Response of non-dicamba-resistant soybean varieties to dicamba at varying doses and application times

by

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Abstract

Introduction and rapid adoption of dicamba-resistant (DR) soybeans led to an increase of post-emergent applications of dicamba for weed control during the soybean growing season, resulting in non-target dicamba injury to non-DR soybean. Two separate field studies were conducted in Manhattan, KS in 2018 and 2019 and in Ottawa, KS in 2019 to (1) determine the response of non-DR soybean to reduced rates, different application timings and multiple exposures of dicamba, and (2) investigate the injury and yield response of soybean varieties with varying herbicide-resistant traits when exposed to dicamba. In first study, soybeans were exposed to 0.56 (1/1000X), 1.12 (1/500X), and 5.6 g ae ha^{-1} (1/100X) rates of dicamba (where 1X rate=560 g ae ha^{-1}) at V3, R1, R3, V3 followed by (*fb*)R1, V3 *fb* R3, R1 *fb* R3, and V3 *fb* R1 *fb* R3 growth stages. In second study, four varieties, including ‘Credenz 3841LL (glufosinate-resistant)’, ‘Credenz 4748LL (glufosinate-resistant)’, ‘Asgrow AG4135RR2Y (glyphosate-resistant)’, and ‘Stine 40BA02’ (glyphosate/isoxaflutole-resistant) were exposed to 5.6 g ae ha^{-1} (1/100X) of dicamba at V3 or R1 growth stages. For both studies, visual soybean injury (%) was evaluated at biweekly intervals throughout the growing season and grain yields were determined at harvest. Injury symptoms, including leaf cupping, brittle leaves, damaged terminal buds, stunting, twisting and pod curling were observed with all dicamba treatments. Soybean injury from dicamba was lower and less persistent when exposed during the V3 than the R1 or R3 growth stages across all dicamba rates tested. Soybean injury symptoms were more severe with increasing dicamba rates and multiple exposures across all site-years. Soybean injury was most severe four weeks after treatment (WAT) and was highest (78% to 81% injury) with the 1/100X rate of dicamba applied at all three timings. Soybean yield reductions were not directly correlated to visual injury and were substantially less than most injury ratings. The highest soybean yield reduction was observed from the 1/100X rate

of dicamba applied at V3, R1, and R3, which resulted in a 53% yield loss. Soybean yield loss was minimal from a single dicamba exposure at the V3 stage regardless of exposure rate, or from the 1/1000X rate, regardless of timing or number of exposures. The greatest soybean yield loss from dicamba occurred with multiple exposures at rates greater than 1/1000X rate of dicamba. In the second study, the greatest injury was observed in ‘Asgrow AG4135RR2Y’ and ‘Stine 40BA02’ when exposed to dicamba at V3 growth stage 4 WAT. Dicamba exposure at the R1 growth stage resulted in the greatest injury to ‘Stine 40BA02’ both four WAT and senescence. At senescence, minimal injury was observed in soybean exposed at V3 and also resulted in minimal yield loss. Exposure at R1 resulted in the least yield loss in ‘Credenz 4748LL’ at 19% and the greatest yield loss in Stin 40BA02 at 34%. Dicamba exposure at R1 resulted in the greatest injury and yield loss, while exposure at V3 resulted in minimal injury and yield reduction across all tested soybean varieties.

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Chapter 1 - Literature Review

History and Formulations

Dicamba (3,6-dichloro-2-methoxybenzoic acid) is a benzoic acid herbicide that inhibits plant growth by mimicking the hormone auxin. Synthetic auxin herbicides were first discovered in the 1940s. The first compounds discovered were 2,4-D and MCPA. The commercialization of 2,4-D transformed agriculture and helped increase crop production. The discovery of 2,4-D lead to the synthesis of other synthetic auxin herbicides, such as dicamba. Dicamba is a weak acid. It has a molecular weight of 221.04 g mol^{-1}, vapor pressure of 4.5×10^{-3} Pa at 25°C, and pKa of 1.87 (Weed Science Society of America 2007). Vapor pressure is the pressure at which a solid or liquid material turns to vapor. The lower the vapor pressure, the less volatile the herbicide is. The strength of an acid is indicated by the pKa . The lower the pKa, the stronger the acid. Dicamba is an inexpensive herbicide and has been extensively used in corn, small grains, and pasture for broadleaf control since the 1960's. Commercial release dates and molecular weights for dicamba formulations can be found in Table 1.1 and chemical structures can be found in Figure 1.1. Of the presently used dicamba formulations, the DMA formulation is the most volatile while the DGA + VaporGrip and BAPMA formulations are the least volatile.

Use of dicamba has historically been associated with off-target movement onto and injury of susceptible plants, including soybeans and cotton (Auch and Arnold 1978, Wax et al. 1969). Dicamba-resistant (DR) cotton and soybean varieties were released in 2015; however, appropriate dicamba formulations were approved for use in 2016. DR crop technology includes resistance to glyphosate and can help soybean producers struggling with herbicide-resistant (HR) weeds, as is it provides an additional option for broadleaf weed control (Behrens et al. 2007). The technology allows new herbicides and mixtures of herbicides in DR crops, which could help producers suppress HR weeds and mitigate the selection of more HR weed biotypes.

Along with the release of the new stacked-trait technology, there was an increase in complaints of off-target injury from dicamba to sensitive plants. In 2017, Dr. Kevin Bradley (Bradley 2017) reported that there were 2,708 complaints of soybeans exhibiting dicamba injury symptoms and approximately 4% of total soybeans acres produced in 2017 were injured by off-target movement of dicamba (NASS 2019). The number of dicamba injury complaints decreased to 605 in 2018 (Bradley 2018), which were associated with approximately 1% of the soybeans acres planted in 2018 (NASS 2019).

The labels of new dicamba formulations (Engenia, Fexapan, and Xtendimax) were reconsidered in 2018 by the Environmental Protection Agency (EPA) and application requirements were modified. Requirements for post-emergence applications including physical buffers, applicator certification, record keeping, spray volume, time of application, rainfall, wind speed, and spray solution pH were modified and in many cases, vary by state. Key dicamba application requirements for Kansas are summarized in Table 1.2. Current labels for all dicamba-containing products for in-season use on DR soybeans will expire in December 2020 (Anonymous).

Mechanism of Action

Dicamba mimics natural auxins by imitating indole-3-yl-acetic acid (IAA). IAA plays a crucial role in several plant physiological processes, including seedling morphology, geotropism, phototropism, apical dominance, leaf senescence, and maturation (Cobb and Reade 2010). Response to dicamba is often dose dependent, and an application of dicamba can either stimulate or inhibit plant growth (Sterling and Hall 1997). Different plant tissues often respond differently to dicamba applications. For example, younger tissues are more sensitive to dicamba application than older tissues. Growth inhibition is attributed to ethylene production, which is initiated once a specific level of auxin is reached. Ethylene production is associated with plant senescence (Sterling and Hall 1997). The application of synthetic auxin herbicides also leads to production of hydrogen peroxide and abscisic acid (ABA). Hydrogen peroxide is linked to tissue damage and ABA is linked to stomatal closure, which ends carbon assimilation (Christoffoleti et al. 2015). The death of a plant treated by synthetic auxin herbicides occurs in a three phase process, all of which include a variety of morphological and physiological responses (Grossman 2010). The stimulation phase (phase one) occurs within the first day after the synthetic auxin herbicide is applied. Plants often respond with abnormal growth which includes tissue swelling, stem epinasty, and leaf epinasty. This phase is also characterized by metabolic activation which includes ethylene production, abscisic acid accumulation, enzyme synthesis, and gene expression (Grossman 2010, Christoffoleti et al. 2015, Cobb and Reade 2010).

The second phase or inhibition phase takes place within a week of application. It is characterized by a variety of physiological responses that includes stomatal closure, reactive oxygen species production, and reduction of transpiration, carbon fixation, and starch formation (Grossman 2010, Christoffoleti et al. 2015, Cobb and Reade 2010). The second phase is also characterized by decreased shoot and root growth and, as well as increased green leaf pigmentation. There is also an observed loss of apical dominance in lateral buds which can lead to increased branching (Grossman 2010).

The third and final phase is the decay phase, which occurs within 10 days after treatment (DAT). Senescence, cell death, and plant death occur in this phase because chloroplasts, membranes, and vascular tissues are disrupted by desaturation of plasma membrane lipids caused by reactive oxygen species (Grossman 2010, Christoffoleti et al. 2015, Cobb and Reade 2010).

Plant and cell death is also attributed to an accumulation of ABA (Christoffoleti et al. 2015). As a result of cell death, the plant will exhibit red discoloration, chlorosis, wilting, and necrosis. (Grossman 2010, Cobb and Reade 2010). Plant and cell death occur when exposed to high doses of synthetic auxin herbicides that are more in line with intentional applications of the herbicide, while off-target movement will result in less severe responses, as discussed in the following section.

Soybean injury

A variety of soybean injury symptoms are associated with off-target dicamba movement and are often dependent on soybean growth stage at the time of exposure (Wax et al. 1969, Auch and Arnold 1978, Weidenhamer et al. 1989). Greater injury is often observed in soybeans exposed to dicamba during early reproductive stages compared to vegetative stages (Auch and Arnold 1978, Wax et al. 1969). In fact, Griffin et al. (2013) numerically characterized the susceptibility of R1 soybeans to be 2.5 times more sensitive to dicamba exposure than soybeans in vegetative growth stages. However, Scholtes et al. (2019) reported that soybeans were most susceptible to dicamba exposure from V4 to R2 and least susceptible at R5 and R6.

Dicamba injury symptoms in soybeans treated during vegetative stages include epinasty of petioles and shoots, malformation of leaves, and chlorosis of terminal buds (Weidenhamer et al. 1989, Al-Khatib and Peterson 1999, Andersen et al. 2004, Kelley et al. 2005, Griffin et al. 2018). Terminal bud death, swollen stems, cracked stems, and axillary bud release, which causes increased branching and branching from cotyledon and unifoliate nodes, can also be observed when soybeans are treated with larger doses of dicamba (Wax et al. 1969, Behrens and Lueschen 1979, Weidenhamer et al. 1989, Griffin et al. 2018, Solomon and Bradley 2014, Robinson et al. 2013). In addition to the aforementioned symptomology, exposure during early reproductive stages can also cause malformed pods and delayed maturity (Auch and Arnold 1978, Wax et al. 1969, Weidenhamer et al. 1989, Griffin et al. 2013, McCown et al. 2018, Jones et al. 2018*a*). Soybeans treated during vegetative stages show more foliar symptomology than those treated during reproductive growth stages, unless the soybeans are indeterminate (Weidenhamer et al. 1989), which have achieved only half their height at flowering (Fehr and Caviness 1977).

Expression and duration of symptomology is dependent on dicamba dose and timing of exposure. Al-Khatib and Peterson (1999) found that injury symptomology from 187 g ae ha^{-1} dicamba could be seen in as little as 3 h after treatment and injury from lower doses, like 5.6 g ae ha^{-1} dicamba, could be seen within 1 d of exposure. Soybeans treated with 17, 56, 187 g ae ha^{-1} dicamba displayed severe leaf cupping and curling, shoot and petiole epinasty, and petiole swelling within the first 7 days after treatment (DAT). Exposure to the highest dose resulted in leaf and shoot tips starting to die 10 DAT and complete death of shoot tips 20 DAT. Scholtes et al. (2019) observed that soybeans at V1 to V2 showed the most injury 7 DAT, V1 to V4 showed the most injury 14 DAT, V2 to R1 showed the most injury 21 DAT, and V3 to R2 showed the most injury 28 DAT.

While soybean injury is influenced by the dicamba dose and timing, there is evidence that suggests that soybean cultivars can also respond differently to dicamba exposure (Weidenhamer et al. 1989, McCown et al. 2018). There are two major types of soybeans, determinate and indeterminate. Determinate soybeans are generally grown in the southern United States and are later maturing varieties. Determinate varieties stop vegetative growth at the onset of flowering. Indeterminate soybeans are generally grown in the mid-western United States and are earlier maturing varieties. Indeterminate varieties continue growth after the initiation of flowering (Ritchie et al. 1985). McCown et al. (2018) observed injury in both indeterminate and determinate varieties as a result of exposure to 2.18 and 8.75 g ae ha^{-1} dicamba at V4, V6, R1, R2, R3, R4, R5, and R6. The most severe injury was observed when soybeans were treated during V4, V6, R1, R2, and R3 in both indeterminate and determinate varieties. However, as the soybeans matured severity of injury decreased. Determinate soybeans treated during R4, R5, and R6 resulted in minimal to no injury, regardless of the dose. The indeterminate variety showed minimal injury as well as from treatment at R4, R5, and R6, regardless of dose. Weidenhamer et al. (1989) observed soybeans treated during late vegetative stages with 80 g ae ha^{-1} dicamba resulted in 62% reduction in soybean height in both an indeterminate and determinate variety, while treatment with the same dose during R2 resulted in 18% and 32% height reduction in a determinate and indeterminate variety, respectively. McCown et al. (2018) observed significant reductions of soybean height in indeterminate and determinate soybean varieties exposed during V4, V6, R1, and R2 to 2.18 and 8.75 g ae ha^{-1} dicamba. Determinate varieties are generally less affected than indeterminate varieties, possibly due to the greater amount of vegetative growth that determinate varieties have.

Vegetative growth stages

Soybean injury as a result of dicamba exposure is dose-dependent. Al-Khatib and Peterson (1999) observed 12 to 66% soybean injury 7 DAT with 5.6 to 187 g ae ha^{-1} dicamba applied at V3/V4. Andersen et al. (2004) observed 35, 43, and 80% injury 6 DAT as a result of treatment with 5.6, 11.2, and 56 g ae ha^{-1} dicamba, respectively applied at V3/V4 growth stage. McCown et al. (2018) reported 12% to 25% soybean injury 7 DAT when exposed to 2.18 and 8.75 g ae ha^{-1} dicamba at V4 growth stage; however, injury was only 9% to 19% when exposed to the same dicamba doses at V6. Griffin et al. (2018) recorded injury ratings 7 DAT at V3 that ranged from 20% to 89% as dicamba doses increased from 4.4 to 280 g ae ha^{-1}. At 14 DAT soybean injury increased to 35% to 97%. Kelley et al. (2005) observed an average of 37% injury 14 DAT when soybeans were exposed to 5.6 g ae ha^{-1} dicamba at V3 and 28% injury when exposed to the same dose at V7. Solomon and Bradley (2014) reported 44% injury 14 DAT when exposed at V3 to 28 g ae ha^{-1} dicamba. Andersen et al. (2004) reported an average of 43, 48, and 85% injury 12 DAT when soybeans were exposed to 5.6, 11.2, 56 g ae ha^{-1} dicamba at V3/V4. Osipitan et al. (2019) observed that visual injury 21 DAT increased from 29% to 75% as dicamba doses increased from 0.56 to 56 g ae ha^{-1} when treated at V2. Kelley et al. (2005) observed 25% injury 42 DAT for soybeans treated at V3 with 5.6 g ae ha^{-1} dicamba and an average of 34% injury when exposed to the same dose at V7. Andersen et al. (2004) observed an average of 55% and 90% injury 48 DAT in soybeans treated with 11.2 g ae ha^{-1} and 56 g ae ha^{-1} dicamba, respectively. Severity of injury increased as dose increased. Injury was persistent to the end of the season when soybeans were treated with higher doses.

Soybeans treated during early vegetative stages with smaller doses of dicamba were able to recover by the end of the season. Al-Khatib and Peterson (1999) noted that plants treated with 5.6 g ae ha^{-1} dicamba started to recover 30 DAT, plants treated with 17 g ae ha^{-1} and 56 g ae ha^{-1} dicamba started to recover 45 DAT, and plants treated with 187 g ae ha^{-1} dicamba had injury that persisted to the end of the season. Soltani et al. (2016) reported recovery for soybeans treated at V2/V3, but it was not noted until 8 weeks after treatment. While it may be possible for soybeans to recover from a dicamba drift event, it is also possible for injury to persist through the end of the season and yield reduction may not be avoided. Soybeans that are exposed during early vegetative stages are more likely to recover and compensate than those exposed during reproductive stages.

Soybean height reduction often responds to the interaction of application timing and dicamba dose (Robinson et al. 2013). Wax et al. (1969) observed severe stunting following application of higher doses of dicamba. Al-Khatib and Peterson (1999) noted height reduction in treatments with severe injury. Foster and Griffin (2018) showed a 5% to 59% reduction in height as dicamba doses increased from 2.2 to 35 g ae ha^{-1} when applied during V3/V4. Kelley et al. (2005) reported 21% to 22% reduction in height after treatment with 5.6 g ae ha^{-1} dicamba at V3 and 25% to 27% reduction in height after treatments with the same dose at V7. Al-Khatib and Peterson (1999) observed height reduction in soybean treated with dicamba doses of 56 and 187 g ae ha^{-1}. Wax et al. (1969) observed a 17% to 22% reduction in soybean height when V3 soybeans were exposed to 2.2 to 70 g ae ha^{-1} dicamba. Osipitan et al. (2019) reported that exposure to 56 g ae ha^{-1} dicamba at V2 resulted in 46% reduction in height. Weidenhamer et al. (1989) found greater height reduction in soybean treated during vegetative stages than those treated during reproductive stages.

Reproductive growth stages

Soybeans are more sensitive to reduced doses of dicamba during reproductive stages than vegetative stages and injury that occurs during reproductive stages has more potential to negatively impact yield. The early bloom or R1 growth stage is the most susceptible growth stage to dicamba injury (Auch and Arnold 1978, Jones et al. 2019, Foster and Griffin 2018). Solomon and Bradley (2014) reported similar injury symptoms when soybeans were treated at R2 as when treated at V3. Dicamba at 28 g ae ha^{-1} during R2 resulted in 18% injury 14 DAT (Solomon and Bradley 2014). Griffin et al. (2013) reported an increase in injury from 19% to 64% as dicamba doses increased from 1.1 to 70 g ae ha^{-1} at 7 DAT and an increase no more than 4% when evaluated 14 DAT at R1. Early season exposure events are often not indicative of soybean yield reduction due to their ability to recover by the end of the rowing season, but soybeans exposed during the reproductive stages resulted in more persistent symptomology which may be a more reliable indicator of yield loss (Al-Khatib and Peterson 1999).

Soybeans have less time before harvest to compensate for injury that occurs during reproductive stages, and yield components, such as pods are more likely to be injured when exposure occurs during reproductive stages. Jones et al. (2018a) reported that exposure to 2.19 and 8.75 g ae ha^{-1} dicamba at R1, R3, and R5 soybeans resulted in an average of 12%, 30%, and 4% pod malformation, respectively. McCown et al. (2018) reported that pod malformation occurred when soybeans were treated at V4 and V6, but greater pod malformation was caused by applications made during the reproductive stages. Soybeans exposed to 2.19 g ae ha^{-1} dicamba at R1, R2, R3, R4, R5, and R6 showed 28, 26, 29, 31, 26, and 21% pod malformation, respectively. Treatment with 8.75 g ae ha^{-1} dicamba at R1, R2, R3, R4, R5, and R6 resulted in 34, 29, 40, 49, 24, and 21% pod malformation, respectively. These results indicate that soybeans treated during pod development and seed fill are more sensitive to reduced doses of dicamba, which may have negative impacts on soybean offspring.

Dicamba exposure has also been shown to result in delays in soybean maturity (Wax et al. 1969; Kelley et al. 2005; Solomon and Bradley 2014; Osipitan et al. 2019). Wax et al. (1969) observed an increase from 4- to 24-day maturity delay as dicamba dose increased from 4.4 to 70 g ae ha^{-1}. Solomon and Bradley (2014) observed 1- and 24-day maturity delays when soybeans were treated with 2.8 and 28 g ae ha^{-1} dicamba, respectively. Kelley et al. (2005) observed a 4- to 6-day maturity delay when exposed to 5.6 g ae ha^{-1} dicamba at V3 and V7, but attributed the delay to adverse climate. Osipitan et al. (2019) observed 16- to 22-day delay in maturity when soybeans were treated at V2, R1, and R2 with 56 g ae ha^{-1} dicamba. Greater delays in maturity were seen when soybeans were exposed during reproductive stages.

Soybean height was not impacted when soybeans were treated from R4 to R7 due to cessation of vegetative growth (Auch and Arnold 1978; McCown et al. 2018; Jones et al. 2018a;

Scholtes et al. 2019). Auch and Arnold (1978) and Scholtes et al. (2019) observed that plant height was reduced by dicamba applications between V4 and R2. Foster and Griffin (2018) showed a 1% to 15% reduction in height as dicamba doses increased from 2.2 to 35 g ae ha^{-1} when applied during R1/R2. Wax et al. (1969) reported that dicamba application during R2 resulted in 25 to 42% reduction in height when treated with 2.2 to 70 g ae ha^{-1} dicamba, respectively. Jones et al. (2018a) showed that dicamba at 2.19 and 8.75 g ae ha^{-1} applied at R1 resulted in an average of 24% height reduction and treatment during R3 with the same doses resulted in an average of 14% height reduction. Osipitan et al. (2019) reported that treatment at R1 and R2 with 56 g ae ha^{-1} dicamba resulted in 65 and 21% reduction in height, respectively. Greater reductions in height were associated with exposure during early reproductive growth stages rather than later reproductive stages, when exposed to higher doses of dicamba.

Soybean yield

Worrisome soybean injury often occurs at doses that are much lower than those required to reduce yield (Al-Khatib and Peterson 1999). Kniss (2018) reported that injury associated with 5% yield reduction was 30% when soybeans were exposed during vegetative stages, but only 12% for exposure during reproductive stages. Al-Khatib and Peterson (1999) noted that the dicamba dose causing at least 50% injury was continually lower than the dose required to cause 50% yield reduction. Similarly, Wax et al. (1969) noted that exposure at V3 required with a dose of 70 g ae ha^{-1} dicamba to reduce yield by 20%, whereas 2.2 g ae ha^{-1} dicamba was required during R2. These results indicate that soybeans in early reproductive stages are 8 times more susceptible than soybean in vegetative stages.

Yield loss is highly dependent on dose and timing of application. Jones et al. (2018a) showed that treatment with 2.19 and 8.75 g ae ha^{-1} dicamba during R1 resulted in an average of

16% reduction in yield and treatment during R3 with the same doses resulted in an average of 7% reduction in yield. Griffin et al. (2013) reported 52% reduction in yield in soybeans treated at V3/V4 with 70 g ae ha^{-1} dicamba. Soybeans treated at R1 with 17.5 g ae ha^{-1} dicamba resulted in 36% yield reduction. Foster and Griffin (2018) showed that treatment with 2.2, 8.8, and 35 g ae ha^{-1} dicamba at V3/V4 resulted in 5, 18, and 54% yield reduction and at R1/R2 resulted in 9, 30, and 76% yield reduction, respectively. Andersen et al. (2004) reported 14% to 34%, 14% to 41%, and 72% to 83% yield reduction after exposure to 5.6, 11.2, and 56 g ae ha^{-1} dicamba at V3, respectively. Kelley et al. (2005) reported 15% to 22% yield reduction after exposure at V3 to 5.6 g ae ha^{-1} dicamba. After exposure at V7 to the same dose, yield was reduced by 12% to 28%. Solomon and Bradley (2014) reported no yield reduction in soybeans with dicamba exposure at V3, but treatment at R2 resulted in an increase in yield reduction from 2 to 67% as dicamba dose increased from 0.028 to 28 g ae ha^{-1}. Yield reductions were greatest when dicamba exposure occurred during early reproductive growth stages and increased as dicamba doses increased.

Soybean yield loss is influenced by dicamba dose and timing, but there is also evidence that suggests that soybean cultivars can respond differently to dicamba exposure (Weidenhamer et al. 1989; McCown et al. 2018). Weidenhamer et al. (1989) reported that treatment of a determinate soybean during vegetative stages and R2 with 80 g ae ha^{-1} dicamba caused 35% reduction in yield, whereas yield of an indeterminate soybean variety treated with the same dose was reduced 40% and 23% when treated during pre-bloom and R2, respectively. As a result of exposure to 2.18 and 8.75 g ae ha^{-1} dicamba, McCown et al. (2018) observed that dicamba exposure during V4, V6, R1, and R2 averaged 8, 9, 14, and 8% yield reduction, respectively in a determinate variety. In an indeterminate soybean variety, dicamba exposure at V4, V6, and R2 averaged 16% yield reduction, respectively and at R1 averaged 19% yield reduction. Again, soybean yields of determinate varieties are generally less affected by dicamba than in indeterminate varieties, possibly due to the increased amount of vegetative growth of determinate varieties.

Yield components

The quantification of soybean yield components is vital in understanding the impacts of dicamba exposure on soybean yield (Robinson et al. 2013). Soybean yield components include seed weight, seeds m^{-2}, seeds pod^{-1}, seeds $plant^{-1}$, pods m^{-2}, pods $plant^{-1}$, pods reproductive $node^{-1}$, reproductive nodes m^{-2}, percentage of reproductive nodes, and nodes m^{-2} (Board and Modali 2005, Robinson et al. 2013, Solomon and Bradley 2014, Soltani et al. 2016). Yield components are categorized into primary, secondary, tertiary, and quaternary traits. Primary traits include seeds m^{-2}, seeds $plant^{-1}$, and seed weight. Secondary traits include pods $plant^{-1}$, pods m^{-2}, and seeds pod^{-1}. Tertiary traits include pods reproductive $node^{-1}$ and reproductive nodes m^{-2}. Finally, quaternary traits include percentage reproductive nodes and nodes m^{-2}. Yield components that are categorized as primary traits have the most effect on yield, while those categorized as quaternary traits have the least effect on yield.

Primary traits

Seeds m^{-2}, seeds $plant^{-1}$, and seed weight are the primary traits that effect yield (Board and Modali 2005). Grain yield is generally more impacted by the number of seeds rather than the weight of the seeds (Elgi 1975). Robinson et al. (2013) reported that seeds m^{-2} was reduced by 5% when applications of 0.186, 1.62, and 1.10 g ae ha^{-1} dicamba were made on V3, V5, and R2 soybeans and by 10% when applications of 11.9, 2.92, and 2.22 g ae ha^{-1} dicamba were made on V3, V5, and R2 soybeans, respectively. Seeds m^{-2} was reduced by 20% when applications of 5.71 and 4.74 g ae ha^{-1} dicamba were made on V5 and R2 soybeans. Soltani et al. (2016) noted that seeds $plant^{-1}$ decreased as dicamba dose increased and predicted dicamba doses of 0.75, 3.2, 10.6, 36.4, and 60 g ae ha^{-1} for V2/V3 and 0.75, 1.5, 3.2, 6.9, and 26 g ae ha^{-1} for R1 would be required to decrease seeds $plant^{-1}$ by 1, 5, 10, 20, and 50%, respectively. Robinson et al. (2013) reported 5% reduction in seed weight when soybeans were treated at V5 with 0.365 g ae ha^{-1} dicamba. Solomon and Bradley (2014) reported 3% reduction in seed weight when exposed to 0.028 g ae ha^{-1} dicamba at V3 and 2% reduction when exposed to 0.28, 2.8, and 28 g ae ha^{-1} dicamba. Kelley et al. (2005) reported 11 to 12% reduction in seed weight when treated with 5.6 g ae ha^{-1} dicamba at V3 and V7. Soybeans seed weight is less sensitive to dicamba exposure during early reproductive stages as compared to during vegetative stages, while seeds m^{-2} and seeds $plant^{-1}$ are more sensitive to applications made during reproductive stages.

Secondary traits

Secondary traits affecting soybean yield are pods $plant^{-1}$, pods m^{-2}, and seeds pod^{-1} (Board and Modali 2005). McCown et al. (2018) observed greater reductions in pods $plant^{-1}$ with dicamba exposure in early reproductive stages than vegetative and late reproductive stages. Robinson et al. (2013) reported 5% reduction of pods m^{-2} when dicamba at 0.025, 2.11, and 1.15 g ae ha^{-1} was applied at V3, V5, and R2, respectively. Pods m^{-2} was reduced by 10% when applications of 3.65 and 2.44 g ae ha^{-1} dicamba were made on V5 and R2 soybeans and by 20% when applications of 7.17 and 5.64 g ae ha^{-1} dicamba were made on V5 and R2 soybeans. Solomon and Bradley (2014) reported 6% reduction in pods $plant^{-1}$ after exposure at V3 to 0.028 and 2.8 g ae ha^{-1} dicamba. Exposure at R2 to 0.028, 0.28, 2.8, and 28 g ae ha^{-1} dicamba resulted in 13, 10, 19, and 73% reduction in pods $plant^{-1}$. Kelley et al. (2005) reported that exposure at V3 to 5.6 g ae ha^{-1} dicamba resulted in 8 to 14% reduction in pods $plant^{-1}$ and at V7 resulted in 6%. Soltani et al. (2016) predicted that dicamba doses from 0.75 to 60 g ae ha^{-1} for V2/V3 soybeans and 1.2, 4.1, 7.7, 14.5, and 42.7 g ae ha^{-1} for R1 soybeans would be needed to reduce pods $plant^{-1}$ by 1, 5, 10, 20, and 50%.

Robinson et al. (2013) observed up to 10% reduction of seeds pod^{-1} when V5 and R2 soybeans were exposed to dicamba at 14.2 and 7.35 g ae ha^{-1}. Solomon and Bradley (2014) reported exposure at V3 to reduce seeds pod^{-1} by 4% when exposed to 0.028 and 0.28 g ae ha^{-1} dicamba, by 5% when exposed to 2.8 g ae ha^{-1} dicamba, and by 3% when exposed to 28 g ae ha^{-1} dicamba. When exposed at R2, reductions increased from 9% to 72% as dicamba dose increased from 0.028 to 28 g ae ha^{-1}. Kelley et al. (2005) reported exposures to 5.6 g ae ha^{-1} dicamba at V3 resulted in 2 to 9% reduction in seeds pod^{-1} and at V7 resulted in 11 to 25% reduction. All the secondary traits are more sensitive to application of dicamba during reproductive growth stages.

Tertiary traits

Tertiary traits that affect pod number within a certain area include pods reproductive $node^{-1}$ and reproductive nodes m^{-2} (Board and Modali 2005). Robinson et al. (2013) noted that dicamba application had no effect on pods reproductive $node^{-1}$. But, reproductive nodes m^{-2} had 2 times greater impact on pods m^{-2} than pods reproductive $node^{-1}$. Board and Modali (2005) showed that as reproductive nodes m^{-2} increased, seed yield increased. Reproductive nodes m^{-2} were shown to be reduced by 5 to 20% when exposed to dicamba at V3, V5, and R2 with doses ranging from 0.073 to 2.72 g ae ha^{-1} (Robinson et al. 2013). The reduction of reproductive nodes m^{-2} can have a direct impact on the development of other yield components, thus resulting in yield reduction.

Quaternary traits

The quaternary traits affect reproductive nodes within a certain area and include percentage reproductive nodes and nodes m^{-2} (Board and Modali 2005). Robinson et al. (2013) showed that the percentage reproductive nodes was reduced by 5% and 10% when soybean was treated at R2 with 10.3 and 16.1 g ae ha^{-1} dicamba. It was shown that nodes m^{-2} were reduced by 5% after exposure to 0.020, 0.847, and 1.14 g ae ha^{-1} dicamba at V2, V5, and R2, respectively. Exposure to 0.162, 1.27, and 2.04 g ae ha^{-1} dicamba at V2, V5, and R2 resulted in 10% reduction, respectively. When V2, V5, and R2 soybeans were exposed to 1.45, 1.94, and 3.82 g ae ha^{-1} dicamba 20% reduction of nodes m^{-2} was observed. Soybean yield components were generally more affected by dicamba exposure at reproductive stages, with the exception of seed weight which was more impacted by exposure during vegetative stages.

Offspring of exposed soybean

The effect of dicamba exposure on soybean offspring is not well understood; but, understanding the impact is crucial for soybean seed production (Jones et al. 2018a). Small doses of dicamba have been shown to reduce germination, emergence, and vigor and injure offspring of soybeans exposed during reproductive stages (Wax et al. 1969, Jones et al. 2018a, Auch and Arnold 1978, Thompson and Egli 1973). Exposure during vegetative stages did not impact soybean offspring (Wax et al. 1969, Auch and Arnold 1978).

Auch and Arnold (1978) observed 19, 23, 25% reduction in germination when parent soybeans were treated during R3 with 11, 28, and 56 g ae ha^{-1} dicamba, respectively. When exposed during R4, germination was reduced by 28, 67, and 61% when treated with 11, 28, and 56 g ae ha^{-1} dicamba, respectively. Wax et al. (1969) only saw reductions in germination when the parent soybeans were treated during R2 with dicamba doses of 35 and 70 g ae ha^{-1}. Thompson and Elgi (1973) reported that parent soybean exposure during R5 and R6 to dicamba doses of 30 to 220 g ae ha^{-1} resulted in 70% of the seed being characterized as dead due to non-germination. Parent soybean exposure during R1 to 30 g ae ha^{-1} dicamba resulted in 36% to 50% reduction in germination. The greatest reductions in germination occurred when parent soybeans were exposed to 30 and 220 g ae ha^{-1} dicamba during later reproductive stages, such as R5 and R6, than exposure during R3 and R4. Jones et al. (2019a) compared the effects of simulated dicamba drift on soybeans at R1, R2, R3, R5, and R6 growth stages and found that a drift event at R5 was more likely to result in reduced germination and emergence, due to the ability of dicamba to rapidly enter seed in already formed pods. Auch and Arnold (1978) reported a 36% and 32% reduction in emergence of offspring at 15 and 21 DAP, respectively when parent soybeans were treated at R3 with 56 g ae ha^{-1} dicamba. When parent soybeans were exposed to 11 g ae ha^{-1} dicamba during R3, there was 19% and 18% reduction in emergence 15 and 21 DAP, respectively. Thompson and Elgi (1973) reported 50% to 54% reduction in emergence in offspring of parent soybeans exposed to 30 g ae ha^{-1} dicamba during R1/R2 and R5/R6. However, the greatest reduction in emergence was seen in offspring of parent soybeans exposed during R5/R6 to 560 g ae ha^{-1} dicamba, which resulted in 74% reduction in emergence. Germination and emergence of soybean progeny were most impacted by drift events that occurred during seed fill and decreased as dicamba doses increased, which could also impact other progeny characteristics.

Seed from exposed parent plants may germinate and emerge, but seedlings may exhibit injury or reduced vigor. Jones et al. (2019a) observed a correlation between pod malformation of parent soybean exposed to dicamba at R3 with reduced vigor of offspring. While there is not a standardized rating system for vigor, Jones et al. (2018a, 2019a) assessed vigor on a 1 to 5 scale with 1 being low vigor, which is characterized by reduced and/or delayed emergence, and 5 being high vigor, which is characterized by quick emergence and normal growth. Reduction in offspring vigor across R1, R3, and R5 when treated with 2.2 and 8.8 g ae ha^{-1} dicamba were also observed. Greater reductions in vigor were observed as the application timing was delayed and dose increased. When parent soybean was exposed to 2.2 g ae ha^{-1} dicamba, there was an 11 to 22%

reduction of vigor in offspring. When exposed to 8.8 g ae ha^{-1} dicamba, there was a 12 to 44% reduction in offspring vigor.

Auch and Arnold (1978) noted that the offspring of parent soybeans treated with dicamba at doses of 11 to 56 g ae ha^{-1} did not show reduced vigor or dicamba injury symptoms. Thompson and Elgi (1973) noted that offspring injury symptoms varied from minimal crinkling and cupping of leaves to complete restriction of leaf expansion. The authors also noted that offspring of soybeans treated during pod-fill showed symptomology in the trifoliate leaves but not unifoliate and it was opposite for the offspring of soybeans treated during flowering. Jones et al. (2019a) observed offspring injury from drift events that occurred during R1, R2, R3, and R5. The height of parent soybean at R1 and R2 was highly correlated to offspring injury. As parent soybean height decreased, offspring injury increased. The authors attribute this relationship to high concentrations of dicamba causing severe stunting in parent soybeans and hypothesized that this caused dicamba to still be present during R5/R6. When exposed during R2, there was a correlation between parent leaf malformation and offspring injury. The injury symptom of parent soybeans most correlated with offspring injury was parent pod malformation following exposure R5. Although the R5 exposure only resulted in 0 to 15% pod malformation, the percentage of injured offspring seedlings increased to 99%; whereas, the percentage of injured offspring as a result of R3 exposure was only 50%. Jones et al. (2018a) reported a similar trend where parent soybeans treated during R5 resulted in 81 and 96% injured plants when treated with 2.2 and 8.8 g ae ha^{-1} dicamba, but when treated during R3 with the same doses there was 34 and 59% injured plants. Thompson and Elgi (1973) also reported severe injury in offspring of parent soybeans treated during R5/R6. Of all the reproductive stages discussed, drift events during R6 may be the most detrimental to the soybean seed production industry because the presence of dicamba was not detected by germination test or emergence tests and dicamba may not be detected until the offspring is at least V1 or V2 (Jones et al. 2019a). Exposure during the reproductive stages of parent soybeans will have a negative impact on the offspring, but the severity is dependent on the exposure dose and timing.

Off-target herbicide movement

Off-target movement is the movement of herbicides away from the intended target (Metzer et al. 1972). The most common sources of off-target dicamba movement are particle drift, vapor drift, and contaminated spray solution (Bish et al. 2019, Behrens and Lueschen 1979, Boerboom 2009). Particle drift occurs when spray particles move away from the nozzle before reaching the target, while vapor drift occurs when herbicide volatilizes and moves in the gaseous from, usually after reaching the application target (Metzer et al. 1972). Both types of movement can cause injury to sensitive plants outside of the application area, and it can be difficult to identify the exact source of herbicide. However, the pattern of damage can often explain the source (Boerboom 2009). Physical drift tends to have more severe injury on the side of the field closest to the application with a gradual decrease in severity the farther away from the application area one moves, while vapor drift shows uniform injury across the field (Jones et al. 2019).

Physical drift

Physical drift is influenced by wind speed and can be minimized when proper nozzles, boom height, pressure, and speed are used (Metzer et al. 1972, Maybank et al. 1978, Carlsen et al. 2005). Nozzles are the primary factor in ensuring proper deposition of chemical on to the target (Azimi et al. 1985). Maybank et al. (1978) reported that initial spray drift values were dependent on nozzle and ranged between <0.5% and 8% of the spray emitted when applying 2,4-D. Gil et al. (2014) reported the least drift from TTI and TD SprayMax nozzles, which had drift protentional values (DPVs) of 2.73 and 2.75, respectively, while XR nozzles had a DPV of 23.73. DPV is a unitless number used to compare drift potential. The DPV is calculated by equation 1.1.

Equation 1.1

$$DPV = \sum_{i=1}^{n} \frac{D_i}{RSD} \times 100 \tag{1.1}$$

Where D_i is the amount of spray solution deposited on a target, n is the number of targets, and RSD is the amount of spray solution intended to fall in the treatment area (Gil et al. 2015). A greater DPV indicates drift is more likely to occur.

Large orifice, flat fan nozzles and cone jet nozzles have less potential than conventional flat fan nozzles to produce driftable fines (spray particles that are 100 µm or less) as these types of nozzles can be operated at lower pressure levels. However, lower spray pressures produce larger droplets that can result in in less surface coverage area of the plant to allow herbicide uptake (Maybank et al. 1974). Greater pressures increase spray pattern uniformity due to increased exit speed of the spray solution and increased angle width, but low pressure fan nozzles are capable of maintaining uniform pattern at low pressures (Azimi et al. 1985). Butts et al. (2019) observed that increased pressure did not adversely affect the spray pattern uniformity of non-venturi nozzles; but, there was a decrease in uniformity when pressure was increased while using dual fan venturi nozzles. Venturi nozzles are a dual orifice nozzle, where one orifice meters pressure and the second orifice creates the spray pattern. Flat fan nozzles with 65° and 80° angles and air induction nozzles produced droplets with volume median diameter (VMD) larger than 300 µm, while flat fan nozzles with 110° angles and hollow cone nozzles produced droplets with VMD smaller than 300 µm (Al Heidary et al. 2014). When nozzle height was set at 900 mm above the ground, there was approximately a 50% reduction in drift when the spray angle decreased from 110° to 80° and approximately an 80% reduction in drift when the spray angle decreased from 110° to 65° (Al Heidary et al. 2014).

Nozzle height also influences drift potential due to the influence on the time it takes spray droplets to travel from nozzle to target (Al Heidary et al. 2014), as well as the influence on spray pattern uniformity (Azimi et al. 1985). Miller et al. (2011) observed a 13.5-fold increase in the amount of driftable spray solution as nozzle height increased from 350 to 850 mm. Physical drift can be mitigated when proper application parameters are followed.

Spraying within the Engenia®, Fexapan®, and Xtendimax® label wind speed guidelines, between 16 km hr^{-1} and 24 km hr^{-1}, is ideal to mitigate physical drift. Conditions with wind speed below 16 km hr^{-1} tend to create a more stable environment and have the potential to have an increased amount of suspended dicamba particles (Bish et al. 2019a). As wind speed increases the amount of drifted spray solution increases, but the amount of airborne spray particles decreases rapidly the farther downwind the spray particles are carried (Maybank et al. 1978). As the wind speed increased from 10 km hr^{-1} to 40 km hr^{-1}, Maybank et al. (1978) observed over a 2.5% increase in the initial drift from a single swath and each subsequent swath adds to the total drift when applying 2,4-D. Gil et al. (2015) reported that wind speeds at or below 3.6 m s^{-1} had little to no effect on DPV. Drift potential values averaged at 65.26 with a frontal wind at ≥5.4 km hr^{-1} and 23.04 with a lateral wind at ≤5.4 km hr^{-1} (Gil et al. 2015).

Vapor drift

Dicamba has a high vapor pressure relative to other herbicides, and is therefore more susceptible to vapor drift. The extent of vapor drift is influenced by formulation and environmental conditions, especially temperature and relative humidity. The BAPMA salt of dicamba has a molecular weight of 366.29 g mol^{-1}, which makes it the heaviest of all the available formulations

(Weed Science Society of America 2018). However, molecular weight alone does not determine the volatility of the molecule. The strength of the bond between the salt and parent dicamba acid molecule impacts the potential for volatility. Stronger bonds between the dicamba parent acid and the salts result in less volatile molecules (Bish et al. 2019a).

The DMA salt of dicamba was believed to eliminate dicamba vapor drift; however, results from several researchers disproved that claim (Behrens and Lueschen 1979; Egan and Mortensen 2012; Mueller et al. 2013;). Similarly, dicamba formulations developed during the 2000's were thought to be a solution to dicamba vapor drift (MacInnes 2017). Once again, research is demonstrating that vapor drift of Engenia, FeXapan, and XtendiMax do occur. For example, Bish et al. (2019a) observed that dicamba can remain in the air for up to 3 days after application with no difference between DGA and BAPMA formulations. However, Jones et al. (2018b) observed that the DGA formulation caused soybean injury two times farther away from the application site than the BAPMA formulation and that the BAPMA formulation is slightly less prone to vapor drift than the DGA formulation. Mueller et al. (2013) observed 2 times more of the DMA formulation than the DGA formulation of dicamba 48 h after application. Farrell et al. (2017) observed 31% and 34% less dicamba present in the air 0.5 to 8 h after an application of the BAPMA formulation in comparison to the DGA formulation. Egan and Mortenson (2012) found that using the DGA formulation instead of the DMA formulation could reduce vapor drift by up to 94%. Formulation selection is critical when attempting to prevent off-target movement, but needs to be paired with other best application practices, such as avoiding application in adverse meteorological conditions.

Temperature and relative humidity have a significant impact on the dicamba volatility because volatilization generally occurs when temperatures are high and humidity is low (Grover 1975; Behrens and Lueschen 1979). Soybean plants placed near a sprayed field up to 3 days after dicamba application expressed injury between 0 and 48%, with the most injury occurring when temperatures were highest and the least injury occurring when rainfall occurred after application. Strachan et al. (2010) observed an increase in volatilization of dicamba when placed on heated surfaces. Behrens and Lueschen (1979) and Mueller and Steckel (2019) recorded significant increases in dicamba volatility as temperatures increased from 15°C to 30°C; but, temperatures up to 40°C resulted in a plateauing effect of vapor injury on sensitive plants due to stomatal closure. Minimal volatilization of dicamba occurred below 15°C (Mueller and Steckel 2019, Behrens and Lueschen 1979). Bish et al. (2019a) observed for every increase of 1°C there was a 1.67 ng m^{-3} increase in dicamba concentrations in the air.

Rainfall after application in amounts as small as 1 mm can significantly lower the chance of volatilization due to rain washing the spray droplets from the leaves of the target plants and increased humidity (Boerboom 2009). Small reductions in humidity can cause large amounts of dicamba vapor activity (Behrens and Lueschen 1979). Behrens and Lueschen (1979) studied two humidity levels, 85% – 95% and 70% – 75% relative humidity. When humidity was reduced from the higher level to the lower level, there was 12% increase in injury following application of the DMA formulation of dicamba. Current restriction on time of day at which application can occur in DR soybean can make it difficult for applicators to avoid high temperatures and low humidity that occur during midday, but it can help avoid other conditions that facilitate off-target movement.

Temperature inversions

Temperature inversions are another concern regarding off-target movement of herbicides, although inversions do not directly cause off-target movement; it is the conditions surrounding inversions that facilitate physical and vapor drift (Enz et al. 2019). Temperature inversions occur when the densest or coolest air is near the earth's surface and no air movement is present to mix the cool air with warmer, less dense air that's farther from the surface (Enz et al. 2019). When inversions occur there is a dewpoint temperature line, where below the line condensation forms in the air due to the temperature being below the dewpoint (Enz et al. 2019). When pesticide applications are made during a temperature inversion, spray droplets below the dewpoint temperature line do not evaporate while the droplets above the line evaporate slowly (Enz et al. 2019). The droplets introduced into the inversion move as the temperature inversion moves and will remain within the inversion until they can evaporate or possibly land on an unintended target (Enz et al. 2019). Higher dewpoint temperatures can hinder dispersion of dicamba molecules by causing a reformation of droplets that can settle out of the atmosphere (Bish et al. 2019a). Baker et al. (1969) reported that midnight was the most common time for inversions to form, further reinforcing dicamba label guidelines of only applying dicamba between 1 hour after sunrise and 2 hours before sunset. Temperature inversions generally end close to sunrise when the lower level air becomes warmer than higher level air (Bish et al. 2019b). The concept of inversions can be difficult to understand, so it is important that education efforts continue to help applicators know when inversions are occurring.

Contaminated spray solution

Contaminated spray solution often originates from contaminated nurse tanks or water, measuring containers, transfer containers, and hoses (Boerboom 2009) or anywhere dicamba has the opportunity to adhere to materials like plastic or rubber (Steckel et al. 2005). Unfortunately, all plant growth regulator herbicides (PGR), including dicamba, are difficult to clean out of spray equipment. Cundiff et al. (2017) demonstrated that different sprayer hose types sequestered varying levels of dicamba. Of the five types tested, the John Deere PMA 4086-08 which was made out of a low-density polyethylene blend showed the least sequestration with and without proper cleanout procedures while the Goodyear hose made out of Versigard synthetic rubber resulted in the most sequestration with and without proper cleanout (Cundiff et al. 2017). For proper clean out, ammonia and water should sit in the tank for several hours or overnight to ensure the PGR is removed from the plastic and rubber surfaces within the spray system (Steckel et al. 2005). A survey conducted in Missouri reported that 65% of commercial applicators and 43% of private applicators surveyed always triple-rinsed the sprayer tanks between herbicide applications; however, 1% of commercial applicators and 3% of private applicators never rinsed between herbicide applications (Bish and Bradley 2017). The survey also reported that 67% of commercial and 44% of private applicators who always triple rinsed sprayers used tank cleanout products, while 56% and 35% of commercial and private applicators that rinsed $\leq$ 3 times between application used tank cleanout products (Bish and Bradley 2017). Boerboom et al. (2009) reported that even when proper clean out procedures were used after applying a field use dose of dicamba samples collected from water in the tank contained 0.02% of a field use dose and water collected from the spray boom had 0.63% of a field use dose. Prevention of contaminated spray solution and following proper cleanout procedure help mitigate off-target movement of synthetic auxin herbicides on to sensitive plants.

Off-target movement of dicamba on to non-DR soybeans negatively impacts soybeans in a variety of ways including severe injury, reduced yield due to adverse effects on yield components, and reduced height. Offspring of exposed soybeans can also experience negative impacts, which would adversely affect the soybean seed industry. Off-target movement of dicamba can be mitigated if proper equipment and dicamba formulations are used, and applications are not made during adverse meteorological conditions. However, research gaps exist in research regarding multiple exposures of dicamba to non-DR soybeans, which will likely increase as the DR soybeans become more widely adopted. It is also important to compare the response of soybean varieties with different herbicide resistant traits as anecdotal accounts suggest that soybeans with certain herbicide resistance traits are more sensitive than others. These gaps have led to the development of the following objectives:

1. determine non-DR soybean response to 1/1000th, 1/500th, and 1/100th field-use dose (560 g ae ha^{-1}) of dicamba;
2. determine non-DR soybean response when exposed to dicamba at V3, R1, and R3 growth stages;
3. determine non-DR soybean response when exposed to dicamba multiple times; and
4. evaluate the response of soybean varieties and traits when exposed to 1/100th field-use dose of dicamba at V3 and R1 growth stages.

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Figure 1.1 Dicamba molecule and salts: (a) dicamba parent acid, (b) dimethylamine salt of dicamba, (c) sodium salt of dicamba, (d) diglycoamine salt of dicamba, (e) diglycoamine salt of dicamba and acetic acid, (f) *N,N* Bis-(3-aminopropyl)methylamine salt dicamba.

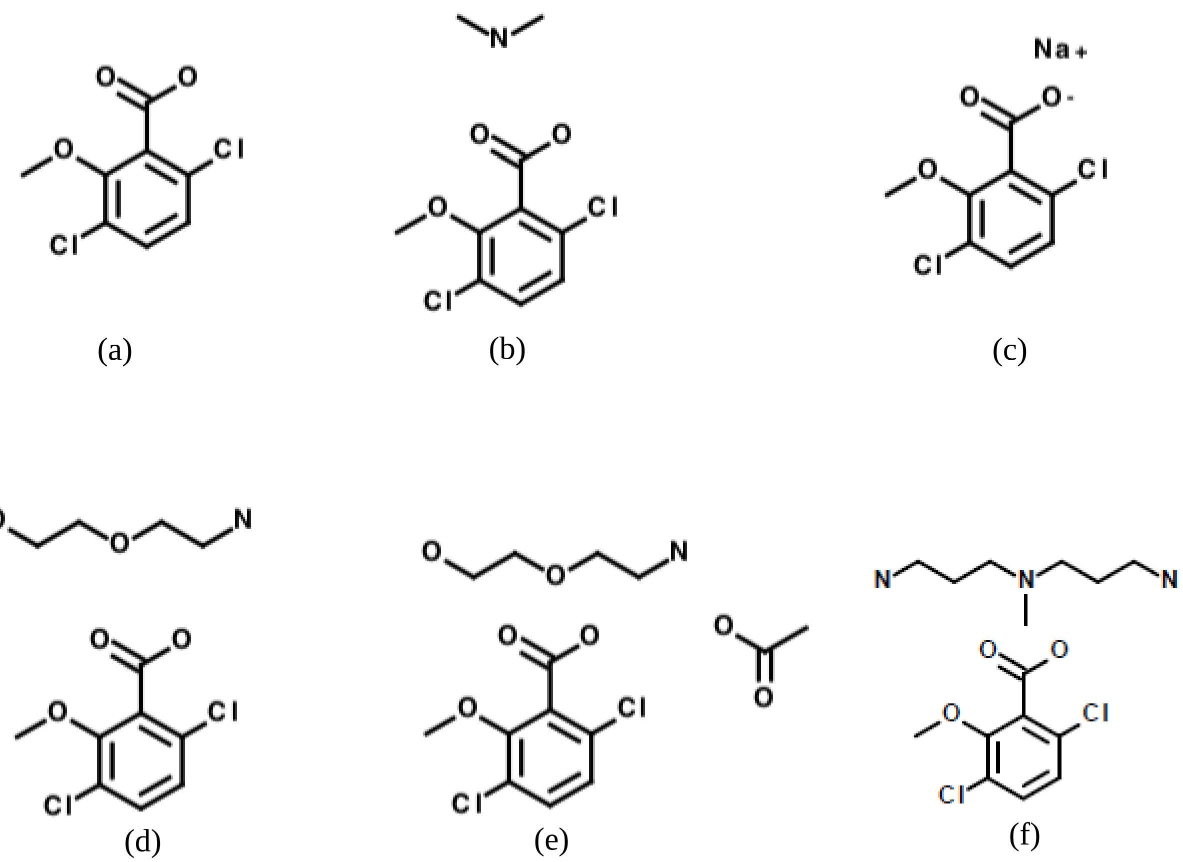

Table 1.1 Dicamba formulations with chemical names, common trade names, commercial release date, and molecular weight.

Dicamba formulation	Chemical Name	Common Trade Name	Commercial Release	Molecular weight (g mol^{-1})
Dicamba Acid	3,6-Dichloro-2-methoxybenzoic acid		1962[e]	221.04
DMA salt	Dimethylamine salt of dicamba	Banvel[a]	1967	266.12
Na salt	Sodium salt of dicamba	Banvel II[b]	1981	243.02
DGA salt	Diglycoamine salt of dicamba	Clarity[b]	1990	326.18
DGA salt + Acetic Acid	Diglycoamine salt of dicamba + Acetic Acid	Xtendimax with Vaporgrip technology[c], Fexapan with Vaporgrip technology[d]	2017	326.18
BAPMA salt	*N*, *N* Bis-(3-aminopropyl)methylamine salt dicamba	Engenia[b]	2017	366.29

[a] Arysta LifeScience, Tokyo, Japan
[b] BASF Agriculture, Florham Park, New Jersey
[c] Bayer Crop Science, St. Louis, Missouri
[d] Corteva Agriscience, Wilmington, Delaware
[e] Discovery of dicamba

Table 1.2 Application requirement additions and/or changes to dicamba labels that went into effect in 2018 to mitigate off-target movement when reduced-volatility formulations are applied to dicamba-resistant soybeans and cotton.[a]

Application Parameter	Requirement
Postemergence application window	• Dicamba tolerant soybeans: 45 days after planting or up to R1 (whichever comes first) • Dicamba tolerant cotton: 60 days after planting or up to Mid-bloom (whichever comes first)
Buffer	• 34 m downwind buffer is always required • If in county where listed endangered species are present fields will require: o 17 m buffer on all sides of the field • Do not apply if sensitive crops or residential area is downwind
Applicator Requirements	• Only certified applicators can purchase and apply
Minimum Spray Volume	• 140 liters ha^{-1}
Application Hours	• 1 hour after sunrise and 2 hours before sunset
Record Keeping Requirements	• Records must be created within 72 hours of the application • Must include planting date and the buffer distance calculation
Rainfall Amount	• Do not apply if the expected rainfall amount over the next 24 hours is expected to produce runoff
Wind Speed Limits	• 4.8 to16 km hr^{-1}
Spray Solution pH	• Use a buffer solution if solution pH is less than 5

[a] Sourced from Kansas Department of Agriculture (2018)

Chapter 2 - Soybean response to multiple dicamba applications

Introduction

Dicamba is a synthetic auxin herbicide that has been used extensively in corn, small grains, and pasture for broadleaf control since the 1960s. Widespread use of dicamba often resulted in off-target movement and injury to sensitive plants, including soybeans and cotton (Auch and Arnold 1978, Wax et al. 1969). Introduction of DR soybean and cotton dramatically increased the application of dicamba in the vicinity of actively growing susceptible soybean and cotton crops. Various injury symptoms, including epinasty of petioles and shoots, leaf malformation, terminal bud chlorosis, malformed pods and delayed maturity are associated with off-target dicamba movement to non-DR soybean (Wax et al. 1969; Solomon and Bradley 2014). Injury severity is often dependent on soybean growth stage time of observation and dicamba dose (Wax et al. 1969; Auch and Arnold 1978; Weidenhamer et al. 1989).

Greater injury is often observed in soybeans exposed to dicamba during early reproductive stages in comparison to vegetative stages (Auch and Arnold 1978, Wax et al. 1969); however, Solomon and Bradley (2014) reported similar injury symptoms when soybeans were either treated at R2 or at V3. Kniss (2018) reported dicamba doses studied in the literature range from 0.028 (Solomon and Bradley 2014) to 560 g ae ha^{-1} dicamba (Huang et al. 2016). Dicamba doses less than 1.0 g ae ha^{-1} caused 3-27% visual injury when applied at vegetative and reproductive growth stages, while dicamba doses between 1.0 and 5.6 g ae ha^{-1} resulted in 36 to 47% injury at vegetative growth stages (Kniss 2018). Solomon and Bradley (2014) reported 21 to 44% and 15 to 18% visual soybean injury 2WAT when exposed at V3 and R2 to 0.28 to 28 g ae ha^{-1}. Visual injury 4WAT ranged from 9 to 12% and 17 to 14% after exposure at V3 and R2 to the same rates.

Soybean injury is not a reliable indicator of yield loss due to the development of uninjured leaves and extended flowering period, which means worrisome injury often occurs at doses that are much lower than those required to reduce yield (Al-Khatib and Peterson 1999). When exposure occurs during vegetative growth stages, soybeans require 30% injury to reduce yield by 5%, but the same yield loss occurs with only 12% injury if exposure occurs during vegetative growth stages (Kniss 2018). Wax et al. (1969) noted that the dicamba dose required to reduce soybean yield by 20% was 70 g ae ha^{-1} at V3 compared to 2.2 g ae ha^{-1} dicamba at R2. Jones et al. (2018a) showed that 2.19 and 8.75 g ae ha^{-1} dicamba at R1 resulted in an average of 16% yield loss; however, those same dicamba doses at R3 resulted in an average of 7% yield loss.

Quantifying soybean yield components provides greater understanding of the impacts of dicamba exposure on soybean yield (Robinson et al. 2013). Solomon and Bradley (2014) reported that dicamba applied at R2 reduced the number of seeds pod^{-1} at all doses evaluated, but no reductions were observed when dicamba was applied at V3. Similarly, pods plant^{-1}was not affected at V3, but was reduced by 2.8 and 28 g ae ha^{-1} dicamba applied at R2; however, no clear trend was observed for soybean seed weight in that experiment.

The effects of off-target dicamba movement on soybean offspring are not well understood; but, understanding the impacts is crucial for soybean seed production (Jones et al. 2018a). Dicamba exposure during reproductive growth stages has been shown to reduce germination, emergence, and vigor of offspring (Wax et al. 1969, Jones et al. 2018a, Auch and Arnold 1978, Thompson and Egli 1973). Dicamba-contaminated soybean seed may have the ability to germinate and emerge, but parent soybean exposure to dicamba may result in reduced seedling vigor and seedling injury. Jones et al. (2018a, 2019a) reported negative impacts following soybean exposure to 2.2 and 8.8

g ae ha^{-1} dicamba at R1, R3, and R5, with greater reductions in vigor and seedling injury observed at later application timings and higher doses, even though no dicamba was detected in the seed.

Despite numerous studies quantifying soybean response to dicamba, there are still research gaps regarding response of non-DR soybeans to multiple dicamba exposures. Therefore, the main objective of this research was to determine the response of non-DR soybeans to single and multiple exposures of dicamba at 0.56, 1.12, and 5.6 g ae ha^{-1} at V3, R1, and R3 growth stages.

Materials and Methods

Field studies were conducted at the Kansas State University Ashland Bottoms Research Farm in Manhattan, Kansas (latitude:39.12 and longitude:-96.63) during 2018 (MHK18) and 2019 (MHK19) and at the Kansas State University East Central Experiment Field in Ottawa, Kansas (OTT19; latitude 38.54 and longitude: -95.25) during 2019. At all site-years, Credenz 3841LL (glufosinate resistant) soybeans (BASF Corp., Research Triangle Park, NC) were planted at approximately 308,880 seeds ha^{-1}, 3.8 cm deep, and in 76.2 cm rows using a 4-row row crop planter. This variety was selected because it is commonly grown in eastern Kansas, is high yielding, and was suspected to exhibit high susceptibility to dicamba in comparison to similar varieties. Seed beds were prepared using a field cultivator on the day of planting in Manhattan; whereas, a field cultivator was used before the early pre-plant herbicide application in Ottawa. Information regarding planting dates, soil properties, and fertilizers for each location is summarized in table 2.1 and pre-emergent herbicide treatments for early-season weed control are summarized in table 2.2. Plots were kept weed free with hand weeding throughout each growing season across all locations.

The *N*,*N*-Bis-(3-aminopropyl) methylamine salt (BAPMA) of dicamba (Engenia, BASF Corp., Research Triangle Park, NC) was applied at three doses on three soybean growth stages in a single or sequential manner. Dicamba doses were 0.56, 1.12, and 5.6 g ae ha^{-1}, which are equivalent to 1/1000X, 1/500X, and 1/100X of a 1X field-use dose (560 g ae ha^{-1} of dicamba, respectively. Applications were made at V3, R1, R3, V3 followed by (*fb*) R1, V3 *fb* R3, R1 *fb* R3, and V3 *fb* R1 *fb* R3 (Fehr and Caviness 1977). Treatments with multiple application timings received the same dose of dicamba at each application timing. Treatments were arranged in a factorial arrangement of dicamba doses and application timings with a randomized complete block (RCB) design and 4 replications at each location. Individual plots were 3 m by 9 m in size. Each block contained a non-treated check plot. Dates of dicamba application and environmental conditions during application for each location are presented in Table 2.3. Environmental conditions were obtained from the Kansas Mesonet (Kansas State University 2019).

Reduced dicamba doses were achieved by initially producing a stock solution of the field use dose (1X = 560 g ae ha^{-1}) and diluting with water to obtain 0.56 (1/1000X), 1.12 (1/500X), and 5.6 g ae ha^{-1} (1/100X). Spray solution was applied directly to plots at a spray volume of 140 L ha^{-1} using a CO_2 powered back pack sprayer and a 4 tip, 1.9 m hand-held boom equipped with TTI110015 nozzles operated at 220.6 kPa. The center 2 rows of each plot received the full dose while the two outer rows received a partial dose and acted as a buffer between treatments.

Soybean injury was visually assessed weekly following the initial dicamba application until the onset of senescence. Soybeans were evaluated on a 0 to 100% crop injury scale with 0% indicating no injury and 100% indicating plant death. Symptomology at lower injury levels included leaf cupping, leaf crinkling, and chlorosis of terminal buds. Symptomology at greater injury levels included the aforementioned symptomology and necrosis of terminal buds, pod malformation, and stunting. To further evaluate soybean response to dicamba applications, the heights of five randomly-selected plants were recorded at the onset of senescence. Yield component data were collected at harvest by harvesting mature soybeans from 1 m row^{-1} from the two center rows of each plot. Yield components collected included: 100-seed weight, number of pods $plant^{-1}$, seeds pod^{-1}, and total main stem nodes $plant^{-1}$. Soybeans were harvested for grain yield with a small-plot combine and moisture was adjusted to 13%. Soybean height, yield, and yield component data were converted to a relative percent of the non-treated check before statistical analysis. Percent reduction was calculated using equation 2.1, where plot data (PLOT) and the non-treated check (NTC) plot within each replication were used.

Equation 2.1

$$1 - ((NTC - PLOT) / NTC) \tag{2.1}$$

To test seed germination, 50 g soybean seed samples were taken from each plot and sent to the Seed Laboratory at the Kansas Crop Improvement for analysis. One-hundred soybean seeds were counted and placed on Kimpack (Anchor Paper Co., St. Paul, MN) which was placed on a food tray and moistened with 500 ml tap water. Soybeans seeds were then covered with 0.6 to 1.3 cm of mason sand. Trays were placed in a germination chamber at 30°C for 8 h (hours) and 20°C for 16 h, where lights came on with the warm cycle. After 8 d (days) of incubation, seedlings were evaluated to determine normal, abnormal, dead or hard seed (AOSA 2019)

To quantify response of offspring, 10 seeds from each plot were planted into 14-cm pots containing Miracle-Gro Moisture Control potting mix (The Scotts Company LLC, Marysville, OH) and grown in the Kansas State University Weed Science greenhouse until V3. Pots were arranged by site-year within the greenhouse. Daytime temperature was 30°C and night time temperature was 22.2°C with a photoperiod of 15 h. The number of injured offspring and percent visual injury of emerged offspring were recorded. Soybeans were evaluated on a 0 to 100% crop injury scale with 0% indicating no injury and 100% indicating plant death. Symptomology at lesser injury levels included leaf cupping, leaf crinkling, and chlorosis of terminal buds.

Statistical analysis

Visual injury ratings, soybean height, soybean yield components, soybean yield, germination, offspring emergence, offspring height, number offspring injured, and visual injury of offspring were subjected to an ANOVA using PROC GLIMMIX in SAS (SAS 9.4, SAS® Institute Inc.). Replication was considered a random effect and site-year, dicamba application timing, and dicamba dose were considered fixed effects. When there was a significant interaction with site-year, site-years were analyzed separately. If there was not an interaction with site-year, data were pooled across site-years. Means were separated using Fisher's protected LSD test (α = 0.05). Visual injury ratings, soybean height, soybean yield components, soybean yield, germination, offspring emergence, offspring height, number of offspring injured, and visual injury of offspring were subjected to PROC CORR in SAS (SAS 9.4, SAS® Institute Inc.). If less than 0.3, Pearson coefficients were considered weak. If greater than 0.3 but less than 0.5, Pearson coefficients were considered moderate. If greater than 0.5, Pearson coefficients were considered strong. Strong Pearson coefficients with levels of significance less than 0.05 will be bolded in correlation tables. Yield and height data were subjected to PROC REG in SAS (SAS 9.4, SAS® Institute Inc.) where a linear model was considered to be the best fit based on r-square values and p-values.

Results and Discussion

Environmental Conditions

Precipitation at Manhattan was more timely and evenly distributed in 2019 than 2018. Rainfall occurring between planting and the onset of senescence totaled 432 mm at MHK18 and 451 mm at MHK19; both were less than the 30-year average for rainfall of 475 mm from May to September (National Climatic Data Center 2011; Figure 2.1 and 2.2). During MHK18, a majority of the rainfall was received after R5/R6 and delayed harvest of the soybean plots. Two large rain events before the onset of the reproductive stages and several smaller events during the reproductive stages at MHK19. At the OTT19 location, the rainfall totaled 667 mm from planting to the onset of senescence, which was much greater than the 30-year average for rainfall of 475 mm from May to September (National Climatic Data Center 2011; Figure 2.3). There were several rain events prior to the onset of the reproductive stages. There were also several smaller rain events that occurred throughout the course of the reproductive and one larger event that took place right before R3 that totaled 131 mm.

Visual Injury

There was an interaction among location, time of application, and dicamba dose for visual injury 2 WAT, 4 WAT, and at the onset of senescence (Table 2.4); therefore, results were analyzed and presented separately for each location.

MHK18

Application of 0.56 g ae dicamba ha^{-1} resulted in 8 to 44% visual injury 2 WAT with the greatest injury occurring from exposure at V3 *fb* R1 *fb* R3 and the least injury from exposure at V3 or R1 (Table 2.5). Similarly, application of 1.12 g ae ha^{-1} dicamba resulted in 9 to 53% soybean injury 2 WAT, with the greatest injury at V3 *fb* R1 *fb* R3 and the least injury at V3 or R1. Exposure to 5.6 g ae ha^{-1} dicamba resulted in 20 to 75% visual injury with the greatest injury at V3 *fb* R1 *fb* R3 and the least injury occurring R1.

At 4WAT, exposure at V3 *fb* R1 *fb* R3 resulted in the greatest injury and exposure at V3 resulted in the least injury, regardless of dose (Table 2.5). Application of 0.56 g ae ha^{-1} dicamba resulted in 17 to 58% visual injury. Injury following application of 1.12 g ae ha^{-1} dicamba resulted in 23 to 64% visual injury. When 5.6 g ae ha^{-1} dicamba were applied, 32 to 80% visual injury was observed.

Visual injury observed at the onset of senescence as a result of treatment with 0.56 g ae ha^{-1} dicamba was 5 to 53% with the greatest injury from exposure at V3 *fb* R1 *fb* R3, while the least injury resulted from exposure at V3 (Table 2.5). Treatment with 1.12 g ae ha^{-1} dicamba resulted in 5 to 64% visual injury with the greatest injury from exposure at V3 *fb* R1 *fb* R3 and R1 *fb* R3 and the least injury from exposure at V3. Application of 5.6 g ae ha^{-1} dicamba resulted in 10 to 76% visual injury with the greatest injury from exposure at V3 *fb* R1 *fb* R3 and the least injury from exposure at V3.

MHK19

Visual injury 2 WAT following application of 0.56 g ae ha^{-1} dicamba was 6 to 38%, with the greatest injury from exposure at V3 *fb* R1 *fb* R3 and the least injury from exposure at V3 and R1 (Table 2.6). Treatment with 1.12 g ae ha^{-1} dicamba resulted in 13 to 54% visual injury with the greatest injury from exposure at V3 *fb* R1 *fb* R3 w and the least injury from exposure at V3 and R1. Following treatment with 5.6 g ae ha^{-1} dicamba, soybeans showed 28 to 76% visual injury with the greatest injury from exposure at V3 *fb* R1 *fb* R3 and the least injury from exposure at V3 and R1.

Application of 0.56 g ae ha^{-1} dicamba resulted in 9 to 40% 4 WAT, with the greatest injury resulting from exposure at V3 *fb* R1 *fb* R3, while the least injury was from exposure at V3 (Table 2.6). When treated with 1.12 g ae ha^{-1} dicamba, soybeans showed 14 to 49% visual injury with the greatest injury from exposure at V3 *fb* R1 *fb* R3 and the least injury from exposure at V3 and R3. Treatment with 5.6 g ae ha^{-1} dicamba resulted in 30 to 78% visual injury with the greatest injury from exposure at V3 *fb* R1 *fb* R3 and the least injury from exposure at R3.

At the onset of senescence, treatment with 0.56 g ae ha^{-1} dicamba resulted in 4 to 41% visual injury with the greatest injury from exposure at V3 *fb* R1 *fb* R3 and R1 *fb* R3 and the least injury from exposure at V3 (Table 2.6). Treatment with 1.12 g ae ha^{-1} dicamba resulted in 5 to 49% visual injury with the greatest injury from exposure at V3 *fb* R1 *fb* R3 and the least injury from exposure at V3. When treated with 5.6 g ae ha^{-1} dicamba, soybeans showed 5 to 78% visual injury with the greatest injury from exposure at V3 *fb* R1 *fb* R3 and the least injury from exposure at V3.

OTT19

Visual injury 2 WAT with 0.56 g ae ha^{-1} dicamba resulted in 3 to 40% with the greatest injury from exposure at V3 *fb* R1 *fb* R3 and R1 *fb* R3 and the least injury from exposure at V3 at OTT19 (Table 2.7). Treatment with 1.12 g ae ha^{-1} dicamba resulted in 7 to 54% visual injury with the greatest injury from exposure at V3 *fb* R1 *fb* R3 and the least injury from exposure at V3. Soybeans treated with 5.6 g ae ha^{-1} dicamba showed 26 to 73% visual injury with the greatest injury from exposure at V3 *fb* R1 *fb* R3 and the least injury from exposure at V3.

At 4WAT with 0.56 g ae ha^{-1} dicamba resulted in 11 to 48% visual injury with the greatest injury from exposure at V3 *fb* R1 *fb* R3 and R1 *fb* R3 and the least injury from exposure at V3 (Table 2.7). Treatment with 1.12 g ae ha^{-1} dicamba resulted in 15 to 64% visual injury with the greatest injury from exposure at V3 *fb* R1 *fb* R3 and the least injury from exposure at V3. Application of 5.6 g ae ha^{-1} dicamba resulted in 32 to 81% visual injury with the greatest injury from exposure at V3 *fb* R1 *fb* R3 and the least injury from exposure at V3.

At the onset of senescence, treatment with 0.56 g ae ha^{-1} dicamba resulted in 5 to 46% visual injury with the greatest injury from exposure at R1 *fb* R3 and the least injury from exposure at V3 (Table 2.7). Dicamba exposure 1.12 g ae ha^{-1} dicamba resulted in 5 to 55% visual injury with the greatest injury from exposure at V3 *fb* R1 *fb* R3 and the least injury from exposure at V3. Similarly, application of 5.6 g ae ha^{-1} dicamba resulted in 5 to 75% visual injury with the greatest injury from exposure at V3 *fb* R1 *fb* R3 and the least injury from exposure at V3.

Multiple dicamba exposure at V3 *fb* R1 *fb* R3 growth stages resulted in the greatest injury across all locations regardless of the evaluation timing or dose. Exposure at V3 *fb* R1 *fb* R3 to 5.6 g ae ha^{-1} dicamba resulted in greater injury across all location and evaluations timings than previously reported, with the exception of Andersen et al. (2004) who observed an average of 43 or 48% injury at about 2 WAT when soybeans were exposed to 5.6 or 11.2 g ae ha^{-1} dicamba at V3/V4. In some cases, visual soybean injury observed following exposure at R1 and R3 was greater than what was observed by McCown et al. (2018) and Jones et al. (2018a) when soybeans were exposed to similar doses. McCown et al. (2018) reported an average of 43 and 18% injury 4 WAT when soybeans were treated at R1 and R4, respectively. Jones et al. (2018a) reported an average of 27% to 37% visual injury in soybeans treated at R1 and an average of 8% to 10% visual injury in soybeans treated at R3 after exposure to 2.19 and 8.75 g ae ha^{-1} dicamba.

Soybeans treated at V3 were able to almost completely recovered. Our results are in agreement with Al-Khatib and Peterson (1999) and Soltani et al. (2016) who observed end-season recovery in soybeans treated at V3 with lower doses of dicamba. While it may be possible for soybeans to recover from a dicamba drift event during early vegetative growth stages, injury may still persist to the end of the season and yield reduction may not be avoided.

Height

For relative soybean height, there was a significant interaction among location, dicamba dose, and time of application (Table 2.8). Results were analyzed and presented separately for each location.

MHK18

Dicamba exposure at 0.56 g ae ha^{-1} dicamba resulted in 11 to 29% reduction in height, with the greatest reduction at V3 *fb* R1 *fb* R3 and the least reduction was observed in soybeans treated at V3, R1, R3, and V3 *fb* R1 (Figure 2.4). Application of 1.12 g ae ha^{-1} dicamba resulted in 11 to 34% reduction in height, with the greatest reduction at V3 *fb* R1 *fb* R3 and R1 *fb* R3 and the least reduction was observed in soybeans treated at V3. Treatment with 5.6 g ae ha^{-1} dicamba resulted in 23 to 62% reduction in height, with the greatest reduction at V3 *fb* R1 *fb* R3 and R1 *fb* R3 and the least reduction was observed in soybeans treated at V3 and R3.

MHK19

Soybean exposure to 0.56 g ae ha^{-1} dicamba resulted in 4 to 19% reduction in height, with the greatest reduction at V3 *fb* R1 *fb* R3 and the least reduction in soybeans treated at V3, R1, R3, and V3 *fb* R1 (Figure 2.5). Treatment with 1.12 g ae ha^{-1} dicamba resulted in 6 to 32% reduction in height, with the greatest reduction at V3 *fb* R1 *fb* R3 and R1 *fb* R3, while the smallest reduction was observed in soybeans treated at V3 and R3. Soybean exposure to 5.6 g ae ha^{-1} dicamba resulted in 13 to 63% reduction in height, with the greatest reduction at V3 *fb* R1 *fb* R3 and the least reduction was observed in soybeans treated at V3 and R3.

OTT19

Applications of 0.56 g ae ha^{-1} dicamba resulted in 5 to 14% reduction in height, with the greatest reduction at V3 *fb* R1 *fb* R3 and R1 *fb* R3 and the least reduction was observed in soybeans treated at V3 and V3 *fb* R3 (Figure 2.6). Soybean exposure to 1.12 g ae ha^{-1} dicamba resulted in 3 to 30% reduction in height, with the greatest reduction at V3 *fb* R1 *fb* R3, while the smallest reduction was observed in soybeans treated at V3. Treatment with 5.6 g ae ha^{-1} dicamba resulted in 8 to 62% reduction in height, with the greatest reduction at V3 *fb* R1 *fb* R3, while the smallest reduction was observed in soybeans treated at V3.

Previous research reported greater reductions in height as result of exposure at V3. Wax et al. (1969) observed a 17% reduction in soybean height when soybeans at V3 were exposed to 2.2 g ae ha^{-1} dicamba. Kelley et al. (2005) observed 21 to 22% reduction in height when soybeans were exposed at V3 to 5.6 g ae ha^{-1} dicamba. However, height reductions as a result of exposures during R1 and R3 to 0.56 and 1.12 g ae ha^{-1} dicamba were similar to those observed by Jones et al. (2018) and McCown et al. (2018). Jones et al. (2018a) and McCown et al. (2018) showed that soybean exposure to 2.19 and 8.75 g ae ha^{-1} dicamba during R1 and R3 resulted in an average of 32 to 35% and 11 to 14% height reduction .

Herbicide exposure at V3 and R1 appear to have the greatest influence on height reductions following multiple exposures because height reductions as a result of a single treatment at R3 were minimal. This is likely due to the cessation of vegetative growth that occurs after R1.

Yield and Yield Components

A significant interaction between dose and time of application occurred for relative yield (Table 2.8). Exposure to 0.56 g ae ha^{-1} dicamba averaged 11% or less yield regardless of application timing across all locations (Figure 2.7). Exposure to 1.12 g ae ha^{-1} dicamba resulted in 4 to 19% yield loss, with the greatest yield loss observed following dicamba exposure at R1 *fb* R3 and V3 *fb* R1 *fb* R3 and the least yield loss observed following dicamba exposure at V3 and V3 *fb* R3. Exposure to 5.6 g ae ha^{-1} dicamba resulted in 3 to 53% yield loss, with the greatest yield loss observed in V3 *fb* R1 *fb* R3 and V3 *fb* R1 and the least reduction in yield was observed in V3 and R3. Multiple dicamba exposures, especially those including R1, and a single application at R1 resulted in the greatest yield losses when treated with both 1.12 and 5.6 g ae ha^{-1} dicamba.

Soybean injury is not a reliable indicator of yield loss because later-developing leaves are not injured and flowering occurs over an extended period of time, which means worrisome injury can occur at doses that are much lower than those required to reduce yield (Al-Khatib and Peterson 1999). Soybean injury greater than 30% was required to cause 5% yield loss when exposure occurred during vegetative stages. However, soybeans exposed during reproductive stages had 5% yield loss when exposed to doses that caused 12% injury (Kniss 2018). Also, Wax et al. (1969) noted that soybeans in early reproductive stages were 8 times more susceptible to yield loss than soybean in vegetative stages. Treatment of soybeans two and three times with 5.6 g ae ha^{-1} resulted in a soybeans being exposed to a total amount of 11.2 and 16.8 g ae ha^{-1} dicamba, respectively. Yield loss as a result of the multiple exposures, especially when treated with 5.6 g ae ha^{-1} dicamba, were similar to previous research where doses greater than 5.6 g ae ha^{-1} were used (Solomon and Bradley 2014; Jones et al. 2018a).

For reduction of pods $plant^{-1}$, there was only a significant effect of dicamba dose (Table 2.8). Soybean exposure to 0.56, 1.12, and 5.6 g ae ha^{-1} dicamba resulted in 1%, 4%, and 7% reduction in pods $plants^{-1}$. These results were consistent with Solomon and Bradley (2014), who reported minimal to no reductions in pods $plant^{-1}$ when exposed to dicamba doses ranging from 0.028 to 28 g ae ha^{-1} dicamba at V3. Kelley et al. (2005) also observed minimal reduction in pods $plant^{-1}$ after exposure at V3 to 5.6 g ae ha^{-1} dicamba; however, exposure at R2 to 2.8 g ae ha^{-1} and 28 g ae ha^{-1} dicamba resulted in 19% and 72% reduction in pods $plant^{-1}$ in that study.

For relative nodes $plant^{-1}$ there was a significant interaction among location, time of application, and dose (Table 2.8). Due to the interaction with location, results were analyzed and presented separately for each location. Exposure at V3 to 0.56 and 1.12 g ae ha^{-1} resulted in 5% or less reduction in nodes $plant^{-1}$ regardless of site-year (Table 2.10). Treatment at V3 *fb* R1 *fb* R3 at

5.6 g ae ha^{-1} resulted in the greatest reduction of nodes $plant^{-1}$ at MHK19 and OTT19 with reductions of 43% and 60%, but at MHK18 V3 *fb* R3 treated with 5.6 g ae ha^{-1} dicamba resulted in the greatest reduction of nodes $plant^{-1}$ with a reduction of 49%. Robinson et al. (2013) reported that nodes m^{-2} decreased in sensitivity as soybeans mature from vegetative stages to reproductive stages. For example, it was shown that nodes m^{-2} were reduced by 5% after exposure to 0.020, 0.847, and 1.14 g ae ha^{-1} dicamba at V2, V5, and R2, respectively. These dicamba doses were similar to the lower doses used in this trial, 0.56 and 1.12 g ae ha^{-1} dicamba, which resulted in increased nodes $plant^{-1}$ after exposure at V3 and V3+R1. Robinson et al. (2013) also observed 20% reduction of nodes m^{-2} when V2, V5, and R2 soybeans were exposed to 1.45, 1.94, and 3.82 g ae ha^{-1} dicamba, which suggests that a larger dose, more similar to 5.6 g ae ha^{-1} dicamba, could result in reductions similar to those observed in this study.

There was a significant interaction between location and dose for relative seed weight (Table 2.8). Results were analyzed and presented separately for each location. However, means separation did not show significant differences among doses (Table 2.11). Robinson et al. (2013) noted that soybean seed weight decreased as dicamba doses increased, but was not as responsive as other yield components such as seed m^{-2}, nodes m^{-2}, and seeds pod^{-1}.

There was significant interaction between site-year and dose, as well as an interaction between dicamba dose and time of application for relative seed pod^{-1}(Table 2.8). At MHK18, soybean exposure at 0.56, 1.12, and 5.6 g ae ha^{-1} resulted in 3, 9, and 12% reduction in seed pod^{-1}, respectively (Table 2.12). There were no significant differences among doses at MHK19. Soybean exposure to 0.56, 1.12, and 5.6 g ae ha^{-1} resulted 8, 10, and 17% reduction in seeds pod^{-1}, respectively. The greatest reduction in seed pod^{-1} was observed in soybeans that were treated at R1, R1 *fb* R3, and V3 *fb* R1 *fb* R3 with 5.6 g ae ha^{-1} dicamba with reductions of 11, 21, and 23%, respectively. Treatment at V3 did not result any reduction of seeds pod^{-1} regardless of dose (Table 2.13). All other treatments had 10% or less reduction of seeds pod^{-1}. Solomon and Bradley (2014) also reported minimal reductions in seeds pod^{-1} after exposure at V3 to doses that ranged 0.028 g ae ha^{-1} to 28 g ae ha^{-1} dicamba. Applications of 5.6 g ae ha^{-1} dicamba at R1 and R1 *fb* R3 resulted in reduction similar reductions reported by Robinson et al. (2013) and Solomon and Bradley (2014) when soybeans were treated at R2, but treatment at V3 *fb* R1 *fb* R3 resulted in significantly higher reductions in seeds pod^{-1}. Robinson et al. (2013) reported 10% reduction in seeds pod^{-1} after exposure to 7.35 g ae ha^{-1} dicamba. Solomon and Bradley (2014) reported 9 to 12% reduction when soybeans were exposed at R2 to doses that ranged from 0.028 g ae ha^{-1} to 2.8 g ae ha^{-1} dicamba. Exposure during early vegetative stages had less impact on seeds pod^{-1} than exposures to larger doses that occur during reproductive stages.

There were strong correlations between injury, height, yield components, and yield loss (Table 2.14). There was also a strong correlation and linear relationship (Figure 2.8) between height reduction and yield loss indicating that greater reductions in height resulted in greater yield losses. As height increased by 1 cm, yield increased by 39 kg ha^{-1} (Equation 2.2).

Equation 2.2

$$y = 503.44845 + 38.73966x \qquad (2.2)$$

Reduction in height was strongly correlated with injury 2 WAT, 4 WAT, at the onset of senescence, and nodes $plant^{-1}$. There was also a correlation between nodes $plant^{-1}$ and injury at senescence. Robinson et al. (2013) noted that seeds m^{-2}, pods m^{-2}, and nodes m^{-2} need to be characterized in order to understand the total effect of dicamba exposures on non-DR soybeans.

Effects on offspring

Soybean germination, emergence, visual injury, number of offspring injured, and height were collected to characterize the effects of parent soybean exposure to dicamba on offspring.

Offspring response for MHK18 could not be characterized due to poor seed quality as a result of poor harvest conditions and soybean diseases, specifically purple stain caused by *Cercospora kikuchii*. However, soybean offspring collected from MHK19 and OTT19 were be characterized (Appendix A.4 and A.5). There were no significant differences observed among location, dose, and timing of application for reduction in germination, reduction in offspring emergence, number of offspring injured, offspring visual injury, and reduction in offspring height (Appendix A.6). Results were not consistent with previous research characterizing the effects of parent soybean exposure to dicamba on offspring, which may be due to differences in application timing and dicamba doses used. Jones et al. (2019a) hypothesized that parent soybean exposure during R5/R6 may be the most detrimental to the soybean seed production industry because visual injury of the parent soybean will be harder to detect, it may not impact germination or emergence, and visual injury of offspring may not be expressed until V1 or later.

Conclusion

Although the addition of DR soybeans gives producers more options to control herbicide resistant weeds, it puts non-DR soybean producers at a greater risk for off-target injury from dicamba treatments. With the common practice of double-crop soybeans, producers of non-DR soybeans are even at more risk for multiple exposures and exposure at later growth stages. Soybeans treated at R1 were more susceptible to dicamba than those treated at V3 and R3. Soybeans exposed multiple times resulted in the greatest injury. Injury 2 WAT, 4 WAT, and at the onset of senescence was correlated with height, as injury increased height decreased. Injury levels at senescence were also strongly correlated with yield loss, which indicates that as injury at the onset of senesce increased yield loss would increase. Of the yield components impacted, the greatest reductions were observed in nodes plant^{-1} and seeds pod^{-1}. Impact on offspring of soybeans exposed to dicamba was minimal and showed no differences among treatments. It is critical that applicators follow mandatory application guidelines to not only protect producers of non-DR soybeans but, to preserve the DR technology.

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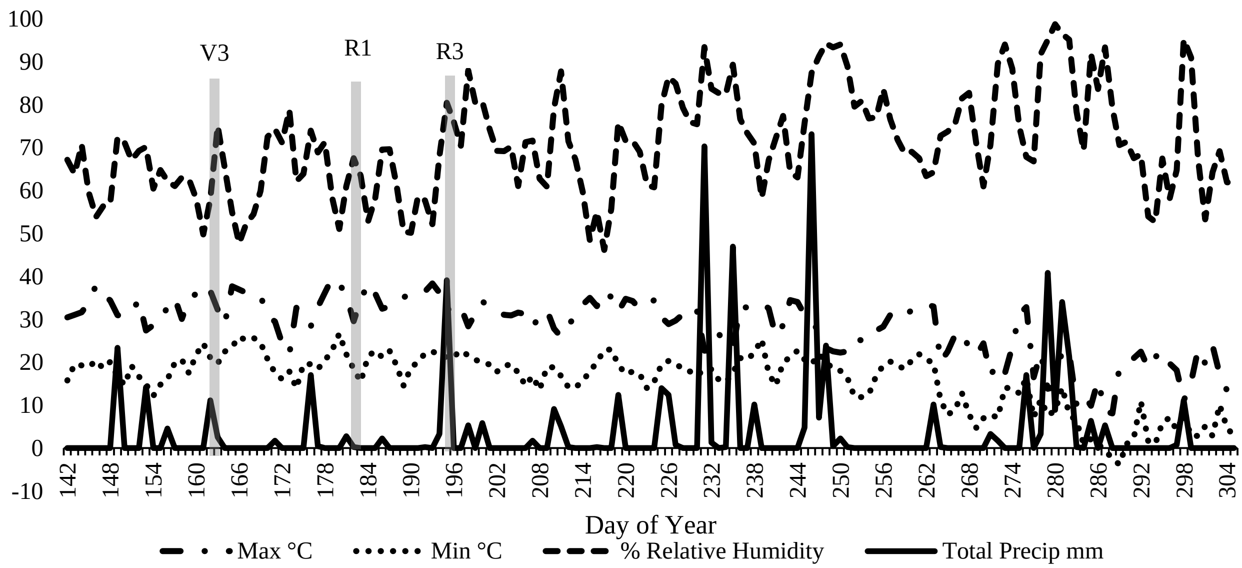

Figure 2.1 Daily maximum temperature, minimum temperature, percent relative humidity, total precipitation from the time of planting to harvest with corresponding application dates at Manhattan, KS (MHK18) in 2018.[a]

[a]Kansas State University (2019)

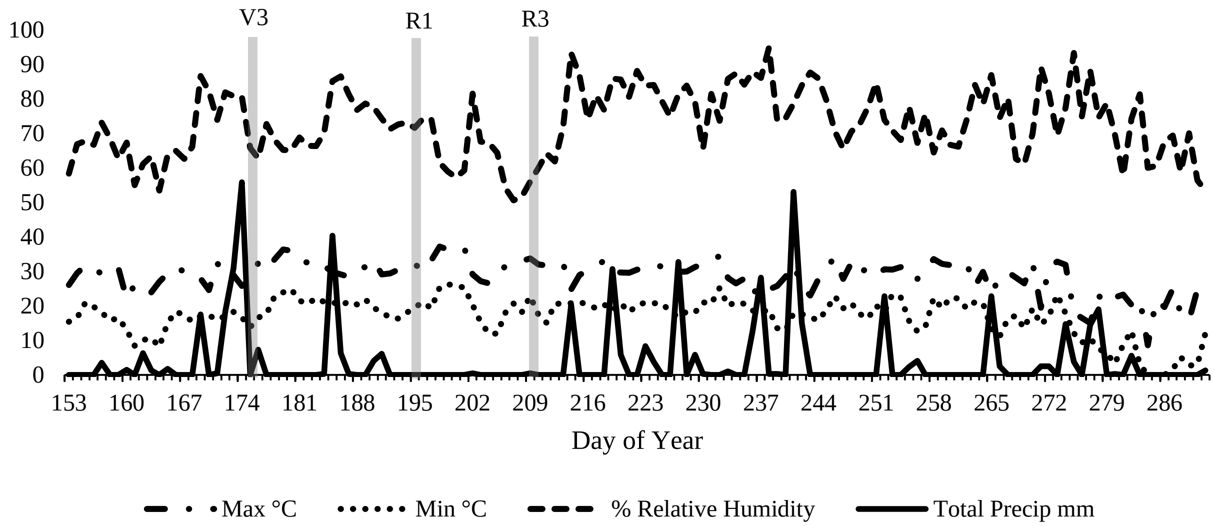

Figure 2.2 Daily maximum temperature, minimum temperature, percent relative humidity, total precipitation from the time of planting to harvest with corresponding application dates at Manhattan, KS (MHK19) in 2019.

[a]Kansas State University (2019)

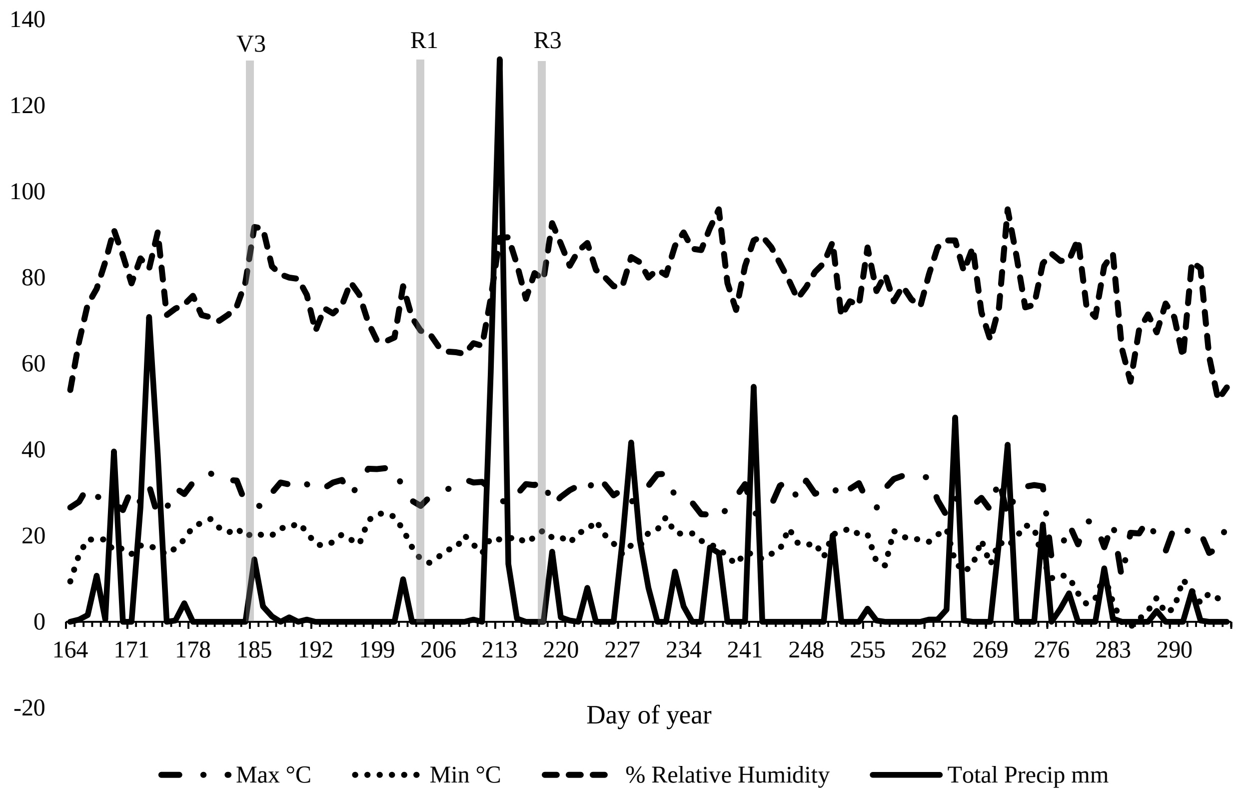

Figure 2.3 Daily maximum temperature, minimum temperature, percent relative humidity, total precipitation from the time of planting to harvest with corresponding application dates at Ottawa, KS (OTT19) in 2019.

[a]Kansas State University (2019)

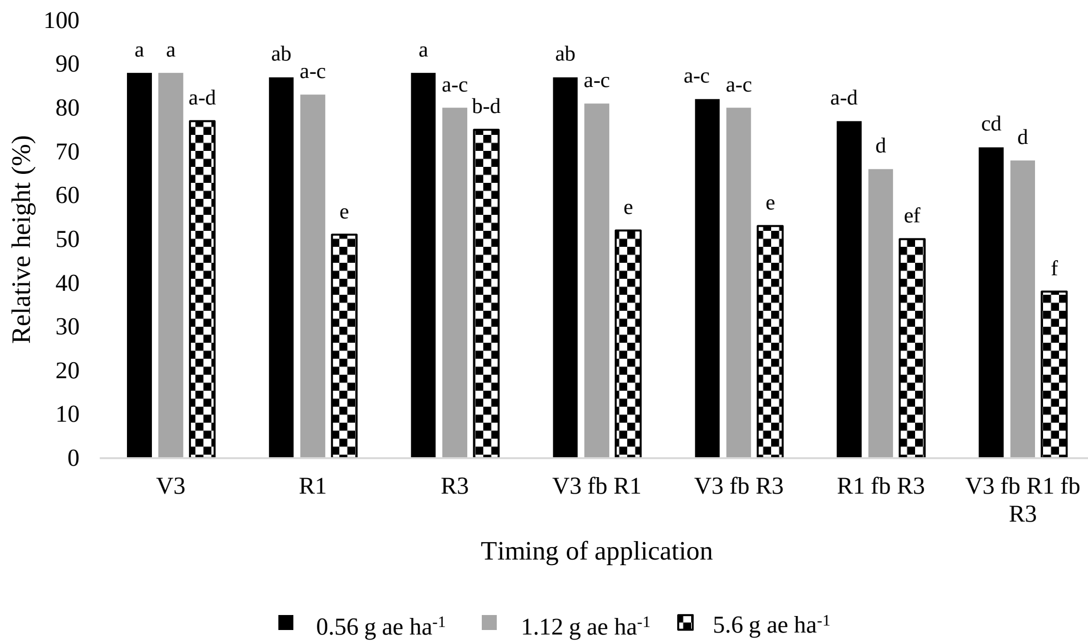

Figure 2.4 Relative soybean height as a result of exposure to reduced doses of dicamba at varying growth stages with single and multiple exposures at Manhattan, KS (MHK18) in 2018.[a,b]

[a] Means followed by the same letter are not statistically different according to Fisher's Protected LSD ($\alpha = 0.05$).
[b] Non-treated check height = 78 cm

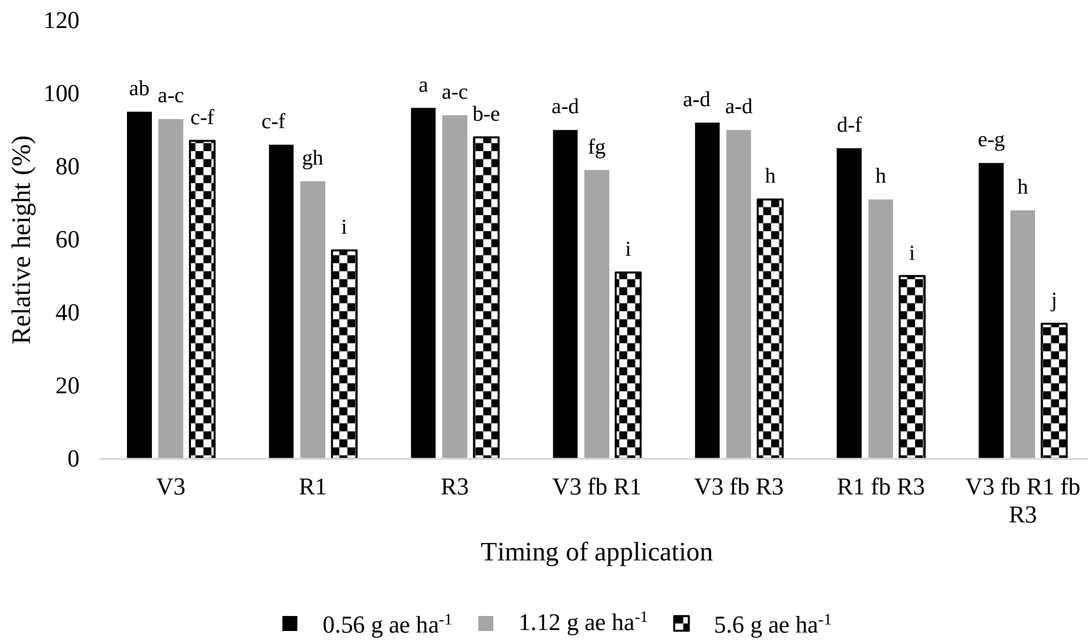

Figure 2.5 Soybean relative height as a result of exposure to reduced doses of dicamba at varying growth stages with single and multiple exposures at Manhattan, KS (MHK19) in 2019.[a,b]

[a] Means followed by the same letter are not statistically different according to Fisher's Protected LSD ($\alpha = 0.05$).
[b] Non-treated check height = 114 cm

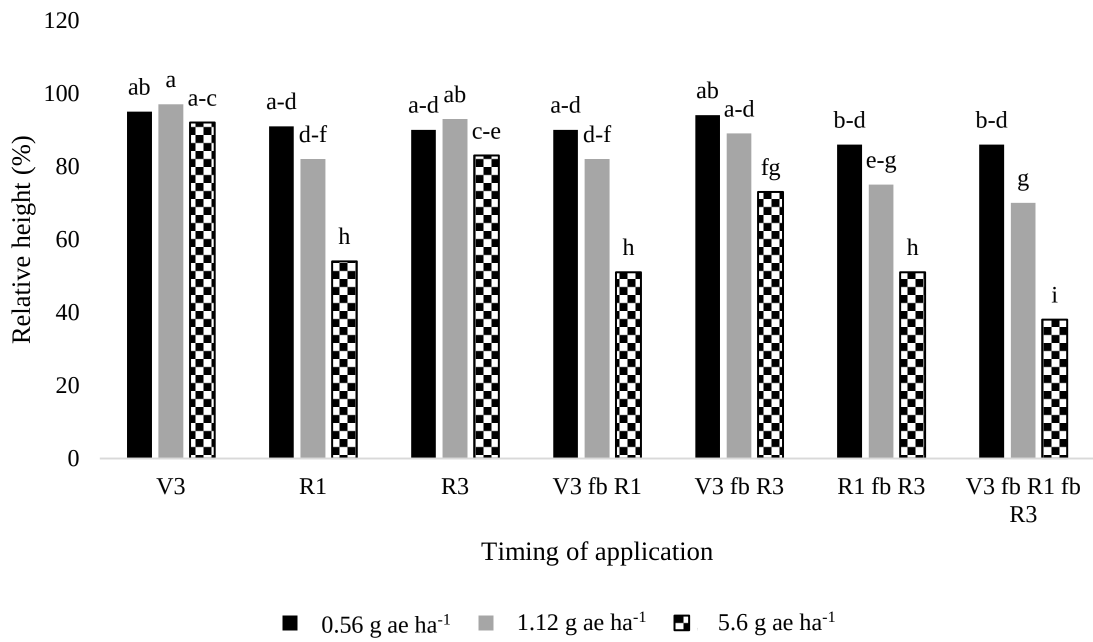

Figure 2.6 Relative soybean height as a result of exposure to reduced doses of dicamba at varying growth stages with single and multiple exposures at Ottawa, KS (OTT19) in 2019.[a,b]

[a] Means followed by the same letter are not statistically different according to Fisher's Protected LSD ($\alpha = 0.05$).
[b] Non-treated check height = 100 cm

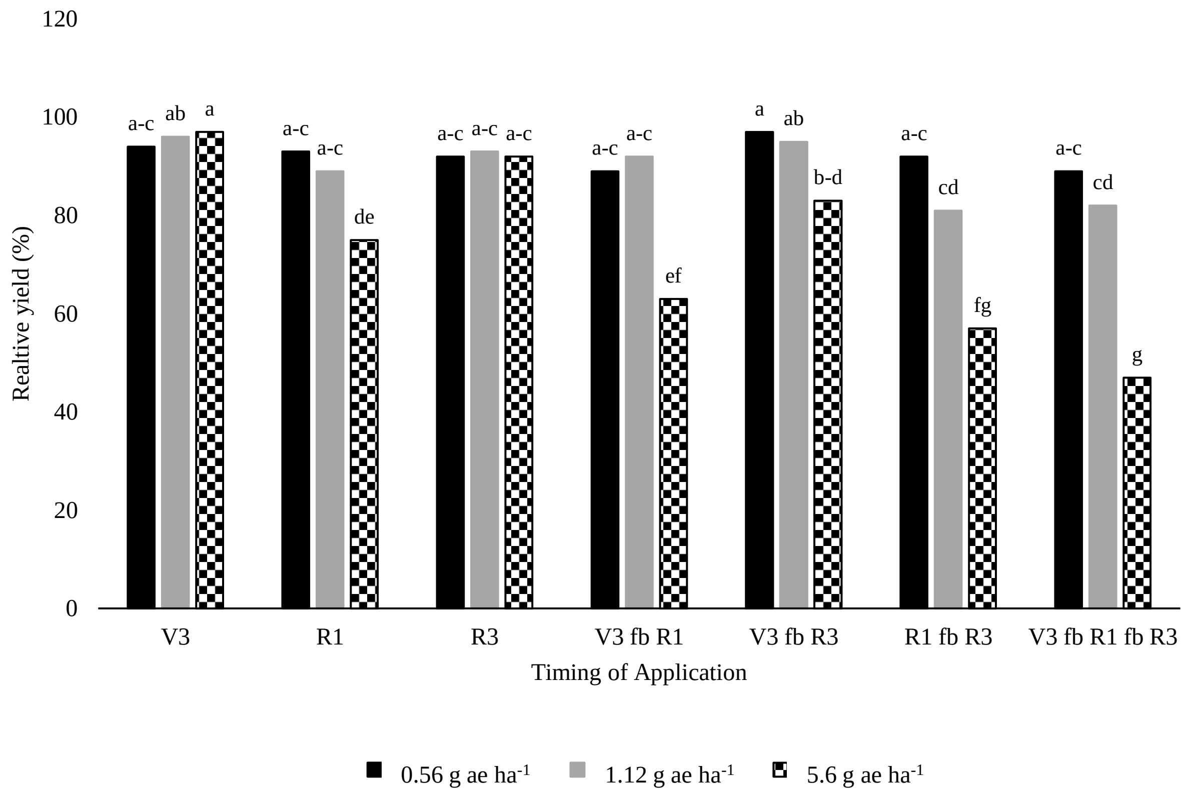

Figure 2.7 Relative soybean yield as a response to reduced doses of dicamba and multiple applications timings in averaged across all site-years.[a,b]

[a]Means followed by the same letter are not statistically different according to Fisher's Protected LSD (α = 0.05).
[b]Non-treated check yield = 3987 kg ha^{-1}

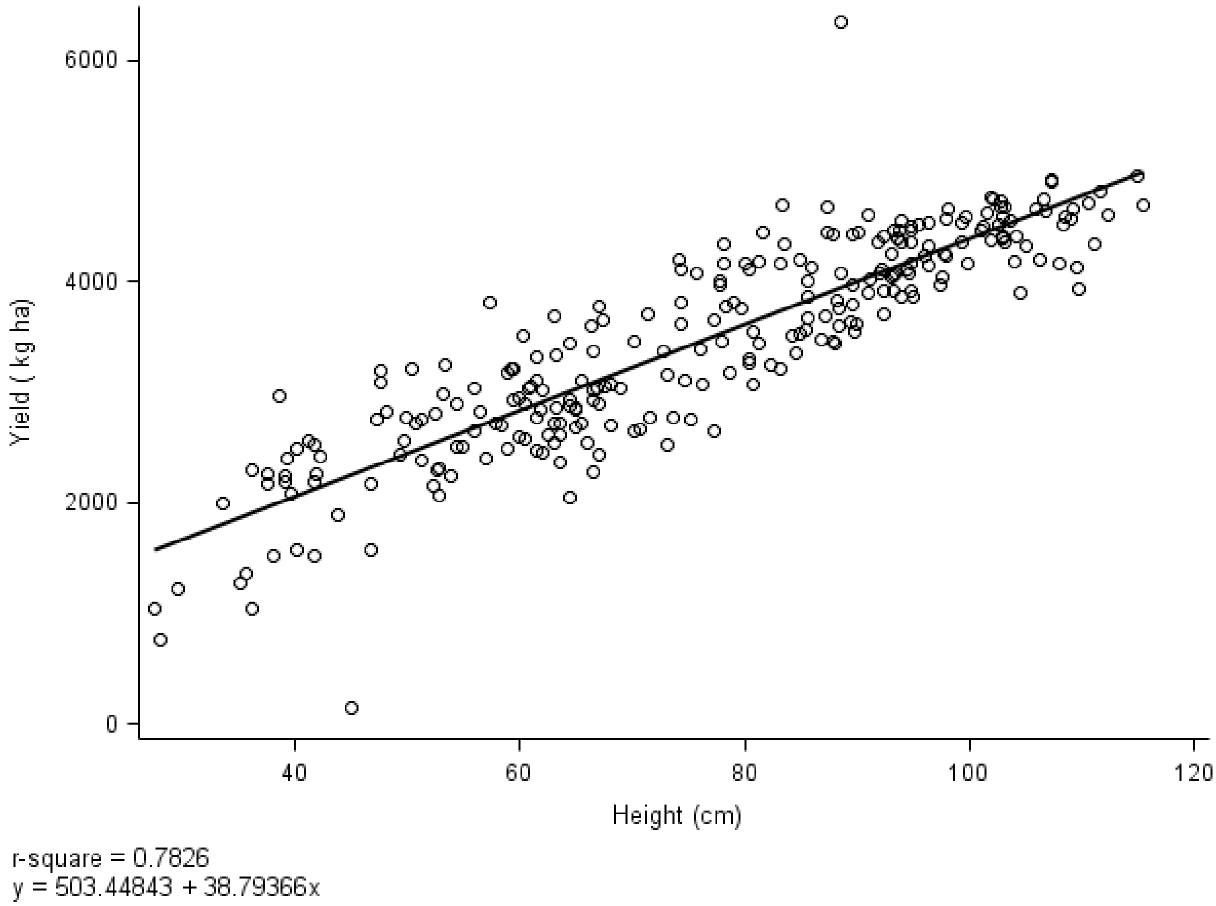

Figure 2.8 Linear regression of yield and height as a response to reduced doses of dicamba and multiple applications timings pooled across all site-years.

Table 2.1 Planting date, soybean variety, soil properties, and total in season rainfall in experiments evaluating dicamba drift injury in 2018 and 2019.

Site-year	Planting Date	Soybean Variety[d]	Soil Temp, °C	Soil pH	OM%	Soil Texture	Total in season precipitation, mm
MHK18[a]	5/22/2018	Credenz[e] 3841LL	24	5.7	2.7	Wymore silty clay loam	584
MHK19[b]	6/2/2019	Credenz[e] 3841LL	29	6.2	2.9	Reading silt loam	541
OTT19[c]	6/13/2019	Credenz[e] 3841LL	29	6.4	3.3	Woodson silt loam	833

[a] Manhattan, KS in 2018
[b] Manhattan, KS in 2019
[c] Ottawa, KS in 2019
[d] Planted at 308,880 seeds ha^{-1}
[e] BASF Agriculture, Florham Park, NJ

Table 2.2 Maintenance herbicide application timing, date, product, and dose used prior to crop emergence in experiments evaluating dicamba injury in 2018 and 2019.

Site-year	Application Timing	Application Date	Product	Dose
MHK18[a]	PRE[d]	5/22/2018	Sulfentrazone + *S*-metolachlor[f]	0.01 + 0.44 liters ha^{-1}
MHK19[b]	PRE[d]	6/2/2019	Sulfentrazone + *S*-metolachlor[f]	0.01 + 0.44 liters ha^{-1}
OTT19[c]	EPP[e]; PRE[d]	5/16/2019, 6/13/2019	Sulfentrazone + chlorimuron[g] & *S*-metolachlor[h]; *S*-metolachlor[h]	0.3 + 0.02 kg ae ha^{-1} & 1.4 liter ha^{-1}; 1.0 liter ha^{-1}

[a] Manhattan, KS 2018
[b]Manhattan, KS 2019
[c]Ottawa, KS 2019
[d]pre-emergent
[e] early pre-plant
[f]Authority Elite (FMC Corporation, Philadelphia, PA) at 0.07 liter ha^{-1}
[g]Authority Maxx (FMC Corporation, Philadelphia, PA) at 0.49 kg ae ha^{-1}
[h]Cinch (Corteva Agriscience, Wilmington, DE) at 1.8 and 1.2 liter ha^{-1}

Table 2.3 Application date and meteorological conditions during all application timings in experiments evaluating dicamba injury in 2018 and 2019.

V3

Site-year	Date of application	Air temp, °C (start, stop)	Soil temp, °C	% Relative humidity (start, stop)	Wind speed (km h^{-1}), direction (start; stop)
MHK18[a]	6/12/2018	30	34	63	6.4, N
MHK19[b]	6/26/2019	21, 21	22	78, 76	6, NNW; 8 NW
OTT19[c]	7/3/2019	21, 22	25	86, 77	8, S; 10, S

R1

Site-year	Date of application	Air temp, °C (start,stop)	Soil temp, °C	% Relative humidity (start, stop)	Wind speed (km h^{-1}), direction (start; stop)
MHK18	7/2/2018	24	31	70	5, E
MHK19	7/16/2019	22, 27	26	88, 80	2, W; 5, W
OTT19	7/23/2019	15,18	22	89	5, N; 6, N

R3

Site-year	Date of application	Air temp, °C (Start,Stop)	Soil temp, °C	% Relative humidity (Start, Stop)	Wind speed (km h^{-1}), direction (start; stop)
MHK18	7/16/2018	29	27	62	5, ENE
MHK19	7/30/2019	21, 27	24	79, 62	0; 7, E
OTT19	8/6/2019	22, 24	26	96, 89	3, S; 3, SW

[a] Manhattan, KS 2018
[b]Manhattan, KS 2019
[c]Ottawa, KS 2019

Table 2.4 Analysis of variance of fixed effects and all interactions for soybean visual injury at 2 WAT, 4 WAT, the onset of senescence at Manhattan, KS in 2018 and 2019 and at Ottawa, KS in 2019 as a result of exposure to reduced doses of dicamba at varying growth stages with single and multiple exposures.

Fixed effects	2WAT[a]	4WAT	At senescence
	--------- P-value---------		
Site-year	0.0060	<.0001	<.0001
Dose	<.0001	<.0001	<.0001
Timing	<.0001	<.0001	<.0001
Site-year by dose	0.0209	0.0501	0.4481
Site-year by timing	<.0001	<.0001	<.0001
Dose by timing	<.0001	<.0001	<.0001
Site-year by dose by timing	<.0001	<.0001	0.0009

[a]WAT = weeks after treatment

Table 2.5 Soybean visual injury at 2 WAT, 4 WAT, and at the onset of senescence at Manhattan, KS in 2018 as a result of exposure to reduced doses of dicamba at varying growth stages with single and multiple exposures.

Dicamba dose (g ae ha^{-1})	Time[a]	2WAT[a]	4WAT	At senescence
		----------------------	%	----------------------
0.56	V3	9 m[b]	17 l	5 h
	R1	8 m	30 jk	35 g
	R3	23 j-l	36 ij	39 fg
	V3 *fb*R1	28 i-k	36 ij	40 e-g
	V3 *fb* R3	30 h-j	43 g-i	36 g
	R1 *fb* R3	34 f-i	53 d-g	50 d-f
	V3 *fb* R1 *fb* R3	44 d-f	58 c-e	53 cd
1.12	V3	17 lm	23 kl	5 h
	R1	9 m	39 h-j	50 d-f
	R3	30 h-j	49 e-h	46 d-g
	V3 *fb* R1	32 g-j	45 f-i	51 de
	V3 *fb* R3	39 e-h	49 e-g	40 e-g
	R1 *fb* R3	44 d-f	63 cd	64 bc
	V3 *fb* R1 *fb* R3	53 cd	64 bc	64 bc
5.6	V3	24 j-l	32 jk	10 h
	R1	20 kl	54 c-f	69 ab
	R3	41 e-g	60 cd	64 bc
	V3 *fb* R1	46 de	74 ab	75 ab
	V3 *fb* R3	66 ab	76 a	71 ab
	R1 *fb* R3	61 bc	75 a	73 ab
	V3 *fb* R1 *fb* R3	75 a	80 a	76 a

[a] fb = followed by; WAT = weeks after treatment

[b] Means separated within each evaluation timing. Means followed by the same letter within a column are not statistically different according to Fisher's Protected LSD ($\alpha = 0.05$).

Table 2.6 Soybean visual injury at 2 WAT, 4 WAT, and at the onset of senescence at Manhattan, KS in 2019 as a result of exposure to reduced doses of dicamba at varying growth stages with single and multiple exposures.

Dicamba dose (g ae ha^{-1})	Time[a]	2WAT[b]	4WAT	At senescence
		---------------------%----------------------		
	V3	6 l[b]	9 k	4 l
	R1	14 jk	30 i	34 hi
	R3	10 kl	12 jk	10 lk
0.56	V3 *fb*R1	21 h-j	38 f-i	38 f-i
	V3 *fb* R3	15 jk	19 j	19 j
	R1 *fb* R3	30 fg	38 f-i	36 g-i
	V3 *fb* R1 *fb* R3	38 ef	40 e-h	41 e-h
	V3	13 kl	14 jk	5 l
	R1	18 i-k	36 g-i	43 e-g
	R3	16 jk	16 jk	14.3 jk
1.12	V3 *fb* R1	30 fg	43 d-g	43 e-g
	V3 *fb* R3	25 g-i	33 hi	30 i
	R1 *fb* R3	44 de	46 d-f	45 ef
	V3 *fb* R1 *fb* R3	54 bc	49 de	49 de
	V3	28 gh	33 hi	5 l
	R1	26 gh	52 cd	56 cd
	R3	30 fg	30 i	31 i
5.6	V3 *fb* R1	56 b	69 ab	68 b
	V3 *fb* R3	48 cd	49 de	46 e
	R1 *fb* R3	55 bc	60 bc	61 bc
	V3 *fb* R1 *fb* R3	76 a	78 a	78 a

[a] fb = followed by; WAT = weeks after treatment

[b] Means separated within each evaluation timing. Means followed by the same letter within a column are not statistically different according to Fisher's Protected LSD (α = 0.05).

Table 2.7 Soybean visual injury at 2 WAT, 4 WAT, and at the onset of senescence at Ottawa, KS in 2019 as a result of exposure to reduced doses of dicamba at varying growth stages with single and multiple exposures.

Dicamba dose (g ae ha^{-1})	Time[a]	2WAT[a]	4WAT	At senescence
		---------------------%----------------------		
0.56	V3	3 j[b]	11 k	5 j
	R1	26 g-i	36 g-j	36 e-i
	R3	23 hi	32 ij	26 g-i
	V3 *fb*R1	30 f-i	38 f-j	31 f-i
	V3 *fb* R3	20 i	28 j	20 ij
	R1 *fb* R3	40 c-e	48 c-f	46 c-f
	V3 *fb* R1 *fb* R3	39 c-f	48 c-f	40 d-h
1.12	V3	7 j	15 k	5 j
	R1	33 e-h	45 e-g	44 d-g
	R3	23 hi	34 h-j	24 hi
	V3 *fb* R1	36 d-g	46 d-g	48 b-f
	V3 *fb* R3	30 d-i	39 f-g	33 f-i
	R1 *fb* R3	46 bc	58 bc	51 b-e
	V3 *fb* R1 *fb* R3	54 b	64 b	55 b-d
5.6	V3	26 g-i	32 ij	5 j
	R1	46 b-d	61 b	65 ab
	R3	31 e-h	43 e-i	35 f-i
	V3 *fb* R1	51 b	56 b-d	63 a-c
	V3 *fb* R3	46 b-d	53 b-e	46 c-f
	R1 *fb* R3	65 a	76 a	74 a
	V3 *fb* R1 *fb* R3	73 a	81 a	75 a

[a] fb = followed by; WAT = weeks after treatment

[b] Means separated within each evaluation timing. Means followed by the same letter within a column are not statistically different according to Fisher's Protected LSD (α = 0.05).

Table 2.8 Analysis of variance of fixed effects and all interactions for soybean height reduction, yield reduction, yield component reduction, and germination reduction at Manhattan, KS in 2018 and 2019 and at Ottawa, KS in 2019 as a result of exposure to reduced doses of dicamba at varying growth stages with single and multiple exposures.[a]

Fixed effects	Relative height	Relative yield	Relative pods plant^{-1}	Relative nodes plant^{-1}	Relative seeds pod^{-1}	Relative seed weight
	---------------------------------P Value ----------------------------------					
Site-year	<.0001	0.0823	0.4316	0.3618	0.0997	0.3044
Dose	<.0001	<.0001	0.0181	<.0001	<.0001	0.9736
Timing	<.0001	<.0001	0.3565	<.0001	<.0001	<.0001
Site-year by dose	<.0001	0.1864	0.1021	0.4822	0.0367	0.0466
Site-year by timing	<.0001	0.0003	0.3317	0.0577	0.2021	0.6892
Timing by dose	<.0001	<.0001	0.1722	<.0001	0.0029	0.3286
Site-year by dose by timing	<.0001	0.1549	0.151	0.0238	0.8644	0.9107

Table 2.9 Relative pods plant^{-1} as a result of exposure to reduced doses of dicamba at varying growth stages with single and multiple exposures in Manhattan, KS in 2018 and 2019 and in Ottawa, KS in 2019.[a,b]

Dicamba dose (g ae ha^{-1})	Relative pods plant^{-1} (%)
0.56	99 a[b]
1.12	96 ab
5.6	93 b

[a] Non-treated check pods plant^{-1} = 44

[b] Means followed by the same letter are not statistically different according to Fisher's Protected LSD (α = 0.05).

Table 2.10 Relative nodes plant^{-1} as a result of exposure to reduced doses of dicamba at varying growth stages with single and multiple exposures in Manhattan, KS in 2018 and 2019 and in Ottawa, KS in 2019.[a,b]

Dicamba dose (g ae ha^{-1})	Time[c]	Site-year		
		MHK18[c]	MHK19[d]	OTT19[e]
		---------------------%---------------------		
0.56	V3	99 a[f]	106 a	96 ab
	R1	93 ab	88 a-c	89 a-d
	R3	88 a-d	91 a-c	90 a-d
	V3 *fb*R1	90 a-d	91 a-c	100 a
	V3 *fb* R3	87 a-d	102 ab	98 ab
	R1 *fb* R3	78 b-f	87 a-c	89 a-d
	V3 *fb* R1 *fb* R3	79 b-f	87 a-c	89 a-d
1.12	V3	95 ab	106 a	101 a
	R1	72 d-g	74 a-e	85 b-e
	R3	77 b-f	94 a-c	93 a-c
	V3 *fb* R1	89 a-d	76 a-d	88 a-d
	V3 *fb* R3	92 a-c	99 ab	93 a-c
	R1 *fb* R3	73 c-g	68 b-e	81 c-e
	V3 *fb* R1 *fb* R3	73 c-g	70 b-e	74 ef
5.6	V3	67 e-h	70 b-e	89 a-d
	R1	62 f-h	69 b-e	61 fg
	R3	83 a-e	94 a-c	86 b-e
	V3 *fb* R1	56 gh	49 de	59 g
	V3 *fb* R3	52 h	64 c-e	78 de
	R1 *fb* R3	58 gh	63 c-e	63 fg
	V3 *fb* R1 *fb* R3	53 gh	41 e	56 g

[a] Non-treated check nodes plant^{-1} values in Manhattan, KS 2018 = 16; Manhattan, KS 2019 = 18; Ottawa, KS = 18
[b] fb = followed by
[c] Manhattan, KS 2018
[d] Manhattan, KS 2019
[e] Ottawa, KS 2019
[f] Means were separated by location and means followed by the same letter are not statistically different according to Fisher's Protected LSD (α = 0.05).

Table 2.11 Relative seed weight 100 seeds^{-1} as a result of exposure to reduced doses of dicamba at varying growth stages with single and multiple exposures in Manhattan, KS in 2018 and 2019 and in Ottawa, KS in 2019.[a]

Dicamba dose (g ae ha^{-1})	Site-year		
	MHK18[c]	MHK19[d]	OTT19[e]
	-----------------%------------------		
0.56	108 a[b]	95 a	98 a
1.12	107 a	94 a	98 a
5.6	105 a	92 a	98 a

[a] Non-treated check seed weight in Manhattan, KS 2018 = 15.8 g; Manhattan, KS 2019 = 17.2 g; Ottawa, KS = 15.9 g

[b] Means were separated by location and means followed by the same letter are not statistically different according to Fisher's Protected LSD ($\alpha = 0.05$).

[c] Manhattan, KS 2018

[d] Manhattan, KS 2019

[e] Ottawa, KS 2019

Table 2.12 Relative seeds pod^{-1} as a result of exposure to reduced doses of dicamba at varying growth stages with single and multiple exposures in Manhattan, KS in 2018 and 2019 and in Ottawa, KS in 2019.[a]

Dicamba dose (g ae ha^{-1})	Site-year		
	MHK18[c]	MHK19[d]	OTT19[e]
	----------------------	%	----------------------
0.56	97 a[b]	99 a	92 a
1.12	91 b	102 a	90 a
5.6	88 b	97 a	83 b

[a] Non-treated check seeds pod^{-1} values in Manhattan, KS 2018 = 2.2; Manhattan, KS 2019 = 1.6; Ottawa, KS = 2.2
[b] Means were separated by location and means followed by the same letter are not statistically different according to Fisher's Protected LSD ($\alpha = 0.05$).
[c] Manhattan, KS 2018
[d] Manhattan, KS 2019
[e] Ottawa, KS 2019

Table 2.13 Relative seeds pod^{-1} as a result of exposure to reduced doses of dicamba at varying growth stages with single and multiple exposures in Manhattan, KS in 2018 and 2019 and in Ottawa, KS in 2019.[a]

	Dicamba dose (g ae ha^{-1})		
Time[b]	0.56	1.12	5.6
	----------------------%-----------------------		
V3	100 ab[c]	99 ab	102 a
R1	94 ab	92 ab	88 b-d
R3	101 a	98 ab	99 ab
V3 *fb* R1	98 ab	92 ab	90 a-c
V3 *fb* R3	97 ab	98 ab	93 ab
R1 *fb* R3	90 a-c	93 ab	77 cd
V3 *fb* R1 *fb* R3	92 ab	88 b-d	76 d

[a] Non-treated check seeds pods^{-1} = 2
[b] fb = followed by
[c] Means followed by the same letter are not statistically different according to Fisher's Protected LSD (α = 0.05).

Table 2.14 Pearson correlation coefficients and corresponding p-values as a result of soybean exposure to reduced doses of dicamba at varying growth stages with single and multiple exposures pooled across all site-years.[a]

	2WAT[b]	4WAT	At senescence	Relative height	Relative yield	Relative pods plant^{-1}	Relative nodes plant^{-1}	Relative seed weight	Relative seeds pod^{-1}
2WAT	1								
4WAT	**0.89938[c] <.0001[d]**	1							
At senescence	**0.78489 <.0001**	**0.91298 <.0001**	1						
Relative height	**-0.79928 <.0001**	**-0.85347 <.0001**	**-0.83399 <.0001**	1					
Relative yield	**-0.67735 <.0001**	**-0.71005 <.0001**	**-0.7022 <.0001**	**0.79404 <.0001**	1				
Relative pods plant^{-1}	-0.21239 0.0007	-0.23013 0.0002	-0.13652 0.0309	0.15656 0.0132	0.11587 0.0685	1			
Relative nodes plant^{-1}	**-0.66248 <.0001**	**-0.69616 <.0001**	**-0.64687 <.0001**	**0.76152 <.0001**	**0.58323 <.0001**	0.38614 <.0001	1		
Relative seed weight	-0.05163 0.4154	0.05267 0.406	0.02962 0.6404	0.01825 0.7736	-0.01687 0.7911	-0.27297 <.0001	-0.0713 0.2614	1	
Relative seeds pod^{-1}	-0.44398 <.0001	-0.47134 <.0001	-0.45325 <.0001	0.42542 <.0001	0.42675 <.0001	0.22975 0.0002	0.35704 <.0001	-0.16238 0.0101	1

[a] Bolded text indicates strong correlations with levels of significance ≤0.05
[b]WAT = weeks after treatment
[c]pearson coefficient; <0.3 = weak correlation, > 0.3 but <0.5 = moderate correlation, > 0.5 = strong correlation
[d] p-value

Chapter 3 - Response of non-dicamba-resistant soybean varieties and traits to dicamba

Introduction

Dicamba is a synthetic auxin herbicide that has been extensively used in corn, small grains, and pasture for broadleaf control since the 1960s. Widespread use of dicamba, often resulted in off-target movement and injury to sensitive plants, including soybeans and cotton (Auch and Arnold 1978, Wax et al. 1969). Introduction of dicamba-resistant (DR) soybean and cotton dramatically increased the application of dicamba in the vicinity of actively growing, susceptible soybean and cotton crops. Various injury symptoms, including epinasty of petioles and shoots, leaf malformation, terminal bud chlorosis, malformed pods, and delayed maturity are associated with off-target dicamba movement to non-DR soybean (Wax et al. 1969; Solomon and Bradley 2014). Greater injury is often observed in soybeans exposed to dicamba during early reproductive stages in comparison to vegetative stages (Auch and Arnold 1978, Wax et al. 1969).

While soybean injury and yield loss are influenced by the dicamba dose and timing, there is also evidence that suggests that soybean cultivars can respond differently to dicamba exposure (Osipitan et al. 2019b; France et al. 2019; Granadino et al. 2019). Osipitan et al. (2019b) observed no differences in injury among conventional, glufosinate-resistant, and glyphosate-resistant soybeans. However, France et al. (2019) exposed 21 different non-DR soybean varieties to reduced rates of dicamba and observed that one glufosinate-resistant variety and three conventional varieties were less injured by reduced rates of dicamba.

Soybean injury is not a reliable indicator of yield loss due to the ability to produce subsequent uninjured leaves and extended flowering period, which means worrisome injury often occurs at doses that are lower than those required to reduce yield (Al-Khatib and Peterson 1999).

Greater yield reductions are observed following dicamba exposure during early reproductive growth stages than vegetative growth stages. Soybeans exposed during vegetative stages to dicamba doses high enough to cause 30% injury were not likely to cause yield reductions greater than 5%. However, exposure during reproductive stages to doses high enough to cause 12% injury were likely to cause greater than 5% yield loss (Kniss 2018). No significant differences have been reported in yield loss among different non-DR soybean varieties (France et al. 2019; Granadino et al. 2019; Osipitan et al. 2019b).

Quantifying soybean yield components provides greater understanding of the impacts of dicamba exposure on soybean yield (Robinson et al. 2013). McCown et al. (2018) and Solomon and Bradley (2014) observed greater reductions in pods plant^{-1} following dicamba application during early reproductive stages compared to vegetative stages. Similarly, Solomon and Bradley (2014) reported dicamba exposure at R2 had reduction in seeds pod^{-1} and node m^{-2} more than exposure at vegetative stages.

The effects of off-target dicamba movement on soybean offspring are not well understood; but, understanding the impacts is crucial for soybean seed production (Jones et al. 2018a). Off-target dicamba movement has been shown to reduce germination, emergence, and vigor, and injure offspring of soybeans treated with dicamba during reproductive stages (Wax et al. 1969, Jones et al. 2018a, Auch and Arnold 1978, Thompson and Egli 1973), with greater impacts on more mature soybeans. In addition, offspring that emerge may have reduced vigor or foliar injury symptoms following dicamba application to the mother plant during reproductive growth (Jones et al. 2018a, 2019a). Of all the reproductive stages discussed, drift events that occur during R6 may be the most detrimental to the soybean seed production industry because the presence of dicamba was not detected by germination test or emergence tests, so dicamba may not be detected until the offspring is at least V1 or V2 (Jones et al. 2019a).

There is considerable evidence describing the harmful effects of dicamba to sensitive soybean varieties. However, little is known about the relative responses of soybean varieties with different herbicide resistance traits. Therefore, the main objective of this research was to determine the response of non-DR soybean varieties with various herbicide-resistant traits when exposed to a reduced dose of dicamba at V3 and R1.

Materials and Methods

Field studies were conducted at the Ashland Bottoms Research Farm, Kansas State University, Manhattan, Kansas (latitude:39.12 and longitude: -96.63) during 2018 (MHK18) and 2019 (MHK19) and at the East Central Experiment Field, Kansas State University, Ottawa, Kansas (latitude 38.54 and longitude: -95.25) during 2019 (OTT19).

Soybeans were planted at approximately 308,880 seeds ha^{-1}, 3.8 cm deep, and in 76.2 cm rows using a 4-row row crop planter across all locations. Seed beds were prepared using a field cultivator on the day of planting at MHK18 and MHK19; whereas, a field cultivator was used before the early pre-plant herbicide application at OTT19. Information regarding planting dates and soil properties for each site-year is summarized in table 3.1. PRE herbicide treatments for early-season weed control are summarized in table 3.2. Plots were hand-weeded as needed to keep weed free throughout the growing season across all site-years. The same soybean varieties were planted across all site-years. Soybean varieties included Asgrow AG4135RR2Y (glyphosate-resistant), Credenz 3841LL (glufosinate-resistant), Credenz 4748LL (glufosinate-resistant), and Stine 40BA02 (glyphosate and isoxaflutole-resistant). Details regarding maturity group, herbicide traits, and company are presented in table 3.3.

The *N*,*N*-Bis-(3-aminopropyl) methylamine salt (BAPMA) of dicamba (Engenia, BASF Corp., Research Triangle Park, NC) formulation was applied to all varieties at V3 and R1 (Fehr and Caviness 1977). Dates of application and environmental conditions during application for each site-year are presented in table 3.4. Dicamba was applied at dose of 5.6 g ae ha^{-1}, which is equivalent to 1/100X of a field-use dose (1X = 560 g ae ha^{-1}). A split-plot design with 4 replications was used. Soybean variety was the main plot and the application timing was randomly assigned to the subplot. Each main plot contained a non-treated check. Individual plots were 3 m by 9 m in size.

The reduced dicamba dose was achieved by initially producing a stock solution of the field use dose (1X = 560 g ae ha^{-1}) and appropriate dilution with water to obtain 5.6 g ae ha^{-1} (1/100X). The spray solution was applied directly to plots with 140 L ha^{-1} spray volume using a CO_2 powered back pack sprayer and a 4-tip, 1.9-m hand-held boom equipped with TTI110015 nozzles at 220 kPa. The center 2 rows of each plot received the full dose while the two outer rows received a partial dose and acted as a buffer between treatments.

Visual soybean injury was visually assessed weekly until the onset of senescence following the initial dicamba application. Soybeans were evaluated on a 0 to 100% crop injury scale with 0% indicating no injury and 100% indicating plant death. Symptomology at lower injury levels included leaf cupping, leaf crinkling, and chlorosis of terminal buds. Symptomology at greater injury levels included the aforementioned symptomology and necrosis of terminal buds, pod malformation, and stunting. To further evaluate soybean response to dicamba applications, the heights of five randomly-selected plants from the center two rows of each plot were recorded at the onset of senescence. Yield component data were collected at harvest by harvesting mature soybeans from 1 m row^{-1} from the two center rows of each plot. Yield components measured included: seed weight, number of pods plant^{-1}, seeds pod^{-1}, and total main stem nodes plant^{-1}. Soybeans were harvested for grain yield from the center two rows of each plot with a small plot combine and moisture was adjusted to 13%. Soybean height, yield, and yield component data were converted to a percent reduction in comparison to the non-treated check before statistical analysis. Percent reduction was calculated using equation 3.1, where plot data (PLOT) and the non-treated check (NTC) plot within each replication were used.

Equation 3.1

$$1 - ((NTC - PLOT)/NTC) \tag{3.1}$$

To test seed germination, 50-g soybean seed samples were taken from each plot and sent to the Seed Laboratory at the Kansas Crop Improvement for analysis. One-hundred soybean seeds were counted and placed on Kimpack (Anchor Paper Co., St. Paul, MN), which was placed on a food tray and moistened with 500 ml tap water. Soybeans seeds were then covered with 0.6 to 1.3 cm of mason sand. Trays were placed in a germination chamber at 30°C for 8 h (hours) and 20°C for 16 h, where lights came on with the warm cycle. After 8 d (days) of incubation, seedlings were evaluated as to whether they are normal, abnormal, dead or hard seed (AOSA 2019)

Response of soybean offspring was characterized by planting 10 seeds from each plot into 14-cm pots containing Miracle-Gro Moisture Control potting mix (The Scotts Company LLC, Marysville, OH) and grown in the Kansas State University Weed Science greenhouse until V3. Pots were arranged by site-year with a split plot design. Daytime temperature was 30°C and night time temperature was 22.2°C with a photoperiod of 15 h. Percent emergence, number of injured offspring, percent visual injury of emerged offspring, and offspring height were recorded. Soybean injury was evaluated on a 0 to 100% crop injury scale with 0% indicating no injury and 100%

indicating plant death. Symptomology included leaf cupping, leaf crinkling, and chlorosis of terminal buds.

Statistical analysis

Visual injury ratings, soybean height, soybean yield components, soybean yield, germination, offspring emergence, offspring height, number offspring injured, and visual injury of offspring were subjected to an ANOVA using PROC GLIMMIX in SAS (SAS 9.4, SAS® Institute Inc.). Replication was considered a random effect and site-year, dicamba application timing, and soybean variety were considered fixed effects. When there was a significant interaction between treatments and site-year, data for each site-year were analyzed separately. If there was no interaction between treatment and site-year, data were pooled across site-years. Means were separated using Fisher's protected LSD test (α = 0.05). Visual injury ratings, soybean height, soybean yield components, soybean yield, germination, offspring emergence, offspring height, number offspring injured, and visual injury of offspring were subjected to PROC CORR in SAS (SAS 9.4, SAS® Institute Inc.). Pearson coefficients were considered weak if less than 0.3, moderate if greater than 0.3 but less than 0.5, and strong if greater than 0.5. Yield and height data were subjected to PROC REG in SAS (SAS 9.4, SAS® Institute Inc.) using a linear model, which was selected as the best fit based on r-square values and p-values.

Results and Discussion

Visual injury

Visual injury 2 WAT had a significant interaction among site-years, time of application, and variety (Table 3.5). At MHK18, visual ranged from 16% to 26%, with the greatest injury observed in ST40B and CR4748 when treated at V3 and the least amount of injury observed in AG4135, CR3841, and CR4748 when treated at R1 (Table 3.6). Visual injury ranged from 26% to

40% at MHK19, with the greatest injury observed in ST40B treated at R1, while all other varieties had similar injury regardless of application timing (Table 3.7). Soybeans treated at R1 had significantly greater injury than soybeans treated at V3 regardless of the variety at OTT19 (Table 3.8).

Soybean injury at the MHK18 was similar to injury reported by McCown et al. (2018) for the V3 application and Solomon and Bradley (2014) for the R1 application. McCown et al. (2018) observed 24% visual injury 2 WAT at V4 to 2.18 g ae ha^{-1} dicamba. Solomon and Bradley (2014) reported dicamba at 28 g ae ha^{-1} at during R2 resulted in 18% injury 2WAT. At MHK19 and OTT19, injury as a result of V3 was similar to what was reported by McCown et al. (2018), who noted an average of 24% visual injury 2 WAT when soybeans were exposed to 2.18 g ae ha^{-1} dicamba at V4 and injury as a result of R1 was similar to what was reported by Andersen et al. (2004), who noted an average of 42.5% visual injury 2 WAT when soybeans were exposed to 5.6 g ae ha^{-1} dicamba at V3/V4.

At 4 WAT, there was also a significant interaction among site-years, variety, and time of application for visual injury (Table 3.5). Visual injury was significantly greater for soybeans treated at R1 than V3. At MHK18, visual ranged from 26% to 64%, with the greatest injury observed in ST40B when treated at R1 and the least amount of injury observed in AG4135, CR3841, CR4748, and ST40B when treated at V3 (Table 3.6). Visual injury ranged from 33% to 64% at MHK19, with the greatest injury observed in ST40B treated at R1 and the least amount of injury observed in AG4135, CR3841, CR4748, and ST40B when treated at V3 (Table 3.7). At OTT19, visual injury ranged from 35% to 69%, with the greatest injury observed in ST40B when treated at R1 and the least amount of injury was observed in CR3841 and CR4748 when treated at V3.

In general, soybeans treated at R1 presented greater visual injury 4 WAT than those treated at V3. Visual injury observed at V3 was similar to what Osipitan et al. (2019a) observed at lower doses of dicamba. Osipitan et al. (2019a) observed that visual injury 3 WAT increased from 29% to 75% as dicamba doses increased from 0.56 to 56 g ae ha^{-1} dicamba when treated at V2. Injury as a result of dicamba exposure at R1 was greater than what was observed by McCown et al. (2018), who reported an average of 43% injury 4 WAT when soybeans were treated at R1 to 2.19 and 8.75 g ae ha^{-1} dicamba. Similarly, Jones et al. (2018a), who reported an average of 27% to 37% visual injury in soybeans treated at R1 after exposure to 2.19 g ae ha^{-1} dicamba. Our data were similar to injury reported by Andersen et al. (2004), even though they did not observe increased injury in soybeans treated with 5.6 g ae ha^{-1} dicamba at V3/V4 48 DAT, but did observe an average of 55% injury from 11.2 g ae ha^{-1} dicamba.

Visual injury at the onset of senescence had a significant interaction for site-years and variety, site-years and time, and variety and time (Table 3.5). For the site-years by variety interaction, the ANOVA indicated significance but there were no differences among varieties within each site-year (Table 3.9). For the site-years by time interaction, V3 application resulted in significantly less visual injury than R1 applications (Table 3.10). Visual injury following application at V3 resulted in 5% to 7% injury, with the greatest injury being at MHK18 and the least amount of injury in MHK19 and OTT19. Applications at R1 resulted in 56% to 65% injury, with the greatest injury at MHK18 and the least injury occurring at MHK19. The variety by time interaction showed that treatment at V3 resulted in 7%, 6%, 5% and 5% visual injury in ST40B, CR3841, AG4135, and CR4748, respectively (Table 3.11). When treated at R1 growth stage, soybean variety ST40B, AG4135, CR3841, and CR4748 had 69, 64%, 62%, and 50%, respectively.

Soybeans treated at V3 were able to almost completely recover by soybean senescence. This result is in agreement with Al-Khatib and Peterson (1999) and Soltani et al. (2016) who observed end of the season recovery in soybeans treated at V3 with lower doses of dicamba. Soltani et al. (2016) observed 20% or less injury 8 WAT in soybeans exposed to 0.75 to 6 g ae ha^{-1} dicamba. Similar to Osipitan et al. (2019b) who observed no differences in injury among conventional, glufosinate-resistant, and glyphosate-resistant soybeans, varieties in this study exposed at V3 showed no differences in injury. However, exposure at R1 revealed differences among varieties where injury coincided with France et al. (2019) after soybean exposure at R1 to dicamba doses similar to the dose used in this study.

Height

There was a significant effect of dicamba application timing on soybean height reduction across varieties (Table 3.12). Dicamba exposure at V3 resulted in 5% reduction in height, while treatment at R1 resulted in 36% reduction in height (Table 3.13). Soybeans treated at R1 were shorter than plants treated at V3. Height reductions as a result of the V3 application were similar to height reductions observed by Foster and Griffin (2018). Greater reductions in height as a result of treatment at R1 were similar to those reported by Kelley et al. (2005). Previous research showed a 1% to 27% reduction in soybean height when exposed to dicamba at similar doses (Wax et al. 1969; Kelley et al. 2005; Jones et al. 2018b; Foster and Griffin 2018), with greatest reduction occurring in soybeans treated at V7 with 5.6 g ae ha-1 dicamba (Kelley et al. 2005) and in soybeans treated at R1 with 2.19 and 8.75 g ae ha-1 dicamba (Jones et al. 2018b), while the smallest reduction in height occurred in soybeans treated at V3 and R1 with 2.2 and 8.8 g ae ha-1 dicamba (Foster and Griffin 2018). There was a significant correlation between height and the final injury rating (Table 3.10). As injury increased from 5% to 70%, there was a 42% reduction in height.

This indicates that greater injury was associated with shorter plants. Al-Khatib and Peterson (1999) noted that reduction in height was seen in treatments with severe injury. Robinson et al. (2013) indicated that height reduction may be a quick way to estimate potential yield loss as a result of dicamba exposure.

Yield and Yield Components

Differences in yield across the locations could be attributed to differences in amount and timing of rainfall. For MHK18, rainfall totaled 596 mm. A majority of the rainfall came after pod and seed formation and negatively impacted harvest of the soybean plots. At MHK19, the total rainfall was 541 mm. There were two large rain event before the onset of the reproductive stages and several smaller throughout the course of the reproductive stages. At OTT19, rainfall totaled 833 mm. There were several rain events prior to the onset of the reproductive stages. There were also several smaller rain events that occurred throughout the course of the reproductive stages and one larger event that occurred right before R3 that totaled 131 mm.

For relative yield, there were significant interactions between site-years and variety and variety and time of application (Table 3.9). For the interaction between site-years and variety, mean separation showed no differences among varieties in terms of yield reduction within locations (Table 3.14). Dicamba exposure at V3 resulted in 5% or less yield loss, regardless of the variety (Figure 3.5). Dicamba exposure at R1 resulted 19% to 34% yield, with greatest yield loss occurring in ST40B and the least yield loss occurring in CR4748. Yield loss observed in this study is most similar to yield loss reported by Foster and Griffin (2018), who reported 5 and 18% yield loss when soybeans were treated with 2.2 and 8.8 g ae ha^{-1} at V3/V4. Similarly, McCown et al. (2018), who reported an average of 8 to 16% yield loss as a result of exposure at V4 to .2 and 8.8 g ae ha^{-1} dicamba. Foster and Griffin (2018) showed that soybeans exposed to 2.2 and 8.8 g ae ha^{-}

^{1} dicamba at R1/R2 resulted in 9 and 30% yield loss and McCown et al. (2018) reported 14% to 19% as result of exposure at R1 to the same doses.

Site-years, variety, or timing of application did not effect on relative pods plant^{-1}, despite later planting dates. Casteel (2011) reported that both pods plant^{-1} and nodes plant^{-1} are negatively impacted by late planting. However, planting date did not affect yield components in this study. Planting dates ranging from May 22nd at MHK18 to June 13th at OTT19.

There was a significant interaction between site-years and variety, site-years and time, and variety and time of application for reduction of main stem nodes plant^{-1} (Table 3.9). Although reduction of main stem nodes plant^{-1} showed a significant p-value from the ANOVA, mean separation showed no significant differences among varieties across the different locations. Treatment at R1 resulted in a greater reduction of main stem nodes plant^{-1} than V3 treated soybeans at OTT19 and MHK18, but the reduction was similar at MHK19 (Table 3.10 and 3.11). All varieties treated at V3 were similar except for CR3841 and AG4135, which had a reduction of main stem nodes plant^{-1} of 22% and 2%, respectively. There was greater reduction in number of main stem nodes plant^{-1} and height when treated during R1 as compared to V3. All varieties treated at R1 had similar reductions of main stem nodes plant^{-1}, ranging from 47% to 31% reduction. These observations are in agreement with Robinson et al. (2013) who observed 5 to 20% reduction of reproductive nodes m^{-2} when treated at V3, V5, and R2 with doses ranging from 0.073 to 2.72 g ae ha^{-1}.

There were no significant differences observed among site-year, variety, and timing of application for reduction in seed weight (Table 3.9, Appendix B.1). While seed size is important, Robinson et al. (2013) noted that soybean seed weight decreased as dicamba doses increased from

0.06 to 22.7 g ae ha^{-1}, but was not as responsive as other yield components such as seed m^{-2}, nodes m^{-2}, and seeds pod^{-1}.

For relative seeds pod^{-1}, there was a significant interaction between site-years and variety (Table 3.9). For the interaction between site-years and variety, the mean separation showed no differences among varieties within site-years (Table 3.18). However, Kelley et al. (2005) who reported exposure to 5.6 g ae ha^{-1} at V3 resulted in 2 to 9% reduced seeds pod^{-1}.

There was a strong, negative correlation between yield and injury levels 4WAT, as well as the final visual injury rating (Table 3.19). There were also a strong correlations between yield components and yield loss. In addition to a strong correlation, a linear relationship between greater yield loss. As height increased by 1 cm, yield increased by 31 kg ha^{-1} (Equation 3.2, Figure 3.6).

Equation 3.2

$$y = 1015.14759 + 31.361645x \qquad (3.2)$$

There was a positive correlation between reduction of main stem nodes $plant^{-1}$ and reduction in height. Nodes $plant^{-1}$ was correlated with the relative height, relative yield, injury 4WAT, and injury at senescence. Height reduction can be associated with relative nodes $plant^{-1}$, pods $plant^{-1}$, and seed weight (Robinson et al. 2013). Relative pods $plant^{-1}$, seed weight, and seeds pod^{-1} were positively correlated to relative yield. Robinson et al. (2013) noted that seeds m^{-2}, pods m^{-2}, and nodes m^{-2} need to be characterized in order to understand the total effect of dicamba exposures on non-DR soybeans.

Effects on Offspring

Data on germination, emergence, visual injury, number injured, and height were collected to characterize the effects of parent soybean exposure to dicamba on offspring (Appendix B.3). These data were not be characterized for MHK18 due to poor seed quality as a result of poor harvest conditions and soybean diseases, specifically purple seed stain caused by *Cercospora* *kikuchii*. However, MHK19 and OTT19 could be characterized (Appendix B.3). There were no significant differences among site-years, variety, and timing of application for reduction in germination, reduction in offspring emergence, number of offspring injured, offspring visual injury, and reduction in offspring height (Appendix B.4). Results were not consistent with previous research characterizing the effects of parent soybean exposure to dicamba on offspring, which may be due to differences in application timing and dicamba doses used. However, Jones et al (2018a) observed less visual injury in parent soybeans than visual injury that was observed in this trial after exposure to similar dicamba doses. Jones et al. (2018a, 2019a) hypothesized that parent soybean exposure during R5/R6 may be the most detrimental to the soybean seed production industry because visual injury of the parent soybean will be harder to detect, it may not impact offspring germination or emergence, and visual injury of offspring may not be expressed until V1 or later.

Conclusion

Although the availability of DR soybean varieties gives producers more options to mitigate development of herbicide resistant weeds, it puts non-DR soybean producers at a greater risk of off-target injury, particularly if an more-sensitive variety is exposed. Soybeans were more sensitive to dicamba when treated at R1 than at V3 and injury from the R1 application generally persisted to the end of the season, whereas injury at V3 was not apparent by the final rating time. Injury at the onset of senescence was strongly correlated to height and yield loss, which indicates that the height of injured soybean at the onset of senesce may be a useful indicator of yield loss when compared to non-injured soybean.

Yield loss due to dicamba exposure was influenced by timing of exposure, but not with trait. Application at V3 resulted in minimal to no yield loss, but application at R1 resulted in 19% to 34% yield loss. The greater yield reductions observed following exposure at R1 could be attributed to the reduced amount of growing time that the plants had between the time of application and the end of vegetative growth in comparison to those treated at V3. Of the yield components impacted, the greatest reductions were observed in nodes plant^{-1}, seeds pod^{-1}, and seeds m^{-2}. Only the R1 applications resulted in reductions greater than 10% in the aforementioned yield components. While the other yield components characterized in this trial showed 10% or less reduction in comparison to the non-treated checks. To avoid damage to non-DR soybeans it is critical that applicators follow mandatory application guidelines to not only protect producers of non-DR soybeans but, to preserve the DR technology. It may also be vital for non-DR producers to communicate with neighboring soybean producers about soybean technology that will be planted and herbicides that will be applied.

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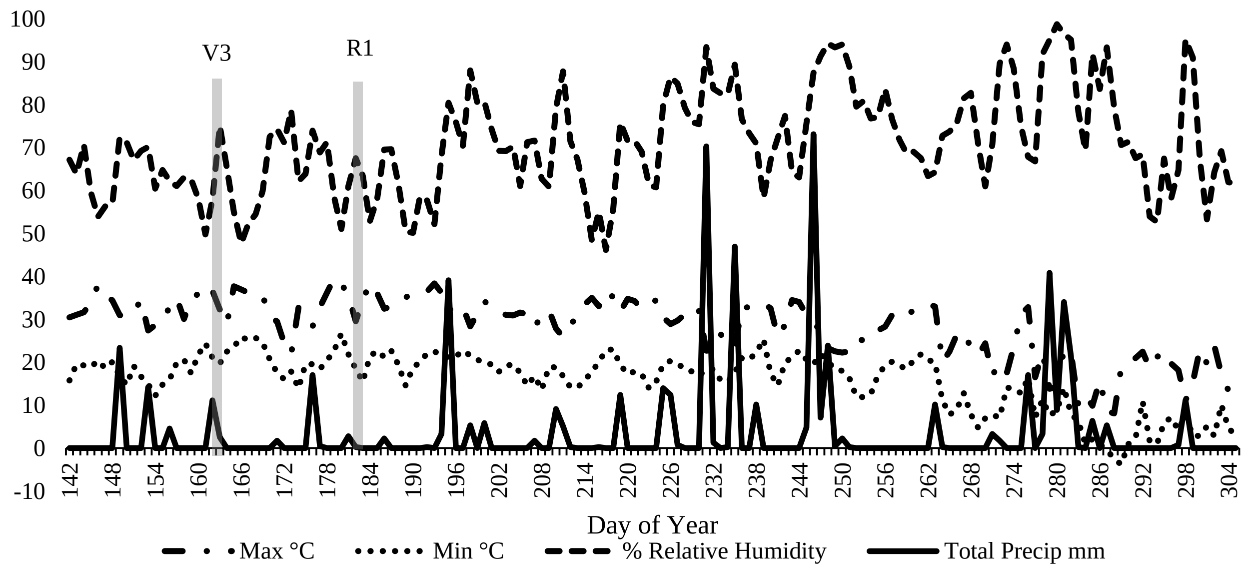

Figure 3.1 Daily maximum temperature, minimum temperature, percent relative humidity, total precipitation from the time of planting to harvest with corresponding application dates at Manhattan, KS in 2018.[a]

[a]Kansas State University (2019)

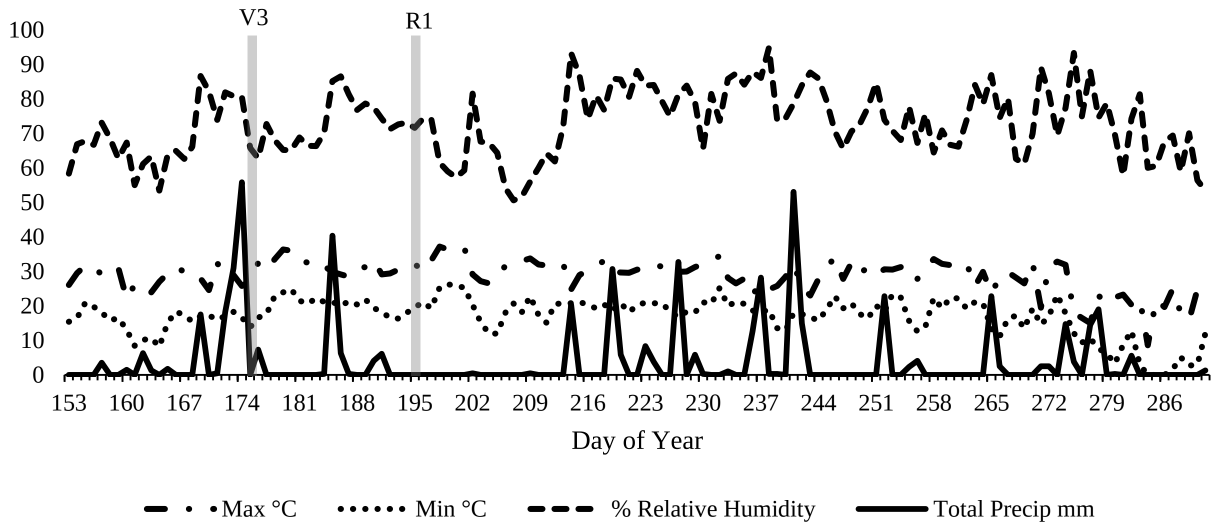

Figure 3.2 Daily maximum temperature, minimum temperature, percent relative humidity, total precipitation from the time of planting to harvest with corresponding application dates at Manhattan, KS in 2019.

[a]Kansas State University (2019)

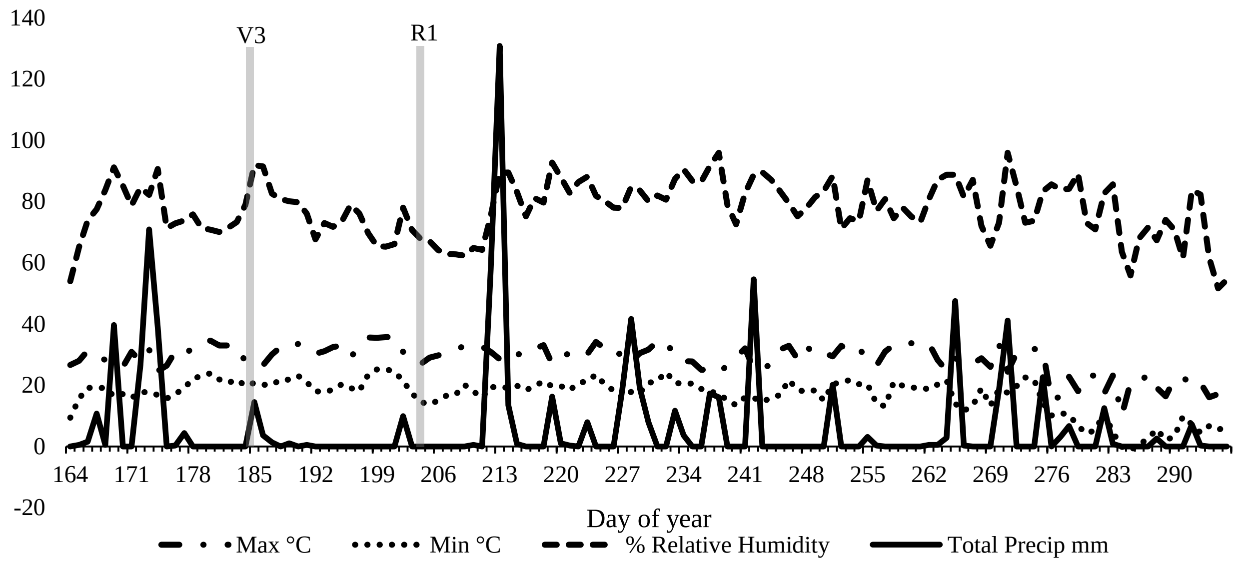

Figure 3.3 Daily maximum temperature, minimum temperature, percent relative humidity, total precipitation from the time of planting to harvest with corresponding application dates at Ottawa, KS in 2019.

[a]Kansas State University (2019)

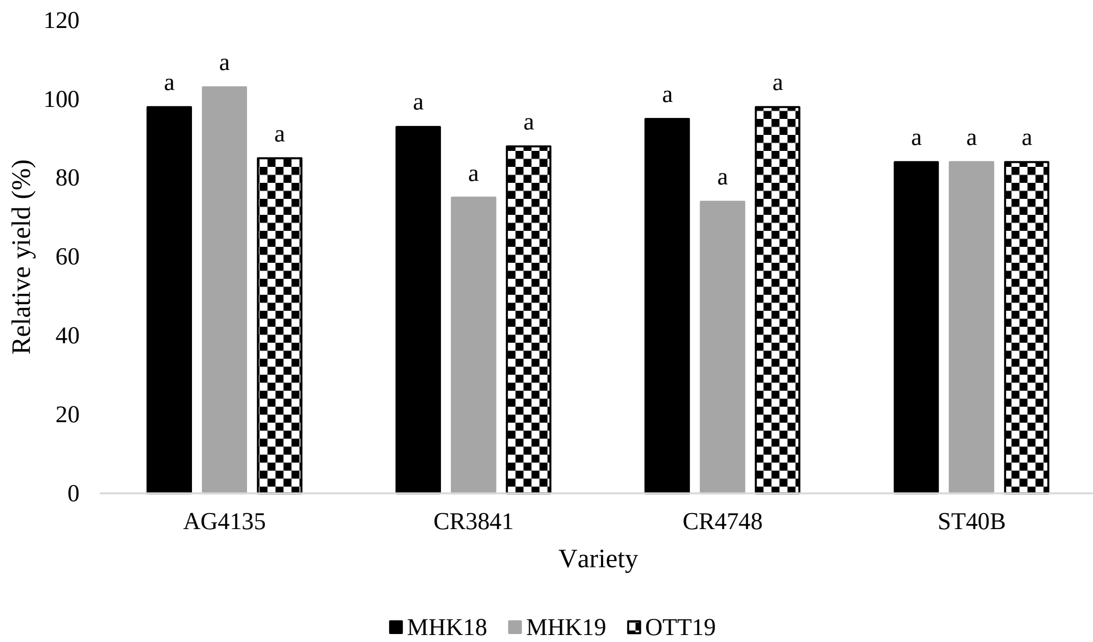

Figure 3.4 Relative soybean yield as a result of varying soybean varieties exposed to dicamba at varying application times at Manhattan, KS in 2018 and 2019 and at Ottawa, KS in 2019.[a,b]

[a]Means followed by the same letter are not statistically different according to Fisher's Protected LSD ($\alpha = 0.05$).

[b] Non-treated check yields: AG4135 = 3820 kg ha^{-1}; CR3841 = 3733 kg ha^{-1}; CR4748 = 3921 kg ha^{-1}; ST40B = 3661 kg ha^{-1}

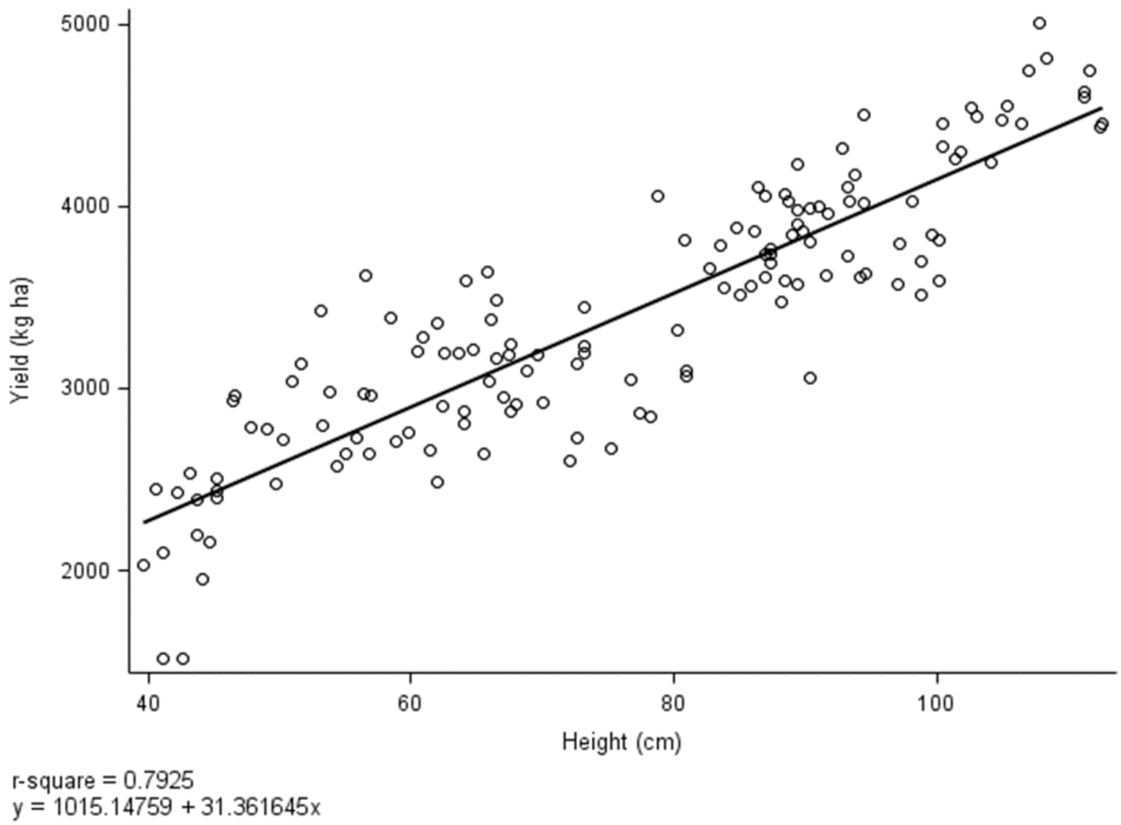

Figure 3.5 Linear regression of yield and height as a result of varying soybean varieties exposed to dicamba at varying application times at Manhattan, KS in 2018 and 2019 and at Ottawa, KS in 2019.

Table 3.1 Planting date, soil properties, and total in season rainfall in 2018 and 2019.

Site-year	Planting Date	Soil Temp, °C	Soil pH	OM%	Soil Texture	Total in season precipitation, mm
MHK18[a]	5/22/2018	24	5.7	2.7	Wymore silty clay loam[d]	584[e]
MHK19[b]	6/2/2019	29	6.2	2.9	Reading silt loam[d]	541[e]
OTT19[c]	6/13/2019	29	6.4	3.3	Woodson silt loam[d]	833[e]

[a] Manhattan, KS 2018
[b]Manhattan, KS 2019
[c]Ottawa, KS 2019
[d]silty clay loam
[e] Kansas State University (2019)

Table 3.2 Maintenance herbicide application timing, date, product, and dose used prior to crop emergence in Manhattan, KS in 2018 and 2019 and in Ottawa, KS in 2019.

Site-year	Application Timing	Application Date	Product	Dose
MHK18[a]	PRE[d]	5/22/2018	Sulfentrazone + *S*-metolachlor[f]	0.01 + 0.44 liters ha^{-1}
MHK19[b]	PRE[d]	6/2/2019	Sulfentrazone + *S*-metolachlor[f]	0.01 + 0.44 liters ha^{-1}
OTT19[c]	EPP[e]; PRE[d]	5/16/2019, 6/13/2019	Sulfentrazone + chlorimuron[g] & *S*-metolachlor[h]; *S*-metolachlor[h]	0.3 + 0.02 kg ae ha^{-1} & 1.4 liter ha^{-1}; 1.0 liter ha^{-1}

[a] Manhattan, KS 2018
[b]Manhattan, KS 2019
[c]Ottawa, KS 2019
[d]pre-emergent
[e] early pre-plant
[f]Authority Elite (FMC Corporation, Philadelphia, PA) at 0.07 liter ha^{-1}
[g]Authority Maxx (FMC Corporation, Philadelphia, PA) at 0.49 kg ae ha^{-1}
[h]Cinch (Corteva Agriscience, Wilmington, DE) at 1.8 and 1.2 liter ha^{-1}

Table 3.3 Soybean varieties planted in Manhattan, KS 2018 and 2019 and in Ottawa, KS in 2019 with corresponding herbicide traits, maturity groups, abbreviations, and companies.

Variety	Herbicide Traits	Maturity Group	Abbreviation	Company
Asgrow AG4135RR2Y	Glyphosate resistant	4.1	AG	Bayer Crop Science[a]
Credenz 3841LL	Glufosinate resistant	3.8	C38	BASF Agriculture[b]
Credenz 4748LL	Glufosinate resisant	4.7	C47	BASF Agriculture[b]
Stine 40BA02	Glyphosate and isoxaflutole resistant	4.0	ST	Stine Seed Co.[c]

[a] Bayer Crop Science, St. Louis, Missouri
[b] BASF Agriculture, Florham Park, New Jersey
[c] Stine Seed Company, Adel, Iowa

Table 3.4 Application date and meteorological conditions during all application timings in experiments evaluating dicamba injury in 2018 and 2019.

V3

Site-year	Date of application	Air temp, °C (start, stop)	Soil temp, °C	% Relative humidity (start, stop)	Wind speed (km h^{-1}), direction (start; stop)
MHK18[a]	6/12/2018	30	34	63	6, N
MHK19[b]	6/26/2019	21, 21	22	78, 76	6, NNW; 8 NW
OTT19[c]	7/3/2019	21, 22	25	86, 77	8, S; 10, S

R1

Site-year	Date of application	Air temp, °C (start, stop)	Soil temp, °C	% Relative humidity (start, stop)	Wind speed (km h^{-1}), direction (start; stop)
MHK18	7/2/2018	24	31	70	5, E
MHK19	7/16/2019	22, 27	26	88, 80	2, W; 5, W
OTT19	7/23/2019	15,18	22	89	5, N; 6, N

[a] Manhattan, KS 2018
[b]Manhattan, KS 2019
[c]Ottawa, KS 2019

Table 3.5 Analysis of variance of fixed effects and all interactions for soybean visual injury as a response of different soybean varieties exposed to a reduced dose of dicamba at varying application timings at Manhattan, KS in 2018 and 2019 and in Ottawa, KS 2019.

Fixed effects	2WAT[a]	4WAT[a]	At senescence
	----------------- P-value-----------------		
Site-year	<.0001	<.0001	<.0001
Variety	0.0401	<.0001	<.0001
Timing	<.0001	<.0001	0.0687
Site-year by variety	<.0001	<.0001	<.0001
Site-year by timing	<.0001	<.0001	0.1127
Timing by variety	<.0001	<.0001	0.0090
Site-year by variety by timing	0.0428	0.0001	0.2787

[a]WAT= weeks after treatment

Table 3.6 Soybean injury at 2 WAT and 4 WAT as a result of varying soybean varieties exposed to dicamba at varying application times at Manhattan, KS in 2018.

Time	Variety	2WAT[a]	4WAT
		---------------------------%---------------------------	
V3	AG4135	24 ab[b]	33 c
	CR3841	23 ab	31 c
	CR4748	25 a	26 c
	ST40B	26 a	32 c
R1	AG4135	18 c	54 b
	CR3841	16 c	50 b
	CR4748	18 c	55 b
	ST40B	20 bc	64 a

[a] WAT = weeks after treatment

[b] Means followed by the same letter within a column are not statistically different according to Fisher's Protected LSD ($\alpha = 0.05$).

Table 3.7 Soybean injury at 2 WAT and 4 WAT as a result of varying soybean varieties exposed to dicamba at varying application times at Manhattan, KS in 2019.

Time	Variety	2WAT[a]	4WAT
		---------------------------%---------------------------	
V3	AG4135	28 b[b]	35 c
	CR3841	27 b	33 c
	CR4748	28 b	34 c
	ST40B	28 b	37 c
R1	AG4135	32 b	59 ab
	CR3841	33 b	55 b
	CR4748	31 b	59 ab
	ST40B	40 a	63 a

[a] WAT = weeks after treatment
[b] Means followed by the same letter within a column are not statistically different according to Fisher's Protected LSD ($\alpha = 0.05$).

Table 3.8 Soybean injury at 2 WAT and 4 WAT as a result of varying soybean varieties exposed to dicamba at varying application times at Ottawa, KS in 2019.

Time	Variety	2WAT[a]	4WAT
		--------------------------%--------------------------	
V3	AG4135	30 b[b]	39 c
	CR3841	26 b	34 d
	CR4748	28 b	34 d
	ST40B	28 b	37 cd
R1	AG4135	45 a	66 ab
	CR3841	44 a	65 b
	CR4748	47 a	65 b
	ST40B	43 a	69 a

[a] WAT = weeks after treatment

[b] Means followed by the same letter within a column are not statistically different according to Fisher's Protected LSD ($\alpha = 0.05$).

Table 3.9 Soybean injury at the onset of senescence as a result of varying soybean varieties exposed to dicamba at varying application times at Manhattan, KS in 2018 and 2019 and in Ottawa, KS in 2019.

	Site-year		
Variety	MHK18[a]	MHK19[b]	OTT19[c]
	----------------------%----------------------		
AG4135	36 a[d]	33 a	36 a
CR3841	38 a	30 a	34 a
CR4748	28 a	26 a	28 a
ST40B	42 a	34 a	38 a

[a] Manhattan, KS in 2018
[b] Manhattan, KS in 2019
[c] Ottawa, KS in 2019
[d] Means separated within site-year and means followed by the same letter within a column are not statistically different according to Fisher's Protected LSD ($\alpha = 0.05$).

Table 3.10 Soybean injury at the onset of senescence as a result of varying soybean varieties exposed to dicamba at varying application times at Manhattan, KS in 2018 and 2019 and in Ottawa, KS in 2019.

Timing of application	Site-year		
	MHK18[a]	MHK19[b]	OTT19[c]
	----------------------	%	----------------------
V3	7 b[d]	5 b	5 b
R1	65 a	56 a	62 a

[a] Manhattan, KS in 2018
[b] Manhattan, KS in 2019
[c] Ottawa, KS in 2019
[d] Means separated within site-year and means followed by the same letter within a column are not statistically different according to Fisher's Protected LSD ($\alpha = 0.05$).

Table 3.11 Soybean injury at the onset of senescence as a result of varying soybean varieties exposed to dicamba at varying application.

Timing of application	Variety			
	AG4135	CR3841	CR4748	ST40B
	----------------------%----------------------			
V3	5 d[a]	6 d	5 d	7 d
R1	64 b	62 b	50 c	69 a

[a] Means followed by the same letter are not statistically different according to Fisher's Protected LSD ($\alpha = 0.05$).

Table 3.12 Analysis of significance of fixed effects and all interactions for soybean trait response to a reduced dose of dicamba at multiple timings.

Fixed effects	Relative yield	Relative height	Relative pods $plant^{-1}$	Relative nodes $plant^{-1}$	Relative seeds pod^{-1}	Relative seed weight
	———————————	———————	——————— P Value	———————————	———————	———————
Site-year	0.42	0.1948	0.5494	0.2641	0.4454	0.5939
Variety	0.4504	0.4513	0.1368	0.1118	0.4775	0.3525
Timing	<.0001	<.0001	0.1637	<.0001	0.0003	0.3257
Site-year by variety	0.0031	0.1061	0.1613	0.0457	0.0353	0.0602
Site-year by timing	0.5026	0.0652	0.9214	0.0003	0.2695	0.9063
Timing by variety	0.0349	0.2491	0.5237	0.0064	0.5048	0.9202
Site-year by variety by timing	0.9914	0.9204	0.3289	0.7964	0.8961	0.9918

Table 3.13 Relative soybean height as a result of varying soybean varieties exposed to dicamba at varying application timings.[a]

Timing of application	Relative height (%)
V3	95 a[b]
R1	64 b

[a] Non-treated check height = 90 cm

[b] Means followed by the same letter are not statistically different according to Fisher's Protected LSD ($\alpha = 0.05$).

Table 3.14 Relative soybean yield as a result of varying soybean varieties exposed to dicamba at Manhattan, KS in 2018 and 2019 and in Ottawa, KS in 2019.

	Site-year		
Variety	MHK18[a]	MHK19[b]	OTT19[c]
	----------------------	%	----------------------
AG4135	98 a[d]	103 a	85 a
CR3841	93 a	75 a	88 a
CR4748	95 a	74 a	98 a
ST40B	84 a	84 a	84 a

[a] Manhattan, KS in 2018; Non-treated check yields: AG4135 = 2928 kg ha^{-1}; CR3841 = 2924 kg ha^{-1}; CR4748 = 3426 kg ha^{-1}; ST40B = 2868 kg ha^{-1}
[b] Manhattan, KS in 2019; Non-treated check yields: AG4135 = 4683 kg ha^{-1}; CR3841 = 4550 kg ha^{-1}; CR4748 = 4474 kg ha^{-1}; ST40B = 4330 kg ha^{-1}
[c] Ottawa, KS in 2019; Non-treated check yields: AG4135 = 3850 kg ha^{-1}; CR3841 = 3723 kg ha^{-1}; CR4748 = 3865 kg ha^{-1}; ST40B = 3848 kg ha^{-1}
[d] Means separated within site-year and means followed by the same letter within a column are not statistically different according to Fisher's Protected LSD ($\alpha = 0.05$).

Table 3.15 Relative nodes plant^{-1} as a result of varying soybean varieties exposed to dicamba at Manhattan, KS in 2018 and 2019 and in Ottawa, KS in 2019.

Variety	Site-year		
	MHK18[a]	MHK19[b]	OTT19[c]
	---------------------%---------------------		
AG4135	89 a[d]	85 a	75 a
CR3841	78 a	81 a	81 a
CR4748	75 a	65 a	72 a
ST40B	82 a	57 a	80 a

[a] Manhattan, KS in 2018; Non-treated check nodes plant^{-1}: AG4135 = 15; CR3841 = 16; CR4748 = 19; ST40B = 16
[b] Manhattan, KS in 2019; Non-treated check nodes plant^{-1}: AG4135 =20; CR3841 = 19; CR4748 =19; ST40B = 20
[c] Ottawa, KS in 2019; Non-treated check nodes plant^{-1}: AG4135 = 17; CR3841 = 17; CR4748 = 18; ST40B = 18
[d] Means separated within site-year and means followed by the same letter within a column are not statistically different according to Fisher's Protected LSD ($\alpha = 0.05$).

Table 3.16 Relative nodes plant^{-1} as a result of varying soybeans varieties exposed to dicamba at multiple application timings at Manhattan, KS in 2018 and 2019 and in Ottawa, KS in 2019.

Timing of application	Site-year		
	MHK18[a]	MHK19[b]	OTT19[c]
	---------------------	%	---------------------
V3	98 a[d]	72 a	95 a
R1	64 b	63 a	59 b

[a] Manhattan, KS in 2018; Non-treated check nodes plant^{-1}= 17
[b] Manhattan, KS in 2019; Non-treated check nodes plant^{-1}= 19
[c] Ottawa, KS in 2019; Non-treated check nodes plant^{-1}= 18
[d] Means separated within site-year and means followed by the same letter within a column are not statistically different according to Fisher's Protected LSD ($\alpha = 0.05$).

Table 3.17 Relative nodes plant^{-1} as a result of varying soybeans varieties exposed to dicamba at multiple application timings.[a]

Timing of application	Variety			
	AG4135	CR3841	CR4748	ST40B
	---------------------%----------------------			
V3	93 a[b]	92 a	93 a	101 a
R1	57 b	69 b	52 b	58 b

[a] Non-treated check nodes plant^{-1}: AG4135 = 17; CR3841 = 17; CR4748 = 19; ST40B = 18
[b] Means followed by the same letter are not statistically different according to Fisher's Protected LSD (α = 0.05).

Table 3.18 Relative seeds pod^{-1} as a result of varying soybean varieties exposed to dicamba at Manhattan, KS in 2018 and 2019 and in Ottawa, KS in 2019.

	Site-year		
Variety	MHK18[a]	MHK19[b]	OTT19[c]
	----------------------	%	----------------------
AG4135	94 a[d]	120 a	94 a
CR3841	100 a	95 a	91 a
CR4748	86 a	94 a	99 a
ST40B	90 a	94 a	90 a

[a] Manhattan, KS in 2018; Non-treated check seeds pod^{-1}: AG4135 = 2; CR3841 = 1.9; CR4748 = 1.9; ST40B = 2.1
[b] Manhattan, KS in 2019; Non-treated check seeds pod^{-1}: AG4135 =1.7; CR3841 = 1.7; CR4748 =1.8; ST40B = 1.8
[c] Ottawa, KS in 2019; Non-treated check seeds pod^{-1}: AG4135 = 2.1; CR3841 = 2.1; CR4748 = 2.2; ST40B = 2
[d] Means separated within site-year and means followed by the same letter within a column are not statistically different according to Fisher's Protected LSD ($\alpha = 0.05$).

Table 3.19 Pearson correlation coefficients and corresponding p-values for soybean trait response to a reduced dose of dicamba at multiple timings at Manhattan, KS in 2018 and 2019 and in Ottawa, KS in 2019.[a]

	2WAT[b]	**4WAT**	**At senescence**	**Relative height**	**Relative yield**	**Relative pods plant^{-1}**	**Relative nodes plant^{-1}**	**Relative seed weight^{-1}**	**Relative seeds pod^{-1}**
2WAT	1								
4WAT	**0.50377[c]** **<.0001[d]**	1							
At senescence	0.26119 0.011	**0.92657** **<.0001**	1						
Relative height	-0.29273 0.0042	**-0.69148** **<.0001**	**-0.72727** **<.0001**	1					
Relative yield	-0.21525 0.0372	**-0.57497** **<.0001**	**-0.58185** **<.0001**	**0.83178** **<.0001**	1				
Relative pods plant^{-1}	-0.17154 0.0983	-0.1433 0.1682	-0.0875 0.4017	0.47974 <.0001	**0.57214** **<.0001**	1			
Relative nodes plant^{-1}	-0.24123 0.0192	**-0.56062** **<.0001**	**-0.5463** **<.0001**	**0.73555** **<.0001**	**0.74813** **<.0001**	**0.51214** **<.0001**	1		
Relative seed weight^{-1}	0.01248 0.905	-0.09158 0.38	-0.10204 0.3277	**0.62875** **<.0001**	**0.608** **<.0001**	**0.53961** **<.0001**	0.49648 <.0001	1	
Relative seeds pod^{-1}	-0.1293 0.2142	-0.28456 0.0054	-0.30914 0.0024	**0.72526** **<.0001**	**0.68875** **<.0001**	**0.53782** **<.0001**	**0.56531** **<.0001**	**0.67142** **<.0001**	1

[a] Bolded text indicates strong correlations with levels of significance $\leq$0.05
[b]WAT = weeks after treatment
[c]pearson coefficient; <0.3 = weak correlation, > 0.3 but <0.5 = moderate correlation, > 0.5 = strong correlation
[d] p-value

Appendix A - Supplemental information for chapter 2

Table A.1 Soybean yield and yield component at Manhattan, KS in 2018 as a result of exposure to reduced doses of dicamba at varying growth stages with single and multiple exposures.

Dicamba dose (g ae ha^{-1})	Time of application[a]	Yield (kg ha^{-1})	Height (cm)	Pods plant^{-1}	Nodes plant^{-1}	Seed weight (g)	Seeds pod^{-1}	Germination (%)
0.56	V3	2689 a-d[b]	69 ab	25 a	16 a	16.7 a	2.2 a	71 a
	R1	2904 a-c	68 bc	29 a	15 ab	16.1 a	2.1 ab	60 a
	R3	2561 a-e	69 ab	22 a	14 a-c	16.3 a	2.2 a	65 a
	V3 *fb*R1	2628 a-e	68 bc	27 a	15 a-c	16.3 a	2.1 ab	67 a
	V3 *fb* R3	3057 ab	64 bc	24 a	14 a-c	16.9 a	2.2 a	70 a
	R1 *fb* R3	2596 a-e	60 b-e	22 a	13 b-e	17.3 a	2.1 ab	63 a
	V3 *fb* R1 *fb* R3	2760 a-d	58 c-e	24 a	13 a-e	16.3 a	2.1 ab	63 a
1.12	V3	2820 a-c	69 ab	24 a	15 ab	16.6 a	2.2 a	69 a
	R1	2796 a-d	65 bc	26 a	12 c-f	16.9 a	2 a-c	58 a
	R3	2639 a-d	63 b-d	23 a	12 b-e	16.8 a	2.1 ab	60 a
	V3 *fb* R1	2974 a-c	63 bc	27 a	14 a-c	16.5 a	2 a-c	68 a
	V3 *fb* R3	2930 a-c	63 b-d	25 a	15 a-c	16.7 a	2.1 ab	64 a
	R1 *fb* R3	2346 c-e	52 e	23 a	12 c-f	16.9 a	2.1 ab	59 a
	V3 *fb* R1 *fb* R3	2414 b-e	53 de	23 a	12 c-f	16.8 a	2 a-c	59 a
5.6	V3	3072 ab	60 b-e	25 a	11 d-g	17.1 a	2.1 ab	67 a
	R1	2338 c-e	40 f	26 a	10 e-g	16.9 a	1.9 cb	64 a
	R3	2536 a-e	59 c-e	26 a	13 a-d	17.3 a	2.1 ab	56 a
	V3 *fb* R1	1899 ef	41 f	28 a	9 gf	16.9 a	2 a-c	72 a
	V3 *fb* R3	2125 d-f	42 f	24 a	8 g	17.5 a	2 a-c	58 a
	R1 *fb* R3	1469 fg	39 fg	22 a	9 gf	16.6 a	1.8 c	57 a
	V3 *fb* R1 *fb* R3	1013 g	30 g	26 a	9 gf	16.2 a	1.8 c	56 a
Non-treated		3121 a	79 a	27 a	16 a	15.8 a	2.2 ab	61 a

[a] fb = followed by
[b]Means followed by the same letter within a column are not statistically different according to Fisher's Protected LSD (α = 0.05).

Table A.2 Soybean yield and yield component at Manhattan, KS in 2019 as a result of exposure to reduced doses of dicamba at varying growth stages with single and multiple exposures.

Dicamba dose (g ae ha^{-1})	Time of application[a]	Yield (kg ha^{-1})	Height (cm)	Pods $plant^{-1}$	Nodes $plant^{-1}$	Seed weight (g)	Seeds pod^{-1}
0.56	V3	4561 ab[b]	108 a-c	72 a	19 a	16.7 ab	1.7 ab
	R1	4426 ab	97 e-h	65 a	16 a-d	15.6 ab	1.5 ab
	R3	4570 ab	109 ab	63 a	17 a-d	16.3 ab	1.7 ab
	V3 *fb*R1	4365 ab	102 b-g	62 a	17 a-d	16 ab	1.7 ab
	V3 *fb* R3	4579 ab	105 b-f	62 a	19 ab	17.1 ab	1.6 ab
	R1 *fb* R3	4295 ab	96 f-h	62 a	16 a-d	16.1 ab	1.5 ab
	V3 *fb* R1 *fb* R3	4380 ab	94 g-i	52 a	16 a-d	16.4 ab	1.5 ab
1.12	V3	4359 ab	106 a-e	61 a	19 a	16.1 ab	1.7 ab
	R1	4479 ab	86 ij	65 a	14 a-e	16.1 ab	1.5 ab
	R3	4520 ab	107 a-d	52 a	17 a-d	16.1 ab	1.7 ab
	V3 *fb* R1	4546 ab	90 hi	60 a	14 a-e	16.1 ab	1.7 ab
	V3 *fb* R3	4521 ab	102 b-g	53 a	18 a-c	16.8 ab	1.7 ab
	R1 *fb* R3	4026 bc	81 j	62 a	12 c-e	16 ab	1.6 ab
	V3 *fb* R1 *fb* R3	4129 bc	77 j	70 a	13 b-e	15.7 ab	1.6 ab
5.6	V3	4289 ab	98 d-h	51 a	12 c-e	15.5 ab	1.9 a
	R1	3607 cd	64 k	58 a	12 c-e	15.4 ab	1.5 ab
	R3	4621 ab	99 c-g	66 a	17 a-d	16.5 ab	1.7 ab
	V3 *fb* R1	3392 de	58 k	50 a	9 ef	15.2 ab	1.6 ab
	V3 *fb* R3	4193 a-c	81 j	62 a	11 d-f	16.3 ab	1.7 ab
	R1 *fb* R3	2887 ef	57 k	59 a	11 d-f	16.5 ab	1.4 b
	V3 *fb* R1 *fb* R3	2530 f	42 l	54 a	8 f	15.1 b	1.4 b
Non-treated		4763 a	114 a	59 a	18 a-c	17.2 a	1.6 ab

[a] fb = followed by

[b]Means followed by the same letter within a column are not statistically different according to Fisher's Protected LSD (α = 0.05).

Table A.3 Soybean yield and yield component at Ottawa, KS in 2019 as a result of exposure to reduced doses of dicamba at varying growth stages with single and multiple exposures.

Dicamba dose (g ae ha^{-1})	Time of application[a]	Yield (kg ha^{-1})	Height (cm)	Pods plant^{-1}	Nodes plant^{-1}	Seed weight (g)	Seeds pod^{-1}
0.56	V3	4096 ab[b]	95 a-c	46 a	18 a	15.8 a-c	2.1 ab
	R1	3798 ab	91 a-d	41 a	16 a-d	15.3 a-c	2.1 a-c
	R3	3973 ab	90 a-d	43 a	16 a-c	15.9 a-c	2.2 a
	V3 *fb*R1	3823 ab	90 a-d	46 a	18 ab	15.5 a-c	2 a-c
	V3 *fb* R3	4047 ab	94 a-c	43 a	18 ab	16 a-c	2 a-c
	R1 *fb* R3	4203 a	87 c-e	46 a	16 a-d	15.8 a-c	1.8 a-e
	V3 *fb* R1 *fb* R3	3619 a-c	87 c-e	46 a	16 a-d	15.2 a-c	2 a-c
1.12	V3	4254 a	97 a	44 a	17 a	15.6 a-c	2.1 a-c
	R1	3399 a-d	82 e-g	46 a	15 b-e	15.5 a-c	2 a-c
	R3	4113 a	93 a-c	43 a	17 a-c	16.1 a-c	2.1 a-c
	V3 *fb* R1	3419 a-d	82 e-g	44 a	16 a-d	15.1 bc	1.9 a-d
	V3 *fb* R3	3987 ab	89 b-e	42 a	17 a-c	15.8 a-c	2.1 ab
	R1 *fb* R3	3457 a-c	76 gh	44 a	14 c-e	15.1 bc	1.9 a-d
	V3 *fb* R1 *fb* R3	3290 a-d	70 h	39 a	13 ef	15.5 a-c	1.8 b-e
5.6	V3	4154 ab	92 a-c	43 a	16 a-d	16.1 a-c	2.2 a
	R1	3043 b-d	54 i	37 a	11 fg	15.1 bc	1.9 a-d
	R3	3890 ab	83 d-f	43 a	15 a-d	16.8 a	2 a-c
	V3 *fb* R1	2313 d	51 i	38 a	11 g	15.3 a-c	1.7 c-e
	V3 *fb* R3	3747 ab	73 hg	39 a	14 de	16.3 ab	2 a-c
	R1 *fb* R3	2605 cd	51 I	39 a	11 fg	14.9 bc	1.6 de
	V3 *fb* R1 *fb* R3	2310 d	38 j	39 a	10 g	14.7 c	1.5 e
Non-treated		4078.52 ab	100.1 a	47 a	18 a	15.9 a-c	2.2 a

[a] fb = followed by
[b]Means followed by the same letter within a column are not statistically different according to Fisher's Protected LSD (α = 0.05).

Table A.4 Response of offspring as a result of parent soybeans exposure to reduced doses of dicamba at varying growth stages with single and multiple exposures at Manhattan, KS in 2019.

Dicamba dose (g ae ha^{-1})	Time of application[a]	Germination (%)	Emergence (%)	Number Injured	Visual Injury	Height (cm)
	V3	82.5 a[b]	82.5 a	1 a	1.3 a	14.6 a
	R1	78.5 a	72.5 a	1.5 a	5.6 a	14.8 a
	R3	77.1 a	70 a	1.8 a	4.6 a	16.6 a
0.56	V3 *fb*R1	83 a	85 a	1.8 a	2.5 a	15.3 a
	V3 *fb* R3	84.3 a	90 a	0.5 a	3.8 a	16.8 a
	R1 *fb* R3	73.4 a	77.5 a	0.75 a	1.3 a	15.9 a
	V3 *fb* R1 *fb* R3	78.8 a	77.5 a	2.5 a	5.6 a	17.2 a
	V3	80.6 a	80 a	0.8 a	3.8 a	15.2 a
	R1	79.1 a	85 a	0.5 a	3.8 a	17.3 a
	R3	79.1 a	82.5 a	0.5 a	1.3 a	17.9 a
1.12	V3 *fb* R1	83.4 a	82.5 a	2.8 a	7.5 a	16.1 a
	V3 *fb* R3	78.1 a	75 a	2.8 a	5.6 a	15.7 a
	R1 *fb* R3	77.9 a	85 a	2 a	7.5 a	17.9 a
	V3 *fb* R1 *fb* R3	77.6 a	80 a	2.3 a	5.9 a	16.6 a
	V3	80.8 a	77.5 a	1 a	4.5 a	15.4 a
	R1	75.4 a	75 a	1.5 a	5 a	16.7 a
	R3	78.4 a	87.5 a	3 a	5.5 a	16.5 a
5.6	V3 *fb* R1	79.5 a	77.5 a	1 a	5.6 a	17.3 a
	V3 *fb* R3	75.9 a	85 a	2.8 a	8.8 a	15.5 a
	R1 *fb* R3	78.8 a	77.5 a	3.3 a	5.8 a	16.9 a
	V3 *fb* R1 *fb* R3	85 a	80 a	2.8 a	6 a	15.2 a
Non-treated		79.8 a	75 a	–	–	16.8 a

[a] fb = followed by
[b]Means followed by the same letter within a column are not statistically different according to Fisher's Protected LSD ($\alpha = 0.05$).

Table A.5 Response of offspring as a result of parent soybeans exposure to reduced doses of dicamba at varying growth stages with single and multiple exposures at Ottawa, KS in 2019.

Dicamba dose (g ae ha^{-1})	Time of application[a]	Germination (%)	Emergence (%)	Number Injured	Visual Injury	Height (cm)
0.56	V3	91 a[b]	87.5 a	0.5 a	1.3 a	15.6 a
	R1	89.5 a	92.5 a	2 a	5.5 a	14.4 a
	R3	87.1 a	82.5 a	1.5 a	6.9 a	15.9 a
	V3 *fb*R1	92.9 a	87.5 a	0.5 a	2.5 a	14.2 a
	V3 *fb* R3	89 a	85 a	1.3 a	2.5 a	16.5 a
	R1 *fb* R3	90.4 a	77.5 a	1.3 a	6.3 a	13.6 a
	V3 *fb* R1 *fb* R3	88.4 a	92.5 a	0.8 a	3.8 a	14.7 a
1.12	V3	90.5 a	91 a	0.3 a	1.3 a	15.2 a
	R1	90.5 a	85 a	1.5 a	4.3 a	14.9 a
	R3	88.5 a	87.5 a	1 a	3.8 a	15.2 a
	V3 *fb* R1	90 a	82.5 a	1 a	7.5 a	14.1 a
	V3 *fb* R3	89.1 a	95.5 a	1.5 a	5 a	15.7 a
	R1 *fb* R3	90.9 a	95 a	2.3 a	5.6 a	15.9 a
	V3 *fb* R1 *fb* R3	89 a	82.5 a	1 a	6.3 a	15.3 a
5.6	V3	92.8 a	92.5 a	0.8 a	3.8 a	16.1 a
	R1	89.3 a	85 a	1 a	4.4 a	13.1 a
	R3	86 a	90 a	2.3 a	6.8 a	15.9 a
	V3 *fb* R1	94.4 a	75 a	0.8 a	3.8 a	12.5 a
	V3 *fb* R3	89.6 a	90 a	2.8 a	6.3 a	14.3 a
	R1 *fb* R3	86.3 a	90 a	1.5 a	7.5 a	12.4 a
	V3 *fb* R1 *fb* R3	86.8 a	77.5 a	1.8 a	6.4 a	13.1 a
Non-treated		91.8 a	92.5 a	–	–	14.8 a

[a] fb = followed by

[b]Means followed by the same letter within a column are not statistically different according to Fisher's Protected LSD ($\alpha = 0.05$).

Table A.6 Analysis of variance of response of offspring as a result of parent soybeans exposure to reduced doses of dicamba at varying growth stages with single and multiple exposures at Manhattan, KS and Ottawa, KS in 2019.

Fixed effects	Reduction in emergence	Reduction of offspring height	Number injured	Offspring injury
	———————————— P-value ————————————			
Site-year	0.2546	0.9202	0.2443	0.9678
Dose	0.9377	0.2707	0.1159	0.0343
Timing	0.9931	0.1961	0.1023	0.0885
Site-year by dose	0.9844	0.7345	0.8146	0.7268
Site-year by timing	0.9871	0.4556	0.6040	0.6463
Timing by dose	0.9648	0.6984	0.2675	0.2117
Site-year by dose by timing	0.9794	0.5783	0.8749	0.9597

Appendix B - Supplemental information for chapter 3

Table B.1 Soybean yield data as a result of varying soybean varieties exposed to dicamba at varying application times at Manhattan, KS in 2018.

Time	Variety	Yield ($kg\ ha^{-1}$)	Height (cm)	Pods $plant^{-1}$	Nodes $plant^{-1}$	Seeds pod^{-1}	Seed weight (g)	Germination (%)
Non-treated	AG4135	2928 a-c[a]	69 bc	29 a-c	15 c	2 a	16.4 a-d	76 ab
	CR3841	2924 a-c	75 ab	23 c	16 bc	1.9 a	17.6 ab	47 de
	CR4748	3426 a	84 a	38 a	19 a	2 a	14.9 cd	69 bc
	ST40B	2868 a-c	65 b-d	30 a-c	16 bc	2.1 a	17.4 ab	70 b
V3	AG4135	3377 a	67 b-d	30 a-c	16 bc	1.9 a	16.4 a-d	88 a
	CR3841	3062 ab	66 b-d	29 a-c	14 c	2 a	17.9 a	53 c-e
	CR4748	3547 a	75 ab	37 ab	19 ab	1.5 a	15.2 b-d	74 ab
	ST40B	3012 a-c	58 cd	33 a-c	16 bc	2 a	16.9 a-d	77 ab
R1	AG4135	2349 b-d	45 ef	29 a-c	10 d	1.7 a	15.9 a-d	77 ab
	CR3841	2325 cd	45 ef	25 bc	11 d	1.9 a	17.3 a-c	45 e
	CR4748	2921 a-c	56 de	38 a	11 d	1.8 a	14.6 d	62 b-d
	ST40B	1821 d	42 f	32 a-c	10 d	1.7 a	15.9 a-d	68 bc

[a]Means followed by the same letter within a column are not statistically different according to Fisher's Protected LSD ($\alpha = 0.05$).

Table B.2 Soybean injury at senescence and yield data as a result of varying soybean varieties exposed to dicamba at varying application times at Manhattan, KS and Ottawa, KS in 2019.

Manhattan, KS

Time	Variety	Yield (kg ha^{-1})	Height (cm)	Pods plant^{-1}	Nodes plant^{-1}	Seeds pod^{-1}	Seed weight (g)
Non-treated	AG4135	4683 a[a]	106 ab	59 ab	20 a	1.7 a	15.5 a
	CR3841	4550 a	107 ab	55 b-d	19 ab	1.7 ab	16.2 a
	CR4748	4474 a	110 a	51 b-d	19 ab	1.8 a	15 ab
	ST40B	4348 ab	106 a-c	66 a	20 a	1.8 a	14.6 ab
V3	AG4135	4273 a	97 bc	52 b-d	13 c	1.8 a	15.4 ab
	CR3841	3728 b	95 c	56 a-d	10 c	1.8 a	15.8 a
	CR4748	3672 bc	98 bc	45 cd	14 bc	1.8 a	15.1 ab
	ST40B	4516 ab	99 bc	61 ab	14 bc	1.8 a	15.1 ab
R1	AG4135	3008 d	60 e	49 b-d	10 c	1.5 ab	14.7 ab
	CR3841	3104 cd	67 e	58 a-c	13 c	1.4 b	15.9 a
	CR4748	2923 d	79 d	44 d	10 c	1.5 ab	15.3 ab
	ST40B	2883 d	63 e	57 a-d	11 c	1.6 ab	13.6 b

Ottawa, KS

Time	Variety	Yield (kg ha^{-1})	Height (cm)	Pods plant^{-1}	Nodes plant^{-1}	Seeds pod^{-1}	Seed weight (g)
Non-treated	AG4135	3850 a	91 a-c	38 c	17 ab	2.1 ab	14.2 bc
	CR3841	3723 a	94 a	37 c	17 ab	2.1 ab	16 a
	CR4748	3865 a	93 ab	36 c	18 a	2.2 a	14.3 bc
	ST40B	3847 a	88 a-c	50 ab	18 a	2 a-c	13.7 c
V3	AG4135	3842 a	86 c	40 bc	16 ab	2.1 ab	14.5 bc
	CR3841	3648 a	88 bc	36 c	15 b	2.1 ab	15.5 ab
	CR4748	3974 a	89 a-c	32 c	17 ab	2.3 a	14.5 bc
	ST40B	3801 a	87 bc	54 a	18 a	2 a-c	13.9 c
R1	AG4135	2731 b	47 f	35 c	10 c	1.8 b-d	13.8 c
	CR3841	2864 b	54 e	41 bc	11 c	1.7 cd	15.3 ab
	CR4748	3569 a	60 d	32 c	9 c	2.1 ab	14.6 bc
	ST40B	2603 b	44 f	42 bc	10 c	1.6 d	14 c

[a]Means followed by the same letter within a column are not statistically different according to Fisher's Protected LSD ($\alpha = 0.05$).

Table B.3 Response of offspring as a result of parent soybeans with varying varieties exposed to dicamba at varying application times at Manhattan, KS and Ottawa, KS in 2019.

Manhattan, KS

Time	Variety	Germination (%)	Emergence (%)	Number injured	Visual injury (%)	Height (cm)
Non-treated	AG4135	83 ab[a]	75 a	-	-	12 b-c
	CR3841	79 ab	80 a	-	-	16 a
	CR4748	82 ab	83 a	-	-	13 a-d
	ST40B	86 a	87 a	-	-	11 cd
V3	AG4135	82 ab	93 a	1 a	5 a	11 cd
	CR3841	80 ab	73 a	1 a	16 a	14 a-c
	CR4748	85 ab	85 a	1 a	10 a	13 a-d
	ST40B	80 ab	77 a	0 a	0 a	11 cd
R1	AG4135	76 ab	70 a	1 a	11 a	10 d
	CR3841	73 b	78 a	2 a	9 a	15 ab
	CR4748	79 ab	75 a	1 a	5 a	11 cd
	ST40B	77 ab	90 a	1 a	5 a	11 cd

Ottawa, KS

Time	Variety	Germination (%)	Emergence (%)	Number injured	Visual injury (%)	Height (cm)
Non-treated	AG4135	90 a-d	95 a	–	–	14 ab
	CR3841	91 a-c	93 a	–	–	17 ab
	CR4748	93 ab	93 a	–	–	13 ab
	ST40B	92 ab	95 a	–	–	13 ab
V3	AG4135	90 a-d	98 a	1 a	5 a	14 ab
	CR3841	90 a-d	88 a	1 a	5 a	18 a
	CR4748	94 ab	95 a	2 a	5 a	15 ab
	ST40B	95 a	98 a	2 a	6 a	13 ab
R1	AG4135	84 d	88 a	2 a	5 a	13 ab
	CR3841	89 b-d	85 a	3 a	6 a	16 ab
	CR4748	93 ab	93 a	2 a	5 a	15 ab
	ST40B	86 cd	83 a	3 a	7 a	12 b

[a]Means followed by the same letter within a column are not statistically different according to Fisher's Protected LSD (α = 0.05).

Table B.4 Analysis of significance of fixed effects and all interactions for response offspring of soybeans with varying traits that were exposed to a reduced dose of dicamba at multiple timings.

Fixed effects	Reduction in emergence	Reduction of offspring height	Number injured	Offspring injury
	------------------------------- P-value-------------------------------			
Site-year	0.5016	0.2109	0.3805	0.7065
Variety	0.5871	0.2277	0.0891	0.0305
Timing	0.2297	0.1632	<.0001	0.1153
Site-year by variety	0.4140	0.0144	0.2045	0.0119
Site-year by timing	0.7948	0.7548	0.8899	0.8584
Timing by variety	0.1385	0.9633	0.0935	0.6646
Site-year by variety by timing	0.1090	0.4998	0.7941	0.2726