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Evaluation of soybean performance following multiple exposures to sub-lethal rates of dicamba

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Evaluation of soybean performance following multiple exposures to sub-lethal rates of dicamba

By

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In 2017, dicamba formulations were labeled for PRE and POST applications and utilized in soybean to control herbicide-resistant weed species. Dicamba-tolerant soybean cultivars were grown in proximity to those representing other herbicide-resistant technologies, creating the potential for problems with off-target movement. Field studies conducted in 2017 and 2018 in Stoneville, MS, characterized the soybean response to exposure to sub-lethal rates of different dicamba formulations and evaluated the performance of soybean cultivars representing different soybean maturity groups following multiple exposures to a sub-lethal rate of dicamba. Other field experiments in 2018 evaluated the performance of soybean following a single exposure to sub-lethal rates of dicamba at different growth stages (Rate and Timing Study) and characterized soybean response to multiple exposures of a sub-lethal rate of dicamba at different growth stages (Multiple Exposures Study).

DEDICATION

I would like to dedicate my hard work to my significate other, Ashley Brown, my father, my mother and my extended family for the constant encouragement throughout this graduate journey. I would also like to dedicate my research to the coaches, teachers and past employers that made an impact on my life. Lastly, I would like to dedicate this research to all the people that have instilled knowledge and love for agriculture in me.

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CHAPTER I

INTRODUCTION

Soybean Production

Soybean [*Glycine max* (L.) Merr.] is a member of the *Leguminosae* family, subfamily *Papilionoidae*, and genus *Glycine* (Probst and Judd 1973). Soybean was domesticated in northeastern China in the 11th century B.C. (Probst and Judd 1973). In 1900, European countries first produced soybean oil, while the U.S. began soybean production for forage, which is mixing soybean with other crops for hay. It was not until 1915 that the U.S. also began using soybean to manufacture oil (Dies 1942). Oil remains the top by-product of soybean; however, soybean are processed for other products such as feed, biodiesel, and building materials (Anonymous 2014).

Soybean production has evolved due to improved genetics and management practices, advances in science, and increased size of markets (Masuda and Goldsmith 2009). World-wide annual production of soybean was 351 million metric tons in 2017 with an average yield of 2.92 metric tons ha^{-1} (USDA-NASS 2017). The U.S. produced 116 million metric tons in 2017 with an average yield of 3.49 metric tons ha^{-1} (USDA-NASS 2017). Soybean production in the U.S. is one of the greatest sources of revenue, currently producing $40.9 billion from 219 million harvested ha (USDA-NASS 2017). Current Mississippi soybean production covers 8.8 thousand ha with an average yield of 3.85 metric tons ha^{-1}(USDA-NASS 2017).

Soybean Physiology

Soybean plants are typically 2 to 3 m tall and are sparsely or densely branched depending on multiple factors such as daylength, row spacing, and fertility (Carlson 1973). Soybean exhibits vegetative and reproductive developmental stages. Processes occurring during vegetative stages include plant emergence and expansion of the unifoliate and trifoliate leaves (Hoeft et al. 2000). Reproductive stages begin when axillary buds grow into clusters consisting of 2 to 35 flowers, continue with pod set and seed formation, and end with plant maturity (Carlson 1973; Hoeft et al. 2000).

Depending on stem growth habit and floral initiation, a soybean can be classified as indeterminate or determinate (Carlson 1973). With indeterminate growth, a plant grows vegetatively as it continues to produce nodes on the main stem after flowering beings. In determinate growth, vegetative growth ceases on the main stem soon after flowering beings (Carlson 1973). Soybean flowering is affected by daylength; therefore, the amount of darkness in a 24-hour period determines when flowering occurs (Hoeft et al. 2000).

Soybean cultivars in the U.S. and Canada are classified into one of 10 maturity groups based on the length of time to reach maturity (Wax 1973). Maturity groups are classed with Roman numerals ranging from 00 to X. Cultivars in maturity groups 00 to IV are classified as having indeterminate growth habit. Cultivars in maturity groups V to X are classified as having determinate growth habitat (Heatherly and Elmore 2004). Maturity groups are utilized in different latitudes because of their different responses to daylength. Maturity groups 00, 0, I and II are utilized in the northern U.S due to shorter daylength; whereas, maturity groups III, IV, V, VI and VII are utilized in southern U.S. as a result of longer daylength (Salmeron et al. 2014; Wax 1973).

Two types of soybean production systems in the southern U.S. include the Conventional Soybean Production System (CSPS) and the Early Soybean Production System (ESPS) (Heatherly 1999a). In the CSPS, cultivars from maturity groups V, VI, and VII are planted in May and June. Theses cultivars are often exposed to drought stress during the reproductive stages from mid-July through mid-September, which can compromise yields. In the ESPS, cultivars from maturity groups III and IV are planted from late-March to early-April, which allows for adequate soil moisture and lower temperatures during the reproductive stages of development (Heatherly 1999a; Wesley and Smith 1991).

Weed Control

A weed is a plant that is objectionable or interferes with the activities or welfare of humans (Heatherly 1999b). Weeds can be classified by morphology, life cycle, and habitat (Heatherly 1999b). Morphological classifications included monocot or dicot. A monocot produces one cotyledon and leaves with parallel vein structure (Radosevich et al. 1997). A dicot, or broadleaf, is an embryo with two cotyledons and netted leaf venation (Radosevich et al. 1997). Types of life cycle classification are annuals, biennials, and perennials. Annuals, which complete their life cycle in one year, are further divided into two groups, winter and summer annuals. A summer annual germinates in spring, grows during summer, reaches maturity by fall, and dies before winter. A winter annual germinates in the fall or winter, grows throughout spring, reaches maturity, and dies in early summer (Heatherly 1999a; Radosevich et al. 1997). Biennial weeds complete their life cycle in two years. Perennials have a life cycle lasting longer than two years. Classification by habitat includes terrestrial or aquatic. Terrestrial plants grow on land. Aquatic plants have the ability to survive in wet or submerged soils. The most common type of weed classification is an annual terrestrial summer broadleaf (Heatherly 1999a).

Weeds are difficult to control because of their efficient growth mechanisms, such as abundant seed production, seed dormancy, rapid growth rate, and root systems (Heatherly 1999b; Ward 2013). Crop growth efficiency is reduced by competition for sunlight, nutrients and space (Heatherly 1999b).

Herbicides are chemicals used to kill or suppress weeds (Radosevich et al. 1997). Preplant herbicides applications target weeds before crop planting (Heatherly 1999b). Preemergence (PRE) herbicides are applied at planting or immediately after planting but prior to crop emergence. Efficacy of PRE herbicides can be affected by amount and timing of rainfall, weed absorption of herbicide, and location of weed seed relative to herbicide placement. Preplant and PRE herbicide applications often do not provide weed control throughout the growing season. Therefore, weeds that escape preplant and PRE herbicide applications are targeted by postemergence (POST) herbicide applications. Postemergence herbicides are applied after crop emergence and often require multiple applications. Efficacy of POST herbicides can be influenced by herbicide mode of action (MOA), weed species, and environmental conditions before, during, or after the application (Heatherly 1999b).

Herbicides are the primary tool for weed management; however, they inevitably select for herbicide-resistant biotypes in targeted weed species (Shaner 2014d). The first reported herbicide-resistant weed populations were in wild carrot [*Daucus carota* (L.)], which evolved resistance to synthetic auxins. Following identification of herbicide-resistant wild carrot, common groundsel [*Senecio vulgaris* (L.)] populations evolved resistance to photosystem II inhibitors. Herbicide resistance continued with four new resistant weed species yr^{-1} evolving resistance to photosystem II inhibitors between 1970 and 1980. Populations of weed species also evolved resistance to microtubule inhibitors and photosystem I inhibitors during this time period.

In 1980, there were 41 herbicide-resistant weeds species, and by 1995, there were 191 resistant weed species (Heap 2019). Weed species evolved resistance to certain older MOA including nucleic acid inhibitors, lipid synthesis inhibitors, mitosis inhibitors, and long chain fatty acid inhibitors (Heap 2019; Shaner 1995). This rapid increase in populations of resistant weed species is correlated with the introduction of Accase and ALS inhibitor herbicides. Weed resistance continued with multiple weed species evolving resistant to EPSP synthase inhibitors, HPPD inhibitors, glutamine synthase inhibitors, and PPO inhibitors between 1995 and 2011 (Shaner 2014b). Currently, 255 weed species have evolved resistance to 23 of the 26 herbicide modes of action. Furthermore, select populations of weed species have evolved resistance to multiple MOA (Heap 2019).

Glyphosate-resistant soybean:

Glyphosate is classified by Weed Science Society of America (WSSA) as a group 9 MOA herbicide (WSSA 2008). Glyphosate is a broad-spectrum, systemic herbicide that inhibits 5-enolpyruvyshikimate 3-phosphate synthase EPSP, a key enzyme in the shikimic acid pathway that synthesizes the amino acids phenylalanine, tyrosine, and tryptophan in plants (Shaner 2014a). Glyphosate translocates to growing points in the meristematic tissues and inhibits the enzymatic process in plants which enables the control of a broad spectrum of annual and perennial weeds (Dill 2010).

In 1996, glyphosate-resistant soybean were registered for use in the U.S (Dill 2010). Glyphosate-resistant soybean was developed by inserting a glyphosate resistant CP4 EPSPS bacterium into plants. Insertion of this bacterium resulted in function of the shikimic acid pathway. Glyposate resistance allowed glyphosate to be applied POST in soybean to control weeds without crop injury (Nandula 2010). Oftentimes, PRE herbicides were not applied in glyphosate-resistant soybean production systems; therefore, weeds were controlled primarily by multiple POST applications of glyphosate (Reddy 2000). Repeated POST applications of glyphosate resulted in weed species evolving resistancae (Nandula 2010). Currently, 45 weed species worldwide, including 16 in the U.S., are resistant to glyphosate (Heap 2019).

Glufosinate-resistant soybean:

Glufosinate is a WSSA group 10 MOA herbicide (WSSA 2008). Glufosinate is a non-selective, contact, broad-spectrum, POST herbicide (Shaner 2014b). Glufosinate works by inhibiting the activity of glutamine synthetase, the enzyme that converts glutamate and ammonia to glutamine (Coetzer 2002). Inhibition of glutamine synthetase causes rapid increase in ammonia concentration within the cell, leading to disruption of chloroplast structure, prevention of normal photosynthesis and photophosphorylation, and eventual cell death (Coetzer 2002). Glufosinate is used to control annual and perennial grasses and broadleaf weeds (Shaner 2014b). In 2009, soybean cultivars resistant to glufosinate became available in the U.S. (Prostko et al. 2013). Glufosinate-resistant soybean was developed using a phosphinothricin acetyltransferase (PAT) gene that encodes for phosphinothricin acetyl transferase (Coetzer 2002). Currently, populations of Italian ryegrass [*Lolium multiforum* (L.)] are the only weed species resistant to glufosinate in the U.S. (Heap 2019).

Dicamba-resistant soybean:

Dicamba is a WSSA herbicide group 4 and is a member of the benzoic acid chemical family (WSSA 2008). This herbicide was registered as a preplant, PRE, and POST herbicide to control broadleaf weeds in corn, pastures, and cereal grains (Monaco et al. 2002). The specific cellular or molecular binding site relevant to the action of IAA has not be identified (Shaner

2014c). Dicamba is readily absorbed by foliage, roots, and stems of treated plants, transported by the phloem and xylem pathways, and is concentrated at the growing points (Shaner 2014c). This herbicide deteriorates rapidly in soil and has low to medium leaching potential (Shaner 2014c). Dicamba affects the life cycle of susceptible plants by damaging cell wall plasticity and nucleic acid metabolism. Dicamba also affects the development of vascular plant tissues, formation of lateral and adventitious roots, and control of apical dominance and tropic responses (Abel 1996). Visual symptomology, which can be observed when a susceptible plant is exposed to dicamba, is leaf cupping, twisting and curling of the stems and petioles (epinasty), stem swelling, and leaf elongation (often known as strapping). Other symptoms include plant wilting, chlorosis, growth inhibition, and necrosis (Grossmann 2000).

In 2016, U.S soybean and cotton [*Gossypium hirsutum* (L.)] cultivars resistant to dicamba received registration (USEPA 2016). Herbicide-resistant cultivars were established with the use of a genetically engineered bacterial gene, dicamba monoooxygenase (DMO). Dicamba monoooxygenase works by encoding a rieske non-heme monoooxygenase gene that is capable of inactivating dicamba when expressed from either the nuclear genome or chloroplast genome of transgenic plants (Behrens et al. 2007).

Dicamba Products:

In 2017, new formulations with proprietary additives received conditional labeling for dicamba-resistant soybean and cotton cultivars in the U.S. (Anonymous 2018a; Anonymous 2018b; Anonymous 2018c). Monsanto™ marketed Xtendimax™ with VaporGrip™ herbicide containing the diglycolamine salt (DGA) of dicamba (Anonymous 2018c). BASF™ marketed Engenia™ herbicide containing the N, N-Bis-(aminopropyl) methylamine salt (BAPMA) of dicamba (Anonymous 2018a). DuPont™ marketed FeXapan™ with VaporGrip™ herbicide containing the DGA salt of dicamba (Anonymous 2018b). A few previously labeled dicamba formulations labled for use in corn (*Zea mays* L.), pastures, and cereal grains (Anonymous 2010a; Anonymous 2010b; Monaco et al. 2002).

Off-Target Herbicide Movement:

Herbicide off-target movement is defined as the unintentional airborne movement of particles in the air soon after application to any off-target location (Henry et al. 2004). Spray particle or vapor movement are types of off-target movement. Spray particle movement is the physical movement of spray droplets to an off-target area at the time or soon after an application. Droplet size, boom height, and wind speed are the main factors influencing the movement of spray particles (Dexter 1995).

Spray droplets are sized by Volume Mean Diameter (VMD). VMD is a value where half of the total volume of the liquid sprayed is made up of droplets with diameters smaller than the mean value and half larger than the mean value. The standard developed by the American Society of Agriculture Engineers classifies spray droplet particle size based of the VMD as extremely fine (<60), very fine (60 to 145 mm), fine (145 to 225 mm), medium (226 to 325 mm), coarse (326 to 400 mm), very coarse (401 to 500 mm), extremely coarse (501 to 650 mm), or Fsusceptible to off-target movement (Anonymous 2017d). Smaller droplet size results in less inertial energy which reduces downward velocity to a target area. Lower boom height reduces the distance from droplet release point to target area; therefore, increasing the amount of smaller droplets reaching the target area. Higher wind speed increases the amount of droplets to an off-target area and increases the distance these droplets can travel. Furthermore, higher wind speeds result in larger droplets moving to an off-target area (Anonymous 2017d; Nelson 2017). Vapor movement occurs when a volatile herbicide changes from a liquid into a gaseous state and moves to an off-target area (Fishel and Ferell 2010). High temperatures and low relative humidity creates environmental conditions for evaporation of particles between the droplet release point and target (Dexter 1995).

Another environmental condition influencing off-target movement of herbicides is temperature inversions. A temperature inversion is a layer of warm air on top of a layer of cool air; therefore, atmospheric temperature profile is inverted from its usual state. (Anonymous 2018). The layer of cool air entraps fine spray particles highly susceptible to lateral movement by horizontal air flow near the earth's surface (Enz et al. 2017) Temperature inversions mostly occur in low wind situations at dawn or dusk (Ellis and Griffin 2002).

Off-target movement of spray particles and vapor can injure susceptible crops. Injury from off-target herbicide movement varies depending on MOA, distance from a susceptible crop, and susceptible crop stage at time of exposure (Henry et al. 2004). Off-target herbicide injury to susceptible crops has been reported and includes paraquat on rice [*Orzya Sativa* (L.)] (Lawrence et al. 2017), rimsulfuron and thifensulfron on soybean (Walker et al. 2017), glufosinate on cotton (Ellis et al. 2002), glyphosate on corn (Ellis et al. 2003), imazethapyr and sethoxydim on grain sorghum [*Sorghum bicolor* (L.)] (Al-Khatib et al. 2003), and glyphosate on wheat [*Tritcum aestivum* (L.)] (Roider et al. 2007).

Dicamba off-target Movement:

Dicamba is susceptible to off-target movement through volatility, physical drift, and sprayer contamination (Solomon and Bradley 2014). Off-target movement of dicamba has been documented in cotton, alfalfa [(*Medicago sativa* L.)], common sunflower [*Helianthus annuus* (L.)], peanut [*Arachis hypogaea* (L.)], wine grape [*Vitis vinifera* (L.)] and many other crops

(Solomon and Bradley 2014). Damage from dicamba off-target movement was documented in more than 1.4 million ha of U.S. soybean in 2017 (Bradley 2017; Kniss 2018). In June of 2017, Mississippi, Nebraska, Missouri, Tennessee, and Virginia reported dicamba injury to various types of tress, ornamental species, garden plants, flowers, and berries (Bradley 2017). In July of 2018, a total of 605 dicamba-related injury investigations across the U.S. were reported by state department of agriculture. Furthermore, university weed scientist estimate 445 thousand hectares of dicamba damage across 13 states (Bradley 2018).

Previous research identified the effects of off-target movement of dicamba on soybean, including visual symptomology and reductions in height and yield (Auch and Arnold 1978; McCown et al. 2016; Scholtes 2014; Wax et al. 1969; Westberg et al. 2017). Al-khatib and Peterson (1999) reported that symptoms of severe epinasty and leaf curling on soybean were observed within 3 hours following dicamba at 186.6 g ha^{-1} and 1 day after exposure for 5.6 g ha^{-1} dicamba rate. Westberg et al. (2017) reported 27% visual soybean injury 2 and 4 wk following exposure of soybean to dicamba at 0.56 g ae ha^{-1} at V1 compared to 13% following exposure at R1. Scholtes (2014) reported dicamba at 0.55 g ha^{-1} resulted in 12 and 16% soybean injury 7 d after treatment (DAT) when exposed at the V3 and R1 growth stages, respectively. Furthermore, a 10% reduction in soybean yield occurred following both V3 and R1 exposures to dicamba at 0.55 g ha^{-1}. Griffin et al. (2013) reported 35% visual soybean injury 2 wk following exposure of soybean to dicamba at 4.4 g ha^{-1} at V3 compared to 25% following exposure at R1.

The effect of dicamba on soybean yield and height varies depending on exposure timing (Auch and Arnold 1978; Scholtes 2014; Wax et al. 1969). Soybean exposure to dicamba in early vegetative stages affected new leaf development and increased branching, specifically with death of apical meristem but not pod and seed formation. Although, apical meristem damage or death during vegetative exposure, plants are able to rapidly produce branches, commonly producing branches from the node below damaged apical meristem (Wax et al. 1989). Injury is typically observed on new forming lateral soybean branches from apical meristem death (Weidnhamer 1989). Soybean exposure to dicamba during flowering or early pod formation caused irregular pod formation and seed development and reductions in yield (Auch and Arnold 1978). Reduction in yield following exposure during reproductive stages is attributed to reduction in pod production, leading to reduced pod number, seed number, and seed weight (Auch and Arnold 1978). Decreased pod production is correlated to decreased development of lateral branches following apical meristem death or damage (Wax et al. 1989). Scholtes (2014) observed ≥40% reduction in soybean yield following soybean exposure to dicamba at 8.75 g ha^{-1} applied at emergence (VE) to late reproductive (R7). Greatest reduction in soybean yield was ≥50% following the V6 exposure (Scholtes 2014). Exposing soybean to dicamba in later reproductive stages R5 to R7 resulted in no significant injury, height, or yield reductions (Scholtes 2014). Griffin et al. (2013) exposed soybean to dicamba in early vegetative stages V3/V4 at 4.4 g ha^{-1} to 17.5 reducing mature height 3 to 9 % and yield 4 to 15%. Wax et al. (1969) observed a 46% reduction in soybean height following dicamba at 17.5 g ha^{-1} during the R1 growth stage. As a result, soybean yield was reduced 52% following the R1 exposure timing. Griffin et al. (2013) also observed 44% mature height and 73% yield reduction following dicamba exposure at 17.5 g ha^{-1} during the R1 growth stage. Auch and Arnold (1978) also reported ≥30 kg ha^{-1} yield reduction following soybean exposure to dicamba at 11 g ha^{-1} during the R1 growth stage. This study also showed exposing soybean to dicamba during the reproductive growth stages can have a greater impact on yield compared to vegetative growth stages (Auch and Arnold 1978). Kniss (2018) reported soybean is two to six times more sensitive to dicamba following exposure during reproductive stage compared to vegetative stage.

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CHAPTER II

PERFORMANCE OF CULTIVARS FROM DIFFERENT SOYBEAN MATURITY GROUPS FOLLOWING EXPOSURES TO A SUB-LETHAL RATE OF DICAMBA

Abstract

In 2017, dicamba formulations were labeled for PRE and POST applications and utilized in soybean to control herbicide-resistant weed species. Dicamba-tolerant soybean cultivars were grown in proximity representing other herbicide-resistant technologies, creating the potential for problems with off-target movement. Field studies conducted in 2017 and 2018 in Stoneville, MS, characterized soybean response to exposure to sub-lethal rates of different dicamba formulations and evaluated the performance of soybean cultivars representing different soybean maturity groups following multiple exposures to a sub-lethal rate of dicamba. In the Formulations Study, no differences were detected for soybean injury, height, or yield following exposure to five different formulations of dicamba. In the Maturity Group Study, soybean injury 7 d after R1 (7 DA-R1) and 7 d after R3 (7 DA-R3) was 8 and 11% greater respectively, for dicamba exposure at V3 followed (fb) R1 compared with V3 fb R3. At 21 DA-R3, soybean injury was at least 8% greater following exposure at R1 fb R3 compared to all other exposure timings. Dry weight of maturity Group V soybean cultivar was 14% greater than for maturity group IV cultivar. However, reduction in dry weight was ≥49% for cultivars of both maturity groups. Exposing cultivars from both maturity groups to dicamba multiple times during reproductive growth stages (R1 fb R3) caused severe injury, height, and yield reductions. Yield

reductions were greater for maturity group IV compared with maturity group V cultivars across all vegetative fb reproductive exposure timings.

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

Keywords: Formulation

Introduction

Increasing pressure from herbicide-resistant weed species along with advancements in herbicide-resistant soybean technology have led to the development of dicamba-resistant soybean (McCown et al. 2018). In 2016, U.S soybean and cotton (*Gossypium hirsutum* L.) cultivars resistant to dicamba received registration (USEPA 2016). Adoption of dicamba-resistant soybean is widespread across the U.S (Kniss 2018). However, since registration of dicamba products for application to dicamba-resistant crops, reports of non-dicamba-resistant soybean exhibiting symptoms of synthetic auxin herbicide damage have been common (Bradley 2017; Kniss 2018). Damage from off-target dicamba movement was documented in more than 1.4 million ha of U.S. soybean in 2017, resulting in approximately 4% of 35 million ha planted exhibiting dicamba injury symptoms (Bradley 2017; Kniss 2018). In July 2018, a total of 605 dicamba-related injury investigations were reported by state departments of agriculture and university weed scientists with an estimated 1.1 million ha of soybean damage from off-target dicamba movement (Bradley 2018).

Dicamba moves off-target through volatility, physical drift, and sprayer contamination (Solomon and Bradley 2014). Distinctive symptoms of soybean exposure to off-target dicamba movement include severe shoot and petiole epinasty, swollen petioles, and leaf cupping and/or strapping (Grossmann 2000; Kelley et al. 2006). Other symptoms include plant wilting, chlorosis, growth inhibition, and necrosis (Grossmann 2000; Kelley et al. 2006). Al-Khatib and Peterson (1999) reported that symptoms of severe epinasty and leaf curling on soybean were observed within 3 hr following exposure to dicamba at 186.6 g ae ha^{-1} and 1 d after exposure to dicamba at 5.6 g ha^{-1}. Damage to soybean terminals following exposure to dicamba can cause significant height and yield reductions (Wax et al. 1969). Robinson et al. (2013) reported dicamba rates $\geq$ 2.3 g ha^{-1} resulted in soybean apical meristem death.

Previous research reported differences in height, injury, and yield in response to varying soybean time of exposure to dicamba (Anderson et al. 2004; Auch and Arnold 1978; Scholtes 2014; Wax et al. 1969). Soybean exposure to dicamba in early vegetative stages affected apical meristems, thereby increasing branching, but pod and seed formation were not affected (Wax et al. 1969). Anderson et al. (2004) reported 30 to 40% injury at 7 and 14 d after exposure (DAT) and 33% yield reduction following soybean exposure to dicamba at 5.6 g ha^{-1} during the V3 growth stage. When dicamba rate was increased to 11.3 g ha^{-1}, injury 24 DAT was 50% and yield was reduced 13 to 41% following V3 soybean exposure timing (Anderson et al. 2004). Soybean exposure to dicamba during flowering or early pod formation caused irregular pod and seed development and reduced yield (Auch and Arnold 1978). Wax et al. (1969) observed a 46% reduction in soybean height and subsequent 52% yield reduction following dicamba at 17.5 g ha^{-1} during the R1 growth stage. Scholtes (2014) also exposed soybean to dicamba at 17.5 g ha^{-1} during R1 growth stage, reducing height 28%. Kniss (2018) reported soybean is two to six times more sensitive to dicamba following exposure during reproductive compared with vegetative growth stages.

Differences in response to dicamba exposure vary among soybean cultivar (McCown et al. 2018; Wax et al. 1969; Weidenhamer 1989). Soybean yield response to dicamba exposure at different growth stages depends on whether cultivars are indeterminate or determinate (Wax et al. 1969). Weidenhamer (1989) reported exposure of an indeterminate soybean cultivar to dicamba during reproductive growth stages resulted in greater negative effects compared with exposure of determinate cultivars. In contrast, injury was 48 and 43% for indeterminate and determinate cultivars, respectively, averaged across dicamba rates of 2.18 and 8.75 g ha^{-1} applied at R1growth stage. Averaged across dicamba rates applied at R1, yield reductions of 19 and 14% were documented for determinate and indeterminate cultivars, respectively (McCown 2018). Auch and Arnold (1978) reported similar yield reductions for indeterminate and determinate soybean cultivars following dicamba exposure at the R1 growth stage.

Registered herbicide formulations can differ in volatility; however, previous research suggests no difference in soybean sensitivity among different formulations of dicamba and 2, 4-D (Miller et al. 2003; Mueller et al. 2013; Thompson et al. 2007; Weidenhamer 1989). Weidenhamer et al. (1989) documented no difference in soybean yield reduction between dimethylamine (DMA) and sodium salts of dicamba. Thompson et al. (2007) reported no difference in soybean response to preplant applications of ester and amine 2,4-D formulations. Miller et al. (2003) also reported no differences between formulations of 2,4-D when evaluating cotton.

In 2017, commercialization of dicamba-resistant crops provided a new system to control herbicide-resistant weeds. In this system, multiple dicamba formulations are registered for PRE and POST application to dicamba-resistant soybean. Furthermore, non-dicamba-resistant soybean cultivars with different growth habits are grown in proximity to dicamba-resistant soybean, creating potential for problems with off-target herbicide movement. Field observations from 2016 through 2018 indicated many non-dicamba-resistant soybean fields were subjected to off-target movement of dicamba multiple times. Previous research demonstrated soybean response to dicamba exposure at different growth stages (Griffin et al. 2013; Scholtes 2014; Weidenhamer et al. 1989; Wax et al. 1996); however, no research has been published on soybean response following multiple exposures to dicamba at different growth stages. Therefore, research was established to (1) characterize the soybean response to exposure to sub-lethal rates of different dicamba formulations and (2) evaluate the performance of soybean cultivars representing different soybean maturity groups following multiple exposures to a sub-lethal rate of dicamba.

Materials and Methods

Formulations Study

A field study was conducted at the Mississippi State University Delta Research and Extension Center in Stoneville, MS, in 2017 (33°25'05.2 "N, 90°54'20.5"W) and 2018 (33°26'27.24 "N, 90°54'29.88"W) to characterize soybean growth and yield following exposure to different dicamba formulations at a sub-lethal rate. Soil in 2017 was a Commerce silty clay loam (Fine-silty, mixed, superactive, nonacid, thermic Fluvaquentic Endoaquepts) with a pH of 7.4 and an organic matter content of approximately 1.5%. Soil in 2018 was a Bosket sandy loam (fine loamy, mixed, active, thermic Mollic Hapludalfs) containing 1.57% organic matter with a pH of 6.8.

Paraquat (Gramoxone SL, 841 g ai ha^{-1},Syngenta Crop Protection, P.O. Box 18300 Greensboro, NC 27419-8300) and *S*-metolachlor plus metribuzin (Boundary 6.5 EC, 1,367 g ai ha^{-1}, Syngenta Crop Protection LLC, P.O. Box 18300 Greensboro, NC 27419-8300) were applied PRE each year. Glyphosate (Roundup PowerMax 4.5 L, 1,1262 g ae ha^{-1}, Monsanto Company, 800 N. Lindburgh Blvd., St. Louis, MO 63167) and *S*-metolachlor plus sodium salt of fomesafen (Prefix formulation, 1,217 g ae ha^{-1}, Syngenta Crop Protection LLC, P.O. Box 18300 Greensboro, NC 27419-8300) were POST-directed in mid-June to maintain experimental sites weed free.

In both years, ‘Asgrow 4632’ (Monsanto Company, 800 N. Lindburgh Blvd., St. Louis, MO 63167) was planted to a depth of 2.5 cm at a seeding rate of 370,600 seeds ha^{-1}, using a small-plot air planter (John Deere 1730, Deer and Company, One John Deere Place Moline, IL, 61265-8098). Soybean were planted in 2017 and 2018 on May 17 and May 10, respectively. Plot size was 4 x 9 m and consisted of four rows spaced 97 cm apart. Rows one and two received treatments while rows three and four remained as a nontreated buffer between adjacent plots.

The Formulations Study was designed as a randomized complete block with four replications. Treatments were different formulations of dicamba and included a DMA salt (Rifle, herbicide, Loveland Products Inc., P.O. BOX 1286, Greeley, CO 80632-1286), a N,N-Bis-(3-aminopropyl) methylamine salt (BAPMA) (Engenia, herbicide, BASF Corporation, 26 Davis Drive Research Triangle Park, NC 27709), and three separate diglycolamine (DGA) salts. The first DGA salt, designated DGA-1 (Clarity, herbicide, BASF Corporation, 26 Davis Drive Research Triangle Park, NC 27709), was a dicamba formulations previously labeled for use in corn (*Zea mays* L.), pastures, and cereal grains (Anonymous 2010). The other DGA salts, designated DGA-2 (FeXapan with VaporGrip, herbicide, Dupont Company, Chestnut Run Plaza, 974 Centre Road, Wilmington, DE 19805), and DGA-3 (Xtendimax with VaporGrip, herbicide, Monsanto Company, 800 N. Lindburgh Blvd., St. Louis, MO 63167) were new formulations with proprietary additives labeled for application in dicamba-resistant crops. Dicamba treatments were applied at 4.4 g ha^{-1}, which is 1/128th of the labeled use rate and based on previous research in Mississippi evaluating soybean response to dicamba (Scholtes et al. 2014).

A nontreated control was included for comparison. Treatments were applied using a CO_2-pressurized backpack sprayer equipped with flat-fan nozzles (Airmix11002 nozzle, Greenleaf Technologies, 230 E Gibson St., Covington, LA 70433) set to deliver 140 L ha^{-1} at 206 kPa at first flower anywhere on the soybean plant (R1) growth stage (Sperry 2019).

Visible estimates of aboveground soybean injury were recorded 7, 14, 21, 28, and 48 d after dicamba exposure (DAT) on a scale of 0 to 100% where 0 indicated no visual effect and 100 indicated complete plant death. Soybean heights were recorded 14 and 28 DAT and at maturity by measuring from the soil surface to the upper soybean terminal and calculating the mean height of five randomly selected plants in each plot. Soybean were harvested in 2017 and 2018 on October 6 and October 1, respectively, using a small-plot combine (Kincaid Equipment, 210 West First St., P.O. Box 400; Haven, KS) and yields were adjusted to a uniform 13% moisture content.

Square roots of visible injury estimates were arcsine transformed. The transformation did not improve homogeneity of the variance based on visual inspection of the plotted residuals; therefore, nontransformed data were used in analyses. Nontreated data were excluded from analysis of injury. Nontransformed data were subjected to ANOVA using the MIXED procedure in SAS 9.4 (SAS Institute Inc. 100 SAS Campus Drive Cary, NC 27513-2414) with year and replication (nested within year) as random effect parameters (Blouin et al. 2011). Type III Statistics were utilized to test the fixed effect of dicamba formulation. Least square means were calculated and mean separation ($p \leq 0.05$) was produced using PDMIX800 in SAS, which is a macro for converting mean separation output to letter groupings (Saxton 1998).

Maturity Group Study

A field study was established at the Mississippi State University Delta Research and Extension Center in Stoneville, MS, in 2017 (33°25'05.2 "N, 90°54'20.5"W) and 2018 (33°25'40.08"N, 90°57'20.88"W) to evaluate the performance of soybean cultivars from different maturity groups following multiple exposures to a sub-lethal rate of dicamba. Soil information, site maintenance, plot size, and planting were as previously described in the Formulations Study.

Treatments in the Maturity Group Study were arranged as a two-factor factorial within a randomized complete block design with four replications. Factor A was soybean maturity group and consisted of cultivars representing maturity groups IV (‘Asgrow 4632’) and V (‘Asgrow 5332’; Monsanto Company, 800 N. Lindburgh Blvd., St. Louis, MO 63167). Factor B was dicamba timing and included dicamba applied to soybean with three trifoliate leaves fully emerged (V3) followed by (fb) first flower (R1), V3 fb 0.48 cm long pod in upper four nodes (R3), and R1 fb R3 growth stages. A nontreated for each cultivar was included for comparison. Dicamba (Clarity, BASF Corporation, 26 Davis Drive Research Triangle Park, NC 27709) treatments were applied at 4.4 g ha^{-1}, or 1/128th of labeled rate, using a CO_2-pressurized backpack sprayer equipped with flat-fan nozzles (Airmix11002 nozzle, Greenleaf Technologies, 230 E Gibson St., Covington, LA 70433) set to deliver 140 L ha^{-1} at 206 kPa.

Visible estimates of aboveground soybean injury were collected on the scale previously described om the Formulations Study at intervals following dicamba timings. Evaluation intervals were7 d after V3 (DA-V3), 7 d after R1 (DA-R1), and 7,14, and 21 d after R3 (DA-R3) dicamba timing. Soybean dry weights in two sub-samples of 1 m^2 were collected from rows one and two in each plot 28 DA-R3. Soybean plant heights were collected 28 DA-R3 and at maturity. Yields were collected as previously described in the Formulations Study. Data for soybean plant height, dry weight, and yield were converted to a percentage of the nontreated control in each replication. Percentage of nontreated control data were calculated by dividing the data from the treated plot by that in the nontreated control plot in the same replication and multiplying by 100. Data analyses were as previously described in the Formulations Study.

Results and Discussions

Formulations Study

No differences in soybean injury were detected among dicamba formulations at any evaluation. However, injury ranged from 15 to 58%. Soybean heights 14 and 28 DAT were reduced at least 25 and 42 cm, respectively, compared with the nontreated following exposure to all dicamba formulations (Table 1). Mature soybean height was reduced at least 41 cm corresponding with a 103 kg ha^{-1} average yield reduction following exposure to dicamba at the R1 growth stage. No differences in soybean sensitivity were detected among the five dicamba formulations. These results are consistent with those from earlier research which reported no difference in soybean sensitivity following exposure to different dicamba formulations (Egan and Mortensen 2012; Jones 2018). Egan and Mortensen (2012) observed no difference in soybean sensitivity to DGA and DMA salts of dicamba. Jones (2018) reported that soybean is equally sensitive to dicamba formulations containing DGA or BAPMA salts.

Maturity Group Study

A main effect of maturity group was detected for soybean injury 7 DA-V3 and dry weight (Table 2). Injury to the maturity group (MG) IV cultivar was 2% greater than for MG V cultivar 7 DA-V3 (Table 3). However, all treatments had not been applied at the 7 DA-V3 evaluation.

Injury differences were detected among dicamba timings at all evaluations. Soybean injury 7 DA-R1 and 7 DA-R3 was 8 and 11% greater, respectively, for dicamba at V3 fb R1 compared with V3 fb R3 (Table 4). No differences in injury were obseved between V3 fb R1 and R1 fb R3 dicamba exposure timings at 7 and 14 DA-R3. By 21 DA-R3, soybean injury was at least 8% greater following dicamba at R1 fb R3 compared with other dicamba timings. Similar to these findings, Griffin et al. (2013) reported soybean injury following dicamba (4.4 g ha^{-1}) exposure at R1 growth stage resulted in 8% greater injury 14 d after exposure compared with exposure during early vegetative growth stages. Kelley et al (2004) also reported 6% greater injury 6 wk after treatment (WAT) following dicamba (5.6 g ha^{-1}) exposure at V2 compared with V7 growth stage. They also observed soybean height reduction was >5% following dicamba at V2 compared to V7 growth stages.

At 28 DA-R3, soybean height following dicamba at V3 fb R1 was 6% greater than those with R1 fb R3 treatments (Table 4). Griffin et al. (2013) also observed 44% mature height reduction and 73% yield reduction following dicamba exposure at 17.5 g ha^{-1} during the R1 growth stage. In the current study, mature height was reduced 68% following dicamba at R1 fb R3 (Table 4). Mature soybean height was greatest following V3 fb R1 and least following R1 fb R3 treatments (Table 4). Weidenhamer et al. (1989) reported 17% greater soybean height following vegetative dicamba exposure compared with reproductive on indeterminate and determinate cultivars. Maturity Group V cultivar dry weight was 14% greater than for MG IV cultivar. However, reduction in dry weight was ≥49% for cultivars of both maturity groups (Table 3).

Soybean yield was influenced by an interaction of maturity group and dicamba timing (Table 2). Exposing cultivars of both soybean maturity groups to dicamba multiple times during reproductive growth stages produced the most severe yield reductions. Yield reductions were at least 6% greater for the maturity group V compared with maturity group IV cultivar with V3 fb R1 and V3 fb R3 dicamba timings. Soybean yield was greatest following V3 fb R3 exposure for maturity group V cultivar. In comparison, Weidenhamer et al. (1989) reported 2% greater yield for determinate compared with indeterminate soybean cultivars following reproductive exposure to dicamba at 5 g ha^{-1}. Similar research stated flexibility in soybean growth results in challenges in generalizing the effect of dicamba (Auch and Arnold 1978). McCown et al. (2018), stated an increased recovery from vegetative exposure is expected for maturity group V cultivars because of longer vegetative growth before initiation of reproductive growth, thereby, increasing leaf area production and nodes for pod production. In the current study, greater recovery was observed for maturity group V cultivar based on greater dry weight 28 DA-R3 compared with maturity group IV cultivar (Table 3). Also, yield of the maturity group V cultivar was greater following all treatments which included dicamba during a vegetative and reproductive growth stages.

Soybean injury, height, and yield were affected regardless of dicamba timing or maturity group. Dicamba timings and cultivar selection can influence soybean growth and yield following multiple exposures to dicamba; however, soybean response to five dicamba formulations was similar. No difference was observed for height between maturity group IV and V soybean cultivars for each dicamba exposure timing, but agronomic performance evidenced in dry weight and yield varied between cultivars. Yield reductions were greater for maturity group IV compared with maturity group V following V3 fb R1 and V3 fb R3 dicamba timings. In addition, maturity group V dry weights were greater pooled across three dicamba timings. Growers should take extreme caution when applying dicamba in proximity to non-dicamba-resistant soybean fields regardless of formulation or soybean growth stage. Future research should focus on determining the responses of soybean following dicamba exposure on diverse germplasms and under different management practices.

Table 2.1 Soybean height 14 and 28 d after exposure (DAT) and at maturity and yield following exposure to different formulations of dicamba applied at 4.4 g ae ha-1 at R1 growth stage in the Formulations Study at Stoneville, MS, in 2017 and 2018a,b.

	Height			
Treatment[b]	14 DAT	28 DAT	Maturity	Yield
	cm			kg ha^{-1}
Nontreated	61 a	97 a	99 a	3735 a
DMA	36 b	51 b	58 b	2693 b
BAPMA	32 b	48 b	60 b	2687 b
DGA-1	34 b	50 b	59 b	2802 b
DGA-2	35 b	53 b	57 b	2686 b
DGA-3	33 b	55 b	58 b	2663 b

[a]Data are pooled over two years. Means followed by the same letter for each parameter are not different at $p \leq 0.05$.
[b]Abbreviation: DMA, dimethylamine salt (Rifle, Loveland Products Inc); BAPMA, N, N-Bis-(aminopropyl) methylamine salt (Engenia, BASF Corporation); DGA-1 diglycolamine salt (Clarity, BASF Corporation); DGA-2, diglycolamine salt (Fexapan with VaporGrip, Dupont Company); DGA-3, diglycolamine salt (Xtendimax with VaporGrip, Bayer Crop Science)

Table 2.2 Significance of soybean maturity group and dicamba timing main effects and interaction for soybean injury following dicamba exposure, height 28 d after R3 dicamba timing and at maturity, dry weight, and yield in the Maturity Group Study at Stoneville, MS, in 2017 and 2018a.

Effects	Injury					Height		Dry weight	Yield
	7 DA-V3	7 DA-R1	7 DA-R3	14 DA-R3	21 DA-R3	28 DA-R3	Maturity		
	p-value								
Maturity group (MG)	0.0124	0.0559	0.0876	0.3838	0.1117	0.8630	0.1084	0.0341	<.0001
Timing	<.0001	<.0001	<.0001	<.0001	<.0001	0.0467	<.0001	0.1955	<.0001
MG*timing	0.0820	0.9786	0.9739	0.8992	0.3966	0.6553	0.4811	0.3569	0.0387

[a]Abbreviation: 7 DA-V3, 7 d after V3; 7 DA-R1, 7 d after R1; 7 DA-R3, 7 d after R3;14 DA-R3, 14 d after R3; 21-DA-R3, 21 d after R3; 28 DA-R3, 28 d after R3.

Table 2.3 Influence of maturity group (MG) on soybean injury 7 d after V3 dicamba timing and dry weight 28 d after R3 dicamba timing in the Maturity Group Study at Stoneville, MS, in 2017 and 2018[a,b].

Maturity group	Injury[b]	Dry weight
	%	% of nontreated
MG IV	8 a	49 b
MG V	6 b	63 a

[a]Data are pooled across three dicamba timings including V3 followed by (fb) R1, V3 fb R3, and R1 fb R3 and two years. Means followed by the same letter for each parameter are not different at $p \leq 0.05$.

[b]All treatments had not been applied at time of evaluation.

Table 2.4 Influence of dicamba exposure timing on soybean injury at different evaluations, height 28 d after R3 dicamba exposure, and at maturity in the Maturity Group Study at Stoneville, MS, in 2017 and 2018[a,b].

	Injury					Height[d]	
Dicamba timing	7 DA-V3[c]	7 DA-R1[c]	7 DA-R3	14 DA-R3	21 DA-R3	28 DA-R3	Maturity
	——%——					——% of nontreated——	
V3 fb R1	14 a	47 a	60 a	59 ab	57 b	53 a	74 a
V3 fb R3	14 a	39 b	49 b	58 b	60 b	49 ab	55 b
R1 fb R3	0 b	11 c	55 a	63 a	68 a	47 b	42 c

[a]Data are pooled across two soybean maturity groups (IV and V) and two years. Means followed by the same letter for each parameter are not different at $p \leq 0.05$.

[b] Abbreviation: 7 DA-V3, 7 d after V3; 7 DA-R1, 7 d after R1; 7 DA-R3, 7 d after R3;14 DA-R3, 14 d after R3; 21-DA-R3, 21 d after R3; 28 DA-R3, 28 d after R3; fb, followed by.

[c]All treatments had not been applied at time of evaluation.

[d]Data for height 28 DA-R3 and at maturity are expressed as a percentage of the nontreated control.

Table 2.5 Effect of maturity group and dicamba timings on soybean yield in the Maturity Group Study at Stoneville, MS, in 2017 and 2018[a,b,c].

Dicamba exposure timing	Maturity Group IV	Maturity Group V
	——% of nontreated——	
V3 fb R1	44 c	50 b
V3 fb R3	47 bc	64 a
R1 fb R3	36 d	42 cd

[a] Data are pooled across two years. Means followed by the same letter are not different at $p \leq 0.05$.
[b] Data are expressed as a percentage of the nontreated control.
[c] Abbreviation: fb, followed by.

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CHAPTER III

SOYBEAN PERFORMANCE FOLLOWING EXPOSURE TO A SUB-LETHAL RATE OF DICAMBA AT MULTIPLE GROWTH STAGES

Abstract

In 2017, dicamba formulations received labeling for PRE and POST applications in dicamba-tolerant crops. Dicamba-tolerant soybean cultivars were grown in proximity to those representing other herbicide-resistant technologies, creating the potential for problems with off-target movement. Field studies conducted in 2018 in Stoneville and Starkville, MS, evaluated the performance of soybean following a single exposure to sub-lethal rates of dicamba at different growth stages (Rate and Timing Study) and characterized soybean response to multiple exposures of a sub-lethal rate of dicamba at different growth stages (Multiple Exposures Study). In the Rate and Timing Study, exposing soybean to dicamba at 4.4 g ae ha^{-1} during V3 or R1 growth stages resulted in greater injury 14 d after treatment (DAT) compared with dicamba at 1.1 g ha^{-1} applied at same growth stages. Dicamba at 4.4 g ha^{-1} at the R1 growth stage caused a 16% reduction in mature soybean height compared with dicamba at 1.1 g ha^{-1} at R1. Soybean yield was 84% of the nontreated following exposure to dicamba during the R1 growth stage and was comparable to that from the V3 dicamba timing. In the Multiple Exposures Study, greatest injury 7 d after R5 application (DA-R5) was 72% from dicamba at V3 followed by (fb) R1 fb R3 fb R5. Mature height was reduced ≥12 cm compared to plots not exposed to dicamba for all treatments except R5 only. Soybean yield and dry weight in plots only exposed to dicamba at R5 were similar to plots with no dicamba exposure. Reproductive growth stage at time of exposure

to dicamba was more indicative of soybean agronomic performance than prior exposure at V3. Soybean was unable to recover following single or multiple dicamba exposures up to the R3 growth stage.

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

Keywords: Drift, injury, off-target movement, yield

Introduction

Herbicides are the primary tool for weed management; however, over use of herbicides select for herbicide-resistant biotypes in targeted weed species (Shaner 2014). Currently, 255 weed species have evolved resistance to 23 of the 26 herbicide modes of action (MOA) (Heap 2019). Furthermore, select populations of weed species have evolved resistance to multiple herbicide MOA (Heap 2019). In 1996, glyphosate-resistant (GR) soybean was registered for use in the U.S. and were widely accepted after commercialization (Dill et al. 2010). Introduction of GR crops offered economic advantages and effective management of weeds without crop injury; however, over reliance on glyphosate resulted in the evolution of GR weed biotypes (Nandula 2010). In 2016, U.S soybean and cotton (*Gossypium hirsutum* L.) cultivars resistant to dicamba received registration (USEPA 2016). Corn (*Zea mays* L.), soybean, and cotton cultivars resistant to 2,4-D became available in the U.S in 2017 (USEPA 2017). In addition, dicamba herbicide products received conditional labeling for PRE and POST applications to dicamba-resistant soybean and cotton cultivars in the U.S. the same year (Anonymous 2018a, b, c).

Pesticide spray drift is the movement of pesticide dust or droplets through the air at time of application or soon after, to any site other than the intended area (USEPA 2017). Droplet size, boom height, and wind speed are the main factors influencing the movement of spray particles (Dexter 1995). Injury from off-target herbicide movement varies depending on herbicide MOA, distance to a susceptible crop, and susceptible crop growth stage at time of exposure (Henry et al. 2004).

There is an increased chance of off-target herbicide movement to adjacent crops due to the introduction of dicamba-tolerant crops and the registration of newly formulated dicamba products (Johnson et al. 2012; Kniss 2018). Dicamba is susceptible to off-target movement through volatility, physical drift, and sprayer contamination (Solomon and Bradley 2014). Different herbicide-resistant crops are frequently grown adjacent to or in proximity to one another, creating susceptibility to off-target herbicide movement (Wax et al 1969). Furthermore, the wide planting window for soybean and the POST application timing of dicamba increases the possibility for off-target herbicide movement to adjacent crops at vulnerable growth stages (McCown et al. 2018).

Soybean sensitivity to dicamba can vary depending on rate and timing of dicamba exposure (Griffin et al. 2013; Scholtes 2014; Weidenhamer et al. 1989). Griffin et al. (2013) reported soybean injury 7 d after treatment (DAT) during early reproductive growth stage (R1) increased from 19% to 64% when dicamba rate increased from 1.1 to 70 g ha^{-1}. In the same study, soybean injury 14 DAT was 10% greater with exposure at R1 compared to V3 for dicamba at 4.4 g ha^{-1}. Weidenhamer et al. (1989) observed a 16% reduction in mature soybean height following dicamba at 1.3 g ha^{-1} during the V3 growth stage. Furthermore, mature height of soybean was reduced 34% following dicamba at 5.0 g ha^{-1} at V3 growth stage (Weidenhamer et al. 1989). Scholtes (2014) observed ≥40% reduction in soybean yield following soybean exposure to dicamba at 8.75 g ha^{-1} applied at emergence (VE) to late reproductive (R7). Soybean yield was reduced ≥50% following exposure to dicamba during the V6 soybean growth stage (Scholtes 2014). Auch and Arnold (1978) exposed soybean to dicamba at 11, 28, and 56 g ha^{-1} at R5 growth stage and observed no reduction in soybean height; however, a yield reduction of 286 kg ha^{-1} was detected following dicamba at 56 g ha^{-1}. They also reported a yield reduction of ≥30 kg ha^{-1} following soybean exposure to dicamba at 1 g ha^{-1} during the R1 growth stage. Griffin et al. (2013) reported soybean is 2.5 times more sensitive to dicamba during early reproductive compared to vegetative growth stages.

In 2017, dicamba formulations received conditional labeling for application to dicamba-resistant crops. Crops with different herbicide-resistant technologies are often grown in proximity to dicamba-resistant crops, which increases likelihood of injury from off-target movement of dicamba. Field observations from 2016 through 2018 indicated many non-dicamba-resistant soybean fields were subjected to off-target dicamba movement at different growth stages multiple times (Bradley 2017, 2018). Previous research demonstrated soybean response to dicamba at different growth stages (Griffin et al. 2013; Scholtes 2014; Weidenhamer et al. 1989; Wax et al. 1969); however, no research has been published on soybean response following multiple exposures to dicamba at different growth stages. Therefore, research was established to (1) evaluate the performance of soybean following a single exposure to sub-lethal rates of dicamba at different growth stages and (2) characterize soybean response to multiple exposures of a sub-lethal rate of dicamba at different growth stages.

Materials and Methods

Rate and Timing Study

A field study was conducted three times in 2018 at the Mississippi State University Delta Research and Extension Center in Stoneville, MS (33°26'27.24"N, 90°54'29.88"W; 33°25'40.08"N, 90°57'20.88"W; and 33°26'20.61"N, 90°54'13.78"W), to characterize soybean response to two sub-lethal rates of dicamba at different growth stages. Soil at two sites was a

Bosket sandy loam (fine-loamy, mixed, active, thermic Mollic Hapludalfs) containing 1.5 to 1.6% organic matter with a pH of 6.5 to 6.8. Soil at the third site was a Sharkey clay (very-fine, smectitic, thermic Chromic Epiaquerts) with a pH of 7.8 containing an organic matter content of 2.4%.

Paraquat (Gramoxone SL, 841 g ai ha^{-1}, Syngenta Crop Protection, P.O. Box 18300 Greensboro, NC 27419-8300) and *S*-metolachlor plus metribuzin (Boundary 6.5 EC, 1,367 g ai ha^{-1}, Syngenta Crop Protection LLC, P.O. Box 18300 Greensboro, NC 27419-8300) were applied PRE immediately after planting in mid-May to control emerged vegetation. Glyphosate (Roundup PowerMax 4.5 L, 1, 262 g ae ha^{-1}, Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, MO 63167) and *S*-metolachlor plus sodium salt of fomesafen (Prefix formulation, 1,217 g ae ha^{-1}, Syngenta Crop Protection LLC, P.O. Box 18300 Greensboro, NC 27419-8300) were applied POST-directed in mid-June to maintain experimental sites weed free.

At all three sites, 'Asgrow 4632' (Monsanto Company, 800 N. Lindburgh Blvd., St. Louis, MO 63167) soybean were planted to a depth of 2.5 cm at a seeding rate of 370,600 seeds ha^{-1} using a small-plot air planter (John Deere 1730, Deer and Company, One John Deere Place Moline, IL, 61265-8098). Soybean were planted on May 10 and May 17, respectively. Plot size was 4 x 9 m and consisted of four rows spaced 97 cm apart. Rows one and two received treatments while rows three and four remained as a nontreated buffer between adjacent plots.

Treatments in the Rate and Timing Study were arranged as a two-factor factorial within a randomized complete block design with four replications. Factor A was dicamba rate and consisted of dicamba (Clarity, herbicide, BASF Corporation, 26 Davis Drive Research Triangle Park, NC 27709) applied at 1.1 and 4.4 g ha^{-1}. Dicamba at 1.1 and 4.4 g ha^{-1} represents 1/128th and 1/512th, respectively, of the labeled rate and were chosen based on previous research in

Mississippi evaluating soybean response to dicamba (Scholtes et al. 2014). Factor B was dicamba timing and included V3 (second trifoliate fully emerged), R1 (first flower present), and R5 (visible seed in pod located on upper four nodes) soybean growth stages. A nontreated control was included for comparison. Treatments were applied using a CO_2-pressurized backpack sprayer equipped with flat-fan nozzles (Airmix11002 nozzle, Greenleaf Technologies, 230 E Gibson St., Covington, LA 70433) set to deliver 140 L ha^{-1} at 206 kPa (Sperry 2019).

Visible estimates of aboveground soybean injury were recorded 7, 14, and 28 d after each treatment (DAT) on a scale of 0 to 100% where 0 indicated no visual effect and 100 indicated complete plant death. Soybean heights were recorded 14 DAT and at maturity by measuring from the soil surface to the upper soybean terminal and calculating the mean height of five randomly selected plants in each plot. Soybean dry weight in two sub-samples of 1 m^2 were collected from rows one and two 28 d after V3, R1, and R5 dicamba timings. Canopeo (Oklahoma State University, Stillwater, OK 74074) was utilized to measure percent green leaf area between treated rows in each plot. Images were recorded weekly from 1 wk following V3 through 42 d after R5 exposure. Canopeo is based on color ratios of red to green (R/G) and blue to green (B/G) and an excess green index (2G– R–B). Soybean were harvested at site one and two on October 1 and site three on October 4 using a small-plot combine (Kincaid Equipment, 210 West First St., P.O. Box 400; Haven, KS), and yields were adjusted to a uniform 13% moisture content. Data for soybean height, dry weight, and yield were converted to a percentage of the nontreated control in each replication. Percentage of nontreated control data were calculated by dividing the data from the treated plot by that in the nontreated control plot in the same replication and multiplying by 100.

Square roots of visible injury estimates were arcsine transformed. The transformation did not improve the homogeneity of the variance based on visual inspection of plotted residuals; therefore, nontransformed data were used in analyses. Nontransformed data were subjected to ANOVA using the MIXED procedure in SAS 9.4 (SAS Institute Inc. 100 SAS Campus Drive Cary, NC 27513-2414) with site and replication (nested within site) as random effect parameters (Blouin et al. 2011). Type III Statistics were utilized to test the fixed effect of dicamba rate and timing and the interaction between these fixed effects. Least square means were calculated and mean separation ($p \leq 0.05$) was produced using PDMIX800 in SAS, which is a macro for converting mean separation output to letter groupings (Saxton 1998).

Multiple Exposures Study

A field study was established in 2018 at two sites at the Mississippi State University Delta Research and Extension Center in Stoneville, MS (33°26'27.24"N, 90°54'29.88"W and 33°25'40.08"N, 90°57'20.88"W), and one site at the R. R. Foil Plant Science Research Center in Starkville, MS (33°28'26.0508"N, 88°46'18.65"W), to evaluate performance of soybean following multiple exposures to a sub-lethal rate of dicamba. Soil at both Stoneville sites was a Bosket sandy loam containing 1.57% organic matter with a pH of 6.85. Soil at Starkville was a Catalpa silty clay loam (fine, smectitic, thermic Fluvaquentic Hapludolls) containing 1.25% organic matter with a pH of 7.2. Soybean at Starkville were planted on May 1. Site maintenance and planting information for Stoneville sites were as previously described in the Rate and Timing Study.

Treatments in the Multiple Exposures Study were arranged as a two-factor factorial in a randomized complete block design with four replications. Factor A was vegetative treatment and consisted of no vegetative treatment and dicamba applied at the V3 soybean growth stage.

Factor B was reproductive treatment and consisted of no reproductive treatment and dicamba applied at the R1, R3, R5, R1 followed by (fb) R3, R1 fb R5, R3 fb R5, and R1 fb R3 fb R5 soybean growth stages. Dicamba (Clarity, BASF Corporation, 26 Davis Drive Research Triangle Park, NC 27709) treatments were applied at 2.8 g ha^{-1} or 1/200th of labeled use rate at designated growth stages using a CO_2-pressurized backpack sprayer equipped with flat-fan nozzles (Airmix11002 nozzle, Greenleaf Technologies, 230 E Gibson St., Covington, LA 70433) set to deliver 140 L ha^{-1} at 206 kPa (Sperry 2019). Visible estimates of aboveground soybean injury were collected 7 d after V3 (DA-V3), 7 d after R1 (DA-R1), 7 d after R3 (DA-R3) and 7, 14, and 21 d after R5 (DA-R5) treatments. Soybean dry weight in two sub-samples of 1 m^2 were collected from rows one and two in each plot 28 DA-R5. Soybean heights were recorded at maturity. Soybean yield components, including node $plant^{-1}$, pod $node^{-1}$, and pod $plant^{-1}$ were measured in two sub-samples of 10 soybean plants collected from rows one and two in each plot at maturity. Yields were collected and data were analyzed as previously described in the Rate and Timing Study.

Results and Discussion

Rate and Timing Study

A main effect of dicamba timing was detected for soybean injury 7 DAT, canopy closure 14 and 28 DAT, dry weight, and yield (Table 1). Soybean injury 7 DAT was 3% greater following the V3 compared to R1 dicamba timing (Table 2). Furthermore, soybean injury with V3 and R1 dicamba timings was at least 8% greater than that for R5 timing. Canopy closure 14 and 28 DAT was reduced 8 to 21% following dicamba at V3 or R1 compared with R5.

An interaction of dicamba rate and timing was detected for soybean injury 14 and 28 DAT and height 14 DAT and at maturity (Table 1). Exposing soybean to dicamba at 4.4 g ha^{-1}

during V3 and R1 growth stages resulted in greater injury 14 DAT compared with dicamba at 1.1 g ha^{-1} applied at same growth stages (Table 3). Additionally, injury 14 DAT was greater with V3 than R1 for both rates of dicamba. Increasing dicamba rate from 1.1 to 4.4 g ha^{-1} increased soybean injury 28 DAT 12 and 21% following V3 and R1 applications, respectively. Injury 28 DAT with dicamba at R1 was 6% greater than that following V3 timing when rate was 4.4 g ha^{-1}. In contrast to injury 14 DAT, soybean injury 28 DAT was similar for V3 and R1 timings with dicamba at 1.1 g ha^{-1} (Table 3). Similarly, Griffin et al. (2013) exposed soybean to dicamba during R1 growth stage at 1.1 and 4.4 g ha^{-1} and observed 19 and 25% injury, respectively, at 14 DAT. Additionally, soybean injury following V3 dicamba exposure at 4.4 g ha^{-1} was 15% at 14 DAT (Griffin et al. 2013).

Soybean height 14 DAT was lower following dicamba at 4.4 compared to 1.1 g ha^{-1} with V3 and R1 timings (Table 3). Mature soybean height was least following dicamba at 4.4 g ha^{-1} applied at R1. A 16% reduction in mature soybean height was observed with R1 timing following dicamba at 4.4 compared to 1.1 g ha^{-1} with the R1 exposure. Similarly, Weidenhamer et al. (1989) reported ≥8% reduction in mature height following dicamba at 5 compared to 1.3 g ha^{-1} following R1 and R3 exposure timings. Current research indicates mature soybean height was greatest following R5 and least with R1 timings for dicamba at 4.4 g ha^{-1} (Table 3).

Soybean dry weight was only 73% of the nontreated following exposure to dicamba during the R1 growth stage (Table 2). Soybean exposure during R1 reduced dry weight 10% compared to V3 exposure. Soybean yield was reduced ≥18% following dicamba at V3 or R1 compared with R5. Soybean yield was 84% of the nontreated following exposure to dicamba during the R1 growth stage and was comparable to that from the V3 dicamba timing (Table 2). Similarly, Griffin et al. (2013) reported a >6% reduction in soybean yield following dicamba at

4.4 g ha^{-1} during R1 compared to V3 exposure timing. Additionally, exposing soybean to dicamba at 8.75 g ha^{-1} at R5 to R7 resulted in no injury, height, or yield reduction (Scholtes 2014). Furthermore, Auch and Arnold (1978) concluded dicamba at 11 g ha^{-1} at R5 soybean growth stage did not result in height and yield reductions. In the current study, no reduction in yield occurred from the R5 dicamba timing (Table 2). Pooled across exposure timings, dicamba at 4.4 g ha^{-1} reduced soybean yield 4% compared to dicamba at 1.1 g ha^{-1} (data not presented). A greater reduction in soybean yield occurred following dicamba at 5 compared to 1.3 g ha^{-1} across vegetative and reproductive exposure timings (Weidenhamer et al. 1989).

Multiple Exposures Study

Pooled across reproductive treatment, soybean injury 7 DA-V3 was 17% greater where soybean were exposed to dicamba at V3 compared to no vegetative treatment (data not presented). Soybean injury was also influenced by the interaction of vegetative and reproductive treatments (Table 4). At 7 DA-R1, injury was ≥43% with prior exposure to dicamba at V3 (Table 6). However, R3 and R5 treatments had not been applied at 7 DA-R1 evaluation. Soybean injury 7 DA-R3 was 11 to 37% greater for all reproductive treatments that had prior exposure at V3; however, treatments including an R5 timing had not been applied. Soybean injury 7 DA-R3 was similar and 40 to 45% with treatments that included R1 treatments but no vegetative treatment. Greatest injury 7 DA-R5 was 72% from dicamba at V3 fb R1 fb R3 fb R5. Soybean exposure to dicamba at V3 only translated into greater injury 14 and 21 DA-R5 following exposure during reproductive growth stages for R5 and R3 fb R5 reproductive treatments. Soybean injury 14 and 21 DA-R5 was actually less for V3 fb R1 compared with R1-only treatments. At 14 DA-R5, soybean injury was 8% greater following R1 only exposure compared to R1 with prior exposure at V3. Soybean injury 14 and 21 DA-R5 was ≥61% with dicamba at R1 fb R3 fb R5 regardless of prior exposure at V3. Soybean injury was ≤9% for R5 only treatment for all evaluations after R5 application.

Mature soybean heights were similar with and without vegetative treatments following R3, R1 fb R5, and R1 fb R3 fb R5 reproductive treatments (Table 7). Mature height was reduced ≥12 cm compared to plots not exposed to dicamba for all treatments except R5 only. Compared with plots not exposed to dicamba, soybean dry weights were reduced at least 767 g m^{-2} with R1 fb R3 fb R5 treatment regardless of prior exposure at V3. Soybean yield was affected more with prior exposure at V3 for all reproductive treatments except R1, R1 fb R3, R1 fb R5. Soybean yield and dry weight in plots only exposed to dicamba at R5 were not affected compared to plots with no dicamba exposure.

Soybean node $plant^{-1}$ was at least 7 nodes greater in plots with no dicamba exposure and those only exposed at R5 compared to all other treatments (Table 7). Soybean pod $node^{-1}$ following R3 with no prior exposure at V3 was 4 pods greater compared to R1 exposure. No difference in node $plant^{-1}$ was detected among reproductive treatments following V3 except for R5 and V3 fb R1 fb R3 fb R5. Pooled across vegetative treatments, reduction in pod $plant^{-1}$ was 8 to 15 pods among R3, R1 fb R3, R3 fb R5, and R1 fb R3 fb R5 treatments (Table 8). Plants treated in the reproductive growth stage produced few pods on the main stem above the point of treatment (Wax et al. 1969). In contrast, plants treated in the vegetative growth stage resulted in increased numbers of branches, pods, and seed per $plant^{-1}$ (Wax et al. 1969). However, following all treatments, seed and pods were developed after death of terminal (Wax et al. 1969). Pooled across reproductive treatments, vegetative treatment increased seed $plant^{-1}$ by 18 seeds compared to no vegetative treatment (data not presented). McCown et al. (2018) stated that following dicamba application at V3, the number of pod $plant^{-1}$ was similar to that of the nontreated control. In contrast, the number of pod plant^{-1} following R2 applicaton was highly influenced by dicamba. Futhermore, Anderson et al. (2004) reported soybean exposed to dicamba at vegetative growth stages resulted in increased lateral development and increased branching following death of terminal; however, pod and seed prodcution were not affected.

Observations from the current and previous research were that soybean plants exposed to dicamba during vegetative growth stages exhibited lateral development and branching, notably following death of the apical meristem (Anderson et al. 2004; McCown et al. 2018;Wax et al. 1969). Therefore, following death of apical meristem, soybean plants began producing branches similar to the mainstem from unifoliate and cotyledonary nodes. In contrast, following death of apical meristem from reproductive (R1) dicamba exposure, a similar meristem branch is not produced. Often, small branches produced many malformed or twisted pods, which did not fully develop (Anderson et al. 2004; McCown et al. 2018; Wax et al. 1969). Soybean was least sensitive to dicamba when exposure only occurred at R5 growth stage based on all parameters measured in the current research. Reproductive growth stage at time of exposure to dicamba was more indicative of soybean agronomic performance than prior exposure at V3. Soybean was unable to recover following single or multiple dicamba exposures up to the R3 growth stage. Growers should take extreme caution when applying dicamba in proximity to non dicamba-tolerant soybean regardless of soybean growth stage. Furthermore, growers should read and follow label instructions when applying dicamba. Future research should focus on evaluating the soybean terminal response following dicamba at multiple rates and timings.

Table 3.1 Significance of the main effects of dicamba rate and timing and interaction between the main effects for soybean injury at different evaluations, canopy closure 14 and 28 d after treatment (DAT), height 14 and 28 DAT, height at maturity, dry weight, and yield in the Rate and Timing Study at Stoneville, MS, in 2018.

	Injury			Canopy closure		Height			
Effects	7 DAT	14 DAT	28 DAT	14 DAT	28 DAT	14 DAT	Maturity	Dry weight	Yield
	p-value								
Rate	0.0664	<.0001	<.0001	0.3581	.8668	<.0001	0.0014	0.5126	0.0448
Timing	<.0001	<.0001	<.0001	<.0001	.0002	<.0001	<.0001	<.0001	<.0001
Rate*Timing	0.5165	0.0032	<.0001	0.7600	.4498	<.0001	.0078	0.3655	0.4972

Table 3.2 Influence of dicamba timing on soybean injury 7 d after treatment (DAT), canopy closure 14 and 28 DAT, dry weight, and yield in the Rate and Timing Study at Stoneville, MS, in 2018[a,b].

	Injury	Canopy closure			
Timing	7 DAT	14 DAT	28 DAT	Dry weight	Yield
	%	% of nontreated			
V3	16 a	77 b	90 b	83 b	88 b
R1	13 b	81 b	93 b	73 c	84 b
R5	5 c	98 a	101 a	96 a	106 a

[a]Data are pooled across two dicamba rates (1.1 and 4.4 g ae ha^{-1}) and three experiments. Means followed by the same letter for each parameter are not different at $p \leq 0.05$.
[b]Data for canopy closure, dry weight, and yield are expressed as a percentage of nontreated control.

Table 3.3 Influence of dicamba rate and timing on soybean injury 14 and 28 d after treatment (DAT), and height at 14 DAT and at maturity in the Rate and Timing Study Stoneville, MS, in 2018a,b.

		Injury		Height	
Dicamba rate	Timing	14 DAT	28 DAT	14 DAT	Maturity
g ae ha^{-1}		— % —		— % of nontreated —	
4.4	V3	49 a	55 b	59 d	85 c
	R1	42 b	61 a	67 c	68 d
	R5	5 d	5 d	100 a	100 ab
1.1	V3	43 b	43 c	78 b	93 b
	R1	33 c	40 c	72 b	84 c
	R5	5 d	5 d	98 a	102 ab

[a]Data are pooled across three experiments. Means followed by the same letter for each parameter are not different at $p \leq 0.05$.
[b]Data for height are expressed as a percentage of nontreated control.

Table 3.4 Significance of the main effects of vegetative and reproductive treatments and interaction between the main effects for soybean injury at different evaluations, mature height, dry weight, and yield in the Multiple Exposures Study at Stoneville and Starkville, MS, in 2018a.

Effects	Injury						Height	Dry weight	Yield
	7 DA-V3	7 DA-R1	7 DA-R3	7 DA-R5	14 DA-R5	21 DA-R5			
	p-value								
Vegetative	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.5812	<.0001	0.0003
Reproductive	0.2377	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Vegetative*Reproductive	0.2377	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

[a]Abbreviation: 7 DA-V3, 7 d after V3 exposure; 7 DA-R1, 7 d after R1 exposure; 7 DA-R3, 7 d after R3 exposure; 7 DA-R5, 7 d after R5 exposure; 14 DA-R5, 14 d after R5 exposure; 21 DA-R5, 21 d after R5 exposure; 28 DA-R5, 28 d after R5 exposure.

Table 3.5 Significance of the main effects of vegetative and reproductive exposure treatments and interaction between the main effects for soybean yield components in the Multiple Exposures Study at Stoneville and Starkville, MS, in 2018

Effects	Node plant^{-1}	Pod node^{-1}	Pod plant^{-1}	Seed plant^{-1}
Vegetative	<.0001	<.0001	0.4116	0.0291
Reproductive	<.0001	<.0001	0.0007	0.1957
Vegetative*Reproductive	<.0001	<.0001	0.3543	0.5149

Table 3.6 Interaction of vegetative and reproductive treatments for soybean injury at different evaluation intervals in the Multiple Exposures Study at Stoneville and Starkville, MS, in 2018[a,b,c].

Vegetative	Reproductive	7 DA-R1	7 DA-R3	7 DA-R5	14 DA-R5	21 DA-R5
		%				
None	None	0	0	0	0	0
	R1	12 d	40 ef	32 fg	30 ef	31 d
	R3	-	19 g	51 d	48 d	47 c
	R5	-	-	3 i	5 i	9 f
	R1 fb R3	15 d	45 cde	60 b	55 c	56 b
	R1 fb R5	15 d	41 ef	41 e	37 e	36 d
	R3 fb R5	-	20 g	50 d	48 d	47 c
	R1 fb R3 fb R5	13 d	43 def	60 b	65 ab	61 ab
V3	None	43 c	37 f	22 h	20 h	20 e
	R1	48 a	55 b	30 fg	22 gh	23 e
	R3	44 bc	52 bc	52 cd	50 cd	47 c
	R5	45 abc	40 ef	26 gh	28 fg	30 d
	R1 fb R3	47 ab	63 a	58 bc	57 c	55 b
	R1 fb R5	45 abc	52 bc	35 ef	37 e	37 d
	R3 fb R5	47 ab	50 bcd	55 bcd	58 bc	56 b
	R1 fb R3 fb R5	45 abc	69 a	72 a	66 a	67 a

[a] Data are pooled across three sites. Means followed by the same letter for each parameter are not different at $p \leq 0.05$.
[b]Abbreviation: 7 DA-R1, 7 d after R1 exposure; 7 DA-R3, 7 d after R3 exposure; 7 DA-R5, 7 d after R5 exposure; 14 DA-R5, 14 d after R5 exposure; 21 DA-R5, 21 d after R5 exposure, fb, followed by.
[c]Treatments not applied: R3, R5, and R3 fb R5 at 7 DA-R1 and R5 at 7 DA-R3.

Table 3.7 Interaction of vegetative and reproductive treatments for soybean mature height, dry weight, yield, node plant^{-1}, and pod node^{-1} in the Multiple Exposures Study at Stoneville and Starkville, MS, 2018[a,b].

Vegetative	Reproductive	Height	Dry weight	Yield	Node plant^{-1}	Pod node^{-1}
		cm	g m^{-2}	kg ha^{-1}	—— no. ——	
None	None	97 a	1,726 a	3,470 a	18 a	14 a
	R1	68 d	1,207 bcd	2,800 b	7 cde	3 cd
	R3	57 ef	1,221 bc	2,740 b	11 b	7 bc
	R5	96 a	1,651 a	3,380 a	18 a	14 a
	R1 fb R3	42 h	917 fg	1,970 ef	8 cd	4 cd
	R1 fb R5	63 de	1,150 cd	2,740 b	8 cd	3 d
	R3 fb R5	56 ef	1,160 cd	2,700 bc	11 b	8 b
	R1 fb R3 fb R5	42 h	959 efg	1,900 f	8 cd	4 cd
V3	None	85 b	1,332 b	2,630 bc	11 b	7 bc
	R1	76 c	1,070 cdef	2,480 bcd	6 de	3 d
	R3	59 ef	1,050 def	2,270 de	9 bc	5 cd
	R5	80 bc	1,139 cd	2,600 bcd	7 cde	4 cd
	R1 fb R3	54 fg	857 g	1,920 f	8 cd	6 cd
	R1 fb R5	67 d	1,100 cde	2,620 bc	6 de	3 d
	R3 fb R5	47 gh	1,070 cdef	2,060 ef	9 bc	5 cd
	R1 fb R3 fb R5	45 h	823 g	2,290 cdef	5 e	3 d

[a]Data are pooled across three sites. Means followed by the same letter for each parameter are not different at $p \leq 0.05$.
[b]Abbreviation: fb, followed by.

Table 3.8 Influence of reproductive treatment on soybean pod $plant^{-1}$ in the Multiple Exposures Study at Stoneville and Starkville, MS, 2018[a,b].

Reproductive treatment	Pod $plant^{-1}$
	no.
None	48 a
R1	42 abc
R3	35 cd
R5	47 ab
R1 fb R3	38 cd
R1 fb R5	42 abc
R3 fb R5	40 bcd
R1 fb R3 fb R5	33 d

[a]Data are pooled across two vegetative treatments and three sites. Means followed by the same letter are not different at $p \leq 0.05$.
[b]Abbreviation: fb, followed by.

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