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Sensitivity of Soybean and Rice to Dicamba

Owen Wesley France
University of Arkansas, Fayetteville

Sensitivity of Soybean and Rice to Dicamba

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Owen Wesley France
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Jason Norsworthy, Ph.D.
Thesis Director

Trent Roberts, Ph.D.
Committee Member

Jeremy Ross, Ph.D.
Committee Member

Tom Barber, Ph.D.
Committee Member

Ed Gbur, Ph.D.
Committee Member

ABSTRACT

Although the integration of dicamba as a postemergence (POST) herbicide has proven useful in controlling many herbicide-resistant weeds, its damage to soybean at low rates, such as supplied by drift and volatility, has been well-documented. Injury to other crops, such as rice, from off-target movement of dicamba and other commonly used herbicides, such as glyphosate, may also occur. Practical options for mitigating injury and yield loss to soybean and other crops from dicamba off-target movement could prove beneficial if dicamba continues to be used during summer months. Experiments were conducted to determine the effects of multiple agricultural practices, including cultivar selection, planting date, irrigation, and fertilization, to soybean injured by a low dose of dicamba, and possible rice injury from dicamba and glyphosate. In an experiment evaluating sensitivity of soybean cultivars to a low dose of dicamba, only 'Eagle DrewSoy' maintained high relative yield and low visible injury, and was, therefore, considered as a likely candidate for enhanced tolerance to dicamba. Soybean exposed to a low dose of dicamba at the V3, R1, or sequentially at V3 followed by R1 growth stages that was planted after mid-June had significant yield loss compared to the nontreated that was planted at the same date. Above-average rainfall in the irrigation experiment may have negated the likelihood of finding differences in yield and injury between regimes. In the fertilization experiment, there was a negative effect of dicamba treatments applied at reproductive growth stages on soybean injury, but not relative yield. Fertilizer applications made shortly after the occurrence of injury did not aid in the recovery of soybean yield. Application timings before reproductive stages were reached could have provided different results by allowing fertilizers more time to aid soybean recovery from dicamba injury. For experiments evaluating the effect of low rates of glyphosate and dicamba on rice, greater crop injury and yield loss occurred when glyphosate and dicamba were mixed than when glyphosate was applied alone. This research shows that soybean injury and yield loss differ among soybean cultivars and planting dates, but not irrigation regimes in a wet year or fertilizer additions at the timings and rates used in this experiment. Rice injury and yield loss from glyphosate off-target movement may be exacerbated by the presence of dicamba in addition to glyphosate.

Nomenclature: Dicamba; glyphosate; rice *Oryza sativa* L.; soybean, *Glycine max* (L.) Merr.

Keywords: Herbicide sensitivity, off-target movement, planting date, irrigation, fertilization, injury, relative yield, rate

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CHAPTER 1

Overview of Dicamba Off-Target Movement and Potential Factors Affecting Movement

Herbicide off-target movement has posed a problem to producers since the commercialization of foliar herbicides. The launch of herbicide-resistant (HR) crops enhanced the issue due to the proximity of susceptible and resistant technologies. Herbicides, as a practical application of weed science, must be as adaptative as the weeds they control. The resurgence of dicamba in recent years, while providing an effective option for weed control, has also posed several new threats to farmers in the form of primary drift and volatility. To combat herbicide-resistant weeds, Bayer's new formulation of dicamba, XtendiMax + VaporGrip®, and BASF's formulation, Engenia, have brought dicamba to the forefront of herbicide controversy with expanded risk of drift and volatility over a longer application period – well into the growing season.

With the introduction of dicamba-resistant cotton (*Gossypium hirsutum* L.) in 2015 and soybean [*Glycine max* (L.) Merr.] in 2016, use of dicamba has increased nationwide. However, increased use of dicamba as a postemergence (POST) herbicide exposes susceptible crops, such as non-dicamba-resistant soybean, to off-target movement of the herbicide (Egan and Mortenson 2012). Although a widespread problem, several potential factors may prove vital in mitigating injury and yield loss among crops affected by dicamba off-target movement. Cultivar tolerance to specific herbicides has already been found and documented (Smith and Caviness 1973; Barrentine et al. 1976). Moreover, due to the increased frequency of soybean injury from dicamba, verification of soybean cultivars found to have enhanced tolerance to dicamba could mitigate yield losses in the crop. Other factors such as planting date, irrigation, and fertilization may also influence injury sustained by soybean from dicamba. Whereas rice is unlikely to have extensive sensitivity to dicamba, applying other herbicides in congruence with dicamba may increase symptomology and injury beyond either herbicide alone. For instance, the impact of glyphosate drift on rice may be compounded by the increased use and off-target movement of dicamba.

Plant Response to Crop Stressors

Crops, as sessile organisms, are constantly under threat from multiple biotic and abiotic stressors. Often, preventing stress to a crop from a single stressor can dissuade other stressors from negatively affecting the crop. For example, there is a direct relationship between abiotic stress and biotic stress incidence (Blaker and MacDonald 1981; Reymond and Farmer 1998; Ochola et al. 2015; Pandey et al. 2017; Tarafdar et al. 2018). A study examining the effect of water stress on disease development found that plants with greater water stress were physiologically weaker and, therefore, more susceptible to Xanthomonas wilt disease (Ochala et al. 2015). Blaker and MacDonald (1981) saw a similar response where plants exposed to either drought conditions or excessive water stress were more susceptible and showed greater disease symptoms. Plants respond to stressors, abiotic or biotic, by releasing various gene-encoded defensive proteins specific to the stressor, many proteins of which have multiple purposes and unique interactions depending on the stress, potentially altering plant physiology (Scherm and Coakley 2003; Pandey et al. 2017). Release of these defense proteins is moderated by altered gene expression; this will ultimately determine the ability of plants to cope with various stressors (Castro et al. 2005). At times, aggressors such as viral, bacterial, or fungal stress can disrupt or amplify certain defense pathways to the benefit of the stressor (Reymond and Farmer 1998). For example, disruption of salicylic acid, a signal pathway to alert the plant of a stressor and cause it to release a defense protein, can cause the plant to become susceptible to viral, fungal, and bacterial pathogens (Reymond and Farmer 1998). Signal pathways and signal molecules, such as salicylic acid, are directly involved in perceiving stress and activating stress-induced genes to initiate various defense protein activity (Wang et al. 2014). Similar to other stressors, plants also initiate defensive mechanisms against herbicides, such as grapevine (*Vitis vinifera* L.) releasing defense proteins in response to flumioxazin (Castro et al. 2005). While little research has been conducted to determine the effect of herbicide stress to plants in conjunction with other stressors, previous research on the response of plants to other types of single and compounding stressors has laid a foundation for researchers to expect a relationship. In addition, studying these relationships for the discovery of a means of mitigating injury and yield loss to crops from contemporary stressors, such as herbicide off-target movement, could lead to beneficial recommendations for producers.

Dicamba

Dicamba (3,6-dichloro-2-methoxybenzoic acid) is a synthetic-auxin herbicide (WSSA group 4) in the benzoic acid family and was patented by S.B. Richter in 1958 (Senseman 2007). First introduced under the tradename Banvel as the dimethylamine (DMA) salt of dicamba, the volatility and drift potential of the herbicide led to a history of attempting to minimize off-target movement (Senseman 2007; Mueller et al. 2013). Since its introduction, dicamba has been re-formulated to minimize volatility several times, most recently as N,N-Bis-(aminopropyl) methylamine (BAPMA), or Engenia ™ and the diglycolamine (DGA) formulated as XtendiMax® which includes a pH modifier to reduce volatility potential of this DGA formulation (Mueller and Steckel 2019). Both of the two new formulations of dicamba can be applied to dicamba-resistant soybean and cotton without resultant injury.

DMA vs. DGA

Dimethylamine salt (DMA) is used in the formulation of Banvel, while diglycolamine salt (DGA) is used in the formulation of XtendiMax as well as another formulation marketed as Clarity (Senseman 2007). The function of the DGA and DMA salts is to increase solubility of the herbicides in water and attraction to plant surfaces while decreasing the volatility of dicamba acid, which is highly volatile without additives, although even within formulations volatility varies (Behrens and Leuschen 1979). According to Mueller et al. (2013), dicamba detected by high-volume air samplers was twice as high for DMA salt than DGA formulated dicamba. Use of older formulations exacerbate the problem of volatility and subsequent widespread injury to sensitive crops such as soybean (Behrens and Leuschen 1979).

Use in Crops

Historically dicamba has been applied as a burndown herbicide prior to crop planting, or during the early stages of corn (*Zea mays* L.) growth and prior to soybean emergence (Weidenhamer et al. 1989; Al-Khatib and Peterson 1999; Anderson et al. 2004). The resurgence of dicamba as a postemergence (POST) herbicide is largely due to its effective control of challenging broadleaf weeds in soybean, including biotypes of weed species that have developed resistance mechanisms to multiple herbicide sites of action (Egan and Mortenson 2012; Mueller et al. 2013). For broadleaf weed control, dicamba was rated at 80 to 90% control of morningglory (*Ipomoea spp.* L.), cocklebur (*Xanthium strumarium* L.), ragweed (*Ambrosia artemisiifolia* L.), common lambsquarter (*Chenopodium album* L.), sicklepod (*Senna obtusifolia* L.), smartweed (*Polygonum spp.* L.), and pigweed (*Amaranthus spp.* L.) in field corn at a rate of 504 g ae ha^{-1} + 0.25% NIS of various dicamba formulations and can be used until the crop is up to 64 cm in height (Scott et al. 2018).

Until recently, dicamba was predominantly applied as a burndown, preplant and preemergence (PRE) application in corn (*Zea mays* L.), grain sorghum (*Sorghum bicolor* L.), and small grains. It was also used in pastures to control broadleaf weeds as dicamba is generally ineffective on grasses (Senseman 2007). In grain sorghum, dicamba can be applied at 280 g ae ha^{-1} up to 20 cm plant height (Scott et al. 2018). For dicamba-resistant soybean and cotton the POST application rate is 560 g ae ha^{-1}. Prior to the advent of dicamba-resistant crops, dicamba use in soybean was limited to a burndown herbicide with a 14-day plant-back interval for soybean at 140 and 280 g ae ha^{-1} use rates and a 28-day plant-back interval for applications greater than 280 g ae ha^{-1} (Scott et al. 2018). When these plant-back restrictions to non-dicamba-resistant soybean are not closely followed, injury from dicamba often results (Thompson et al. 2007). For non-dicamba-resistant cotton, subsequent planting is recommended at 21 days after dicamba burndown while corn can be planted immediately after dicamba application (Scott et al. 2018). Banvel (DMA) and Clarity (DGA) are both registered for use in corn and grain sorghum (Scott et al. 2018).

Dicamba Off-Target Movement

Off-target movement of dicamba is the primary mode by which non-dicamba-resistant soybean are injured (Behrens and Leuschen 1979; Scumbiato et al. 2004). Multiple modes of herbicide off-target movement exist, including movement with irrigation water (Willet et al. 2019), dust particles, or even landscape wood chips sourced from trees applied with certain herbicides (Patton et al. 2013). Physical spray drift and vapor drift are both concerns with dicamba use (Behrens and Leuschen 1979).

Physical Drift

Physical spray drift is the off-target movement of spray droplets projected from the spray equipment at the time of application (Maybank et al. 1978). Incidence of dicamba injury being observed on soybean more often may be explained by the high sensitivity of soybean to dicamba (Egan et al. 2014). Behrens and Leuschen (1979) found that, compared to 2,4-D, dicamba volatility on soybean was reported 8 times as often and with less than half as many acres of POST dicamba sprayed; this research was conducted using the older, DGA formulation of dicamba. Herbicide drift is highly influenced by wind and atmospheric turbulence at the time of spraying as well as temperature inversions (Maybank et al. 1978; Womac et al. 1997). Furthermore, proper selection of spray nozzle tips is critical to reducing off-target movement of herbicide due to compounding factors such as tip size, operating pressure, and spray liquid, all playing a role in droplet size, and therefore drift occurrence (Womac et al. 1997). According to Womac et al. (1997), droplet size plays a focal role in drift potential with larger, heavier droplets typically being more resistant to drift.

The simplest means to reduce the number of droplets that drift easily is by upgrading nozzle technology (Ramsdale and Messersmith 2001). One study reported a decrease of 30% driftable droplets exuded from a nozzle to only 2% driftable droplets just by changing nozzle type and maintaining output pressure (Ramsdale and Messersmith 2001). Spray droplets smaller than 100 to 200 μm have the greatest drift potential as they require more time to reach the ground and they are blown off target by minimal air movements and prone to evaporation. The use of air-induction nozzles has been found to reduce physical drift by injecting spray droplets with air (Womac et al. 1997; Alves et al. 2017).

Volatility

Although all herbicides are prone to particle drift, volatility can vary depending on the herbicide and formulation (Maybank et al. 1978; Behrens and Leuschen 1979; Griffin et al. 2013). Off-target movement from volatility, as opposed to physical drift movement, is caused by the evaporation of herbicide active ingredients into the atmosphere as a gaseous compound following application. The vapor from volatilized compounds can move up to several miles and injure crops far removed from the initial application site. Different types of surfaces can also influence the emissions of volatile dicamba (Behrens and Leuschen 1979). Until recently, dicamba volatility has been primarily combated by formulating the products as salts (Senseman 2007). Most volatilization occurs within the first 12 hours after application; however, continued volatilization can occur for up to four days and likely longer (Behrens and Leuschen 1979; Senseman 2007; Mueller et al. 2013). Two of the primary factors affecting volatilization are air and soil surface temperature and relative humidity (Mueller et al. 2013). Egan and Mortenson (2012) found that higher volatilization occurred at lower humidity levels, and volatilization increased as temperature increased; however, volatilization plateaued when temperatures increased beyond 40°C (Behrens and Leuschen 1979; Mueller et al. 2013). Fluctuations in air movement and wind directions after application make it difficult to predict where the volatilized compound will travel. As both temperature and humidity fluctuate in a predictable manner throughout the day, timing of application may prove a significant dynamic in reducing volatilization (Mueller et al. 2013).

Tank-contamination

The final stage in pesticide applications is cleaning the spray through repeated rinsing of equipment to dilute and dislodge any remaining solution (Osborne et al. 2015). In the U.S., a triple-rinse strategy using water is recommended for adequate removal and dilution of pesticide solution from the spray equipment to a concentration of 1% or less of the initial concentration. One study found that dicamba was removed with 100% efficiency to a concentration of 1% or less (average 0.16%) of the initial tank solution, whereas 2,4-D was removed only at 98% efficiency when the spray tank was triple rinsed (Osborne et al 2015). A fourth rinse may be needed to adequately remove pesticide residues from the spray tank and eliminate capability of unintended injury to particularly susceptible crops (Osborne et al. 2015). In the case of dicamba, as little as 1% of the dicamba use rate for dicamba-resistant soybean can easily cause symptomology on non-dicamba-resistant soybean cultivars (Anderson et al. 2004; Boerboom 2004; Johnson et al. 2012; Griffin et al. 2013). In a study by Bales and Sprague (2019) simulating tank-contamination rates of dicamba and 2,4-D to dry bean (*Phaseolus vulgaris* L.), an application rate of 1% the use rate of dicamba to Xtend™ soybean (5.6 g ae ha^{-1}), simulating rates remaining after sprayer cleanout, injured dry bean by 20% at 14 days after treatment (DAT). This level of injury declined to only 8% by 28 DAT. For 2,4-D applied at 1% of the use rate for Enlist™ soybean (11.25 g ae ha^{-1}), injury was minimal, at 2% or less 14 DAT (Bales and Sprague 2019).

Factors Impacting Dicamba Off-Target Movement

Taking advantage of stacked traits, such as glyphosate- and dicamba-resistant technology, is an efficient way to control a variety of weeds, and it is common for producers to apply glyphosate and dicamba together to achieve greater weed control (Mueller and Steckel 2019);

however, as a result of mixing dicamba and glyphosate, changes of the spray solution occur that could result in undesirable effects (Mueller and Steckel 2019). Research conducted by Alves et al. (2017) found that when spraying dicamba and glyphosate together, droplet size was much more varied compared to either herbicide alone, no matter the nozzle type. Mixing of dicamba and glyphosate leads to an increase in number of very small spray droplets exuded from nozzles, known as driftable fines, and subsequent increase in risk for physical drift. Research also suggests that spraying dicamba plus glyphosate increased drift events compared to dicamba alone for AIXR and XR nozzles, whereas coarser droplets were produced from dicamba alone than dicamba in solution with glyphosate when using air-induction nozzles (Alves et al. 2017). In addition to effects of spray droplet size a recent study by Mueller and Steckel (2019), dicamba treatments in their experiment that contained glyphosate had consistently lower pH values than dicamba alone, regardless of dicamba formulation. Moreover, potential of dicamba to volatilize is known to increase as pH decreases (Hemminghaus et al. 2017; Mueller and Steckel 2019). According to Hemminghaus et al. (2017) dicamba-containing solutions with a pH lower than 5 are more highly associated with off-target movement. When Mueller and Steckel (2019) analyzed the effects of formulations of dicamba with and without glyphosate and other compounds, the final pH was always above 5 only when glyphosate was not included in the solution. As early as 2005, a study by Kelley et al. (2005) noted an interaction between dicamba and glyphosate on GR soybean with increased visible injury from V3 and V7 applications when dicamba was applied at 5.6 g ae ha^{-1} and glyphosate at 1250 g ae ha^{-1} versus when dicamba was applied alone at the same rate.

Soybean

U.S. soybean production began in 1765 as a forage crop for livestock. It was not until 1941 that soybean production for grain, as *Glycine max*, became predominant and a staple in American agriculture (Gibson and Benson 2005). The U.S. was the world's leading soybean producer with 120.58 metric tons harvested in 2018 (USDA-NASS 2020). With 96.6 million metric tons harvested by the U.S. in 2019, Brazil is expected to pass the U.S. as the highest world soybean producer (USDA-NASS 2020). In 2018, Arkansas was the 11th largest producer of soybean in the United States at 4.49 million metric tons of soybean harvested (USDA-NASS 2020). In the U.S., 60% of the soybean grain produced is exported. Soybean grain not exported is processed into meal, oil, and other forms for use in human and animal nutrition, and into biofuel for multiple industrial uses (USB 2020).

Herbicide-Resistant Soybean

Weeds that thrive in agriculture do so because of their ability to adapt to changing environments, including the methods of control imposed on them; therefore, to preserve efficacy, methods of weed control must be just as adaptative or resistance to controls will persist. Soybean weed management is dominated by herbicide use with heavy reliance on herbicide-resistant traits to manage weeds and overcome herbicide-resistance among various weed species, such as Palmer amaranth [*Amaranthus palmeri* (S.) Watson] and horseweed [*Conyza canadensis* (L.) Cronq.]. Palmer amaranth is a common weed of soybean that is resistant to 8 separate sites of action in the U.S. and horseweed is resistant to 4 sites of action in the U.S (Heap 2020). In addition, both Palmer amaranth and horseweed contain biotypes resistant to more than a single site of action (Heap 2020). This type of resistance buildup is common when methods of control are not rotated properly (Norsworthy et al. 2012). Glyphosate-resistant traits in cotton and soybean were the first herbicide-resistant crops available. Following the introduction of herbicide-resistant crop technology, intense pressure from heavy glyphosate use by producers allowed for rapid selection of glyphosate-resistant weeds (Owen 2016). Herbicide resistance among weeds is not a new concept, and repeated use of a single herbicide has long been suspected as a contributor to resistance occurrences among some weed scientists (Harper 1956). Besides heavy selection, producers seeking to reduce production costs by lowering the rates of effective herbicides, such as glyphosate, also contributed to herbicide-resistance among weeds, particularly non-target site resistance (Owen 2016). Currently there are 521 unique cases of herbicide-resistant weeds globally, represented by 262 species (Heap 2020). As of 2017, Palmer amaranth was listed as the most problematic weed in Arkansas, Louisiana, southeast Missouri, Mississippi, and Tennessee (Schwartz-Lazaro et al. 2017). Much of this is due to the resistance of Palmer amaranth to multiple sites of action making it extremely difficult to control though chemical means, especially with herbicide programs containing a single site of action. As an alternative to control resistant weed species, dicamba-resistant cotton and soybean have been made available to producers as of 2015 and 2016, respectively; however, there are issues with off-target movement of dicamba applications during the growing season.

Dicamba Effects to Soybean

Synthetic auxin herbicides mimic the action of natural auxin hormones used to regulate growth within plants (Cobb and Reade 2010). As plants metabolize synthetic auxins applied in concentrated doses, a condition of auxin overdose causes rapid unregulated growth within the plant. An additional effect of the herbicide is production of reactive oxygen species that ultimately kill the plant generally over several weeks. Different species, and even different cultivars within a species, react differently to synthetic auxins. Thompson and Egli (1973) noted that soybean had greater sensitivity to dicamba due to easier translocation of dicamba within the plant and higher volatility compared to 2,4-D. Due to the herbicide site of action, dicamba has representative symptomology for plants treated with synthetic auxins (Cobb and Reade 2010).

Symptomology

Dicamba injury at drift rates manifest on soybean as symptomology indicative of a synthetic auxin with varying degrees of leaf cupping and crinkling on mature leaves, more so than is typical of other synthetic auxins (Wax et al. 1969). Extensive damage manifests at terminal leaflets where it can cause leaf cupping, epinasty, and apical bud death at higher rates (Wax et al. 1969; Al-Khatib and Peterson 1999; Solomon and Bradley 2014). Cracked and swollen stems, as well as severe shoot and petiole epinasty, swollen petioles, and terminal or plant death are also common (Wax et al. 1969; Al-Khatib and Peterson 1999; Solomon and Bradley 2014). Increased branching, especially at rates where injury was enough to kill the terminal bud, was noted by Wax et al. (1969), among others (Behrens and Leuschen 1979; Weidenhamer et al. 1989; Anderson et al. 2004). High rates of dicamba were found to cause curled and malformed pods as well, with leaf margin injury and size reduction also common (Weidenhamer et al. 1989; Anderson et al. 2004). Although younger plants are often more susceptible to synthetic auxin injury, an equivalent rate of dicamba closer to reproductive stages can cause greater yield loss due to disruption of flowering and pod set, which can continue for up to three weeks depending on the herbicide rate, as dicamba continues to manifest at plant terminals (Al-Khatib and Peterson 1999; Solomon and Bradley 2014).

Yield

Yield loss from herbicide injury, influenced by application timing, is of primary concern when considering the consequences of off-target herbicide movement. Yield reduction by off-target dicamba movement can be substantial as multiple studies using non-lethal and extremely low rates of dicamba have documented. At respective application rates, Anderson et al. (2004) noted that yield and application rates of dicamba were negatively correlated, whereas only the highest equivalent rate of 2,4-D resulted in yield loss. Dicamba, among other synthetic auxins, has the highest yield loss potential when applied in the early reproductive stages; however, yield loss can still occur when applied during vegetative growth (Anderson et al. 2004; Griffin et al. 2013). At one-fiftieth (11.2 g ae ha^{-1}) and one-tenth (56 g ae ha^{-1}) the labeled use rate in corn, Anderson et al. (2004) found that dicamba applied to soybean at the V3 growth stage reduced yield by 41 and 83% that of the control, respectively. When COC was added to the 56 g ae ha^{-1} rate, yield dropped to 93% of the nontreated (Anderson et al. 2004). Similar yield reduction in soybean at a one-fiftieth use rate of dicamba was reported for 2,4-D at 20% the use rate in corn, the use rate in corn being 560 g ae ha^{-1} (Anderson et al. 2004). Applications at such low rates of dicamba can be very injurious to soybean and capable of causing yield loss, which correlates with more recent research (Solomon and Bradley 2014). Compared to vegetative treatments of dicamba, Wax et al. (1969) observed a 549 kg ae ha^{-1} decrease in mean yield when dicamba was applied at very early (R1-R2) reproductive stages. Data collected by Weidenhamer et al. (1989) paralleled this finding where dicamba at 9 to 11 g ae ha^{-1} applied during bloom caused an equivalent yield loss in pre-bloom application of 56 to 70 g ae ha^{-1}; however, actual yield data are not mentioned. Auch and Arnold (1978) also noted yield reduction at the early bloom stage while no yield reduction occurred with the same rate applied pre-bloom.

Injury

Several studies have reported greater visible dicamba injury at vegetative versus reproductive stages. At rates of 4.4, 8.8, 17.5, and 35 g ae ha^{-1}, soybean injury at 14 days after application was highest for V3/V4 application versus the R1 application (Griffin et al. 2013). Greater injury at vegetative stages is likely because soybean is still extending nodes and new trifoliates, allowing for higher visible manifestation of dicamba injury, whereas by R1 growth stage, growth is slowing as the plant prepares to produce seed and pods, allowing instead for higher yield reduction at these timings. Egan et al. (2014) also notes that higher yield loss is likely to occur from dicamba drift to soybean during flowering stages as opposed to earlier growth stages. Solomon and Bradley (2014) note that symptoms on R2-applied soybean occurred predominantly on newer tissues, resulting in lower injury ratings while V3-applied soybean showed more injury as plants produced more vegetation. At a dicamba rate of 0.56 g ae ha^{-1} applied at V3, V7, and R2 growth stages, soybean injury was rated highest at V3 with an injury rating of 37% and ratings of 31 and 25% for V7 and R2 treatments, respectively (Kelley et al. 2005). Kelley et al. (2005) also observed an intensification of injury until two weeks after treatment, after which visible injury gradually declined; this phenomenon has been recorded by previous research as well (Al-Khatib and Peterson 1999).

Maturity

Another common correlation with injury is a delay in maturity. Delayed maturity could have a negative effect on yield and result in later harvest dates, increasing producer risk. Dicamba plus picloram severely delayed soybean maturity when applications were made during reproductive growth stages and to a lesser extent for pre-bloom applications (Wax et al 1969; Solomon and Bradley 2014). At a dicamba rate of 28 g ae ha^{-1}, soybean maturity was delayed 5 to 8 days versus R2 applications that delayed maturity 23 to 26 days (Solomon and Bradley 2014). At a dicamba rate of 2.8 g ae ha^{-1}, maturity was delayed 1 to 4 days by the V3 application and 1 to 16 days by the R1 application (Solomon and Bradley 2014).

Height

Height reduction accompanies symptomology common to dicamba injury, increases with injury, and is more common when injury occurs during vegetative growth (Solomon and Bradley 2014). Weidenhamer et al. (1989) noted that height reduction often accompanied yield loss. At dicamba rates less than 1.3 g ae ha^{-1}, Weidenhamer et al. (1989) found that growth was not severely affected by dicamba application, while higher rates, such as 20, 40, and 80 g ha^{-1} decreased plant height by as much as 62% for pre-bloom treatments. Weidenhamer et al. (1989) also stated that height reduction was more severe during dryer conditions where rainfall could not facilitate recovery. However, Solomon and Bradley (2014) found that, at 28 g ae ha^{-1}, soybean height reduction from dicamba was greater from R2 treatments than V3 treatments at 80 and 74% reduction, respectively. In a study by Kelley et al. (2005) involving multiple auxin herbicides, all herbicides except dicamba reduced soybean height at the lowest rate for the R2 application, with the exception of dicamba plus diflufenzopyr. For all auxin herbicides tested, the greatest height reductions occurred at the V7 application for all rates (Kelley et al. 2005).

Soybean Herbicide Tolerance

Incidence of crop tolerance to specific herbicides has been noted in various research. In a study of the response of 44 soybean cultivars to metribuzin, two cultivars were only slightly injured and two cultivars were killed (Barrentine et al. 1976). Additionally, in testing the effects of metribuzin on total oil content of different soybean cultivars, Hardcastle et al. (1974) found that oil concentration was significantly impacted in four cultivars and oil quality impacted in one cultivar compared to the non-treated soybean. Other studies have examined the genetic background of tolerance exhibited by select cultivars. Barrentine et al. (1976) deduced that a single recessive gene was responsible for the tolerance of soybean to metribuzin. Although not specific to yield, these experiments illustrate the potential for variable effects from a stressor across cultivars of a crop. Cultivar differences in relation to yield and herbicide injury were observed in response to 2,4-D and 2,4,5-T as early as the 1950s (Fribourg and Johnson 1955).

Rice

Rice is an important and unique commodity crop to the United States with only several states producing rice, including Arkansas, California, Mississippi, Texas, and Louisiana most commonly (Norman, personal communication 2019). In 2019, 1,000,810 ha of rice were harvested in the U.S. with almost half harvested in Arkansas (USDA-NASS 2020). Arkansas has been the largest rice producing state for many years with California in second place (USDA-NASS 2020). Rice is currently grown in 40 of the 75 counties in Arkansas, with most produced in eastern Arkansas (Hardke 2018). Most Arkansas rice is grown in a delayed-flood system with drill-planting done near the end of April and flooded at the 4- to 5-leaf stage (Hardke 2018). Rice is not drought tolerant and thrives in flooded conditions. However, flooding primarily is used to reduced weed pressure (Hardke 2018; Smith and Fox 1973).

Glyphosate Injury to Rice

Glyphosate is one of the most frequently used herbicides in the world because it has a broad spectrum of activity, can be used in glyphosate-resistant (GR) crops, and is used in agriculture and industrial settings outside of strictly agricultural usage. With the widespread and relatively high use of glyphosate, opportunity for drift of glyphosate is also elevated. Until recently, dicamba drift on rice is believed to cause negligible to no injury alone (Castner et al. 2021); however, glyphosate can be very injurious to rice at drift rates (Ellis et al. 2003). Glyphosate is a non-selective herbicide that controls plants by inhibiting 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase (WSSA group 9) in plants, resulting in general symptomology of foliar chlorosis and necrosis, as well as shikimate accumulation in plants, such as rice, which are highly susceptible to glyphosate (Koger et al. 2005; Senseman 2007; Hensley et al. 2013). Koger et al. (2005) documented different tolerance to glyphosate between two rice cultivars. Shikimate accumulation results from inhibition of EPSP synthase and the subsequent unregulated flow of carbon into the shikimate pathway (Koger et al. 2005; Hensley et al. 2013). At a comparable rate of glyphosate, 'Cocodrie' yield was reduced by 92% versus 'Priscilla' which was reduced by only 60%. Shikimate concentration between cultivars corresponded with yield loss, with higher levels of shikimate corresponding with further reduced yield; however, more injury was observed in 'Priscilla' than 'Cocodrie', indicating visible injury is not a good indicator of yield according to this study (Koger et al. 2005). Following a treatment of glyphosate to rice at boot stage, Hensley et al. (2013) noted symptomology such as multiple shoots arising from secondary nodes and the flag leaf and secondary nodes appearing wrinkled, contorted, or rolled. According to Hensley et al. (2013) when glyphosate is applied at the one-tiller stage, yield reduction on rice is higher than at any other application timing. Applications of glyphosate at boot and one tiller reduced yield by 35 to 52% of the nontreated, respectively (Hensley et al. 2013).

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CHAPTER 2

Effect of Cultivar and Planting Date on Soybean Response to Dicamba

Abstract

Off-target movement of dicamba has been blamed for damaging millions of hectares of soybean in the United States since registration of the herbicide for use in dicamba-resistant cotton and soybean. Understanding the effect of a low dose of dicamba on non-dicamba-resistant soybean across multiple cultivars, growth stages, and planting dates could help producers better understand the implication of current management practices on yield loss from dicamba in fields where non-dicamba-resistant soybean are grown. A field experiment was conducted in 2019 in Fayetteville and Stuttgart, Arkansas, to evaluate the impact of planting date on response of soybean to a low dose of dicamba. The planting date experiment hypothesis was that soybean injury and yield loss will differ depending on planting date and dicamba application timing. Additionally, an experiment was conducted in 2018 and 2019 in Fayetteville to assess whether cultivars differ in sensitivity to dicamba. The cultivar experiment hypothesis was that genetic differences of soybean cultivars will allow for differential tolerance to dicamba. In the cultivar experiment, 'Eagle DrewSoy' was identified as having enhanced tolerance to dicamba based on reduced injury (47% at R1 and 26% at V3) over both experimental years and locations. Soybean height in this experiment was affected only by application timing. In the planting date experiment, planting after mid-June resulted in significantly reduced yields from dicamba injury. Dicamba exposure reduced yield at the July planting date (61% reduction from nontreated) more severely when compared to dicamba-treated plots of other planting dates (94% average relative yield among other planting dates), indicating that the negative effects of dicamba are increasingly deleterious for soybean planted later in the growing season. Maximum injury

manifestation was generally delayed at later planting dates, indicating that dicamba may have been metabolized more slowly.

Nomenclature: Dicamba; soybean, *Glycine max* (L.) Merr.; cotton, *Gossypium hirsutum* (L.)

Keywords: Herbicide sensitivity, tolerance, off-target movement

Introduction

Selection and cross-breeding have been used to modify crops and create new cultivars with unique advantages for thousands of years; meanwhile, utilization of crops based on differing response to herbicides is a staple of modern agriculture, such as the use of herbicide-resistant cultivars. Discovery of existing cultivars with differential tolerance to herbicides could help farmers make practical decisions when faced with modern challenges such as herbicide off-target movement. For example, a study of postemergence applications of bentazon at 3.4 kg ha^{-1} to several hundred cultivars of soybean saw little effect to most soybean cultivars tested; however, all plants of the cultivar 'Hurrelbrink' were killed, and 10 other cultivars were extremely sensitive (Wax et al. 1974). Research such as this proves variability in crop response to herbicides may exist across cultivars.

Herbicide off-target movement to sensitive crops at reduced rates is common. In a study focusing on off-target movement of propanil to soybean, a differential injury response was noted among soybean cultivars tested, with yield of the more highly injured cultivars also significantly reduced compared to most other cultivars tested (Smith and Caviness 1973). Specific to synthetic auxins, as early as 1978, it was reported that soybean cultivars may differ in yield loss in response to a low rate of dicamba (Auch and Arnold 1978). In this research, one cultivar did not experience yield reduction to an early-bloom-stage application of dicamba whereas four others did. The extent of injury to soybean from dicamba exposure may also differ among cultivars

(Alves et al. 2020). One study saw equal yield reductions from a dicamba application to two cultivars of non-dicamba-resistant soybean, but height reduction as a result of dicamba application varied between the cultivars (Auch and Arnold 1978). Further research in differential tolerance of soybean to herbicides could help provide practical data for better management decisions via cultivar selection.

Planting date is one of the most important factors of crop production. Research into the effects of planting date in conjunction with the effect of other factors to crops, such as herbicide injury, could provide practical information for farmers when making production decisions. Planting date impacts many aspects of soybean production, including growth (Bastidas et al. 2008), development (Chen and Wiatrak 2010), yield (Zhang et al. 2010), and even grain quality (Hu and Wiatrak 2012). Planting date can have an impact on crop emergence, with faster emergence representative of later plantings (Zhang et al. 2010). Later-planted soybean typically experiences shorter intervals between planting and initial flowering (Parker et al. 1981). Chen and Wiatrak (2010) found that the shorter vegetative and reproductive growth stages of late-planted soybean occurred in response to increased radiation interception, such as during mid- and late summer (Zhang et al. 2010), whereas soybean planted earlier usually yield higher due to a longer duration of vegetative and reproductive stages (Chen and Wiatrak 2010). Later sowing dates for soybean extend the time between R6 and seed maturity, and in general, later sowing dates result in lower yields, although this can vary some depending on cultivar (Zhang et al. 2010).

Grain yield is highly dependent upon planting date, with later planting dates typically resulting in lower yields (Zhang et al. 2010). Yield decline from late planting is mostly due to a reduction in pod number (Zhang et al. 2010) and total nodes (Bastidas et al. 2008). Under longer photoperiods associated with late planting, the length of soybean vegetative and reproductive stages decreases, contributing to yield loss (Hu and Wiatrak 2012). In addition to photoperiod, increased temperature and decreased precipitation can also influence the yield and growth of late-planted soybean (Hu and Wiatrak 2012). Maturity group can also affect optimum planting date depending on region; for example, among soybean with maturity groups V-VIII, the optimum planting date on the Georgia Coastal Plain ranged from May to early June (Parker et al. 1981). The optimum planting date ranges from late May to early June for soybean planted in the Midwest, Upper South, and Deep South (Egli and Cornelius 2009).

Plant biomass, height, and other yield-related factors can also be impacted by late-planting soybean (Weaver et al. 1991). One study found that soybean height was reduced by 19 cm at a mid-June planting date compared to an early-May planting (Bastidas et al. 2008). Later planting dates have also been shown to negatively impact the oil and protein content of soybean (Hu and Wiatrak 2012). With the recent introduction of dicamba-resistant technology, taking into consideration the sensitivity of non-dicamba-resistant soybean to dicamba, as well as the potential for off-target movement of the herbicide, understanding the impact of management factors such as cultivar selection and planting date on sensitivity of the crop to the herbicide and the ability of the crop to recover from injury may allow growers to better understand risks and expectations when injury to soybean occurs. Therefore, field experiments were conducted to evaluate the effect of planting date and cultivar selection on soybean injury and yield in response to a low dose of dicamba.

Materials and Methods

General Methodology. For all experiments, the design was a randomized complete block with treatments in a split-plot arrangement with four replications. A non-treated control was included for comparison. The dicamba rate for each experiment was 2.2 g ae ha^{-1} or 1/256th of a 1X rate (560 g ha^{-1}) for dicamba-resistant soybean and cotton. Dicamba was applied using CO_2-pressurized backpack sprayers calibrated to deliver 140 L ha^{-1} at 276 kPa using AIXR 110015 nozzles (Teejet Technologies, Springfield, IL, 62703). All field sites were disked and field cultivated prior to forming raised beds for planting. Herbicides labeled for use in conventional soybean were used throughout the season as well as row cultivation and hand weeding as needed. Each trial was furrow-irrigated approximately once weekly if less than 2.5-cm of rainfall occurred over a 7-day period using polytube irrigation equipment (Polytube™, Delta Plastics of the South, Stuttgart, AR, 72160). Grain was harvested from the center two rows of each 4-row plot using a small-plot combine (Almaco™, Nevada, IA, 50201) following maturity. Grain moisture was measured and corrected to 13% moisture. Relative yield was calculated for each cultivar by comparing the yield of treated and nontreated plots (treated yield/nontreated yield*100).

Cultivar Experiment

Field experiments were conducted in 2018 and 2019 at the Milo J. Shult Arkansas Agricultural Research and Extension Center in Fayetteville, AR (36.1° N, 94.1° W). The experiment evaluated tolerance of several commercial cultivars of non-dicamba-resistant soybean to a low rate of dicamba. For the split-plot arrangement, the whole-plot factor was cultivar and the split-plot factor was soybean growth stage at time of dicamba application. The cultivars were chosen from ones having a maturity group (MG) of 4.6 to 5, which represent the optimum MG range for Arkansas (Heatherly 1999). The soil series for the trials in 2018 and 2019 was a Leaf silt loam (Fine, mixed, active, thermic Typic Albaqualts), with the 2018 trial having 25% sand, 64% silt, and 11% clay, 1.67% organic matter (OM), and a pH of 6.0, and the 2019 trial having 17% sand, 74% silt and 9% clay, 1.75% OM, and a pH of 6.6. The experiment included a V3 and an R1 timing for dicamba application with a total of 21 cultivars evaluated in 2018. Some cultivars were discontinued and thus not available in 2019, leaving a total of 15 cultivars for evaluation. Cultivars included in the 2018 trial year were: Pioneer P47A76L, Progeny P4930 LL, UA 5014C, GoSoy 4912 LL, GoSoy 49L17, GoSoy 5115 LL, GoSoy 51C17, Ireane, Leland, Eagle DrewSoy, Asgrow AG4835, Schillinger 495, Pioneer 47B17, GoSoy 49G16, GoSoy 50G17, Delta Grow DG4790, Delta Grow DG4970, Delta Grow DG4670, Delta Grow DG4880, Delta Grow DG4977, Delta Grow DG4967 (Table 2). The 2019 trial did not include GoSoy 5115 LL, Asgrow AG4835, Schillinger 495, GoSoy 47B17, Delta Grow DG4970, or Delta Grow DG4670.

The experiments were planted on May 22 in 2018 and on May 27 in 2019. All soybean cultivars were planted at a 2.5-cm depth and a row spacing of 91 cm. Each four-row plot was 6.1 m long with a 1.5-m alley. Dicamba (Clarity™, BASF Corporation, Research Triangle Park, NC) was applied at the V3 or R1 stages of growth with 2.2 g ae ha^{-1} of dicamba.

Visual estimates of injury were rated 21 days after treatment (DAT) on a 0 to 100 scale, where 0 = no injury and 100 = plant death. At soybean maturity (R8), the height of five plants per plot was measured from the soil surface to the terminal in centimeters and reported relative to the nontreated for each cultivar. Possible differences in crop maturity caused by dicamba were evaluated by recording the date soybean in each plot reached maturity (R8) and reporting each relative to the corresponding nontreated. Because of the different number of cultivars tested between years, 2018 and 2019 data were analyzed and are presented separately; therefore, fixed effects include year. Injury was analyzed as a beta distribution while relative height, maturity date, and relative yield were analyzed as a gamma distribution using ANOVA with SAS 9.4 using PROC GLIMMIX (Gbur et al. 2012) with means separated using Fisher's LSD (α=0.1). Differences between treatments that the researcher believes are real were not captured with a restricted alpha value of 0.05, therefore, the alpha value was adjusted to 0.1. This decision helps account for genetic differences between cultivars among injury and yield data which were often biologically large but not significant at an alpha value of 0.05. The pre-existing genetic differences among soybean cultivars were unique to this experiment, thus, an alpha value of 0.05 was preserved among other experiments.

In 2019, Cercospora leaf blight (*Cercospora kikuchii*) (CLB) was observed on soybean at the end of the growing season in both the cultivar and planting date experiments in Fayetteville. Symptomology of CLB appeared more common on dicamba-treated than nontreated plots. Disease ratings for CLB were taken in both trials when soybean was at, or very near, the R6 growth stage. Ratings consisted of visible assessment of disease incidence per plot (how much of each plot showed CLB symptomology) on a scale from of 0 to 3, with 0 being no incidence and 3 being 67% or more of the plot showing symptoms. All ratings were analyzed as multinomial distributions using separation tests via contrast statements with the ratings analyzed as cumulative logit data in SAS 9.4 using PROC GLIMMIX (Gbur et al. 2012). Because contrast statements were used, obtaining letter separation data with a 'least squares means' statement in SAS was not possible.

Planting Date Experiment

A field experiment was conducted in 2019 at the Milo J. Shult Arkansas Agricultural Research and Extension Center in Fayetteville, AR, and at the Rice Research and Extension Center near Stuttgart, AR (34.3° N, 91.3° W). At both locations, the late MG 4, glufosinate-resistant cultivar 'Credenz CZ 4820LL' (BASF Corporation, Raleigh, NC) was planted at 346,000 seed ha^{-1} in four-row plots 7.6 m in length having a row width of 76 cm. The whole-plot factor was planting date (mid-April, mid-May, mid-June, and mid-July) and the split-plot factor was growth stage at the application of dicamba (Xtendimax™ herbicide, Bayer CropScience, St. Louis, MO) (none, V3, R1, and V3 followed by R1). Actual planting dates in Fayetteville were April 11, May 15, June 13, and July 15. Plots were 6.1 meters in length with a 1.5-meter alley between replications.

Ratings of visual injury were taken on a scale of 0 to 100% with 0 representing no injury and 100 complete plant death. Injury ratings were taken at 14, 21, and 28 DAT on a scale of 0 to 100% and were analyzed as a beta distribution in a repeated measures analysis using the first order autoregressive (AR[1]) covariance structure. Possible differences in crop maturity caused by dicamba were evaluated by recording the date soybean in each plot reached maturity (R8) and reporting each relative to the nontreated check within each planting date. Yield data were taken at harvest and made relative to the nontreated within each planting date. The effect of replication was nested within year as a random effect to account for variability between years, while the effects of planting date, growth stage at application, and the interaction of each were analyzed as fixed effects. Injury ratings were analyzed as a beta distribution, while maturity date and relative yield were analyzed as gamma distributions using ANOVA with SAS 9.4 using PROC GLIMMIX (Gbur et al. 2012) with means separated using Fisher's protected LSD (P=0.05).

Results and Discussion

Cultivar Experiment. To ensure all real effects were captured, the alpha level used in the analysis of all data in this experiment was changed from 0.05 to 0.1 (see Materials and Methods under "Cultivar Experiment"). Treatment of soybean with a low dose of dicamba did not negatively affect soybean maturity in either year (Table 1). The level of injury caused by the V3 dicamba application differed by year. In 2018, soybean injury following the V3 application was less than injury following the R1 application comparing within each cultivar (Figure 1), an occurrence that is contrary to most literature. Soybean in vegetative growth stages typically responds to dicamba treatments by manifesting greater symptomology or more visible injury than soybean treated with dicamba at reproductive stages (Behrens and Leuschen 1979; Griffin et al. 2013; Kniss 2018; McCown 2018). The injury difference is attributed to a precipitation event totaling 0.45 cm that occurred 4 hours after the V3 application in 2018, reducing dicamba absorption due to wash-off and injury at this timing.

In addition to differing injury of V3-treated soybean between years, there was a greater range of injury for V3 and R1 treatments across the interaction of cultivar and application timing (e.g., 22 to 63% in 2018; 53 to 70% in 2019), and of relative yield across cultivars (Figures 1, 2, and 3). This difference was evident both numerically and in the number of statistical differences between treatments for each year (Figures 1, 2 and 3). Soybean cultivars are known to differ in tolerance to disease (Li et al. 2010), iron deficiency (Aksoy et al. 2017), cold (Tian et al. 2014), drought (Thu et al. 2015), flood (Rhine 2010), soil pH and its correlation with multiple nutrients' availability (Pierce and Warncke 2000; Yang et al. 2012), and even ozone content (Bulbovas et al. 2014), among many other potential environmental factors.

Injury. A significant interaction of cultivar and application timing occurred for injury in both 2018 and 2019 (Table 1). In 2018, injury of V3 soybean was numerically lower than R1 for all cultivars; however, the effect of cultivar did not statistically separate across application timing, with 14 V3-treated cultivars not different from cultivars treated at the R1 timing and 20 R1-treated cultivars not different from cultivars treated at the V3 timing (Figure 1). Among the least injured cultivars, (within the lowest letters indicating significance based on the least significant difference) are 'Leland', 'Eagle DrewSoy', and 'Progeny P4930 LL' (22, 26, and 31% injury, respectively) (Figure 1). Injury data collected in 2019 revealed a significant interaction of growth stage and cultivar, similar to 2018; however, injury of V3 soybean was higher as there was no precipitation event following either application timing, and there was less total variation in injury for the experiment compared to 2018 (Table 1; Figures 1 and 2). In 2019, every cultivar treated at V3 had significantly higher injury than cultivars treated at R1 (Figure 2). Due to the decreased range in observed injury, there are more cultivars within each LSD with a total of 9 cultivars having a similar level of injury in 2019 (Figure 2). Among these 9 cultivars, all treated at R1, only 'Eagle DrewSoy' (55% injury) was among the least injured cultivars in 2018 (Figures 1 and 2). These findings suggest that 'Eagle DrewSoy' manifested less visible injury across years regardless of vegetative or reproductive application timing, and that genetic factors may be less important than environmental factors in dictating the extent of injury observed on most soybean cultivars.

Relative Height. The relative height of soybean at harvest was significantly affected by application timing in both 2018 and 2019 (Table 1). For 2018, relative height of soybean treated at V3 (96%) was greater than soybean treated at R1 (89%; data not shown). The opposite was true in 2019, where relative height of V3-treated (86%) was less than R1-treated soybean (93%)

(data not shown). Typically, the height of soybean is reduced more by vegetative dicamba applications as opposed to reproductive applications (McCown et al. 2018). The difference in relative height between years is attributed to a decrease in herbicide efficacy due to the precipitation event after the V3-application in 2018 that also impacted soybean injury (data not shown). Interestingly, there was not a significant cultivar effect for height.

Relative Yield. The effect of cultivar was significant for relative yield in 2018 and 2019 (Table 1). Although there was a significant effect of cultivar in 2018 (Table 1), when averaged over V3 and R1 application timings, no cultivar was different from the relative yield of the nontreated; therefore, relative yield data in 2018 are not discussed (data not shown). The absence of a cultivar-by-application timing interaction indicates that there were no differences in response among cultivars for yield at the application timings and the dicamba rate tested. A higher rate of dicamba could have led to differences among cultivars, if there truly were potential differences in the ability of cultivars to recover from dicamba exposure. In future experiments, multiple applications of dicamba should also be considered to elicit possible relative yield differences among cultivars.

The purpose of this study was to determine if soybean cultivars with enhanced tolerance to dicamba exist and if tolerance is consistent across application timings. Findings indicate that the relative yield of 13 out of the 15 cultivars in 2019 were not different from the nontreated with only ‘Delta Grow DG4967’ and ‘GoSoy 51C17’ having reduced relative yield (Figure 3). In 2019, Delta Grow DG4790’, ‘Delta Grow DG4880’, ‘Progeny P4930 LL’, ‘Eagle DrewSoy’, ‘GoSoy 49G16’, ‘GoSoy 49L17’, ‘Ireane’, ‘Pioneer P47A76L’, and ‘UA 5014C’ had the highest relative yields compared to the other treated cultivars (Figure 3); however, only ‘Eagle DrewSoy’ was among the least injured cultivars across both years (Figures 1 and 2).‘Eagle

DrewSoy' is the only cultivar exhibiting consistent enhanced tolerance to dicamba and is most likely to continue to show increased tolerance if studies were continued. In other research evaluating over 300 soybean genotypes for differences in soybean sensitivity to dicamba, consistency in ranking of cultivar tolerance to the herbicide was seldom observed across environments (L.C. Purcell and J.K. Norsworthy, personal communication).

Cercospora Leaf Blight. Cecrospora leaf blight was observed on the cultivar experiment in 2019. A significant main effect of application timing occurred with incidence of CLB ($P<0.0001$) (data not shown). Cecrospora leaf blight infected a higher percentage of plots exposed to dicamba at the R1 growth stage than either a V3 exposure or nontreated (Figure 4). There were 44 incidence ratings of 4 among the R1-treated soybean plots compared to only 7 among V3-treated plots and no incidence on any non-treated cultivar. Despite varying incidence of CLB by application timing, there was no effect of application timing to relative yield (Table 1), therefore, it appears that CLB had no effect on yield.

Planting Date Experiment. There was a significant three-way interaction among planting date, application timing, and rating date for injury (Table 3). Considering the high number of significant interactions, only those most relevant to planting date are discussed.

Injury. Ultimately, soybean exposed to dicamba at the V3 stage experienced a delay in maximum injury expression as planting dates became later. Among soybean planted in April and May, greatest injury was seen at 14 DAT (47 and 51%, respectively; Figure 5). In June, injury did not differ between 14 (44%) and 21 DAT (39%), and by July, greatest injury was observed at 21 DAT (50%) (Figure 5). The less-than-ideal conditions caused by later planting dates (June and July) delay maximum injury expression of the V3-treated soybean to 21 and 28 DAT, versus

April and May significantly reaching maximum visible injury at the earlier 14 DAT rating date. The conditions that late-planted soybean encounter include the effect of a long photoperiod (Zhang et al. 2010) and high heat, causing abbreviated vegetative and reproductive growth stages (Chen and Wiatrak 2010), reducing soybean photosynthesis and growth (Hu and Wiatrak 2012), and often decreasing plant size at each application stage versus soybean planted earlier (Weaver et al. 1991).

Similar to soybean treated with dicamba at V3, the R1-treated soybean experienced a delay of maximum injury expression across rating date as planting dates became later. Unlike the V3 treatment, greatest injury among earlier planting dates was noted among the latest rating dates. Among the April planting date, highest injury was observed at 28 DAT (49%), whereas by May injury was not different between 21 and 28 DAT (38 and 43%, respectively (Figure 5). By June and July, injury did not differ significantly across rating date (Figure 5); however, among the July planting date, the injury at each rating date (52, 51, and 56% for 14, 21, and 28 DAT, respectively) was numerically greater than the injury of R1-treated soybean at any other rating date within other planting dates (Figure 5). Similar to the V3 treatment, the increase in injury of R1-treated soybean for July at 14, 21, and 28 DAT is attributed to the effect of the sub-par conditions at this late planting date compared to earlier plantings (Chen and Wiatrak 2010). The conditions at the later planting date also led to smaller plant size, due to shorter plant height, and fewer nodes (Weaver et al. 1991; Bastidas et al. 2008), allowing for greater visible injury manifestation at the remaining nodes for this planting date.

Injury of soybean treated sequentially – at V3 followed by R1 – saw a gradual postponement of symptoms, with greatest injury at later rating dates (21 and 28 DAT) among the later planting dates (Figure 5). At 14 DAT of the sequential application, a combination of the effects of the V3 treatment, at which greatest injury was expressed at earlier rating dates, and the R1 treatment, at which greatest injury at later rating dates, occurs. This combination manifests as no significant difference in injury of the sequential treatment for April and May (Figure 5); however, at later planting dates, maximum injury expression is delayed. This trend is similar to the delayed maximum injury seen among V3-treated soybean. By June, injury of the sequentially-treated soybean at 14 DAT (47%) was significantly lower than injury at 21 and 28 DAT (57 and 58%, respectively; Figure 5). For soybean planted in July, injury of the sequentially-treated soybean significantly increased with each subsequent rating date (38, 56, and 71% at 14, 21, and 28 DAT, respectively; Figure 5).

The trend observed among the sequentially treated soybean is attributed to less-than-ideal conditions at the later planting dates that caused increased soybean injury and a delay of visible injury manifestation as the planting date becomes later. As mentioned, late planting leads to smaller plant size at application (Weaver et al. 1991) and greater herbicide efficacy following application relative to earlier plantings, also extending the activity of dicamba within the plant as the herbicide metabolizes more slowly in these smaller plants. This would result in greater visible injury over time, thus explaining the greater injury at later rating dates among June and July plantings.

Maturity. Soybean maturity date was significantly affected by the main effect of planting date (Table 4). Within the April planting date soybean treated with dicamba matured 6 days later than the nontreated, and significantly later than soybean treated with dicamba at any other planting date (Table 5).

Relative Yield. Only the main effects of planting date and application timing affected relative yield (Table 4). The April, May, and June (103, 101, and 80% relative yield, respectively) planting dates were not different, but the July date had significantly lower relative yield at 39% of the nontreated (Table 6). Planting at later dates does commonly result in lower yields versus earlier, more optimal, planting dates (Zhang et al. 2010; Hu and Wiatrak 2012). Optimal planting dates of soybean with maturity groups of V to VIII is May through early June (Parker et al. 1981), with yield rapidly declining for soybean planted after these optimal dates (Egli and Cornelius 2009). In this experiment, the yield reduction was primarily due to the later planting date causes shorter intervals for each growth stage relative to earlier planting dates (Chen and Wiatrak 2010). Additionally, the latest planting date had reduced total biomass present at the time of dicamba application, causing greater injury to the smaller plants and negating much of the recovery potential of the soybean plant. Comparing application timings, only soybean treated at the V3 timing yielded similar to the nontreated, whereas soybean in plots treated at both R1 and the V3 plus R1 stages yield only 70 and 56% of the nontreated, respectively (data not shown). Greater yield loss among soybean exposed to dicamba at reproductive stages as compared to vegetative stages is common (Griffin et al. 2013).

Practical Implications. The results of this research help determine the effect of multiple cultivars and multiple planting dates to non-dicamba-resistant soybean when injured by dicamba at a low rate, such as supplied by off-target movement. According to the results of the cultivar experiment, the cultivar ‘Eagle DrewSoy’ exhibited high relative yield and low visible injury compared to other cultivars tested across both year and location and is a likely candidate for enhanced tolerance to dicamba compared to other soybean cultivars. Isolation of the gene(s) responsible for this tolerance could lead to the production of soybean cultivars with enhanced tolerance to dicamba. Within the planting date experiment, soybean treated with a low-dose of dicamba following the July planting experienced a greater reduction in yield compared to other planting dates. This indicates that dicamba injury to late-planted soybean causes greater yield loss compared to soybean exposed to dicamba at optimum planting dates. In addition, CLB was noted in both experiments in Fayetteville of 2019, with greater incidence among dicamba-treated soybean; however, it is unlikely that yield was affected by CLB.

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Figures

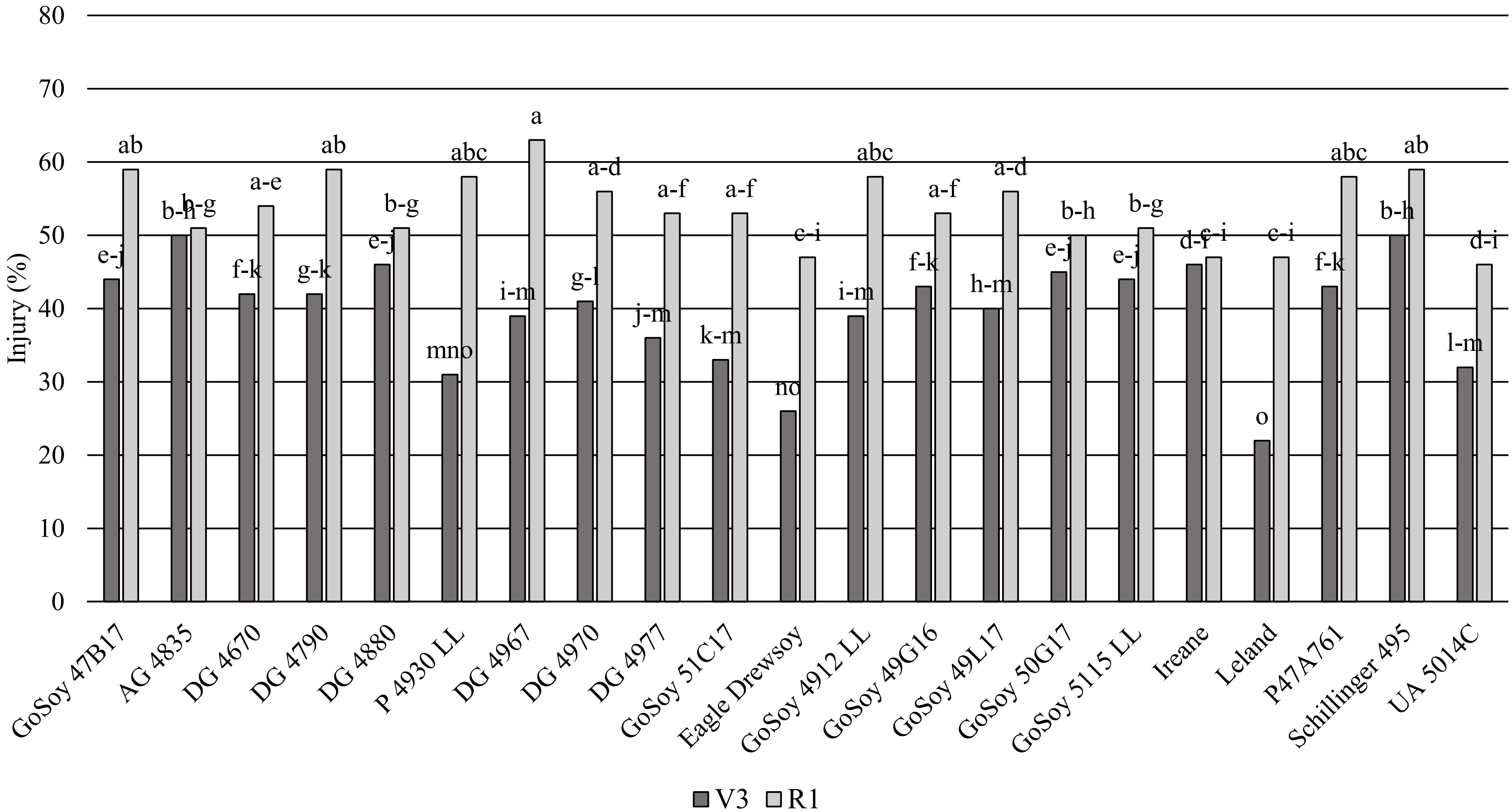

Figure 1. Injury at 21 DAT according to the interaction of cultivar and application timing for the cultivar experiment conducted in Fayetteville, AR, in 2018. Treatments with the same lowercase letter are not significantly different according to Fisher's least significant difference α=0.1.

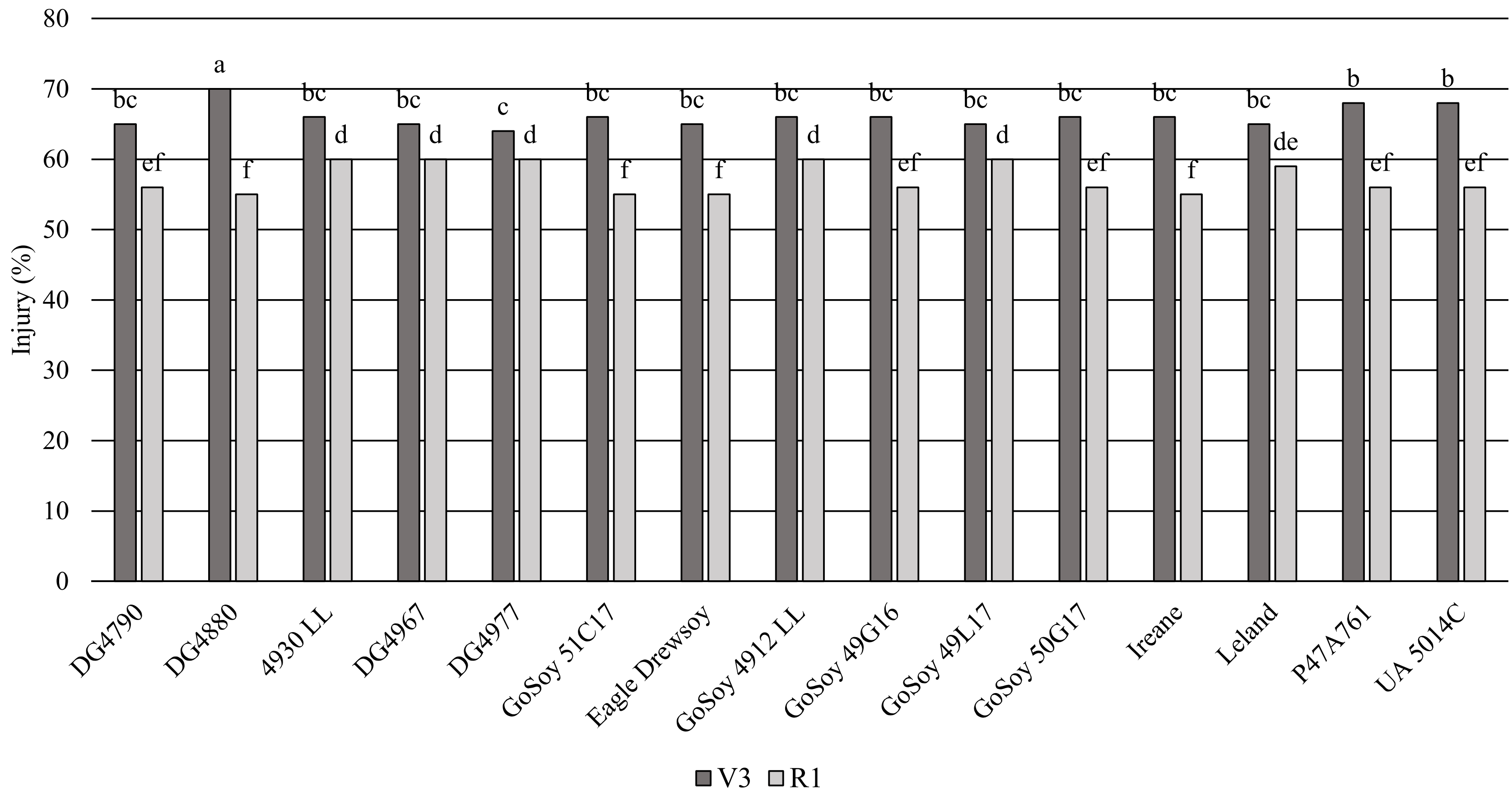

Figure 2. Injury at 21 days after treatment according to the interaction of cultivar and application timing for the cultivar experiment conducted in Fayetteville, AR in 2019. Treatments with the same lowercase letter are not significantly different according to Fisher's least significant difference α=0.1

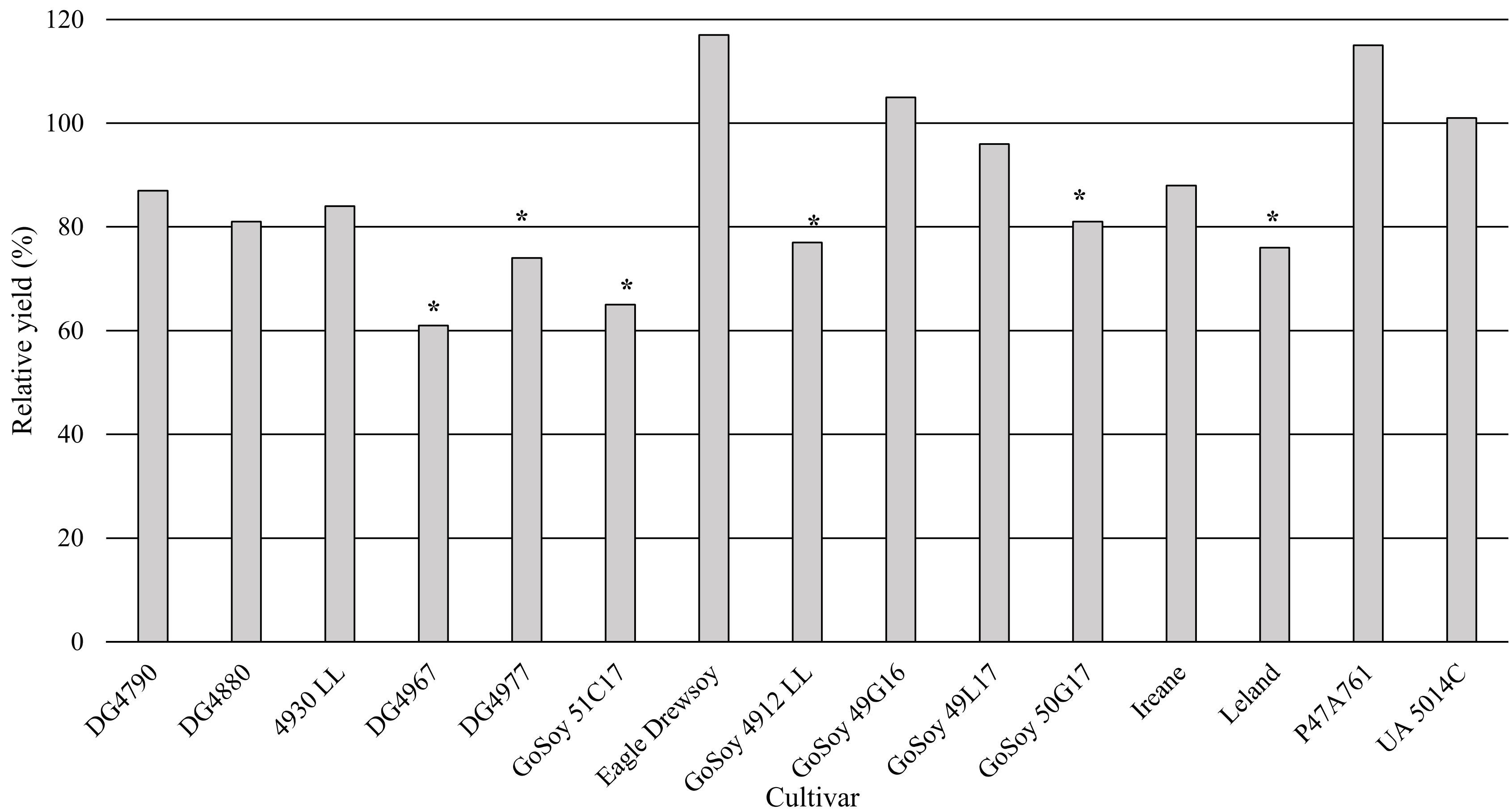

Figure 3. Relative yield of soybean cultivars in Fayetteville, AR, in 2019, averaged over low-dose dicamba exposure at the V3 and R1 stages of soybean. Treatments with the same lowercase letter are not significantly different according to Fisher's least significant difference α=0.1. Treatments marked with an asterisk indicates significant yield loss relative to the cultivar's corresponding nontreated check.

Figure 4. (a) Image of Cercospora leaf blight (*Cercospora kikuchii*) on a soybean plot treated with dicamba at the V3 application timing versus (b) a plot treated with dicamba applied at the R1 application timing. Images taken while soybean were in the R6 growth stage.

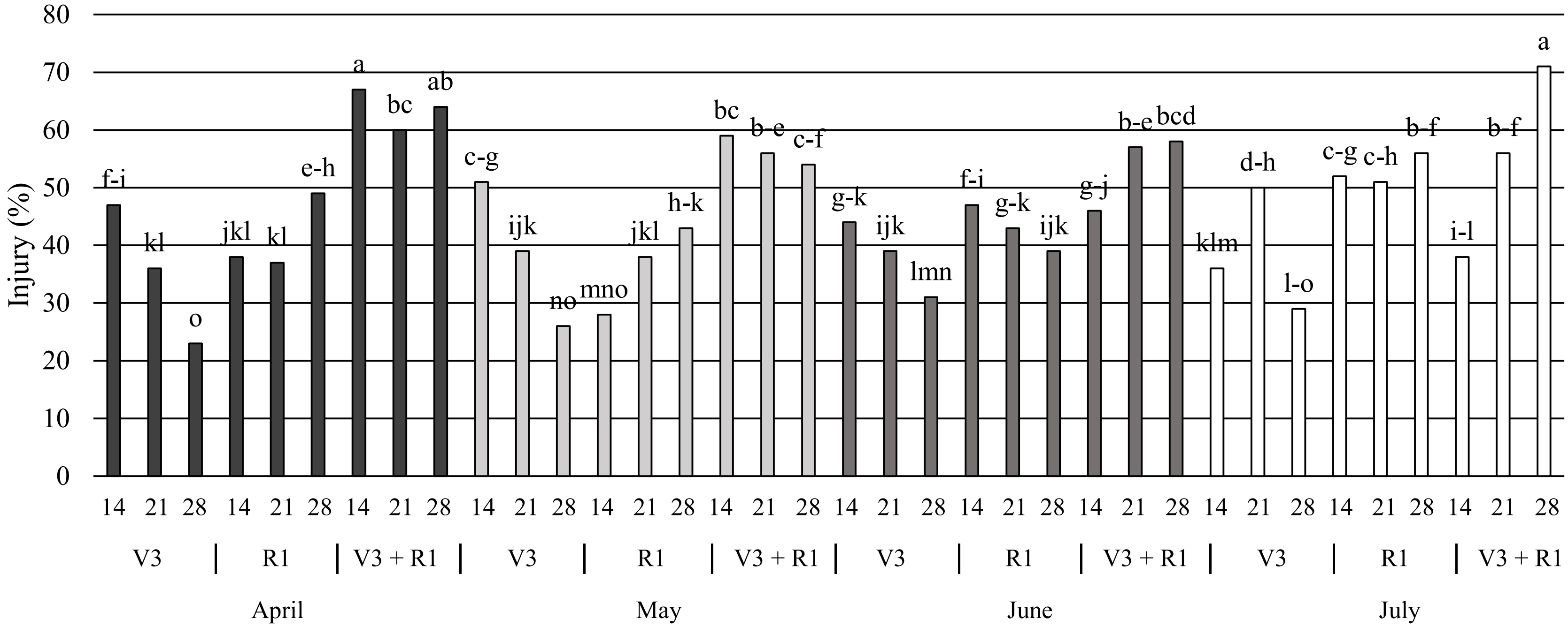

Figure 5. Visual estimates of soybean injury at 14, 21, and 28 days after a low-dose dicamba exposure at two growth stages (V3 and R1) for April, May, June, and July planting dates in Fayetteville, AR, in 2019. Treatments with the same lowercase letter are not significantly different according to Fisher's protected least significant difference α=0.05.

Tables

Table 1. Effects of cultivar and application timing for injury, relative height, relative maturity, and relative yield (relative to nontreated) of soybean in at Fayetteville, AR, in 2018 and 2019.

	2018				2019			
Factors	Injury (%)	Height (%)	Maturity	Yield (%)	Injury (%)	Height (%)	Maturity	Yield (%)
	---p-values---							
Cultivar	**0.0005**	0.9972	0.1049	**0.0867**	0.1501	0.7275	0.5408	**0.0523**
Application timing	**<0.0001**	**0.0185**	0.8555	0.6234	**<0.0001**	**0.0216**	0.2176	**0.0520**
Cultivar*application timing	**0.0691**	0.8851	0.1062	0.7974	**<0.0001**	0.7459	0.2622	0.2131

[a]P-values less than 0.1 considered significant as shown in bold.

Table 2. Soybean cultivars, maturity groups, and traits as well as manufacturer and address.

Cultivars	Maturity group	Trait	Manufacturer	Location
DG4790	4.7	RoundupReady2®	Delta Grow Seed Company, Inc.	England, AR
DG4880	4.8	RoundupReady1®	Delta Grow Seed Company, Inc.	England, AR
DG4967	4.9	LibertyLink®	Delta Grow Seed Company, Inc.	England, AR
DG4977	4.9	LibertyLink® STS	Delta Grow Seed Company, Inc.	England, AR
Eagle Drewsoy	5.0	Conventional	Eagle Seed Company, Inc.	Weiner, AR
GoSoy 49G16	4.9	RoundupReady1®	Stratton Seed Company	Stuttgart, AR
GoSoy 49L17	4.9	LibertyLink®	Stratton Seed Company	Stuttgart, AR
GoSoy 4912LL	4.9	LibertyLink®	Stratton Seed Company	Stuttgart, AR
GoSoy 50G17	5.0	RoundupReady1®	Stratton Seed Company	Stuttgart, AR
GoSoy 51C17	5.1	Conventional	Stratton Seed Company	Stuttgart, AR
Ireane	4.9	Conventional	Stratton Seed Company	Stuttgart, AR
Leland	5.0	Conventional	Stratton Seed Company	Stuttgart, AR
P47A76L	4.7	LibertyLink®	Pioneer	Johnston, IA
P4930 LL	4.9	LibertyLink®	Progeny Ag. Products	Wynne, AR
UA 5014C	5.0	Conventional	University of Arkansas	Fayetteville, AR

Table 3. Effects of planting date, application timing, and rating date to injury analyzed as a repeated measures analysis for the planting date experiment conducted in Fayetteville and Stuttgart, AR, in 2019.

Factors	Injury[a]
	p-values
Planting date	0.0454
Timing	<0.0001
Rating date	0.0179
Planting date*timing	<0.0001
Planting date*rating date	<0.0001
Timing*rating date	<0.0001
Planting date*timing*rating date	<0.0001

[a]P-values at or smaller than 0.05 level considered significant.

Table 4. Effects of planting date and application timing to maturity date and relative yield for the planting date experiment conducted at Fayetteville and Stuttgart, AR in 2019.

Factors	Maturity date	Relative yield
	---------------------p-values---------------------	
Planting date	**0.0196**	**<0.0001**
Application timing	0.6790	**<0.0001**
Planting date*application timing	0.7911	0.2522

[a]P-values at or smaller than 0.05 level considered significant as shown in bold.

Table 5. Days delay until soybean maturity relative to the nontreated check averaged over a low-dose dicamba exposure at V3, R1, and V3 followed by R1 stage of soybean.

Planting date	Soybean maturity delay[a]
April	6.0 a
May	2.4 b
June	1.6 b
July	2.4 b

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

Table 6. Relative yield of soybean at four planting dates averaged over a low-dose dicamba exposure at V3, R1, and V3 followed by R1 stage of soybean.

Planting date	Relative yield (%) [a]
April	103 a
May	101 a
June	80 a
July	39 b

[a]Means within a row followed by the same lowercase letter are not different according to Fisher's protected LSD (α=0.05).
Yield of the nontreated check reported in kg ha^{-1} by month as follows: 3670, 3550, 5130, 3420 for April, May, June, and July, respectively.

CHAPTER 3

Effect of Irrigation Regime and Fertilization on Recovery of Dicamba Injured Soybean

Abstract

With the release of the dicamba-resistant crop technology and subsequent increase in dicamba off-target movement to non-dicamba-resistant crops, such as soybean, discovering means of mitigating yield loss through studying dicamba injury to soybean and interactions with factors such as irrigation regime and fertilization would prove beneficial. The hypothesis for the irrigation experiment was that soybean receiving irrigation would have greater recovery from dicamba injury than non-irrigated treatments. The hypothesis for the experiment where fertilizer was applied to soybean was that soybean receiving fertilizer would experience greater recovery, with greatest recovery achieved by applications of both fertilizers. Field experiments were conducted in 2019 in Fayetteville and Colt, AR, to evaluate the effect of irrigation regime to non-dicamba-resistant soybean that was injured by dicamba at a low dose at multiple timings. Another experiment was conducted in Fayetteville in 2019 and 2020 evaluating the impact of nitrogen (N) and potassium (K) fertilization on soybean recovery following injury by dicamba at multiple reproductive stages. Visible injury in both experiments was affected by application timing, with the greatest injury occurring for sequential applications and the latest reproductive application timings having the least visible injury. Within the irrigation regime experiment, yields were decreased by dicamba applications; however, more soybean yield from plants treated with dicamba came from branches than from the mainstem when compared to nontreated plants. Averaged across irrigated and non-irrigated soybean, dicamba applications decreased mainstem pod and seed number by 55% and 59%, respectively. Soybean compensated for dicamba injury to the mainstem with a branching increase of 105%, averaged across irrigated and non-irrigated plots. Soybean yield harvested from plant branches increased relative to treatments not receiving dicamba, including pods (73%) and seeds (54%) counted on branches. In the fertilization experiment, soybean treated with a low dose of dicamba that received N fertilization tended to have reduced biomass compared to treatments receiving no fertilizer (14% lower) or K alone (10% lower), with the greatest biomass reduction tending to occur among treatments receiving both N and K (37% lower than treatments not receiving fertilizer). Total grain yield was not affected by either irrigation regime or fertilization.

Nomenclature: Dicamba; soybean, *Glycine max* (L.) Merr.

Keywords: Herbicide sensitivity, fertilization, irrigation regime

Introduction

The mid-southern agricultural region has unique characteristics allowing for high potential soybean yields, such as a wide planting window, which in turn allows for wide cultivar and maturity group (MG) selection, and manipulation of yield-affecting factors to optimize yield (Salmeron et al. 2014). By understanding the interaction between manipulatable factors that affect soybean growth and yield, such as the impact of planting date, irrigation, or additional fertilization on herbicide injury sustained by soybean, recovery may be augmented and yields safeguarded when faced with stressors such as off-target herbicide injury.

Irrigation is a practice proven to increase yields over non-irrigated cropland. Recent USDA-NASS (2020) data reports that for Arkansas in 2018, average non-irrigated soybean yield for the state was 2448 kg ha^{-1} whereas the average irrigated soybean yield was 3618 kg ha^{-1}. The difference in yield is due to a common seasonal moisture deficit for traditional soybean production (planted in May and later), occurring when soybean is in its reproductive stages and when moisture deficit is most detrimental (Heatherly and Spurlock 1999). For MG IV, V, and VI soybean planted in April and May, yields for irrigated and non-irrigated were substantially different, with non-irrigated fields yielding 42% lower.

Along with yield impact, the impact of irrigation on soil moisture can also influence herbicide activity. An experiment evaluating the impact of soil moisture on glyphosate efficacy on junglerice (*Echinochloa colona* L.) across four different soil moisture contents found that, regardless of rate, junglerice seedlings applied at 100% field capacity all died earlier than seedlings applied with glyphosate at lower field capacities (Tanpipat et al. 1997). At 29% field capacity, seedlings receiving a higher glyphosate rate died earlier than those receiving a lower rate, and all seedlings at 29% field capacity died later than those applied at 100% field capacity, suggesting that glyphosate is more easily translocated within the plant when there is adequate soil moisture (Tanpipat et al. 1997). In addition, Miller and Norsworthy (2018) found that soils with higher moisture content increased efficacy of a synthetic auxin herbicide, florpyrauxifen-benzyl, on the weed species barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.], yellow nutsedge (*Cyperus esculentus* L.), and hemp sesbania (*Sesbania herbacea* Mill.) by increasing absorption, translocation, and metabolism within each weed species. Also, Weidenhamer et al. (1989) noted that in a year with greater drought-stress a dicamba rate as low as 0.4 g ae ha^{-1} caused a 10% yield reduction in non-dicamba-resistant soybean, whereas in a year with adequate rainfall, dicamba at 15 g ae ha^{-1} was needed to obtain a similar level of yield loss.

Aside from soybean grain yield, yield components can also be affected by drought and herbicide stress. One cause of yield reduction under droughty conditions is reduced seed yield on branches (Frederick et al. 2001). Seed yield of the soybean mainstem is usually unaffected when the stressor is drought alone; however, yield of branches can be greatly reduced, accounting for most of the yield reduction of soybean under droughty conditions (Linkemer et al 1998; Frederick et al. 2001). Furthermore, dicamba at sufficient rates can restrict plant height (Weidenhamer et al. 1989; Kelley et al. 2005; Robinson et al. 2013; Solomon and Bradley 2014). Height restriction results from dicamba injury to, or termination of the soybean apical meristem, which restricts seed yield of the mainstem and forces the plant to rely on seed production from axillary nodes, or branches (Robinson et al. 2013). In addition, Robinson et al. (2013) postulated that drought stress may inhibit detoxification of dicamba within soybean due to reduced translocation. The compounding stress of drought and dicamba injury potentially leads to even greater yield loss as yield components are affected. Some commonly considered soybean yield components include pod and seed number (Wax et al 1969; Kelley et al. 2005; Robinson et al. 2013).

The impact of fertilization on plant response to a herbicide is a little-studied topic; however, it may be important to understand to safeguard soybean yields as well as further the current understanding of plant processes. In a study focused on the influence of N fertilization timings and its effect on how rice (*Oryza sativa* L.) responds to multiple herbicide sites of action, Langaro et al. (2018) found that N applied to rice pre-flood favors plant recovery from an application of bentazon, whereas N applied post-flood delays recovery from bentazon injury. The opposite behavior was found in the case of bispyribac-sodium, which caused greater injury to rice when all N was applied pre-flood, indicating that the interaction between herbicide injury and fertilization may be different depending on the herbicide site of action. Cathcart and Chandler (2004) noted that under low N fertility conditions, herbicide efficacy would likely be reduced on weeds in areas with higher fertility. Additionally, in a study evaluating the efficacy of mesotrione as influenced by various N fertilization factors, crabgrass (*Digitaria sanguinalis* L.) with high aboveground N concentrations experienced greater herbicide injury/weed control versus crabgrass with lower N concentrations, indicating injury decreased as days between N application and mesotrione application increased (Beck et al. 2015). The higher N concentrations were believed to allow an increase of mesotrione translocation and, therefore, activity (Nizampatnam et al. 2015).

Specific to soybean, several researchers have explored how the interaction of herbicide injury and fertilization affects multiple factors that can impact final yield. For example, soybean injury from synthetic auxins can reduce legume nodulation, decreasing N fixation, which may partially account for yield reduction (Nizampatnam et al. 2015). Van de Stroet et al. (2019)

determined that the application of foliar and broadcast N in addition to synthetic auxins applied at low rates impacted soybean rhizobia nodulation, therefore decreasing biomass. Following dicamba application and foliar-applied N, a significant decrease in yield was noted but not when soil-applied broadcast N was used (Van de Stroet et al. 2019). At one location soybean nodulation was not affected while at another location, nodulation was decreased by 35% for plants treated at V3 and R1 with dicamba (Van de Stroet et al. 2019). At 1 g ae ha^{-1} of dicamba applied at R1 alone and V3 + R1 to soybean, biomass was reduced as much as 25% when applied with foliar N 7 days following the R1 dicamba application; biomass reduction was only 10% when treated with foliar N 20 days following the R1 application of dicamba (Van de Stroet et al. 2019). For soybean not treated with N, biomass reduction averaged 20% (Van de Stroet et al. 2019). Addition of N to dicamba injured soybean does not allow for dicamba recovery; however, weekly irrigation of dicamba injured soybean can result in appreciable soybean recovery in terms of injury level, height, and yield (Dintelmann et al. 2021). Specific fungicide applications, plant-growth hormone treatments, and micro-nutrient treatments were also ineffective at allowing soybean recovery in the same experiment (Dintelmann et al. 2021). These experiments demonstrate how multiple events or management decisions can compound to affect distinct crop responses. Converse to research investigating relations between herbicide use and N fertilization, little research has been conducting concerning the effect of herbicide use and K fertilization on plants.

The results of crop response to fertilizers following herbicide injury are largely due to the role of nutrients in the crop. N, absorbed as nitrate (NO_3^-) and ammonium (NH_4^+) by plants, plays a role in the creation of amino acids and proteins, chlorophyll formation, energy transfer, and overall increased vegetative growth (Havlin et al. 2016). K absorbed as a positive ion (K^+)

by plants is responsible for cell water and transpiration rate regulation, carbohydrate transfer and amino acid synthesis, and is also known to aid rhizobium activity in legumes and improve plant drought resistance (Havlin et al. 2016). With the recent introduction of dicamba-resistant technology and increase in off-target movement, research was conducted to understand the interaction of dicamba applied to soybean at low doses and the interaction of subsequent injury with either irrigation regime or application of fertilizers.

Materials and Methods

Separate field experiments were conducted in 2019 to evaluate the effect of irrigation regime and fertilizer addition to soybean injured by dicamba applied at drift rates.

General Methodology. Experiments were initiated on a tilled and bedded bare-ground field, and herbicide treatments were applied using a CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} at 276 kPa using TTI 110015 spray tips. The trial was kept weed-free with herbicides labeled for conventional soybean as well as through use of row cultivation and hand weeding as needed. Visual estimates of percentage injury were recorded at 14, 21, and 28 days after each application (DAA) on a scale of 0 to 100%, with 0 representing no injury and 100 representing complete plant death. Soybean grain was harvested at maturity, and grain moisture was measured and corrected to 13% moisture. Relative yield was calculated for each plot by comparing yield of treated plots to the nontreated plots (treated yield/nontreated yield*100). Plots were 6.1 meters in length with a 1.5-meter alley between plot rows. All injury data were analyzed as a beta distribution in a repeated measures analysis using the first order autoregressive (AR[1]) covariance structure.

Irrigation Experiment. A field experiment was conducted in 2019 at the Milo J. Shult Arkansas Agricultural Research and Extension Center in Fayetteville and at the Pine Tree Research Station near Colt, Arkansas, to determine the impact of irrigation regime to soybean injured with a low-dose rate of dicamba. The soil series at the Fayetteville site was a Leaf silt-loam soil (Fine, mixed, active, thermic Typic Albaqualts) with 25 % sand, 64% silt, and 11% clay, 1.67% organic matter (OM), and a pH of 6.0. The soil series in the trial near Colt was a Calloway silt-loam soil (Fine-silty, mixed, active, thermic Aquic Flaglossudalfs). Rainfall events and irrigation were recorded at each trial location. The section of the trial receiving irrigation for each location was furrow irrigated as needed if at least 2.5 cm of rainfall did not occur over a seven-day period, with irrigation occurring on August 14th and 18th, and September 7th, 10th, and 12th in Fayetteville. At the Colt site, irrigation occurred on August 6th, 14th, and 20th, and on September 3rd, 9th, and 16th. The glufosinate-resistant soybean cultivar 'Credenz CZ 4819LL' was planted at 346,000 seed ha^{-1} in 4-row plots of 7.6 m in length and row width of 91 cm at Fayetteville and 76 cm at Colt. This trial was planted on May 27, 2019 for the Fayetteville location and on June 18, 2019 for the location near Colt.

The trial design was a randomized complete block design with treatments set up in a split-plot arrangement with the whole-plot factor as irrigation regime and split-plot factor as growth stage at exposure with dicamba. The irrigation regime consisted of non-irrigated versus furrow-irrigated plots and growth stages at dicamba application being V5 and R1 separately as well as a sequential application at V5 and R1. The application rate of dicamba was 2.2 g ae ha^{-1}, which is a 1/256x rate for over-the-top use in dicamba-resistant cotton and soybean (Clarity™ herbicide, BASF Corporation). In addition to injury ratings and relative yield data, yield component data, including number of branches per plant, pods per plant, pods on the main plant stem, pods on plant branches, and seed weight per plot were taken as an extra data measurement in Fayetteville by removing 10 plants from each plot and counting by hand.

Replication was analyzed within location and made random while the effects of cultivar, growth stage at application, and the interaction of each main effect were analyzed as fixed effects within the analysis. A beta distribution was used to analyze injury data and a gamma distribution for relative yield, seed weight, number of branches, and pods per plant data. Injury, relative yield and yield component data were subjected to analysis of variance using PROC GLIMMIX in SAS 9.4 (Gbur et al. 2012).

Fertilization Experiment. A field experiment was conducted in 2019 and 2020 at the Milo J. Schult Agricultural Research and Extension Center in Fayetteville, Arkansas, to determine the impact of broadcasting fertilizers following the manifestation of dicamba symptomology on soybean. A glufosinate-resistant soybean cultivar 'CZ 4820LL' was planted at 346,000 seed ha^{-1} in 4-row plots of 6.1 m in length and row width of 91 cm. The trial was planted on May 16 in 2019 and on May 22 in 2020. The experiment was irrigated as needed if at least 2.5 cm of rainfall did not occur over a 7-day period. The experimental design was a randomized complete block with a two-factor factorial of dicamba (Xtendimax™ herbicide, Bayer Crop Science) application timing as factor A (R1, R3, R1 fb R3) and factor B as fertilizer applied following dicamba application (none, N only, K only, N + K). Nitrogen was applied as urea (46% N) at 50 kg ha^{-1} and K as potassium chloride (50% K) at 67 kg ha^{-1}. Dicamba was applied at 3.73 g ae ha^{-1} or a 1/150x rate, with a 1x rate for over-the-top use in dicamba-resistant crops being 560 g ae ha^{-1} (Xtendimax™ herbicide, Bayer Crop Science). Row cultivation and hand weeding were used if necessary. All dicamba treatments were applied to the two middle rows of each four-row plot. During application, shields were used to prevent physical drift onto the outside rows of each four-row plot. Fertilizer rates were calculated for entire plot area and all fertilizer treatments were hand-spread over the entire four-row plot 1 week after the R1 dicamba application. The V5 dicamba and fertilizer treatments were made on June 24 and July 2, respectively, in 2019, and on July 13 and July 20, respectively, in 2020. Soybean biomass was collected when soybean reached the R6 growth stage from 1 m of row in each dicamba-treated plot and the adjacent nontreated row; this allowed the biomass of each treatment to be made relative to biomass of the nontreated within the same plot. Collected biomass was dried for at least 7 d at 55 C, weighed, and reported as relative biomass compared to the nontreated adjacent row.

Injury data were subjected to analysis of variance and analyzed as repeated measures as first order autoregressive data within SAS using the PROC GLIMMIX statement. Biomass, seed weight, nutrient analysis data from tissue samples, and relative yield were subjected to analysis of variance using PROC GLIMMIX in SAS 9.4. Replication nested within year as a random effect while the effects of fertilizer type, growth stage at application, and the interaction of each were analyzed as fixed effects within the analysis. A beta distribution was used to analyze injury and seed weight data, and a gamma distribution was used for relative yield and relative biomass data.

Results and Discussion

Irrigation Experiment. The summer of 2019 was characterized by above-average rainfall. According the National Weather Service (2020), Fayetteville received a total of 99 cm of rainfall from April through September in 2019, with the average rainfall of the past 30 years being 67 cm for the same months combined. A total of 27.8 cm of rainfall occurred within the first 4 weeks after planting at the Fayetteville location and 18.0 cm of rainfall within the 4 weeks following planting at the Colt location (Figure 1). In addition, for the 4 weeks following the V5 application, precipitation totaled 12.5 and 13.2 cm for Fayetteville and Colt, respectively, and a total of 10.1 and 8.6 cm at Fayetteville and Colt, respectively, for the 4 weeks following the R1 application (data not shown). In Fayetteville, irrigation was only needed 28 days after the R1 application (August 14, 2019), in addition to other irrigation timings (refer to Materials and Methods under "Irrigation Experiment"). At the Colt site, the trial was irrigated at 20 days after the R1 application (August 6, 2019), in addition to later irrigation timings. For much of the growing season, there was no need for irrigation at either location due to frequent rainfall.

Injury. Among injury evaluations, there was a significant interaction between application timing and rating date (Table 1), but no effect of irrigation regime. For the V5 application timing injury peaked at 21 DAT with 48% injury (Figure 2). Injury, averaging 60% or more, was greatest at 21 and 28 DAT following sequential dicamba applications at the V5 and R1 growth stages (Figure 2). Overall, less injury within a rating date was observed following dicamba applied at R1 than at the V5 growth stage, which is similar to findings of others (Solomon and Bradley 2014). Plant growth lessens as soybean enters reproductive development, thus less herbicide symptomology caused by dicamba is generally observed when exposure occurs during reproductive stages rather than vegetative stages. Decreased visible injury to soybean exposed to dicamba during reproductive development may also be attributed to decreased translocation of the herbicide to vegetative portions of the plant. Because irrigation was not needed until late in the growing season, with trials irrigated at mid-August through early September for both locations, no significant effect of irrigation to visible soybean injury occurred.

Yield Components. Analysis of yield component data illustrates the compounding effects of both irrigation and herbicide injury to soybean. For both pod and seed data from the mainstem, there was a significant interaction of irrigation regime and application timing, whereas data for branches was impacted only by main effects (Table 2). The significance of irrigation regime to yield component data is due to the timing of data collection. Yield component data were collected at harvest, after irrigation events occurred. Alternatively, injury data, which was not affected by irrigation regime, was collected before irrigation was needed as a result of the frequent early season rainfall.

Pod and seed number per main stem were two of the most sensitive soybean yield components impacted by dicamba. Soybean plants receiving dicamba at the V5 timing had a significant reduction in pods present on the main stem, with the reduction ranging from 51 to 90% relative to nontreated plants (Table 3). Similarly, three of the four dicamba applications at the V5 and V5 and R1 growth stages significantly reduced seeds per mainstem, with as much as a 91% reduction observed under non-irrigated conditions. Due to a high degree of variability among individual plants, a significant reduction in seed or pod numbers following the R1 application of dicamba was not detected, albeit there were 35% fewer pods per mainstem and 35 to 47% fewer seeds per mainstem relative to non-treated plants (Table 3). These findings suggest that low-dose dicamba injury to reproductive soybean had less effect on mainstem yield components than vegetative exposure. Based on the extent of the reduction in pod and seed number per mainstem, it appears that irrigated soybean had greater potential for recovery from the V5 exposure of dicamba than did non-irrigated plants. These differences are largely a result of the late-season irrigation events, albeit it is unknown whether there were fewer flowers on the mainstem or whether pods failed to form. Non-irrigated soybean had greater yield loss on the mainstem likely because of reduced detoxification or sequestration of dicamba even if less visible injury is present as reported elsewhere (Robinson et al. 2013).

Dicamba exposure to soybean tended to cause the soybean plants to increase in branching (Table 3). Applications of the low-dose of dicamba to V5 soybean resulted in more than a 2-fold increase in branches per plant. Conversely, the R1 application timing did not significantly increase branching in either irrigation regime, likely because of minimal new branches forming after the R1 stage of soybean as resources begin to shift toward reproductive development. Soybean plants receiving the sequential application of dicamba had more branches than nontreated plants within each irrigation regime (5.8 average branches irrigated and 7.1 average branches non-irrigated) (Table 3). The greater branching of non-irrigated soybean was likely due to the reduced translocation of dicamba, and therefore reduced detoxification of the soybean plant resulting in greater injury to the apical meristem. The greater axillary node growth compensated for the greater apical meristem injury, as postulated by Robinson et al. (2013) in a similar experiment. Under both irrigated and non-irrigated conditions, soybean compensated for a single exposure to dicamba at the V5 growth stage by increasing pod and seed number per branch. In regards to seed weight, it was only affected by application timing, with lower seed weight following sequential dicamba exposure at the V5 and R1 stages (Table 3).

Relative Yield. A significant effect of application timing occurred for grain yield (kg ha^{-1}) where nontreated plots had significantly greater yield than the V5 and V5 followed by R1 application timings (data not shown), but no effect of irrigation regime occurred (Table 2). Among treatments not receiving dicamba, yield was 3925 kg ha^{-1} for irrigated plots and 2631 kg ha^{-1} for non-irrigated plots (Table 3)), supporting the research of Heatherly and Spurlock (1999) that irrigated soybean yields often exceed those of non-irrigated soybean. Final grain yield of all treatments receiving dicamba were not different (data not shown), indicating that dicamba applications reduced yield regardless of irrigation events or application timings used for this experiment, and despite initial differences in injury between application timings and differences in irrigation and application timing among yield component data.

Fertilization Experiment.

Injury. All fertilizer applications were make 1 week following the R1 dicamba application. The main effect or interactions involving fertilizer were never significant for soybean injury, indicating that the fertilizer treatments did not hasten recovery of soybean symptoms caused by dicamba (Table 4). There was an interaction between dicamba application timing and rating dates for soybean injury when the later factor was analyzed as a repeated measure (Table 4). For ratings dates of 14, 21, and 28 DAT, injury was greatest following sequential exposure to dicamba at R1 and R3 stages than a single exposure at either of these stages (Figure 3). Exposure to dicamba at the R1 and R3 stages caused 65% injury to soybean by 14 DAT of the later exposure, with the level of injury increasing further by 21 DAT.

Biomass, 100-Seed Weight, and Relative Yield. Soybean biomass production was affected by the interaction of fertilizer applied and dicamba application timing (Table 6). In the absence of dicamba, neither N, K, nor the combination of the two nutrients positively or negatively affected biomass production (Figure 4). There was no treatment of N, K, or the combination of the two nutrients that improved soybean biomass production over a dicamba application timing in the absence of additional nutrient fertilization. Surprisingly, N plus K applied to soybean sequentially exposed to dicamba at the R1 followed by R3 stages and the R1 stage alone had less biomass than when dicamba was applied in the absence of additional nutrients. The cause of the biomass reduction beyond that in the absence of the nutrients is unknown. Van de Stroet et al. (2019) observed a biomass reduction following a foliar application of N to soybean and determined reduced rhizobia nodulation as the cause of biomass reduction. In addition, Dintelmann et al. (2021) observed a reduction in height among soybean treated with a low dose of dicamba followed by hand-spread urea fertilizer compared to soybean treated only with a low dose of dicamba at the R2 growth stage. Foliar necrosis following urea applications is cited as a possible cause of height reduction to dicamba-treated soybean (Dintelmann et al. 2021).

Weight of 100 seed was significantly affected only by application timing with the treatments applied at R1 alone (15.8 g) and R3 alone (14.9 g) not different from the nontreated (14.9 g) (Figure 5). However, treatments applied at both R1 followed by R3 had reduced seed weight (12.9 g), likely due to increased stress at reproductive timings that prevented soybean plants from compensatory growth.

Similar to seed weight, relative yield was significantly affected by application timing, with all timings significantly different, except for treatments receiving dicamba at R1 alone (94% relative yield), which were not different from the nontreated plots (Figure 6). Soybean plants treated at R1 had a relative yield of 69% and treatments receiving dicamba at both R1 and R3 stages yielded only 24% of the nontreated (Figure 6). Differences in injury between application timings partially mirrored yield as the greatest injury was seen among treatments receiving both application timings of dicamba (Figure 3); however, fertilizer treatments did not translate to yield differences.

Practical Implications. Under ideal growing conditions, ceteris paribus, irrigated crops will often yield higher than non-irrigated, and nutrient-stressed soybean will respond to fertilization with higher yields. According to these experiments, final yields of soybean injured by dicamba at the late vegetative and early reproductive stages prevent sufficient recovery and yield improvement of the crop regardless of irrigation or fertilization following dicamba injury. In the irrigation regime experiment, above-average rainfall early in the season may have played a role in diminishing the differences observed between irrigated and non-irrigated treatments and the failure to detect an interaction between dicamba application timing and use of irrigation. In the fertilization trial, the impact of N and K addition to dicamba-injured soybean generally caused reductions in biomass and significant, albeit biologically small, differences in injury. However, soybean yields following dicamba were not improved with a subsequent application of N or K. Dicamba exposure(s) during reproductive development may have contributed to the inability of soybean to recoup yield loss due to the shortened period of injury manifestation until maturity.

Typical dicamba injury to soybean includes damaged or killed apical meristems (Weidenhamer et al. 1989; Kelley et al. 2005; Robinson et al. 2013; Solomon and Bradley 2014), and while in the vegetative growth stages, soybean will attempt to compensate for injury with greater axillary stem growth (Robinson et al. 2013). In addition, soybean with main-stem nodes removed was best able to recover when injury occurred at early vegetative stages, such as V2 (Conley et al. 2009); therefore, evaluation of an early vegetative application stage could provide different results. Regardless, according to this research, neither irrigation nor N or K aided in soybean recovery from dicamba injury under the conditions present in these field trials when injured at late vegetative and early reproductive growth stages.

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Figures

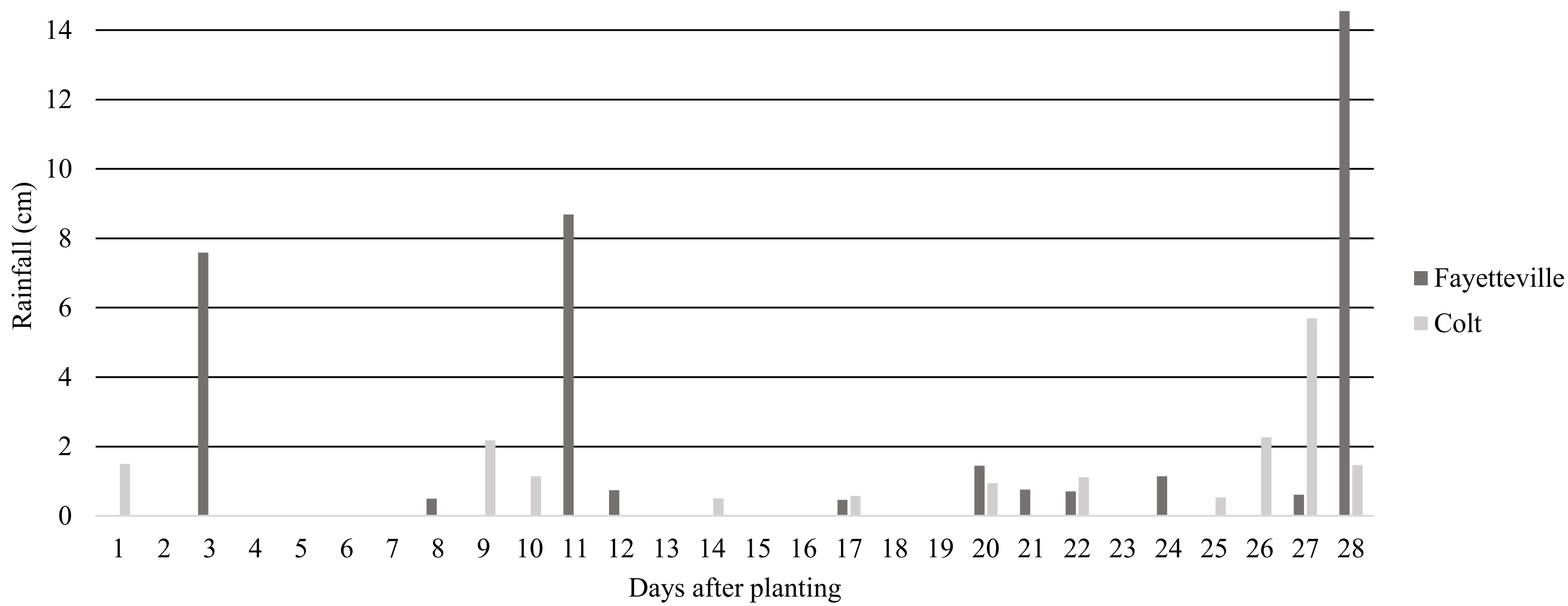

Figure 1. Precipitation history 28 days following planting of irrigation experiment conducted at Fayetteville and Colt, AR, in 2019.

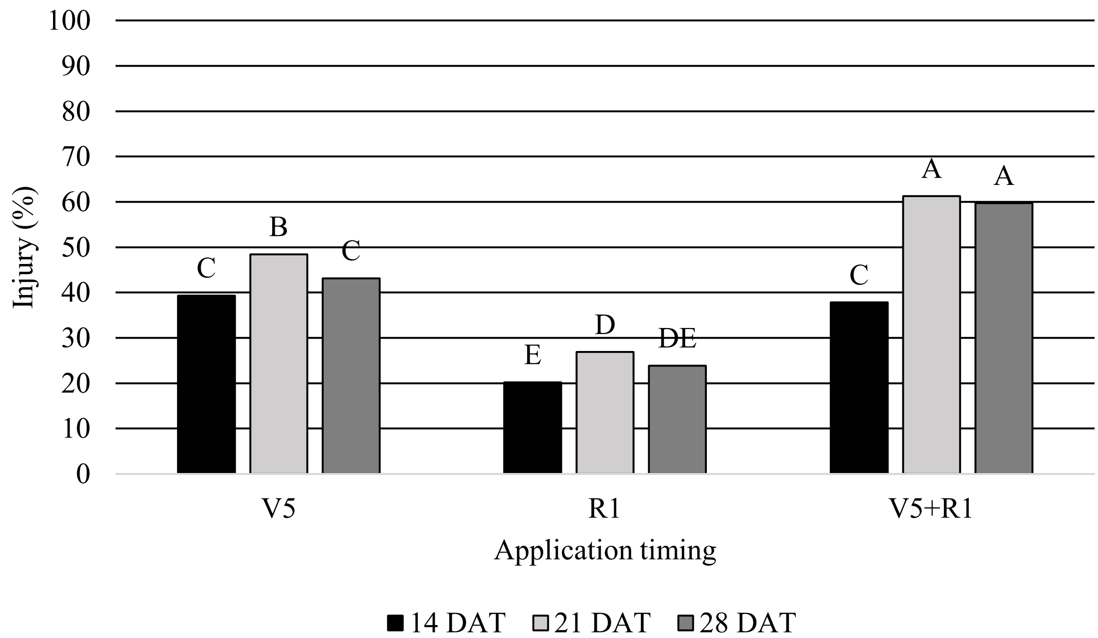

Figure 2. Injury of soybean according to the interaction of application timing and rating date in days after treatment (DAT) for the irrigation experiments conducted in Fayetteville and near Colt, AR in 2019. Treatments with the same uppercase letter are not significantly different according to Fisher's protected least significant difference α=0.05.

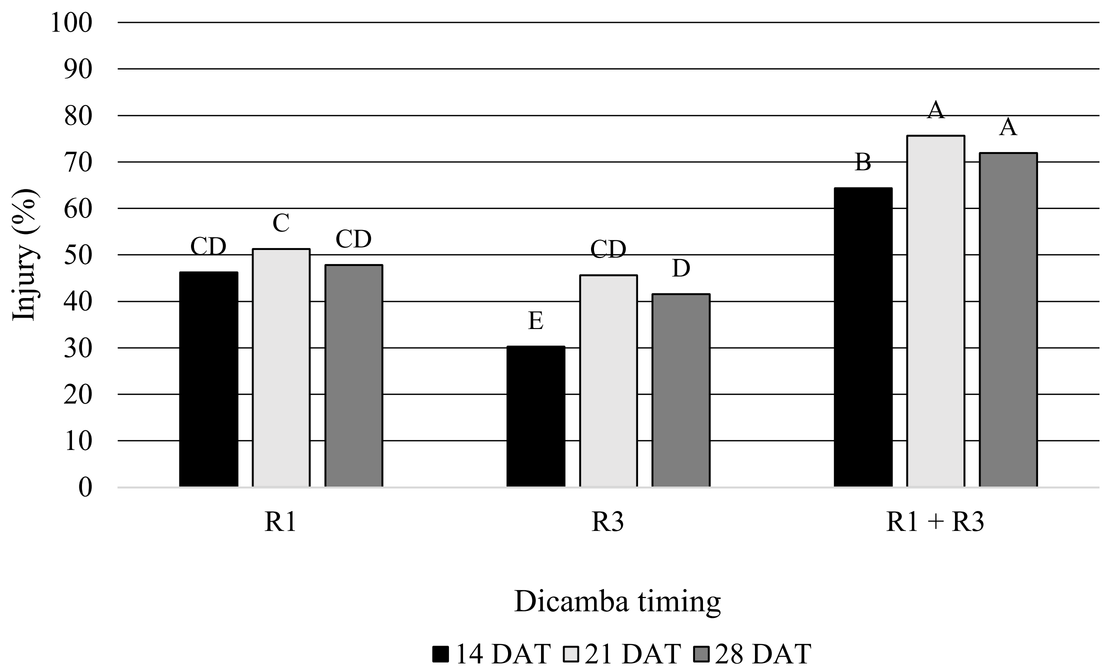

Figure 3. Injury of soybean according to the interaction of application timing and rating date in days after treatment (DAT) for the fertilizer experiment conducted in Fayetteville, AR, in 2019 and 2020. Treatments with the same uppercase letter are not significantly different according to Fisher's protected least significant difference α=0.05.

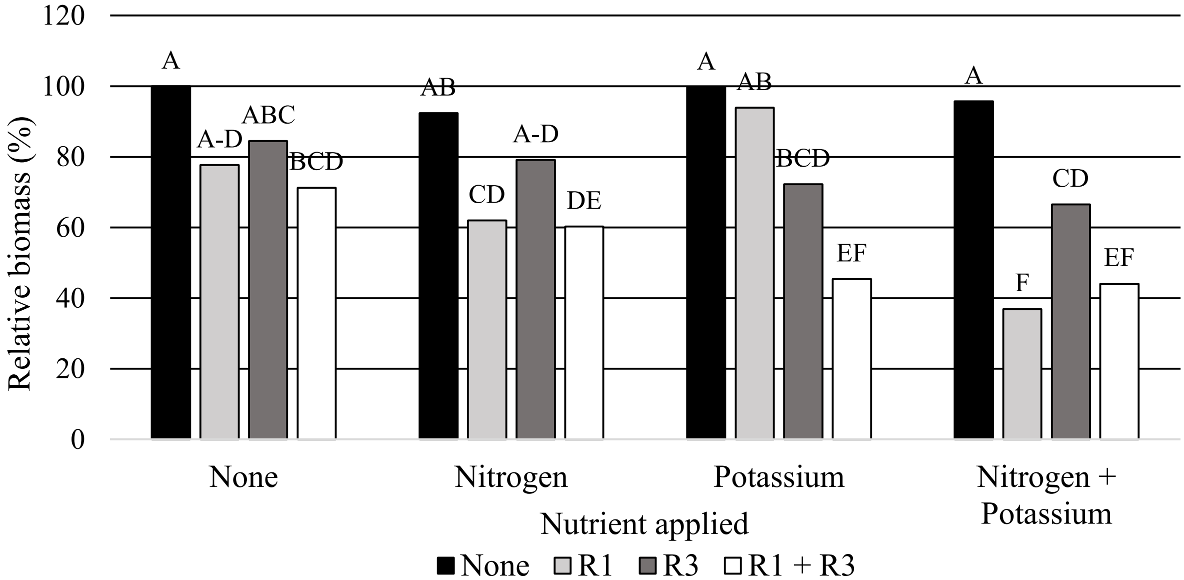

Figure 4. Relative biomass of soybean according to the interaction of application timing and fertilizer type for the experiment conducted in Fayetteville, AR, in 2019 and 2020. Treatments with the same uppercase letter are not significantly different according to Fisher's protected least significant difference α=0.05.

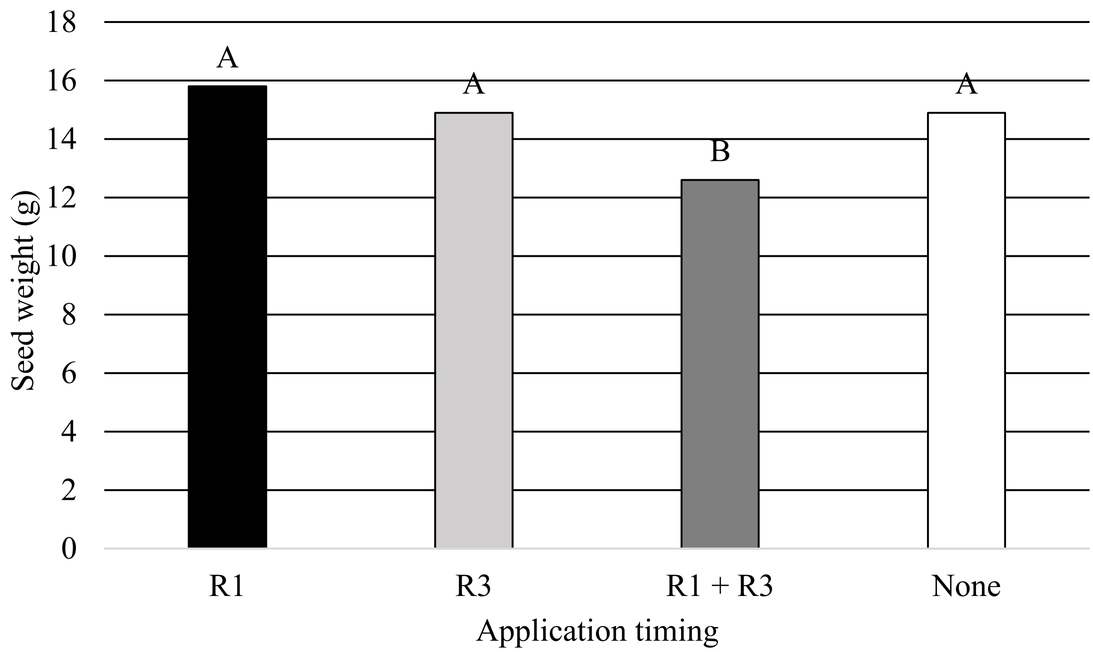

Figure 5. 100-Seed weight of harvested soybean according to the main effect of application timing for the fertilizer experiment conducted in Fayetteville, AR, in 2019 and 2020. Treatments with the same uppercase letter are not significantly different according to Fisher's protected least significant difference α=0.05.

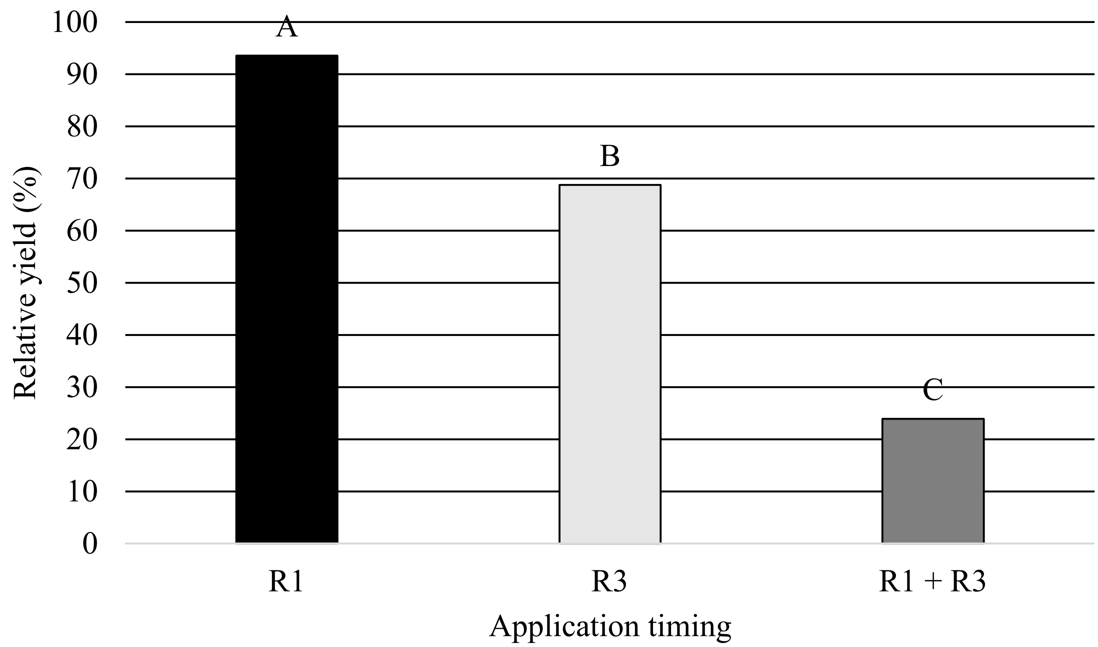

Figure 6. Relative yield of soybean according to the main effect of application timing for the fertilizer experiment conducted in Fayetteville, AR, in 2019 and 2020. Treatments with the same uppercase letter are not significantly different according to Fisher's protected least significant difference α=0.05.

Tables

Table 1. Effects of irrigation regime, application timing, and rating date to injury analyzed as a repeated measures analysis for the irrigation experiments conducted in Fayetteville and Colt, AR in 2019.

Factors	Injury
	p-values
Irrigation regime	0.0696
Application timing	<0.0001[a]
Irrigation regime*application timing	0.4765
Rating date	<0.0001
Irrigation*rating date	0.0864
Application timing*rating date	0.0003
Irrigation regime*application timing*rating date	0.5209

[a]P-values at or smaller than 0.05 level considered significant.

Table 2. Effects of dicamba, irrigation regime, application timing, and the interaction of these effects on yield components and grain yield associated with soybean for the irrigation experiments conducted in Fayetteville and near Colt, AR in 2019.

	Data collected						
Factors	Pods on mainstem	Seeds on mainstem	Total branches	Pods on branches	Seed on branches	100-Seed weight[a]	Grain yield
	-p-values-						
Irrigation regime	**<0.0001**	**0.0007**	**0.0460**	0.0734	**0.0175**	0.2163[a]	0.4168
Application timing	**<0.0001**	**<0.0001**	**<0.0001**	**0.0009**	**0.0061**	**0.0142**	**0.0282**
Application timing* irrigation regime	**0.0042**	**0.0014**	0.9452	0.3439	0.7249	0.4962	0.3893

[a]P-values at or smaller than 0.05 level considered significant as shown in bold.

Table 3. Effects of irrigation regime and application timing on yield components and grain yield collected for evaluation in the irrigation experiments conducted in Fayetteville and Colt, AR in 2019.

Yield components		Pods/Main[ab]		Seeds/Main[b]		Total branches[bd]	Pods/Branches[bd]	Seeds/Branches[bd]	Seed weight[cd]	Yield[d]
									g	kg ha^{-1}
Irrigated	V5	16.2	abc	28.7	abc	4.5	23.3	43.4	15.4	2059
	R1	20.7	abc	34.5	abc	3.1	15.1	26.1	15.3	2618
	V5 + R1	11.6	cd	21.0	cd	5.8	18.1	30.7	12.9	2019
	None	31.8	a	53.4	a	2.2	14.1	25.4	15.4	3925
Non-irrigated	V5	2.3	e	4.6	e	5.5	37.1	68.0	15.5	2350
	R1	14.8	bcd	27.5	bcd	3.7	20.6	42.3	15.5	2376
	V5 + R1	7.1	d	15.0	d	7.1	23.2	39.7	14.5	2018
	None	22.6	ab	52.1	ab	2.6	12.3	28.6	15.6	2631

[a]Means within a column followed by the same lowercase letter are not different according to Fisher's protected LSD (α=0.05).
[b]Yield component data other than seed weight taken as actual counted amounts averaged within each treatment.
[c]Seed weight data collected as grams (g) per 100 seed per plot averaged within each treatment.
[d]These data are included for informational purposes. Only some main effects are significant. Discussion of main effects are included in the text.

Table 4. Effects of application timing, fertilizer applied, and rating date to soybean injury of fertilizer experiment conducted in Fayetteville, AR in 2019 and 2020.

Effects	P-values[a]
Application timing	0.0138
Fertilizer applied	0.1473
Rating date	0.0197
Application timing*fertilizer applied	0.3331
Application timing*rating date	0.0477
Fertilizer applied*rating date	0.3236
Application timing*fertilizer applied*rating date	0.1718

[a]P-values at or smaller than 0.05 level considered significant.

Table 5. Effects of fertilizer applied and application timing to the nutrient content of samples gathered at R5 growth stage for the fertilizer experiment conducted in Fayetteville, AR in 2019 and 2020.

Effects	K	P	Mg	Ca	S
	-----p-values-----				
Fertilizer applied	**0.0037**	0.9483	**0.0017**	**0.0056**	0.7936
Application timing	0.4266	**<0.0001**	0.0777	**<0.0001**	0.1198
Fertilizer applied* application timing	0.1799	0.1119	0.1721	0.9072	0.7238

[a]P-values at or smaller than 0.05 level considered significant as shown in bold.

Table 6. The effects of application timing, fertilizer type applied, and the interaction of these effects on injury, relative biomass, seed weight, and relative yield for the experiment conducted in Fayetteville, AR in 2019 and 2020.

	Data collected		
Factors	Relative biomass[a]	100-Seed weight	Relative yield
	----- p-values -----		
Application timing	**<0.0001**	**0.0012**	**<0.0001**
Fertilizer applied	**0.0002**	0.7403	0.7097
Application timing* fertilizer applied	**0.0031**	0.9266	0.8969

[a]P-values at or smaller than 0.05 level considered significant as shown in bold.

CHAPTER 4

Sensitivity of Rice to Low Rates of Glyphosate and Glyphosate plus Dicamba at Multiple Growth Stages

Abstract

Glyphosate, a commonly used herbicide in glyphosate-resistant crops, can be severely injurious to adjacently grown rice, and glyphosate is routinely mixed with dicamba and applied to crops having resistance to both herbicides. This study was conducted to evaluate the effects of low rates of glyphosate and glyphosate plus dicamba on rice at multiple application timings. The hypothesis was that higher rates of glyphosate and glyphosate plus dicamba would cause greater rice injury and yield loss, with injury manifestations unique to glyphosate injury on rice observed on treatments receiving both glyphosate and dicamba. Separate field experiments were conducted in 2018 and 2019 near Stuttgart, AR. The first experiment evaluated rice response to glyphosate alone and another experiment evaluated rice response to glyphosate applied as a mixture with dicamba over a range of rice growth stages. The rates of glyphosate alone evaluated were 1.6, 3.3, 6.6, 13.1, and 26.3 g ae ha^{-1}, which are lower than those previously tested. The mixtures of glyphosate plus dicamba were evaluated at 3.5 plus 1.8, 14.1 plus 7, and 56.3 plus 28 g ae ha^{-1}, respectively. These rates correspond to $1/320^{th}$, $1/80^{th}$, and $1/20^{th}$ of a labeled rate for glyphosate and dicamba in Xtend cotton and soybean. Unique symptomology and greater yield loss were observed in the experiment evaluating glyphosate mixed with dicamba than in the experiment evaluating glyphosate alone. Visible injury to rice from glyphosate was no more than 3% at the highest rate of 26.3 g ae ha^{-1}, averaged over application timings. There was no effect of application timing on rice injury caused by glyphosate, nor a reduction in rough rice yield observed following any treatment of glyphosate alone. For the glyphosate plus dicamba

experiment, rice injury was not significant for glyphosate plus dicamba rates or application timings, with no more than 13% injury from any treatment. A 1-cm reduction in rice height occurred for the highest rate of glyphosate plus dicamba averaged over timings, and for the earliest timing (tiller) averaged over glyphosate plus dicamba rates. Even though a substantially visible deleterious effect to the crop was not noted in the injury or height assessments, rough rice grain yield was reduced by 21% relative to the non-treated for the highest rate of glyphosate plus dicamba, averaged over application timings. Based on findings from the glyphosate plus dicamba experiment, it is likely that dicamba contributed to this yield loss. Based on these results, care should be taken to prevent off-target movement of mixtures of glyphosate plus dicamba to neighboring rice.

Nomenclature: Dicamba; glyphosate; rice, *Oryza sativa* L.; soybean, *Glycine max* (L.) Merr.; cotton, *Gossypium hirsutum* (L.)

Keywords: Herbicide sensitivity, rate, rice growth stage, off-target movement

Introduction

Events such as spray-tank contamination or off-target movement of glyphosate and dicamba are more likely to occur as dicamba use increases, especially considering that dicamba plus glyphosate spray mixtures are commonly used for weed control (Butts et al. 2018b). In Arkansas, over half of the 77 confirmed cases of glyphosate drift to rice from 2010 to 2020 occurred since 2017, the year that dicamba was allowed for over-the-top use in glyphosate/dicamba-resistant cotton (*Gossypium hirsutum* L.) and soybean (personal communication, Ms. Susie Nichols of the Arkansas State Plant Board). Rice grown in the southern USA is commonly drill-seeded in April through early May and is in late vegetative stages when glyphosate-resistant crop cultivars, such as corn (*Zea mays* L.), cotton and soybean are typically treated with glyphosate. Glyphosate is injurious to rice due to shikimate accumulation from inhibition of EPSP synthase (Senseman 2007). Because of this, glyphosate can be very injurious and cause great yield loss in rice (Kurtz and Street 2003; Ellis et al. 2003; Hensley et al. 2013). Exposure of rice to glyphosate at later application timings typically results in greater yield loss to rice versus earlier timings (Kurtz and Street. 2003; Davis et al. 2011; Hensley et al. 2013). Glyphosate applied to rice in low doses can cause injury such as stunted growth and chlorotic tissue discoloration, along with leaf and inflorescence malformation (Koger et al. 2005; Hensley et al. 2013). Most commonly, experiments evaluate rates of glyphosate to rice that are above 54 g ae ha^{-1}. Kurtz and Street. (2003) evaluated rates of glyphosate to rice at 70 through 1120 g ae ha^{-1} at three- to four-leaf, mid-tiller, panicle initiation (PI), and boot (BT) rice growth stages. Davis et al. (2011) evaluated rates of glyphosate at 109, 218, and 435 g ae ha^{-1} at three- to four-leaf rice and at PI. Hensley et al. (2013) evaluated rates of glyphosate at 54 and 108 g ae ha^{-1} at the one-tiller tiller, post-directed, BT, and physiological mature rice growth stages. Martin et al. (2018) only evaluated glyphosate to rice at 126 g ae ha^{-1} at the two- to three-leaf growth stage. Only Ellis et al. (2003) and Koger et al (2005) used rates of glyphosate to rice lower than 54 g ae ha^{-1}. The experiments discussed herein used rates of glyphosate lower than previously evaluated.

With later application timings allowed for dicamba, the risk of off-target movement of dicamba to rice is increased and could occur in early reproductive growth stages, whether applied with glyphosate or alone. Dicamba is a synthetic auxin herbicide that controls plants by inducing abnormal growth through the production of endogenous auxin hormones, specifically indole acetic acid, resulting in leaf cupping, epinastic bending of stems and petioles, stem swelling and elongation, and leaf curling in grasses (Senseman 2007). Dicamba and most synthetic auxins are typically used to control broadleaf weeds and most are insufficient to control grass weeds when applied alone. Dicamba is not labeled for use in rice, but there have been no published reports of injury to rice caused by the herbicide until recently where it was determined that respective rates of 56 and 560 g ae ha^{-1} of dicamba alone resulted in 3 and 12% injury to rice and yield reductions of 78 and 57%, respectively, from the nontreated (Castner 2021).

One effect of applying glyphosate and dicamba together is a change in spray droplets produced. When applying dicamba with glyphosate versus use of the herbicides alone, droplet size is much more varied, specifically due to the inclusion of glyphosate, no matter the nozzle type (Alves et al. 2017). For example, coarser droplets are produced from dicamba alone than when sprayed with glyphosate when using air-induction nozzles, increasing the chance of drift (Alves et al. 2017). Additionally, dicamba has increased efficacy when applied to weeds as smaller versus larger droplets (Butts et al. 2018a; Meyer et al. 2016), such as when dicamba is applied in a mixture with glyphosate. Conversely, glyphosate has greater adsorption and translocation when applied with larger droplets (Feng et al. 2009), although both glyphosate and dicamba are systemic herbicides. Specific to off-target movement, spraying dicamba plus glyphosate increases drift events compared to dicamba alone for AIXR and XR nozzles (Alves et al. 2017). Efficacy of glyphosate and dicamba as a mixture for weed control in regards to droplet size differs with plant characteristics (i.e. flat, horizontal versus narrow, vertical leaf structure) and environmental factors (i.e. temperature, wind speed); therefore, a site-specific approach to applications of mixtures of these herbicides may prove most efficacious (Butts et al. 2018b).

Dicamba at low rates is not reported to cause visible injury to rice outside of impacting panicle grain fill (Castner 2021); however, soybean is very sensitive to dicamba (Thompson and

Egli 1973; Behrens and Leuschen 1979). According to a study by Kelley et al. (2005), dicamba applied in combination with glyphosate on glyphosate-resistant (GR) soybean increased visible injury at V3 and V7 applications, with dicamba at 5.6 g ae ha^{-1} and glyphosate at 12.7 g ae ha^{-1}. Jones et al. (2018) also noted increased injury to soybean when dicamba was applied in combination with glyphosate compared to dicamba alone, with greater leaf malformation and pod malformation, symptoms typical of dicamba injury to soybean. Similar to GR soybean, GR corn injury is increased by as much as 19% and yield is reduced by 12% when glyphosate is applied as a mixture with 2,4-D, a synthetic auxin similar to dicamba (Soltani et al. 2018). Additionally, 2,4-D, although labeled in rice, when applied to rice can reduce the rate of adventitious root growth, possibly reducing oxygen and nutrient uptake by the rice plant (Lin and Sauter 2019).

The research herein reported was conducted to determine if a glyphosate rate lower than those previously evaluated would elicit symptomology or yield reduction on rice and to determine whether low rates of glyphosate plus dicamba mixtures would cause injury and yield loss in rice over a range of application timings or exposures.

Materials and Methods

General Methodology. Separate field experiments were conducted in 2018 and 2019 at the Arkansas Agricultural Research and Extension Center in Stuttgart, AR, to evaluate the effect of glyphosate alone and glyphosate plus dicamba applied at sublethal rates to rice. The soil series near the Stuttgart trial site was a Dewitt silt-loam soil (Fine, smectic, thermic Typic Albaqualfs) containing 27% sand, 54% silt, and 19% clay with a pH of 6.8 and 1.7% organic matter. Both experiments were initiated simultaneously on a tilled, bare-ground field where the rice cultivar "CL153" was drilled at 70 seeds per meter of row into 9-row plots at a 1.5-cm depth on April 19, 2018, and April 2, 2019. Rows were spaced 18 cm and 5.2 m long with 1-meter alleys. The trials were grown in a delayed-flood system and managed according to specifications in the Arkansas Rice Production Handbook (Hardke 2019). Weeds in each trial were controlled with herbicides labeled for rice as well as through use of hand weeding as needed. All herbicide applications, including the evaluated treatments, were made using a CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} at 276 kPa using AIXR 110015 spray tips. Visible crop injury in both experiments was rated at 21 days after treatment (DAT) on a 0 to 100 scale, with 0 being equivalent of no injury and 100 being plant death. Date of 50% heading of each plot was recorded and reported relative to the corresponding non-treated control plots. Rough rice grain was harvested using a small-plot combine, and grain moisture was corrected to 12%. Relative rough rice grain yield was calculated for each cultivar by comparing the yield of treated to those of nontreated plots. The effect of replication was nested within year as a random effect while the effects of application rate, growth stage at application, and the interaction of each were analyzed as fixed effects for both experiments.

Glyphosate Experiment

This experiment was set up as a randomized complete block with a 2 x 5 factorial arrangement of treatments and four replications. Factor A was growth stage at application, and factor B was application rate. At the one-tiller and late-boot growth stages, glyphosate (Roundup PowerMax™, Bayer CropScience) was applied at the following rates: 1.6, 3.3, 6.6, 13.1, and 26.3 g ae ha^{-1}. These rates represent a 1/683x, 1/341x, 1/171x, 1/85x, and 1/43x rate of glyphosate at 1,120 g ae ha^{-1}, which is commonly applied as the 1x rate to glyphosate-resistant crops.

Injury data were analyzed as a beta distribution; date of 50% heading and relative yield data were analyzed as a gamma distribution using ANOVA with SAS 9.4 using PROC GLIMMIX (Gbur et al. 2012) with means separated using Fisher's protected LSD (P=0.05).

Glyphosate plus Dicamba Experiment

The experiment was designed as a randomized complete block with a 3 x 3 factorial arrangement of treatments with four replications. Factor A was growth stage at application and factor B was rate of glyphosate plus dicamba applied. At one tiller, half-inch internode elongation, and boot growth stages, respectively, rice plants were sprayed with the following rates of glyphosate (Roundup PowerMax™, Bayer CropScience, St. Louis, MO) and dicamba (Clarity™, BASF corporation), respectively: 3.52 and 1.75, 14.1 and 7, and 56.25 and 28 g ae ha^{-1}. These rates represent a 1/320x, 1/80x, and 1/20x respective rate of glyphosate (1120 g ae ha^{-1}) and dicamba (560 g ae ha^{-1}) labeled for POST use on Xtend™ crops. Rice height was measured in centimeters at grain maturity. Injury data were analyzed as a beta distribution while height, date of 50% heading, and relative yield data were analyzed as a gamma distribution using ANOVA with SAS

9.4 using PROC GLIMMIX (Gbur et al. 2012) with means separated using Fisher's protected LSD (P=0.05).

Results and Discussion

Rice Response to Low Glyphosate Rates. Only the main effect of glyphosate rate impacted rice injury (Table 1), even though there was no more than 3% injury at the highest rate of glyphosate (26.3 g ha^{-1}) when averaged over growth stages at application (Table 2). This low level of injury had no measurable effect on rice heading date or rough rice grain yield (Table 1).

The 3% injury to rice at a glyphosate rate of 26.3 g ha^{-1} in this trial is not surprising considering that no more than 3% injury was observed in other research when glyphosate at 9 to 35 g ae ha^{-1} was applied to rice at panicle differentiation (Ellis et al. 2003). Injury to rice averaging 8% resulted from glyphosate at 70 g ae ha^{-1} at the panicle initiation (PI) growth stage (Kurtz and Street 2003). Although yield loss was not observed in this research, others have noted reductions of 7 and 5% when glyphosate was applied to rice at the two- to three-leaf or panicle differentiation (PD) growth stages, respectively, at 18 or 35 g ae ha^{-1} (Ellis et al. 2003). Additionally, neither curvature of the rice hulls nor blanking of rice grains, symptoms of glyphosate injury to rice associated with yield loss (Hensley et al. 2013; Koger et al. 2005; Kurtz and Street 2003) occurred in this experiment. Differential sensitivity of rice cultivars to glyphosate has been reported (Ellis et al. 2003; Koger et al. 2005); thus, the lack of yield loss could be due to relatively greater tolerance of "CL153" to glyphosate compared to other rice cultivars. In addition, studies reporting yield loss at similarly low rates of glyphosate (Ellis et al 2003; Koger et al. 2005) used lower spray volumes than those used in these studies. Lower spray volumes allow for better simulation of herbicide off-target movement. This study utilized a spray volume typically used in conventional herbicide applications; therefore, this study was analogous to a tank-contamination event, a difference that may also have impacted the yield loss results.

Compared to other research investigating the effect of glyphosate at non-lethal rates to rice, this research applied relatively low rates of glyphosate. For example, the highest rate of glyphosate used in this experiment (26.3 g ae ha^{-1}) was equivalent to the second-lowest rate (26 g ae ha^{-1}) used in an experiment by Koger et al. (2005). Rice injury in that study ranged from 1 to 10% following glyphosate at 26 g ae ha^{-1} (Koger et al. 2005). In experiments examining rice response to glyphosate conducted by Kurtz and Street (2003), Davis et al. (2011), Hensley et al. (2013), and Martin et al. (2018), no rates as low as those used here were tested. Only Ellis et al. (2003) used rates of glyphosate lower than 26 g ae ha^{-1} to test rice response. Use of these low rates decreased visible differences of injury among application timings and were responsible for the low injury overall. The low rates also resulted in comparatively less physiological stunting versus higher rates and, thus, no effect to date of 50% heading and no significant effect to relative yield.

Rice Response to Glyphosate plus Dicamba. For rice injury, there was no significant effect of rate (P=0.0905) or growth stage at application (P=0.9612) (Table 3), with the highest observed injury being 13% following the application of glyphosate plus dicamba at the highest rate at the half-inch internode elongation growth stage (data not shown). Similarly, in other work, injury ranged from 1 to 18% at a rate of 26 g ae ha^{-1} of glyphosate alone at 21 DAT (Koger et al. 2005). Injury appeared as varying degrees of leaf malformation and chlorosis, typical of glyphosate injury to rice (Ellis et al. 2003; Hensley et al. 2013; Koger et al. 2005). Symptomology on rice following glyphosate plus dicamba at the half-inch internode elongation was unique among this research compared to other studies in that it manifested as bent secondary leaf blades whereas stems remained upright (Figure 1). The symptomology manifested only at the highest two rates of the herbicides. Rice exposure to glyphosate near the PD stage is translocated to the developing flagleaf (Senseman 2007) and typically results in a stunted flagleaf (Davis et al. 2011); however, this symptomology was not observed in this experiment. Additionally, rice was injured more by low-dose glyphosate applications near the PD stage than at other growth stages (Kurtz et al. 2003). However, glyphosate injury does not explain the unique symptomology observed in this experiment, which may be due to the addition of dicamba.

Rice height in 2018 was significantly affected by herbicide rate and growth stage at application, although there was no interaction (Table 4). The highest rate of glyphosate plus dicamba decreased height (86 cm) compared to the two lower rates (87 and 87 cm), which were not different (Table 4). The application made at the one-tiller growth stage decreased rice height significantly (86 cm) compared to the half-inch internode elongation (87 cm) and boot (87 cm) application stages (Table 5). Similar height reductions at a similar rate of glyphosate alone have been observed previously (Hensley et al. 2013). Although differences were found in rice height, it is unlikely of biological significance. Height reductions averaging only 10 and 2% at the two- to three-leaf and PD growth stages, respectively, were reported for rates of glyphosate alone at 70 and 35 g ae ha^{-1} (Ellis et al. 2003), indicating that rice height is not always greatly reduced by glyphosate injury.

Glyphosate plus dicamba rate had no effect on heading date but did negatively affect yield (Table 3). Furthermore, the main effect or interactions with application timing were not significant for maturity date or relative yield (Table 3). The highest rate of glyphosate plus dicamba reduced yield by 21%, whereas other treatments were not different from the nontreated (Table 6). A yield reduction averaging 28% at a similar rate (53 g ae ha^{-1}, compared to 56 g ae ha^{-1} in this experiment) was previously observed (Hensley et al. 2013). Also, curvature and blanking of kernels were not noted in this experiment, suggesting that dicamba may have played a role in the yield loss observed through a reduction in grain test weight. Curvature and blanking of rice grain are common symptoms of glyphosate injury to rice and associated with yield loss and reduced grain quality (Hensley 2013; Koger 2005; Kurtz and Street 2003). Height and yield reduction were noted in corn treated with a mixture of glyphosate and 2,4-D, a synthetic auxin similar to dicamba (Soltani et al. 2018).

Practical Implications. At the rates evaluated in the glyphosate-alone experiment, no yield loss occurred to rice as a result of exposure to the herbicide. However, yield loss and height reduction were observed in the experiment evaluating applications of both glyphosate and dicamba to rice, albeit glyphosate was evaluated at a higher rate in that trial. Additionally, symptomology unique to rice exposed to glyphosate was noted when rice plots were treated with low rates of glyphosate plus dicamba at the half-inch internode elongation stage. The symptomology was common at the highest rate of glyphosate plus dicamba and observed to a lesser extent at the second-highest rate. For the glyphosate-only trial, no bent leaf blades were observed from the highest rate of the herbicide whereas this symptomology was present in the trial that contained glyphosate plus dicamba at an equivalent glyphosate rate. Considering the trials were adjacent to each other in both years and were planted on the same dates, it appears likely that dicamba contributed to the unique symptomology observed. Additionally, considering the rates of herbicide used in these trials, it is likely that rice will need to be in close proximity to spray applications of glyphosate and dicamba for a high risk for negative crop impact to occur. With soybean resistant to glyphosate and dicamba being grown in close proximity to rice fields in the midsouthern U.S. and considering the relatively low rates of herbicide applied in this experiment, extreme care should be taken to ensure that off-target movement of glyphosate plus dicamba onto neighboring rice fields does not occur.

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Figures

Figure 1. (a) Image of nontreated plot versus (b) a plot treated with the highest rate of glyphosate plus dicamba applied at the half-inch internode elongation application timing. Images taken 21 days after the half-inch internode elongation application timing.

Tables

Table 1. Effects of rate and application timing to injury, maturity date, and relative yield of rice in experiment conducted near Stuttgart, AR in 2018 and 2019.

Factors	Injury (%)	Relative maturity	Relative yield (%)
	--	p-values	--
Rate	**0.0253**	0.3788	0.5741
Application Timing	0.7956	0.9799	0.7188
Rate*Application Timing	0.3084	0.9403	0.3152

[a]P-values at or smaller than 0.05 level considered significant as indicated in bold.

Table 2. Effect of glyphosate rate on rice injury 21 days after treatment, averaged over application timing near Stuttgart, AR in 2018 and 2019.

Glyphosate rate	Percent injury
g ae ha^{-1}[a]	%
26.3	3 a
13.1	1 b
6.56	0 b
3.28	0 b
1.64	0 b

[a]Means followed by the same letter within a column are not statistically different at $\alpha = 0.05$ according to Fisher's protected least significant difference.

Table 3. Effects of rate glyphosate plus dicamba and application timing to injury, relative maturity date, and relative yield of rice in 2018 and 2019, and height data taken only in 2018, in experiment conducted near Stuttgart, AR.

Factors	Injury (%)	Height (cm)	Relative maturity (d)	Relative yield (%)
	------------------------	---------p-values---------	------------------------	
Rate	0.0905	**0.0031**	0.5940	**0.0102**
Application timing	0.9612	**0.0172**	0.0930	0.7380
Rate*application timing	0.5397	0.2996	0.6370	0.4328

[a]P-values at or smaller than 0.05 level considered significant as indicated in bold.

Table 4. Effect of glyphosate and dicamba rate on rice heights in experiment conducted near Stuttgart, AR in 2018.

Rate of glyphosate plus dicamba	Nontreated	56.3 + 28 g ae ha^{-1}	14 + 7 g ae ha^{-1}	3.5 + 1.8 g ae ha^{-1}
Height in centimeters	87 a	86 b	87 a	87 a

[a] Means followed by the same letter within a row are not statistically different at α = 0.05 according to Fisher’s protected least significant difference test.

Table 5. Effect of herbicide application timing and dicamba to rice heights in experiment conducted near Stuttgart, AR in 2018.

Application timing	Nontreated	One tiller	½-inch internode	Boot
Height in centimeters	87 a	86 b	87 a	87 a

[a] Means followed by the same letter within a row are not statistically different at α = 0.05 according to Fisher’s protected least significant difference test.

Table 6. Effect of rate of glyphosate and dicamba to rice relative yield in experiment conducted near Stuttgart, AR in 2018 and 2019.

Rate of glyphosate plus dicamba	56.3 + 28 g ai ha^{-1}	14 + 7 g ai ha^{-1}	3.5 + 1.8 g ai ha^{-1}
Percent relative yield	79 b	98 a	96 a

[a] Means followed by the same letter within a row are not statistically different at α = 0.05 according to Fisher's protected least significant difference test.

GENERAL CONCLUSIONS

Off-target movement of dicamba poses a problem to non-dicamba-resistant (NDR) soybean producers, with very low rates of exposure capable of causing significant injury and yield loss in NDR soybean. Where dicamba is applied during the growing season, mitigation of injury and yield loss is a viable option for NDR soybean growers. Off-target movement of dicamba, in conjunction with glyphosate, may negatively impact grain crops, such as rice, as well.

Planting of soybean cultivars with greater tolerance to dicamba may be viable with one out of fifteen cultivars tested exhibiting consistently reduced injury and yield responses from dicamba exposure. Continued research may lead to a greater understanding of factors responsible for increased tolerance of certain soybean cultivars. Soybean exposed to dicamba across multiple planting dates alters both manifestation of dicamba injury and yield loss, with later planting dates of soybean manifesting greater injury and more yield loss when exposed at the same growth stages. Exposure of soybean to dicamba at later planting dates intensifies yield loss.

According to an assessment of the effect of irrigation regime to soybean exposed to low-dose dicamba, no difference in visible injury between irrigated and non-irrigated soybean was noted and final yields between irrigated and non-irrigated soybean were not different. Fertilization of soybean recently injured by dicamba did affect biomass of soybean, but not injury or yield when exposed to dicamba during reproductive stages. Due to the observed effect on biomass, soybean exposed to dicamba and fertilized in vegetative growth stages might experience greater recovery versus non-fertilized soybean.

Applications of dicamba as a mixture with glyphosate, or either herbicide separately, are common throughout the growing season; therefore, off-target movement of both to crops are likely. The effect of very low rates of glyphosate alone to rice was minimal, with no effect to yield. When a similarly low rate of glyphosate is inadvertently applied as a mixture with dicamba to rice, unique injury symptoms and yield loss occurred, indicating that rice in close proximity to applications of dicamba and glyphosate is at risk for negative effects from off-target movement of these herbicides together.