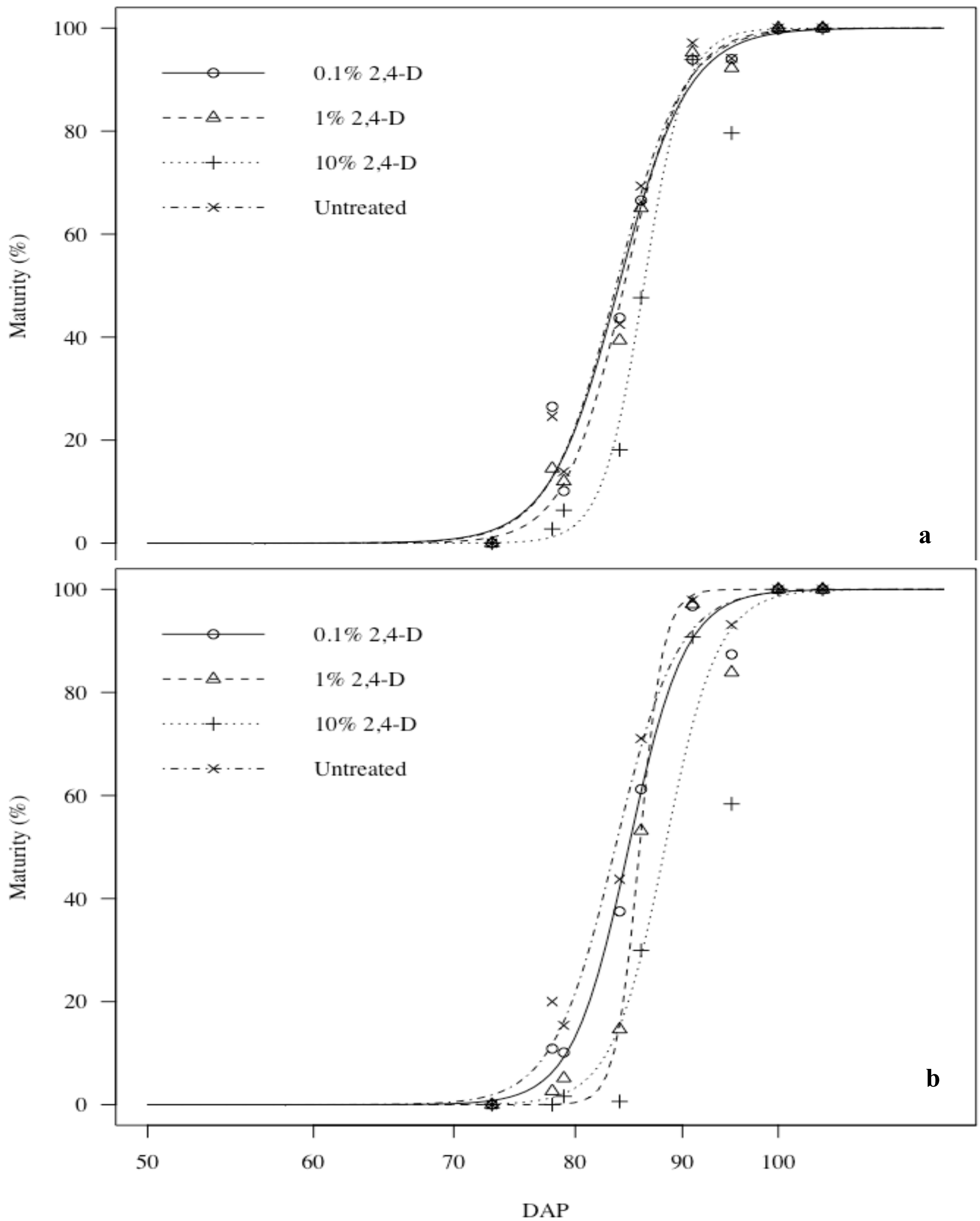


Figure 2.5. Dry bean maturity for the interaction between herbicide treatment and application timing in Richville from applications of multiple rates of 2,4-D at V2 (a) and V8 (b) when data were combined over dry bean class and years

## APPENDIX C
## Greenhouse Experiments

'Zenith' black bean seed was planted 3.8 cm deep at one seed per 10 by 10 cm pot filled with potting media (Suremic™ Perlite, Michigan Gower Products, Galesburg, MI). When dry bean plants were at the V2 stage, between 12-18 cm tall, herbicide applications were made. Dicamba and the choline salt of 2,4-D were each applied at eight different rates: 0, 0.01, 0.5, 1, 5, 10, 50, 100% of the labeled use rates for POST applications in dicamba- and 2,4-D-resistant soybean, respectively. Dicamba rates were 0, 0.56, 2.8, 5.6, 28, 56, 280, and 560 g ae $ha^{-1}$ and rates of the choline salt of 2,4-D were 0, 1.4, 7, 14, 71, 142, 712, and 1425 g ae $ha^{-1}$. Dry bean plants were evaluated for injury 3, 7 and 14 DAT. At 14 DAT aboveground biomass was also harvested, dried for 5 d, and weighed. The experimental design was a completely randomized design with 5 replications and repeated in time.

Injury and biomass for greenhouse experiments were analyzed in the drc package in R. Curves were fitted to the data using a three-parameter log-logistic model estimating the effective herbicide dose for 50% plant injury and 50% reductions ($ED_{50}$) in plant biomass in compairsion to the untreated control. $ED_{50}$ values were compared using paired t-test at $p \leq 0.05$.

In the greenhouse, both dicamba and 2,4-D caused plant injury and reduced plant biomass. When dry bean injury was evaluated at 3 and 7 DAT, greater injury was documented from 2,4-D than dicamba (Figure 2.6). However, when compairing dry bean injury between dicamba and 2,4-D, 14 DAT, differences no longer existed (Figure 2.6). Dry bean biomass at 14 DAT was reduced by up to 60 and 75% from 100% rates of dicamba and 2,4-D, respectively. However, effective doses to reduce biomass by 50% were not different between dicamba and 2,4-D (Table 2.7; Figure 2.6). Findings from greenhouse experiments indicate that dry bean respond similarly to dicamba and 2,4-D, contrasting findings in the field experiments. However, if evaluations were continued beyond 14 DAT it is expected that results may differ due to the slow speed of herbicide activity of both dicamba and 2,4-D.

Table 2.7. The effective dose to cause 50% injury to dry beans and reduce biomass by 50% in dry bean exposed to dicamba or 2,4-D at the V2 growth stage in the greenhouse. Data were combined over replications and time.

| Herbicide | Injury | | | Biomass |
|---|---|---|---|---|
| | 3 DAT | 7 DAT | 14 DAT | |
| | —%— | —%— | —%— | —%— |
| Dicamba | 60.9 b[a] | 78.5 b | 12.7 a | 21.3 a |
| 2,4-D | 11.9 a | 8.9 a | 8.0 a | 10.1 a |

[a]Means within the same column followed by the same letter are not significantly different $\alpha \leq 0.05$.

[b]Percentages are based on the 1X field use rate of 560 and 1120 g ae $ha^{-1}$ for dicamba and 2,4-D, respectively.

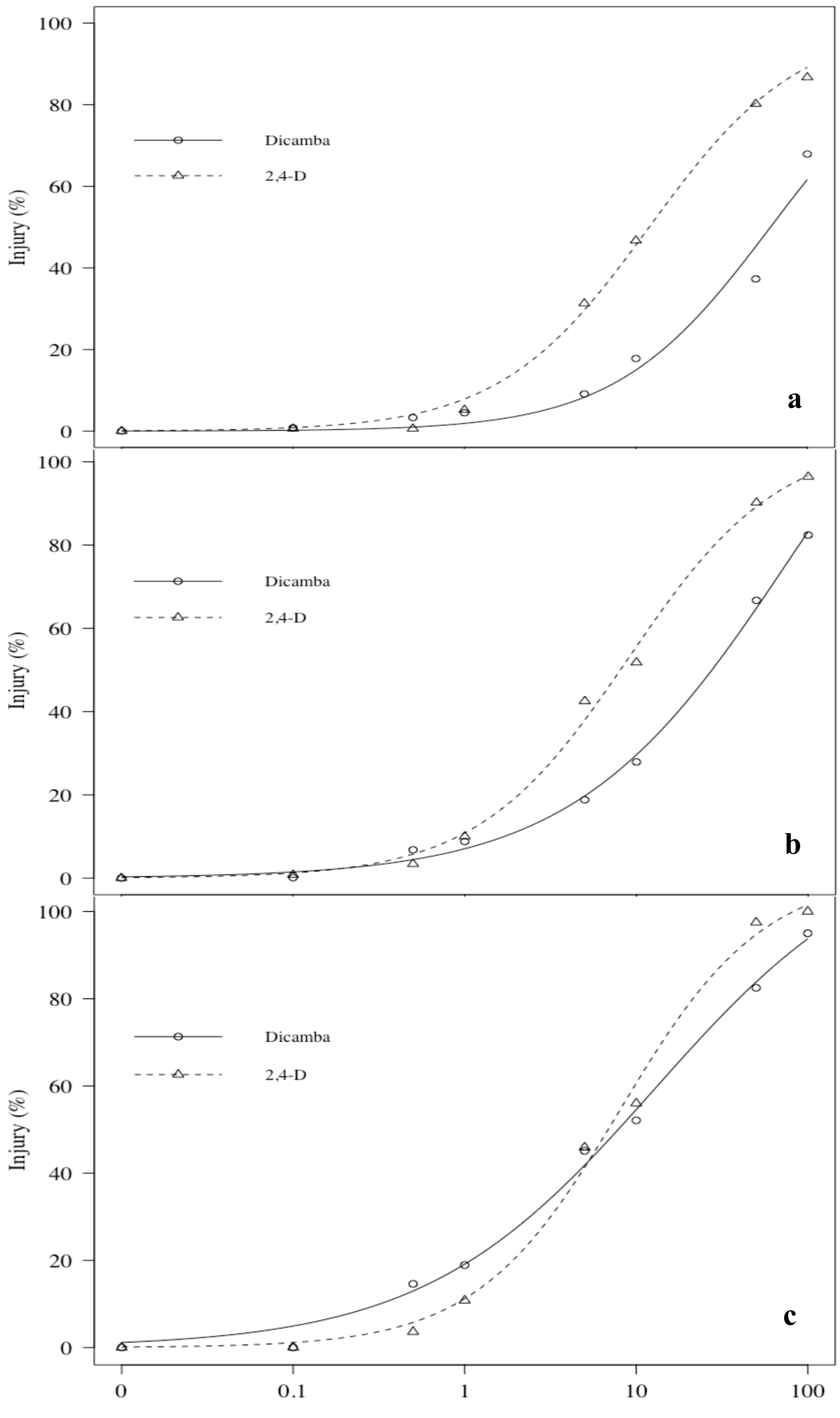


Figure 2.6. Dry bean injury at 3 (a), 7 (b), and 14 (c) days after treatment (DAT) in a greenhouse from V2 applications of dicamba and 2,4-D at 0-100% of the field use rates of 560 and 1120 g ae ha$^{-1}$, respectively. Data were combined over replications and time.

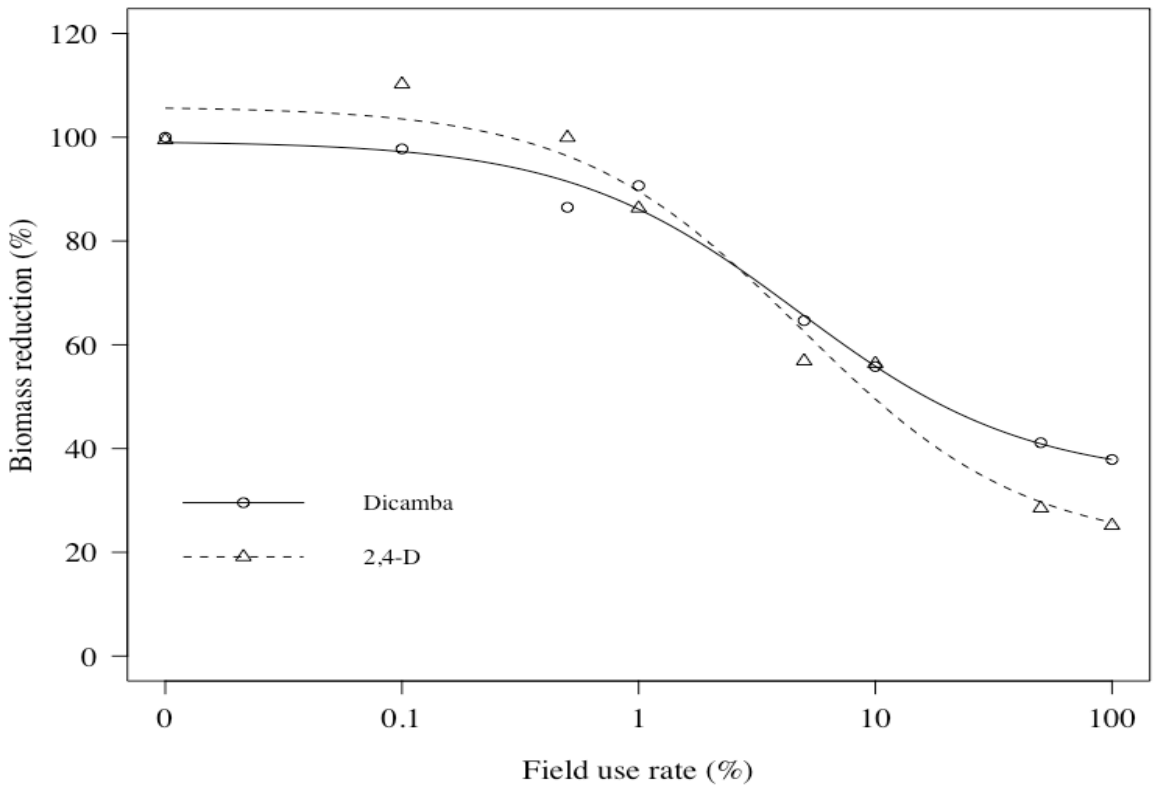


Figure 2.7. Dry bean biomass as a percentage of the untreated control harvested 14 days after V2 applications of dicamba and 2,4-D at 0-100% of the field use rates of 560 and 1120 g ae $ha^{-1}$, respectively. Data were combined over replications and time.

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## LITERATURE CITED


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# CHAPTER III

# TANK-CONTAMINATION OF DICAMBA AND 2,4-D INFLUENCES DRY EDIBLE BEANS


## Abstract

The occurrence of tank-contaminations of dicamba or 2,4-D will likely increase with the recent commercialization of dicamba- and 2,4-D-resistant soybeans. High valued sensitive crops, including dry beans will be at higher risks of exposure. In 2017 and 2018, two separate field experiments were conducted in Michigan to understand how multiple factors may influence dry beans response to dicamba and 2,4-D herbicides, including: (1) the interaction between POST dry bean herbicides and dicamba or 2,4-D and (2) the impact of low rates of glyphosate with dicamba or 2,4-D. Dry bean injury of 20 and 2% was recorded 14 DAT for dicamba (5.6 h ae $ha^{-1}$) and 2,4-D (11.2 g ae $ha^{-1}$) applied alone, respectively. The addition of glyphosate (8.4 g ae $ha^{-1}$) did not increase dry bean injury from dicamba or 2,4-D. Over two-site years the addition of dry bean herbicides to dicamba or dicamba plus glyphosate (8.4 g ae $ha^{-1}$) increased dry bean injury and reduced yield 6-10% more than when dicamba or dicamba plus glyphosate was applied alone. Tank-contamination of 2,4-D (11.2 g ae $ha^{-1}$) had little effect of dry beans with or without dry bean herbicide. Through this work it was documented that synergy occurs between dicamba and dicamba plus glyphosate and both common dry bean herbicide programs tested, imazamox (35 g $ha^{-1}$) + bentazon (560 g $ha^{-1}$) and fomesafen (280 g $ha^{-1}$). The synergy between dry bean herbicide and dicamba and dicamba plus glyphosate can increase plant injury, delay maturity and reduce yield to a greater extent than dicamba or dicamba plus glyphosate alone. This work emphasizes the need for proper sprayer clean out after the applications of dicamba to reduce the risk of exposure to other crops.

## Introduction

Dry bean (*Phaseolus vulgaris* L.) is an economically important crop for Michigan farmers. On average, Michigan harvests 106,000 ha of dry beans per year that equates to a farm-gate value of $140 million (USDA-NASS 2018). Ranking second in total production, Michigan plants 8 of the 13 different dry bean classes grown in the United States (NASS-USDA 2018). Black and navy beans are the top two classes of dry beans grown in Michigan with 48,500 and 29,900 ha harvested in 2017, respectively (USDA-NASS 2018). This unique high value crop is typically planted in June and is harvested in 85 to 100 days (Kelly and Cichy 2013). Soybean (*Glycine max* L.), corn (*Zea mays* L.), wheat (*Triticum aestivum* L.) and sugarbeet (*Beta vulgaris* L.) are other crops in Michigan that are grown adjacent to, or in rotation with dry beans (Christenson et al. 2000).

Herbicide resistance in multiple weed species has become a management challenge for farmers in Michigan and throughout the United States. The increased occurrence of glyphosate resistance and weeds resistant to multiple herbicide sites of action have limited options for control in several different crops (Heap et al. 2017). To help combat this issue, two new soybean technologies have been developed that are resistant to dicamba or 2,4-D (Behrens et al. 2007, Wright et al. 2010). Historically, these herbicides have been used for selective control of broadleaf weeds in corn, small grains, and pastures (Peterson et al. 2016, Shaner 2014). These new technologies provide alternative options for control of glyphosate and multiple resistant weeds in soybean (Byker et al. 2013; Craigmyle et al. 2013; Kruger et al. 2010; Norsworthy et al. 2008; Spaunhorst et al. 2014).

Michigan producers have diverse crop rotations and often use application equipment in multiple crops each season. Issues with herbicide tank-contamination can be high especially with dicamba and 2,4-D, since many broadleaf crops grown in Michigan are sensitive to these herbicides, including dry bean and sugarbeet (Hatterman-Valenti et al. 2017; Lyon and Wilson 1986; Probst 2018). If application equipment is traveling across multiple crops in a season tank-contamination is a risk. However, applying dicamba and 2,4-D elevates this risk because research has documented that even after recommended clean out procedures dicamba and 2,4-D residues were not completely removed from the tank (Boerboom 2004; Osborn et al 2015). This can lead to the unintentional application of the remaining herbicide residues in the next spray application at rates high enough to cause sensitive crop injury (Boerboom 2004). Spray equipment can be difficult to clean completely as there are many components that require attention including: tanks, booms, and inductors. Even after a complete spray system cleanout with an ammonia-water tank cleaning solution, dicamba levels of 0.63% of an initial concentration of 560 g ae $ha^{-1}$ were detected in rinse water (Boerboom 2004). Similarly, when commercial applicator tanks were rinsed with water three times, average concentrations of 0.16% dicamba and 1% 2,4-D were found in the third rinse (Osborn et al. 2015). While these studies reported residual levels of dicamba and 2,4-D it is also possible that residual levels of glyphosate would remain in the spray system, since glyphosate is often applied with both of these herbicides.

Sensitive crop response to these rates has been previously researched, however little is known on the interaction of other herbicides with dicamba and 2,4-D in the event of tank-contamination. Previous research examining tank-contamination levels of dicamba applied with imidazolinone or diphenylether herbicides reported that synergistic responses on soybean are possible (Brown et al. 2009; Kelley et al. 2005). As a result the synergist effect increased plant injury, reduced soybean yield, and altered soybean maturity in comparison to dicamba applied alone. This is of concern for Michigan dry bean producers as both imidazolinone and diphenylether herbicides are commonly used for weed control in dry beans (Sprague and Burns 2018). When these herbicides are used in dry beans they are applied with additional adjuvants, including a crop oil concentrate (COC) and in some cases ammonium sulfate (AMS). Research in soybean also has shown that the addition of a COC to tank-contamination rates of dicamba can increase plant injury and reduce yield (Brown et al. 2009).

With the recent commercialization of soybean varieties resistant to dicamba and 2,4-D, we expect an increased use of these herbicides in Michigan. This change will put sensitive crops in Michigan at an elevated risk of exposure by tank-contamination. Previous research has documented the negative effects that dicamba and 2,4-D can have on dry bean yield and quality (Lyon and Wilson 1986; Hatterman-Valenti et al. 2017). However, information is not known about the interaction between postemergence dry bean herbicides and tank-contamination levels of dicamba or 2,4-D and if the addition of tank-contamination levels of glyphosate influence these results. As we build our knowledge base on the response of dry beans to dicamba and 2,4-D, we have several research questions that remain to be answered with confidence on modern dry bean cultivars and market classes that are economically important to Michigan. Therefore, the objectives of this research were to understand how multiple factors may influence dry beans response to tank-contamination levels of dicamba and 2,4-D herbicides, including: (1) the interaction between postemergence dry bean herbicides and dicamba or 2,4-D, and (2) the impact that the addition of tank-contamination levels of glyphosate has in combination with dicamba or 2,4-D.

## Materials and methods

Field experiments were conducted in 2017 and 2018 at the Michigan State University (MSU) Agronomy Farm in East Lansing, Michigan (42.71 N, -84.47 W) and the MSU Saginaw Valley Research and Extension Center near Richville, Michigan (43.399 N, -83.697 W). The soil types at East Lansing were a Colwood-Brookston loam (fine-loamy, mixed, mesic typic haplaquolls) with pH 7.0 and 2.7% organic matter in 2017, and a pH 6.0 and 3.4% organic matter in 2018. Soils at the Richville location were a Tappan-Londo clay loam (fine-loamy, mixed, active, calcareous, mesic typic epiaquolls) with pH 7.0 and 2.7% organic matter in 2017, and a pH 7.7 and 2.5% organic matter in 2018. Dry beans were planted into conventionally tilled soils. Soil preparation consisted of either fall chisel or moldboard plowing followed by two passes of a soil finisher in the spring prior to planting. Fertilizer applications were standard for dry bean production in Michigan. In East Lansing, 19-19-19 (N-P-K) at 112 kg $ha^{-1}$ was applied with the planter 5 cm down and 5 cm over from the seed. At Richville, 336 kg $ha^{-1}$ of 17-8-15 (N-P-K) fertilizer containing 1.5% manganese and 1.5% zinc was broadcast applied prior to spring tillage.

'Zenith' black beans (Michigan Crop Improvement Association, Okemos, MI), a Type II (upright indeterminate vine) variety, were planted in 76 cm rows at 269,000 seeds $ha^{-1}$ at all locations. Dry beans were planted on June 6, 2017 and June 5, 2018 in East Lansing, and on June 8, 2017 and June 19, 2018 in Richville. Plots were four rows wide by 9.1 m long.

Separate field studies were established for the dicamba and 2,4-D tank-contamination experiments. Dicamba tank-contamination experiments were conducted for 3 site-years (individual locations and years), Richville 2017, Richville 2018, and East Lansing 2018. The 2,4-D tank-contamination experiment was conducted for two site-years, East Lansing 2017 and East Lansing 2018. The diglycolamine salt of dicamba (XtendiMax ® Bayer Crop Science, Research

Triangle Park, NC) and the choline salt of 2,4-D (Enlist One ® Dow AgroSciences LLC, Indianapolis, IN) were applied at 1% rate of their standard use rates of 560 and 1120 g ae $ha^{-1}$ labeled in dicamba and 2,4-D resistant soybean, respectively. These rates were 5.6 and 11.2 g ae $ha^{-1}$ for dicamba and 2,4-D, respectively. Dicamba and 2,4-D were applied alone, and in combination with a 1% field use rate of glyphosate (8.4 g ae $ha^{-1}$) (Roundup PowerMax ® Bayer Crop Science, Research Triangle Park, NC). Dicamba, dicamba + glyphosate, 2,4-D, and 2,4-D + glyphosate were tank-mixed with two commonly used postemergence herbicide treatments in dry beans; 1) imazamox at 35 g ai $ha^{-1}$ (Raptor ® BASF, Research Triangle Park, NC) + bentazon at 560 g ai $ha^{-1}$ (Basagran ® WinField United, St. Paul, MN) + crop oil concentrate (COC) at 1% v $v^{-1}$ + ammonium sulfate at 2.5% w $w^{-1}$, and 2) fomesafen at 280 g ai $ha^{-1}$ (Reflex ®, Syngenta Crop Protection, Greensboro NC) + COC at 1% v $v^{-1}$. Herbicide treatments were applied at two different stages of growth, V2 (two-trifoliate) and V8 (pre-bloom) dry bean. Herbicides were applied using a tractor-mounted, compressed air sprayer calibrated to deliver 177 L $ha^{-1}$ at 206 kPa using AIXR 11003 nozzles (TeeJet Technologies, Wheaton, IL).

Experiments were arranged in a split-plot design with three (East Lansing) or four replications (Richville). The main plot factor was application timing and the subplot factors were arranged in a two-factor randomized complete block design. Factor A was dry bean herbicide treatment and Factor B was tank-contaminant. All field experiments were maintained weed-free throughout the season with between-row mechanical cultivation supplemented by hand weeding.

Dry bean injury was evaluated 14 and 28 days after treatment (DAT) on a scale from 0-100%, with 0% equivalent to no plant response and 100% representing complete plant death. Dry bean maturity was evaluated weekly for 6 weeks prior to harvest. A 0-100% scale was used, with a 0 indicating all green tissue and 100 indicating complete plant maturity (no green tissue).

Dry bean plots were mechanically harvested after natural plant senescence. Plots were direct harvested using a small-plot research combine (Massey-Ferguson 8XP, AGCO, Duluth, GA) with a 1.5 m header. Excess rainfall near harvest at East Lansing in 2018 lead to dry bean regrowth requiring the use of a chemical desiccant prior to harvest. Dry beans were desiccated with saflufenacil (Sharpen® BASF Corporation, Research Triangle Park, NC) at 50 g $ha^{-1}$ plus 1% v $v^{-1}$ methylated seed oil 14 d prior to harvest. Dry bean yield was adjusted to 18% moisture. Samples of harvested dry bean seed were taken from each plot during harvest. Seed samples were sorted for mechanical damage and weight per 100 seeds was recorded on undamaged seeds.

**Statistical analysis.** Data analysis was conducted using PROC MIXED procedure in SAS ® 9.4 (SAS institute, Cary, NC). The statistical model consisted of dry bean herbicide treatment, tank-contaminants, application timing and their interaction as fixed effects. Site-year (individual year and location), replication nested within site-year, and the interaction between application timing and replication nested within site-year as random effects. When analyzing dry bean maturity and yield application timing was removed from the model as analysis was conducted separately for each application timing. Replications were used as an error term for testing the effects of site-year, when an interaction of site-year by one of the fixed effects was significant, data was analyzed separately. Normality assumptions were checked by examining histogram and normal probability plots of the residuals. Unequal variance assumption was assessed by visual inspection of the side-by-side box plot of the residuals followed by the Levene's test for unequal variances.

Further analysis of dry bean maturity was conducted using nonlinear regression with the drc package in R (R, Version 3.0) (Knezevic et al. 2007). Model fit was confirmed using the ModelFit function in R. Nonlinear regression was also used to predict the number of days from planting to 50% dry bean maturity ($MR_{50}$). The non-linear model used was the three-parameter log-logistic model [1]

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

In the log-logistic model for $MR_{50}$ values *y* represented dry bean maturity, *x* represents days after planting, *c* is the lower limit, *b* is the slope of the line around *e* and *e* is the $MR_{50}$. $MR_{50}$ values were analyzed using the PROC MIXED procedure as previously described separately for each site-year and application timing.

Joint activity between dicamba, 2,4-D, or the combinations of glyphosate with dicamba or 2,4-D and the dry bean herbicide treatments was evaluated using the method described by Gowing (1960) [2], where E is the expected injury value of the herbicide combination, A is the observed percent injury from the dry bean herbicide alone, and B is the observed injury from the tank-contaminants alone. The expected values and observed values for each herbicide combination were compared using paired t-test in PROC MIXED. If the observed value is significantly greater than the expected, the interaction would be synergistic ($\alpha \leq 0.05$). If there was no difference between the observed value and the expected the interaction would be additive, and if the observed value was less than the expected the combination would be antagonistic.

$$E = A + \left(\frac{B(100-A)}{100}\right) \qquad [2]$$

## Results and discussion

**Dicamba tank-contamination.** Dry bean injury symptoms from dicamba consisted of leaf crinkling and cupping and were consistent among site-years. Injury symptoms were consistent with those described by Lyon and Wilson (1986). At peak injury, dicamba at the tank-contamination rate of 1% (5.6 g ae $ha^{-1}$) caused 20% injury (Table 3.1). By 28 DAT, dry beans had started to recover from dicamba injury was only 8%. The addition of glyphosate at 1% (8.4 g ae $ha^{-1}$) to dicamba did not affect dry bean injury. Hatterman-Valenti et al. (2017) also reported that the addition of glyphosate to dicamba at tank-contamination rates did not increase injury compared with dicamba alone on pinto and navy beans.

Dry bean injury was low when imazamox (35 g ai $ha^{-1}$) plus bentazon (560 g ai $ha^{-1}$) was applied with the labeled adjuvants of COC (1% v $v^{-1}$) plus AMS (2.5% w $w^{-1}$). Marginal leaf burning and crinkling was evident as soon as 1 DAT; however this injury was transient and was not evident by 14 DAT (Table 3.1). Similarly, injury from applications of fomesafen (280 g $ha^{-1}$) plus COC (1% v $v^{-1}$) was also low. Fomesafen caused less than 5% plant injury 7 DAT (data not shown), and symptoms consisted of foliar leaf spotting, bronzing, and crinkling. These symptoms were consistent with fomesafen injury on dry beans described by Wilson (2005). Injury was transient and was not apparent by 14 DAT.

The combination of either of the dry bean herbicide treatments, imazamox plus bentazon or fomesafen, with the dicamba contaminant resulted in greater injury than the dicamba contaminant alone (Table 3.1). This increase in dry bean injury was still apparent 28 DAT, even after dry beans started to recover. These combinations also resulted in synergistic responses 14 DAT, when comparing expected with actual dry bean injury values. Dry bean injury was also greater when the dry bean herbicide treatments were contaminated with dicamba plus glyphosate (Table 3.1). Injury was 6% higher than dicamba plus glyphosate alone and 3% higher than the combinations of the dry bean herbicides and the dicamba contaminant 14 DAT. These combinations also resulted in synergistic responses when comparing expected injury with actual injury. By 28 DAT, dry bean injury was still higher when the dicamba plus glyphosate contaminant was applied with the dry bean herbicides. However, only the combination of imazamox plus bentazon with the dicamba plus glyphosate contaminant resulted in a synergistic response 28 DAT. Previously synergistic responses with respect to dicamba or dicamba plus glyphosate contaminants have not been reported with combinations of herbicides on dry beans. However, our work does support previous research interactions conducted in soybean. Kelley et al. (2005) reported that imazamox (44 g ai $ha^{-1}$), imazethapyr (71 g ai $ha^{-1}$), and fomesafen (330 g ai $ha^{-1}$) contaminated with dicamba (5.6 g ae $ha^{-1}$) had higher soybean injury compared with the dicamba contaminant alone. They found that there was a synergistic response with herbicides that needed to be metabolized to avoid injury were applied with dicamba. As where when dicamba was applied with glyphosate, no synergistic response was documented as glyphosate-resistant soybeans have target-site resistance. The addition of a crop oil concentrate to the dry bean herbicide treatments may have also contributed to the synergistic responses that we observed when dicamba or dicamba plus glyphosate were added. Brown et al. (2009) reported that soybean injury was greater from tank-contamination rates of dicamba when COC was added.

In addition to herbicide treatment differences, the main effect of application timing influenced dry bean injury 28 DAT, but not 14 DAT. At 28 DAT, combined over all herbicide treatments the V8 (16%) application timing resulted in more injury than V2 (8%) application timing ($P=0.001$). Differences in injury between the two application timings may be due to slower growth from V8 stage, as dry beans transition from vegetative to reproductive stages (Fageria and Santos 2008).

In general, the number of days delayed to 50% maturity ($MR_{50}$) was mostly affected by the main effects of tank contaminant and dry bean herbicide (Table 3.2). The one exception was at the V2 application timing at Richville 2017, when dicamba and dicamba plus glyphosate applied alone caused a 10 and 13 day delay to 50% maturity, respectively compared with the no herbicide control ($P<0.0001$). All other site-years and application timings were mostly affected by the dicamba and dicamba plus glyphosate tank-contaminants. The longest delays in maturity were from the V8 applications in Richville 2017, where the contaminants of dicamba and dicamba plus glyphosate delayed $MR_{50}$ by 27 days compared to the control (Table 3.2). At this site-year, the main effect of dry bean herbicide also impacted maturity. Dry bean herbicides delayed $MR_{50}$ by 19, 18, and 18 days for imazamox plus bentazon, fomesafen, and the no herbicide control, respectively, when combined over tank-contaminants (Table 3.2). Both locations in 2018 had shorter delays in maturity than Richville 2017. At both 2018 locations dry bean maturity was only affected by tank-contaminants and not by dry bean herbicide. At these locations dicamba, and dicamba plus glyphosate delayed dry bean maturity by 1 to 5 days compared with the no herbicide control (Table 3.2). The addition of glyphosate to dicamba did not delay maturity any longer than dicamba alone.

Other researchers have reported delays in maturity when dry beans have been exposed to dicamba (Lyon and Wilson 1986; Hatterman-Valenti et al. 2017). Hatterman-Valenti et al. (2017) reported delays in maturity that resulted in crop loss when pinto beans were exposed to dicamba (4.48 g ae $ha^{-1}$) or dicamba (4.48 g ae $ha^{-1}$) plus glyphosate (10 g ae $ha^{-1}$) at R1 stage of growth. They also reported that navy bean maturity was delayed when R1 beans were exposed to dicamba up to 44 g ae $ha^{-1}$. However, the addition of glyphosate to dicamba did not significantly affect dry beans response.

Dry bean yield was not affected by tank-contaminant or dry bean herbicide in 2017, even though there were significant delays in maturity (Tables 3.2 and 3.3). However, in 2018 when data was combined over the Richville and East Lansing locations both tank-contaminants and dry bean herbicides affected yield independently (Table 3.3). Tank-contamination of dicamba reduced yield up to 8 and 15% for V2 and V8 application timings, respectively (Table 3.3). The addition of glyphosate to dicamba did not reduce dry bean yield further. The effect of dry bean herbicide also affected dry bean yield in 2018. When pooled over dicamba tank-contaminants, both imazamox plus bentazon and fomesafen reduced yield compared to no dry bean herbicide. Differences between imazamox plus bentazon and fomesafen however did not exist. Yield reductions from the addition of dry bean herbicide to dicamba tank-contaminates reduced yield by up to 10% compared with no dry bean herbicide (Table 3.3).

These findings support research conducted by Kelley et al. (2005) in soybean. They found that dicamba at rates of 5.6 g ae $ha^{-1}$ did not always reduced soybean yield, but when combined with other herbicides such as imidazoline herbicides and fomesafen, yield losses were much more likely to occur. Additionally, we found that the addition of low rates of glyphosate to dicamba did not further increase yield loss, which is supported by Hatterman-Valenti et al. (2017) findings in that the addition of glyphosate to dicamba did not further affect pinto and navy bean yield.

Dry bean seed weight was affected by the main effect of tank-contaminant (Table 3.3). Combined over years and locations dicamba alone at V2 and V8 application timing and dicamba plus glyphosate at V8 reduced black bean seed weight. Seed weight was reduced 2.5% at the V2 timing and 3.8% at the V8 application timings. Seed weight was not affected by the main effect of dry bean herbicide. This data supports Kelley et al. (2005) who also found soybean seed weight could be reduced from dicamba applications of 5.6 g ae $ha^{-1}$.

**2,4-D tank-contamination**. Overall dry bean injury from tank-contaminations of 2,4-D at 11.2 g ae $ha^{-1}$ was extremely low (2% or less) (Table 3.4). Similar to dicamba, adding glyphosate at 8.4 g ae $ha^{-1}$ to 2,4-D did not increase injury when there was no dry bean herbicide present. However, when 2,4-D plus glyphosate was applied with either imazamox plus bentazon or fomesafen dry bean injury was 11 and 8%, respectively, 14 DAT. The higher injury from these treatments was still apparent 28 DAT, however dry bean injury was greatest when applied with imazamox plus bentazon. This treatment also showed a synergistic response when comparing expected with actual dry bean injury values, 14 and 28 DAT.

Dry bean maturity was only affected in one year of this experiment 2017 (Table 3.4). In most cases, delays to 50% maturity were 1 to 2 days when any herbicide was applied at the V2 stage. However, greater delays of 11 and 6 days were observed when the 2,4-D plus glyphosate contaminant was applied with either imazamox plus bentazon or fomesafen, respectively. These delays in maturity also occurred at the V8 application timing. At this timing delays were also greatest (12 days) when the 2,4-D plus glyphosate contaminant was applied with imazamox plus bentazon. Over the two application timings, this delay in maturity was 6 days longer than any other herbicide combination. This was the only interaction where a imazamox plus bentazon delayed maturity longer than fomesafen when applied with the same tank-contaminant. The longer delays caused by imazamox supports findings by Bauer et al. (1995) who reported delays in pinto bean maturity by imazethapyr, a herbicide that is in the same chemical family (imidazolinone) as imazamox.

Dry bean yield and seed weight were not affected by 2,4-D or 2,4-D plus glyphosate in either year of this experiment (Table 3.5). However, in 2017 an interaction occurred between the factors of contaminants and dry bean herbicides applied at V2. Results of this interaction showed that the application of imazamox plus bentazon with no contaminant had lower yields than any other treatment. Dry bean yield was reduced 17% with this treatment compared with the no herbicide control (data not shown). Others have reported that injury from imidazolinone herbicides can sometimes delay dry bean maturity and reduce yield (Bauer et al. 1995; Blackshaw et al. 1996, Soltani et al. 2017). While not statistical increase, yield results in 2017 and 2018 showed a slight numerical yield increase of 3-9% when 2,4-D at 11.2 g ae $ha^{-1}$ was added applied when compared to treatments without 2,4-D. While research on the possibility for hormesis in soybeans has provided little evidence of a positive effect from exposures to 2,4-D, future research could examine dry edible beans response (Egan et al. 2014). However, the possibility of delays in maturity from low rates of 2,4-D would likely negate any benefit from the herbicide.

Based on this research we have found that both dicamba and 2,4-D at tank-contamination rates can have a synergistic interactions when applied with postemergence dry bean herbicides. Dicamba tank-contamination was more severe than 2,4-D and the addition of glyphosate to dicamba had very little effect on dry beans with or without common postemergence dry bean herbicides. The tank-contamination rates of 2,4-D had very little effect on dry beans and would likely not be an issue for dry bean farmers. However, dicamba at the 1% rate caused high levels of injury and reduced yield. This is concerning for dry bean producers as the rates tested for dicamba were similar to possible residue levels found in commercial sprayers after cleanout (Boerboom 2004; Osborn et al. 2015). Producers should avoid using application equipment in dry beans directly following applications of dicamba to reduce the risk of this exposure.

APPENDICES

APPENDIX A
Tables

Table 3.1. Dry bean injury, 14 and 28 DAT, from dicamba and dicamba plus glyphosate contaminants alone and in combination with common postemergence dry bean herbicide programs. Data is combined over application timings, V2 and V8, and the three site-years of the experiment. Interactions between combinations were also tested for synergistic responses between expected and observed values.[a]

| | | | Injury | |
|---|---|---|---|---|
| Contaminant[b] | Herbicide program[c] | Rate | 14 DAT | 28 DAT |
| | | — $g\ ha^{-1}$ — | — % — | — % — |
| None | None | - | - | - |
| | Imazamox + bentazon | 35 + 560 | 0 d[d] | 0 d |
| | Fomesafen | 280 | 0 d | 0 d |
| Dicamba | None | 5.6 | 20 c | 8 c |
| | Imazamox + bentazon | 5.6 + 35 + 560 | 23 b (+) | 11 b |
| | Fomesafen | 280 + 5.6 | 23 b (+) | 11 b |
| Dicamba + glyphosate | None | 5.6 + 8.4 | 20 c | 10 bc |
| | Imazamox + bentazon | 5.6 + 8.4 + 35 + 560 | 26 a (+) | 15 a (+) |
| | Fomesafen | 5.6 + 8.4 + 280 | 26 a (+) | 14 a |

[a] Expected injury values were calculated using Gowing's equation (1960) and were compared with observed injury values using t-tests at $\alpha \leq 0.05$. The (+) symbol indicates a synergistic response from the combination.
[b] Dicamba and dicamba + glyphosate contaminants were applied at 1% the recommended use rates.
[c] A crop oil concentrate (COC) at 1% $v\ v^{-1}$ plus ammonium sulfate (AMS) at 2.5% $w\ w^{-1}$ was included with the imazamox plus bentazon tank-mixture and a COC at 1% $v\ v^{-1}$ was applied with fomesafen.
[d] Means followed by the same letter within a column are not significantly different ($\alpha \leq 0.05$).

Table 3.2. P-values and main effects for dry bean maturity, measured in days delayed to 50% ($MR_{50}$), from contaminants of dicamba and dicamba plus glyphosate contaminants alone and in combination with common postemergence dry bean herbicide programs. Data is presented separately by the V2 and V8 application timing for each of the three site-years of the experiment.

| | Richville | | | | | |
|---|---|---|---|---|---|---|
| | 2017 | | 2018 | | East Lansing | |
| | V2 | V8 | V2 | V8 | V2 | V8 |
| *Effects (p-values)* | | | | | | |
| Herbicide program | <0.0001 | 0.0008 | 0.4300 | 0.7800 | 0.9800 | 0.4200 |
| Contaminant | <0.0001 | <0.0001 | 0.0036 | 0.0003 | <0.0001 | 0.0024 |
| Herbicide program x contaminant | <0.0001[a] | 0.2100 | 0.5700 | 0.9400 | 0.9000 | 0.3200 |
| | d delayed | | | | | |
| *Contaminants (Main effect)* | | | | | | |
| None | 0 b[b] | 0 b | 0 b | 0 b | 0 b | 0 b |
| Dicamba | 13 a | 27 a | 1 a | 2 a | 4 a | 6 a |
| Dicamba + glyphosate | 15 a | 27 a | 2 a | 2 a | 5 a | 5 a |
| *Herbicide program (Main effect)*[c] | | | | | | |
| None | 7 c | 18 b | 1 a | 1 a | 3 a | 3 a |
| Imazamox + bentazon | 12 a | 19 a | 1 a | 1 a | 3 a | 4 a |
| Fomesafen | 9 b | 18 b | 1 a | 1 a | 3 a | 5 a |

[a] The was an interaction among the main effects of contaminant and dry bean herbicide program for the V2 application timing at Richville 2017. In general, the interaction followed the main effects, with one exception dry bean maturity was delayed 13 and 10 days from dicamba + glyphosate and dicamba with no herbicide program, respectively.

[b] Means followed by the same letter within a column for each main effect are not significantly different ($\alpha \leq 0.05$).

[c] A crop oil concentrate (COC) at 1% v $v^{-1}$ plus ammonium sulfate (AMS) at 2.5% w $w^{-1}$ was included with the imazamox plus bentazon tank-mixture and a COC at 1% v $v^{-1}$ was applied with fomesafen.

Table 3.3. P-values and main effects for dry bean yield and seed weight from contaminants of dicamba and dicamba plus glyphosate contaminants alone and in combination with common postemergence dry bean herbicide programs. Data is presented separately by the V2 and V8 application timings. Seed weight is combined over the three site-years of the experiment

| | Yield | | | | Seed weight | |
|---|---|---|---|---|---|---|
| | Richville 2017 | | Richville & E.L. 2018[a] | | | |
| | V2 | V8 | V2 | V8 | V2 | V8 |
| *Effects (p-values)* | | | | | | |
| Herbicide program | 0.08 | 0.27 | 0.001 | 0.0300 | 0.68 | 0.42 |
| Contaminant | 0.08 | 0.15 | 0.002 | <0.0001 | 0.04 | 0.01 |
| Herbicide program x contaminant | 0.80 | 0.32 | 0.380 | 0.5100 | 0.88 | 0.41 |
| | kg $ha^{-1}$ | | | | g 100 $seeds^{-1}$ | |
| *Contaminants (Main effect)* | | | | | | |
| None | 2519 a[b] | 2510 a | 4318 a | 4393 a | 22.25 a | 22.48 a |
| Dicamba | 2715 a | 2309 a | 3992 b | 3753 b | 21.69 b | 21.62 b |
| Dicamba + glyphosate | 2702 a | 2171 a | 3941 b | 3690 b | 21.86 ab | 21.62 b |
| *Herbicide program (Main effect)*[c] | | | | | | |
| None | 2658 a | 2319 a | 4318 a | 4142 a | 22.02 a | 21.99 a |
| Imazamox + bentazon | 2746 a | 2205 a | 3891 b | 3816 b | 21.83 a | 21.67 a |
| Fomesafen | 2532 a | 2476 a | 4054 b | 3879 b | 21.96 a | 22.07 a |

[a] Data is combined over the Richville and East Lansing 2018 locations.

[b] Means followed by the same letter within a column for each main effect are not significantly different ($\alpha \leq 0.05$).

[c] A crop oil concentrate (COC) at 1% v $v^{-1}$ plus ammonium sulfate (AMS) at 2.5% w $w^{-1}$ was included with the imazamox plus bentazon tank-mixture and a COC at 1% v $v^{-1}$ was applied with fomesafen.

Table 3.4. Dry bean injury, 14 and 28 DAT, and dry bean maturity, measured in days delayed to 50% ($MR_{50}$), from 2,4-D choline and 2,4-D choline plus glyphosate contaminants alone and in combination with common postemergence dry bean herbicide programs. Injury data is combined over application timings, V2 and V8, and the three site-years of the experiment. Interactions between combinations were also tested for synergistic responses between expected and observed values.[a] Maturity data is only from East Lansing 2017

| | | | Injury | | Maturity | |
|---|---|---|---|---|---|---|
| Contaminant[b] | Herbicide program[c] | Rate | 14 DAT | 28 DAT | V2 | V8 |
| | | — g $ha^{-1}$ — | — % — | — % — | — d — | — d — |
| None | None | - | - | - | 0 d | 0 d |
| | Imazamox + bentazon | 35 + 560 | 0 c[d] | 0 c | 1 c | 1 c |
| | Fomesafen | 280 | 0 c | 0 c | 1 c | 0 d |
| 2,4-D | None | 11.2 | 2 b | 0 c | 2 c | 3 c |
| | Imazamox + bentazon | 11.2 + 35 + 560 | 2 b | 0 c | 2 c | 3 c |
| | Fomesafen | 11.2 +280 + 5.6 | 2 b | 0 c | 2 c | 5 b |
| 2,4-D + glyphosate | None | 11.2 + 8.4 | 2 b | 0 c | 2 c | 5 b |
| | Imazamox + bentazon | 11.2 + 8.4 + 35 + 560 | 11 a (+) | 11 a (+) | 11 a | 12 a |
| | Fomesafen | 11.2 + 8.4 + 280 | 8 a | 4 b | 6 b | 5 b |

[a] Expected injury values were calculated using Gowing's equation (1960) and were compared with observed injury values using t-tests at $\alpha \leq 0.05$. The (+) symbol indicates a synergistic response from the combination.
[b] 2,4-D and 2,4-D + glyphosate contaminants were applied at 1% the recommended use rates.
[c] A crop oil concentrate (COC) at 1% v $v^{-1}$ plus ammonium sulfate (AMS) at 2.5% w $w^{-1}$ was included with the imazamox plus bentazon tank-mixture and a COC at 1% v $v^{-1}$ was applied with fomesafen.
[d] Means followed by the same letter within a column are not significantly different ($\alpha \leq 0.05$).

Table 3.5. P-values and main effects for dry bean yield and seed weight from contaminants of 2,4-D choline and 2,4-D choline plus glyphosate contaminants alone and in combination with common postemergence dry bean herbicide programs. Data is presented separately by the V2 and V8 application timings. Seed weight is combined over the three site-years of the experiment.

| | Yield | | | | Seed weight | |
|---|---|---|---|---|---|---|
| | 2017 | | 2018 | | | |
| | V2 | V8 | V2 | V8 | V2 | V8 |
| *Effects (p-values)* | | | | | | |
| Herbicide program | 0.06 | 0.57 | 0.46 | 0.70 | 0.47 | 0.84 |
| Contaminant | 0.08 | 0.86 | 0.55 | 0.24 | 0.92 | 0.61 |
| Herbicide program x contaminant | 0.01[a] | 0.11 | 0.99 | 0.06 | 0.63 | 0.61 |
| | kg $ha^{-1}$ | | | | g 100 $seeds^{-1}$ | |
| *Contaminants (Main effect)* | | | | | | |
| None | 2243 ab | 2232 a | 3272 a | 3525 a | 20.81 a | 20.65 a |
| 2,4-D | 2446 a | 2288 a | 3427 a | 3773 a | 20.88 a | 21.20 a |
| 2,4-D + glyphosate | 2121 b | 2173 a | 3565 a | 3821 a | 20.69 a | 20.70 a |
| *Herbicide program (Main effect)*[c] | | | | | | |
| None | 2093 b[c] | 2098 a | 3235 a | 3791 a | 20.58 a | 21.04 a |
| Imazamox + bentazon | 2255 ab | 2287 a | 3497 a | 3649 a | 21.14 a | 20.89 a |
| Fomesafen | 2464 a | 2307 a | 3538 a | 3673 a | 20.65 a | 20.70 a |

[a] The interaction for dry bean yield in 2017 for the V2 timing was a result of lower yields with imazamox + bentazon alone (2018 kg $ha^{-1}$) compared with the no herbicide control (2435 kg $ha^{-1}$).

[b] Means followed by the same letter within a column for each main effect are not significantly different ($\alpha \leq 0.05$).

[c] A crop oil concentrate (COC) at 1% v $v^{-1}$ plus ammonium sulfate (AMS) at 2.5% w $w^{-1}$ was included with the imazamox plus bentazon tank-mixture and a COC at 1% v $v^{-1}$ was applied with fomesafen.

APPENDIX B
Supplemental figures

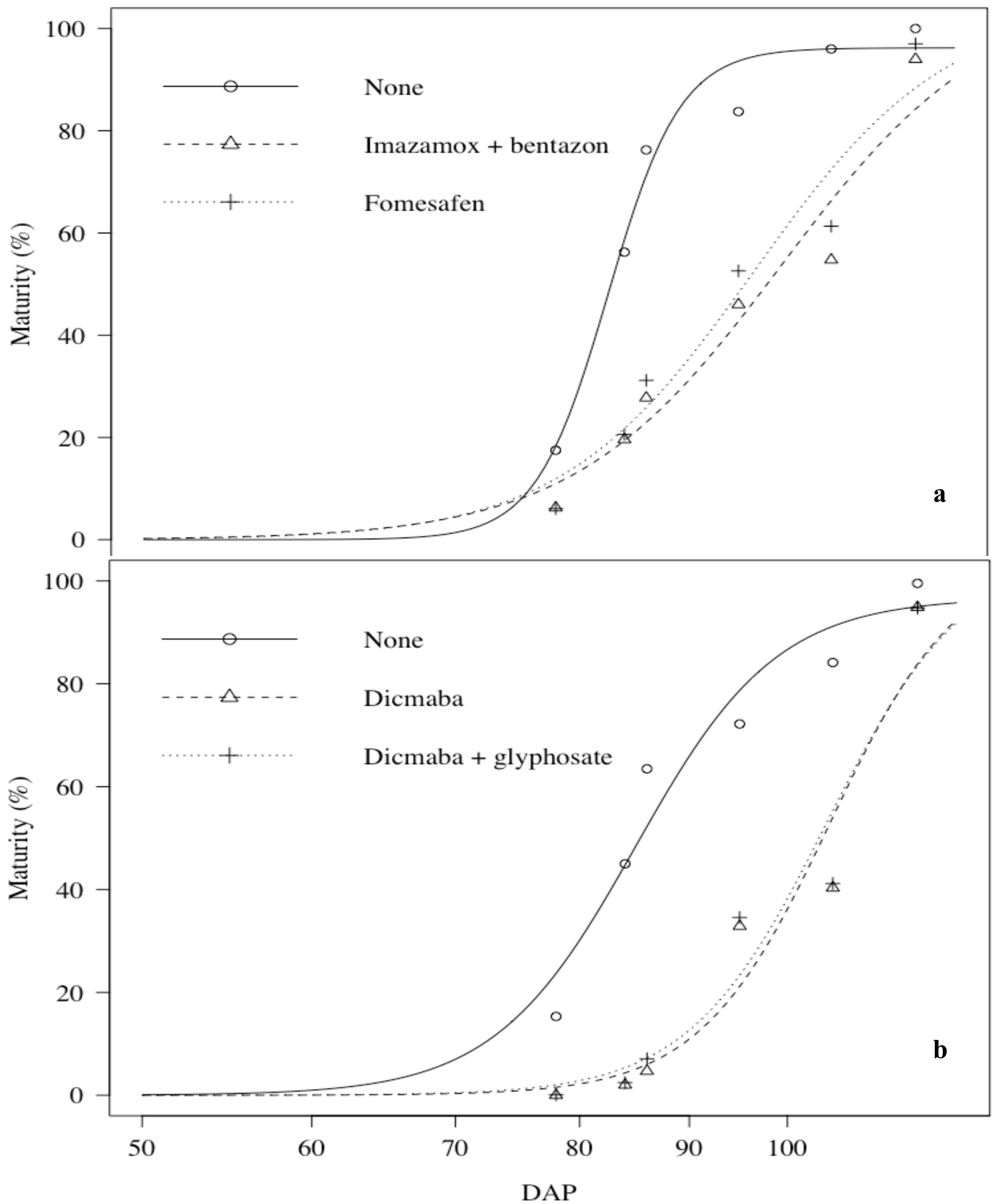


Figure 3.1. Dry bean maturity as affected by the main effects of dry bean herbicide program (a) and dicamba tank-contaminants (b) in Richville 2017 when combined over application timings.

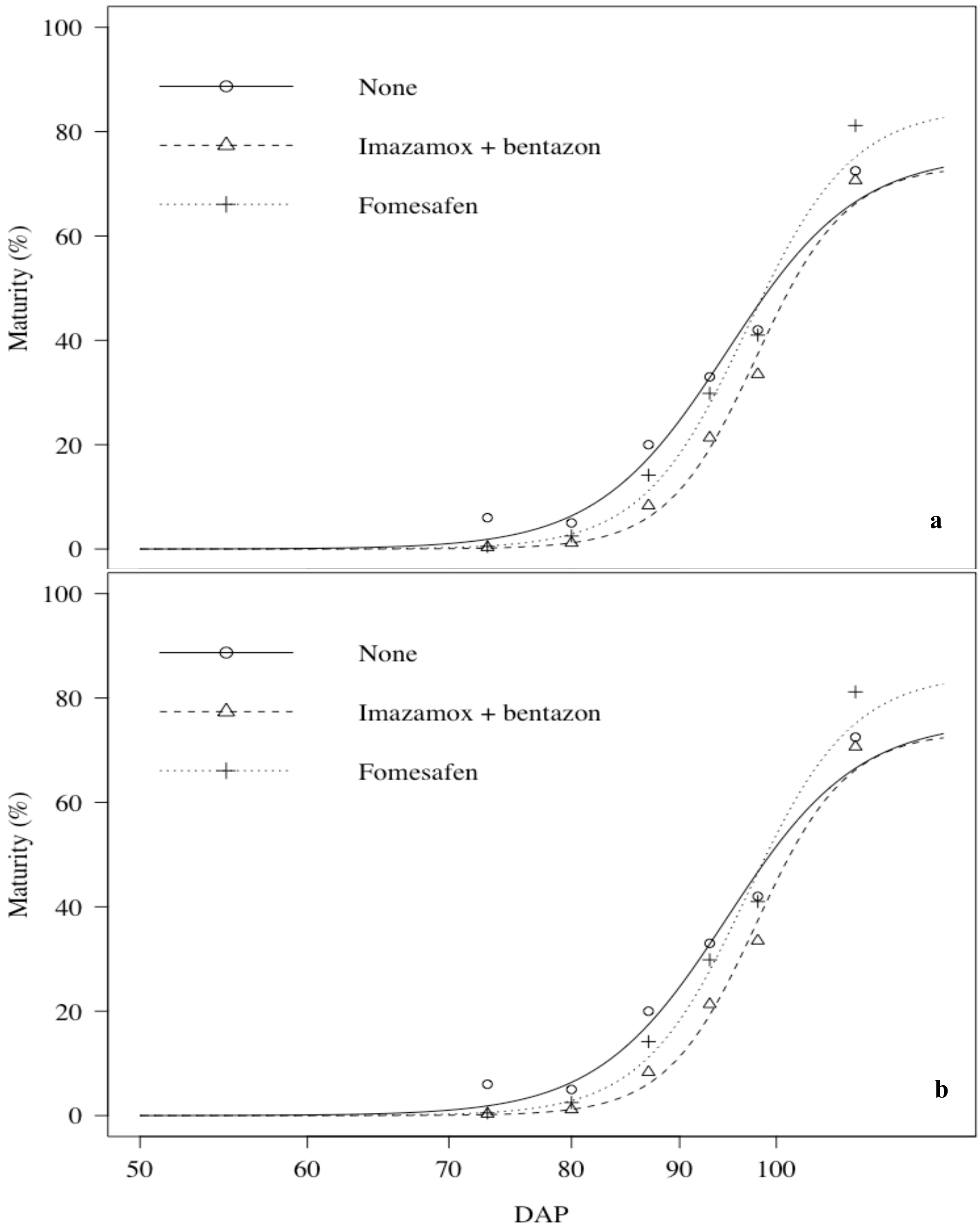


Figure 3.2. Dry bean maturity as affected by the main effects of dry bean herbicide program (a) and dicamba tank-contaminants (b) in East Lansing 2018 when combined over application timings.

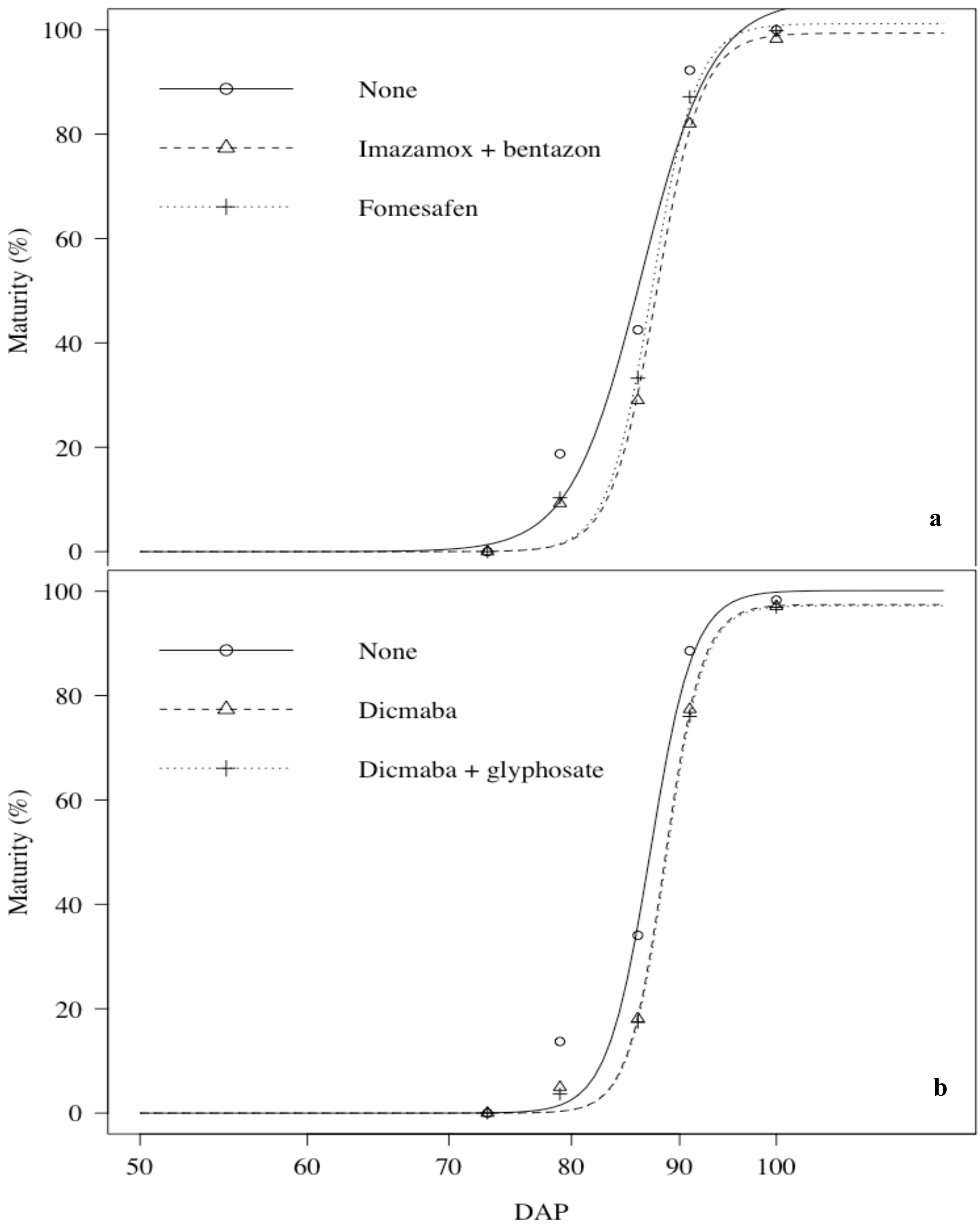


Figure 3.3. Dry bean maturity as affected by the main effects of dry bean herbicide program (a) and dicamba tank-contaminants (b) in Richville 2018 when combined over application timings.

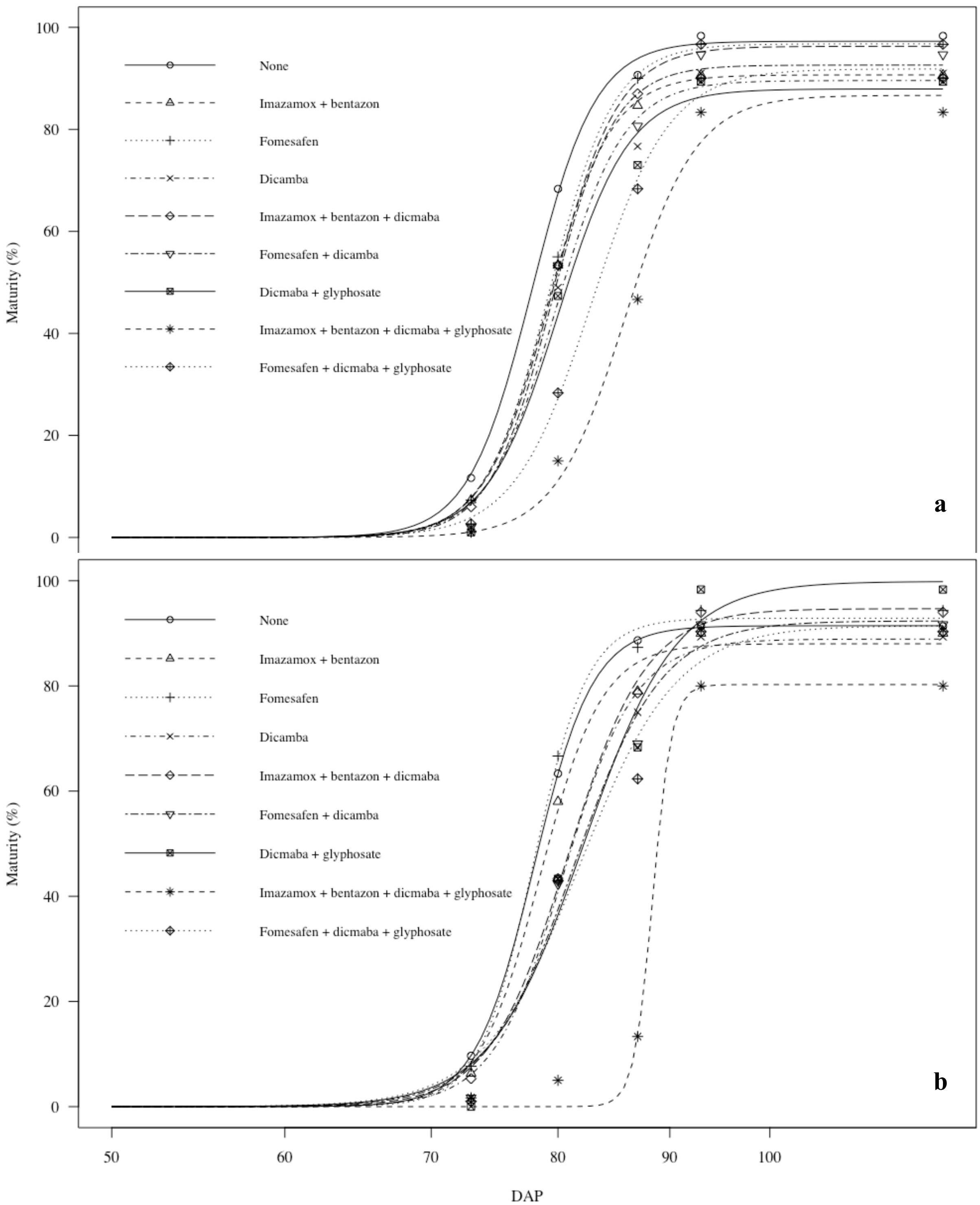


Figure 3.4. The interaction between dry bean herbicide program and tank-contaminants affect of dry bean maturity in East Lansing 2017 when applied at V2 (a) and V8 (b).

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