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Evaluation of Safening Effects to Herbicides Conferred via Insecticide Seed Treatments in Soybean (Glycine max) and Grain Sorghum (Sorghum bicolor)

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Evaluation of Safening Effects to Herbicides Conferred via Insecticide Seed Treatments in Soybean (*Glycine max*) and Grain Sorghum (*Sorghum bicolor*)

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Abstract

Interactions between herbicides and insecticides in crop production have been documented for a number of years. Research has shown that applications of some organophosphate insecticides at planting can reduce cotton injury following applications of the soil-applied herbicide clomazone. Additionally, recent research has shown that, when applied as seed treatments prior to planting, some neonicotinoid insecticides can safen rice to drift from both glyphosate and imazethapyr. Since insecticide seed treatments are commonly used in many crop production systems throughout the Midsouth, exploring their ability to reduce injury from herbicides in other crops besides rice is of great interest. Presently no research exists examining the potential for insecticide seed treatments to reduce herbicide injury in soybean or grain sorghum, important rotational crops in Arkansas. Research contained herein investigates the possibility for commonly-used neonicotinoid insecticide seed treatments to reduce injury from herbicides via drift and soil application in both crops, in addition to applications of postemergence herbicides in soybean that typically cause injury. Results from these studies indicate that injury from herbicide drift may be reduced through the use of insecticide seed treatments in both crops. Injury from seven of the eight herbicides evaluated in soybean, and three of three herbicides in grain sorghum, was reduced in at least one of four site years. Additionally, safening to soil-applied herbicides was seen in five of nine herbicides evaluated in soybean in one or more site years. Injury from soil-applied herbicides in grain sorghum was not reduced in any of the four herbicides evaluated, nor was a safening effect seen in applications of postemergence herbicides in soybean. The amount of injury reduction varied substantially among site years, indicating a strong environmental effect on level of safening. However, based on the fact that insecticide seed treatments are incorporated across a wide array of environmental

conditions each spring, it seems likely that some growers will see the benefits of reduced injury following herbicide exposure.

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Chapter 1

General Introduction and Review of Relevant Literature

Soybean Production

On a global scale, soybean (*Glycine max* (L.) Merr) is one of the most important agricultural commodities. Soybean is an excellent source of both protein and oil and, as such, can be consumed directly by humans, fed to animals, or processed to make fuel. According to the USDA-WASDE (2017), soybean production in 2016 totaled 314 million metric tons globally, with the three largest producers being the United States (US), Brazil, and Argentina. In the US, soybean was planted on over 33 million hectares in 2016 and will account for around $35 billion of income for American growers (USDA-NASS 2016b). In Arkansas, soybean is grown on 1.3 million hectares and provides the state 1.7 billion dollars of income annually (USDA-NASS 2015c). Average national soybean yields are typically around 3,000 kg ha^{-1}, with Arkansas averaging slightly higher at 3,300 kg ha^{-1} in 2015. Planting soybean in Arkansas is typically recommended whenever soil temperatures reach 13 C for three consecutive days, resulting in initial planting in April for much of the state (Ross et al. 2015). Based on a number of factors such as planting date and latitude, growers across Arkansas choose from cultivars that range from mid-maturity group (MG) IV to early-MG V to take full advantage of growing conditions for the daylength-dependent crop (Purcell et al. 2014). Factors such as drainage and soil texture result in a variety of different planting practices for soybean, ranging from drill planting in 19 cm rows to bedded systems, where rows are typically 76 to 97 cm wide. In Arkansas, soybean damage from early season insect pests, such as three-cornered alfalfa hoppers, grape colaspis, and wireworms, can result in yield loss at the end of the season (Lorenz et al. 2014). A common method for controlling these pests is the use of neonicotinoid insecticide seed treatments, which is practiced on approximately 75 percent of soybean planted in Arkansas (G.M. Lorenz, personal communication).

Grain Sorghum Production

Grain sorghum (*Sorghum bicolor* L.) is an important cultivated crop, which accounted for 2.6 million planted hectares in the US in 2016 (USDA-NASS 2016a). Grain sorghum is used for animal feed, direct human consumption, and biofuel production (McGeeney 2015). Following trends based on global demand, grain sorghum area planted in Arkansas typically fluctuates annually. In 2015, over 200,000 hectares were planted to grain sorghum in the state, but in 2016 that number fell to about 68,000 hectares (USDA-NASS 2016c). In 2015, the average yield in Arkansas was 6,400 kg ha^{-1}, resulting in over $187 million of producer income (USDA-NASS 2016c). Grain sorghum prefers warmer soils and planting is usually not initiated until soil temperatures reach 18 C for three consecutive days. This usually occurs during mid-May to early June in Arkansas and once the conditions are met, sorghum is planted in 19- up to 102 cm-wide rows; albeit, wide rows are more typical (Espinosa and Kelley 2004). Protection against early-season insect pests is important for maintaining grain sorghum yields in Arkansas. Aphids, chinch bugs, cutworms, white grubs, and wireworms are all pests that attack seedling grain sorghum and often require management (McLeod and Greene 2004). Similarly to soybean production, control of early-season pests is often achieved through the use of neonicotinoid insecticide seed treatment prior to planting. In Arkansas, this results in approximately 60 percent of grain sorghum planted receiving an insecticide seed treatment each year (G.M. Lorenz, personal communication).

General Weed Control

While insect control, and water and nutrient management are important for maximizing crop yields, competition from weeds for these resources can cause significant reductions in productivity. Without management, Oerke (2006) estimated that weeds alone can reduce yields nearly 50% in certain crops. Weed control is primarily achieved via mechanical methods, such as tillage or cultivation, or by chemical control using herbicides (Oerke 2006). The use of herbicides plays a key role in weed control, with over 95% of US corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), and soybean acreage receiving at least one chemical application per year (Gianessi 2005). This widespread adoption of chemical weed control results in significant economic benefits for producers, as it provides the labor equivalence of approximately 70 million workers (Gianessi and Reigner 2007).

In the US, the widespread adoption of herbicide-resistant (HR) crops has had a profound impact on weed control. In 2016, approximately 94% of all soybean hectares in the US were planted to crops having a HR trait (USDA-ERS 2016). The two most common HR traits are RoundupReady® (Monsanto Company, St. Louis, MO) and LibertyLink® (Bayer Crop Science, Research Triangle Park, NC), which provides a crop resistance to the herbicides glyphosate and glufosinate, respectively. These technologies allowed for applications of broad-spectrum, postemergence herbicides with no damage to the crop, and since their introduction, have improved farmer profits by over $28 billion (Brookes and Barfoot 2012).

Weed Control in Soybean

Since soybean is a dicot, it follows that some of the most problematic and difficult-to-control weeds in soybean cropping systems are also dicots. A survey of weeds in the US and

Canada showed dicots make up more than 90% of the list of most troublesome weeds in soybean production (Van Wychen 2016). Some of the most frequently-appearing weeds in the same survey were amaranths (*Amaranthus* spp.) and morningglories (*Ipomoea* spp.). Amaranths, or ‘pigweeds’, such as redroot pigweed (*Amaranthus retroflexus* L.), waterhemp (*Amaranthus rudis* L.), and Palmer amaranth (*Amaranthus palmeri* L. Wats.) are especially problematic in soybean, with Palmer amaranth being the most competitive among the three. Bensch et al. (2003) reported that at a density of 8 plants m^{-1} row of soybean, Palmer amaranth reduced yield by as much as 78%. The same study also demonstrated that yield loss can be lessened but not eliminated if weed control is effective during the early part of the growing season, highlighting the importance of season-long weed management.

In order to achieve season-long control, a number of herbicide programs exist. Herbicidal recommendations usually include a burndown application to remove weeds before planting, a herbicide with residual activity applied very close to when the crop is planted, and a selective postemergence herbicide to remove any plants that survive previous applications and tillage (Scott et al. 2017). Commonly included in these recommendations for soybean production is an application of glyphosate or glufosinate. As previously mentioned, crops with traits that express resistance to these chemicals (Roundup Ready® and LibertyLink®) are popular and they allow producers to kill weeds in a given field without damaging the crop. However, one issue that accompanies the use of these herbicides is the evolution of herbicide-resistant weeds. Contrary to information presented by Bradshaw et al. (1997), which suggested that a glyphosate-only control program in Roundup Ready® crops would not lead to resistant weed populations, resistance to the herbicide occurred and rapidly became widespread within the first ten years of its introduction. Current reports list 37 species worldwide that are resistant to glyphosate alone, and nearly another 150 resistant weed species span 23 of the 26 known sites of action (SOAs), including many that are resistant to more than one of these sites (Heap 2017).

The widespread evolution of resistant weeds presents obvious challenges for growers. As the frequency of resistant weeds increases, the need for more diverse forms of control becomes exceedingly more important. Norsworthy et al. (2012) concluded that best management practices (BMPs) that mitigate the evolution of herbicide-resistant weeds must be widely implemented to ensure the continued effectiveness of herbicides. Among these recommended BMPs was the use of multiple effective herbicide sites of action in conjunction with non-chemical weed control methods. Powles (2008) also puts forth many of the practices promoted by Norsworthy et al. (2012), including diversification of weed management programs and a focus on reducing the soil seedbank.

As herbicide programs become more diverse and incorporate multiple application timings and SOAs, the risk of crop damage to adjacent crops from misapplication, drift events, or via carryover inherently increases. For example, this increased risk can be seen in non-transgenic crops such as conventional soybean. These varieties have no tolerance to non-selective herbicides, like glyphosate and glufosinate; thus, they are susceptible to drift from nearby fields that utilize these herbicides. Additionally, attempts to deter herbicide resistance have resulted in movement toward using more soil-applied herbicides with residual activity. Injury from soil-applied herbicides essentially comes from two sources: carryover from herbicide applications made in a previous crop/burndown program and injury to emerging seedlings from preemergence herbicides.

Weed Control in Grain Sorghum

Although grain sorghum is a member of the grass (Poaceae) family, both grass and broadleaf weeds pose significant yield-reducing threats to the crop. Grain sorghum is affected by a wide range of weeds due in large part to a lack of labeled herbicides available compared to corn and soybean (Scott et al. 2017). Some particularly troublesome weeds in grain sorghum include Palmer amaranth, johnsongrass (*Sorghum halepense* L.), and morningglories (Espinosa and Kelley 2004). Of particular interest among these weeds is johnsongrass. Due to its high level of genetic similarity to grain sorghum, there is a relatively high potential for crop/weed hybridization. It has been observed that viable sorghum/johnsongrass hybrids could be found at distances up to 100 m, depending on wind and other weather conditions (Schmidt 2013). For this reason, among others, no transgenic HR varieties of grain sorghum currently exist.

The lack of HR traits in grain sorghum creates a number of challenges for growers and eliminates the use of glyphosate or glufosinate as viable weed control options other than a pre-plant burndown application. While a number of effective chemical control options still exist, the lack of broad-spectrum, postemergence options, such as glyphosate and glufosinate, makes early season weed control more important in grain sorghum than in soybean and corn. Early season weed control is typically achieved with a preemergence (PRE) application of atrazine in combination with a chloroacetamide, such as *S*-metolachlor (Dual II Magnum® Syngenta Crop Protection, Greensboro, NC) (Smith and Scott 2004). Unfortunately, the use of chloroacetamides in grain sorghum can result in subsequent crop injury. However, through the use of a safener that is added in combination with the herbicide, this injury can be reduced to acceptable levels in order to avoid yield loss as a result of crop injury (Spotanski and Burnside 1973; Nielsen 2008). With the necessity for reducing crop injury to grain sorghum, a common practice is to use a safener applied as a seed treatment. Two of the most common safeners added to seed to aid against chloroacetamide damage in grain sorghum are Screen® and Concep® (Monsanto Company, St. Louis, MO and Syngenta Crop Protection, Greensboro, NC, respectively). Since seed treatment safeners are already commonly used in grain sorghum production, examining the potential for insecticide seed treatments to provide increased crop tolerance to herbicides could provide substantial benefits for growers. If their use reduces crop injury from herbicides, commercially available insecticide seed treatments would further benefit producers. Improved herbicide tolerance, decreased insect pressure, and suppression of soil-borne fungi can improve crop stands, ultimately resulting in increased yield at the end of the growing season (Sharma et al. 2015). With these benefits, particularly in improved tolerance to herbicide applications, additional herbicides that are not currently labeled for use in grain sorghum production could potentially be applied.

Crop Risks Associated with Chemical Weed Control

While the use of herbicides in agriculture has proven to be a vital resource for producers, a major drawback is the potential for crop injury associated with the application of chemicals. Crop injury due to herbicide application can take on a number of forms, including stunting, chlorosis, epinasty, leaf cupping, root malformation, and necrosis. These symptoms come from a variety of herbicide classes and are typically representative of specific SOAs (Boerboom 2005). Some common reasons for crop injury due to herbicides include sprayer misapplication, chemical persistence in the soil, off-target drift to sensitive crops, and varietal sensitivity to some herbicides. With an increased prevalence of weeds that are resistant to postemergence herbicides, soil-applied herbicides will become increasingly popular among growers (Hager et al. 2011).

With an increase in soil-applied herbicide treatments, reducing crop injury associated with these applications appears to be of great importance to growers. Additionally, with the introduction of crops with resistance to drift-prone herbicides such as 2,4-D and dicamba, it can reasonably be assumed that an increase in prevalence of drift-related crop injury will be seen in the near future.

Although potential exists for herbicide-related crop injury, a number of methods exist for mitigating these risks. One such method includes labeling HR traits in the field through use of flags (Scott et al. 2015), which helps to reduce risk of misapplication and bring awareness to potential off-target herbicide movement. When applying drift-prone herbicides, following best management practices such as those appearing on the Enlist Duo label (Anonymous 2016) can greatly reduce damage to susceptible crops and off-target plants. In addition, observing and understanding plant-back restrictions such as those presented by Barber et al. (2015) is critical for reducing injury caused by soil-applied herbicides. Aside from the above listed methods, the use of safeners has proven to be highly effective in some situations for improving crop tolerance to herbicides.

Plant Metabolism of Herbicides

The success and widespread adoption of herbicides is mainly due to their selective nature, which destroys susceptible plants and leaves crops intact. Current research suggests that herbicidal selectivity is due in large part to a lack of the specific enzymes needed for metabolism, such as cytochrome P450 monooxygenases (P450s) and glutathione *S*-transferase enzymes (GSTs) (Riechers et al. 2010). Additionally, selectivity is dependent upon two critical components: plant uptake and plant metabolism, and is affected by variations in physiology from plant to plant (Cobb and Reade 2010). Cobb and Reade (2010) describe three generalized phases of chemical metabolism once a herbicide has penetrated the waxy leaf surface of plants: metabolic attack, conjugation, and sequestration. In metabolic attack, plant enzymes known as P450s help to hydroxylate the chemically active parts of herbicidal compounds (eg. aromatic rings and alkyl groups). This P450-mediated hydroxylation is an important mechanism of plant metabolism of a number of herbicide families such as sulfonylureas, chloroacetamides, and imidazolinones (Cobb and Reade 2010). As such, the importance of this class of enzymes cannot be understated.

Conjugation describes the joining of the hydroxylated product from metabolic attack to other cell metabolites such as amino acids and sugars. This step forms a conjugate with higher solubility and less phytotoxicity compared to the original herbicidal molecule. A well-understood conjugation reaction is the pairing of herbicidal compounds to the antioxidant glutathione via GSTs. This pairing helps plants transition to phase three of metabolism, sequestration, as the pairing with glutathione directs the herbicidal compound towards the plant vacuole for storage. Once in the vacuole, the compound can be stored for long periods of time and presents a much smaller phytotoxic risk to the plant.

Herbicide Safeners

Chemicals that can amplify a crop's ability to metabolize herbicides without compromising efficacy in target weeds are referred to as “antidotes” or “safeners”, and are of great interest from a commercial standpoint. While the mechanisms responsible for reducing crop injury have proven to be very complex on a cellular level, two of the best-understood reasons for the success of crop safeners are their structural similarity to herbicides and their ability to increase plant metabolism of the chemicals (Davies and Caseley 1999). Safeners with structural similarity to herbicidal compounds can aid in the prevention of crop damage by competitively binding to target enzymes, preventing the herbicide from binding to its active site. Alternatively, safener molecules that are structurally dissimilar to the chemicals they protect against help to improve the three phases of metabolism described by Cobb and Reade (2010). Some safeners have been shown to cause an increased production of P450s and other enzymes associated with Phase 1 of metabolism, further increasing the crop's ability to detoxify the herbicide and reduce overall injury. In Phase 2 of metabolism, some safener molecules act by inducing production of enzymes that promote herbicide conjugation to glutathione, glucose, and other endogenous molecules (Davies and Caseley 1999). The result from improving Phase 2 reactions is an overall loss of phytotoxicity of the herbicide, as the conjugated molecule does not bind to the original active site of the chemical.

Herbicide safening activity is most commonly seen in monocot crops such as grain sorghum, corn, rice, and winter cereals; however, a few examples exist in dicots such as cotton (Jablonkai 2013). In the grass crops, commercial safeners effectively protect against applications of chloroacetamides, thiocarbamates, sulfonylureas, imidazolinones, and aryloxyphenoxypropionate herbicides. These safeners can be applied to seeds of the crops or are sprayed in conjunction with the herbicidal compound and act as mediators that only allow sub-lethal amounts of herbicide to reach the target site (Hatzios and Wu 1996). A widely studied safening effect in dicots can be seen with the use of clomazone and some organophosphate insecticides in cotton. Clomazone is a soil-applied, preemergence herbicide that is labeled for use in a number of crops, including cotton; however, in order to ensure adequate crop tolerance in cotton, an insecticidal compound must be applied with the herbicide to reduce injury (Ferhatoglu et al. 2005). Riechers et al. (2010) reinforced that safening activity is not as prevalent in dicots, and DeRidder and Goldsbrough (2006) suggest that this may be due to the organ-specific nature of production of metabolic enzymes. As mentioned by Nielsen (2008), the use of safeners in grain sorghum production is vital for reducing crop injury to commercially acceptable levels when using chloroacetamide herbicides. If an effective safening method could be consistently displayed in dicots such as soybean, similar utility could be expected and could potentially result in more herbicides labeled for use in soybean production.

Insecticides as Potential Safeners

Because of broad-spectrum insect pest control, the neonicotinoid class of insecticide seed treatments has rapidly expanded since introduction in 1991 (Elbert et al. 2008). Neonicotinoids are highly effective, even at low use rates, and are translocated systemically throughout the plant (Elbert et al. 2008). As such, neonicotinoids are often applied as seed treatments to protect plants from early-season pests. The use of insecticide seed treatments has increased significantly since the early-2000s, and in 2011, nearly 80% of all US corn acreage and over a third of the soybean acreage utilized insecticide seed treatments (LaJeunesse 2015). While use of these seed treatments has improved yield due to insect control, Miller et al. (2016) discovered an additional benefit in rice production. Field trials in 2013 and 2014 confirmed that conventional rice varieties having a CruiserMaxx® insecticide/fungicide seed treatment had both lower visible crop injury and higher yield compared to non-treated seeds following applications of drift rates of both glyphosate and imazethapyr. Although the mechanisms behind this particular safening effect are not yet understood, similar instances where applications of insecticides altered crop tolerance to herbicide application have been reported (Clarkson 2014; Kreuz and Fonne-Pfister 1992; York et al. 1991).

Interactions between herbicides and insecticides can cause unexpected outcomes when the two are used in combination with one another. For instance, it has been noted that tolerance to primsulfuron (a sulfonylurea herbicide) was reduced in corn when the plants were sprayed with malathion (an organophosphate insecticide) (Kreuz and Fonne-Pfister 1992). Conversely, in cotton, York et al. (1991) found that when disulfoton and phorate (both organophosphate insecticides) were applied in-furrow at planting, in combination with clomazone, injury and death to cotton seedlings was greatly reduced compared to applications of clomazone alone. Culpepper et al. (2001) sought to explain this herbicide/insecticide interaction in cotton and ultimately found that the insecticide reduced overall metabolism of clomazone, meaning a product of clomazone metabolism is likely the cause of crop injury in cotton. Further understanding of these interactions and how specific herbicides cause phytotoxicity, coupled with greater knowledge of plant metabolism of insecticides, may improve the utility of insecticides for use as safeners.

As seed treatments continue to remain popular in both grain sorghum and soybean production systems, providing the extra utility of acting as a safener could be a significant additional benefit to growers, particularly as drift-prone herbicides such as dicamba and 2,4-D become increasingly popular. From an industry standpoint, seed treatments are already labeled for use in a wide variety of crops; for this reason, little additional product labeling would be needed to further increase adoption of the products as safeners. It can be reasoned that this combination, if proven to be effective, would provide an added economic benefit for producers and chemical companies alike. Thus, field trials were conducted as part of a two-year study to evaluate the potential safening effect to herbicides of insecticide seed treatments in soybean and grain sorghum.

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Chapter 2

Use of Insecticide Seed Treatments as Safeners to Drift Rates of Herbicides in Soybean and Grain Sorghum

Abstract

Previous research has shown that some insecticide seed treatments provide safening effects in rice following exposure to drift rates of the herbicides glyphosate and imazethapyr. However, no such research has been conducted to determine whether a similar effect may be seen in soybean or grain sorghum, two important rotational crops in Arkansas, and across the Midsouth. In order to evaluate a similar result in these two crops, field trials were conducted in Marianna, Arkansas in 2015 and 2016, and in Keiser, Arkansas and near Colt, Arkansas in 2016. In soybean, glyphosate, glufosinate, 2,4-D, dicamba, halosulfuron, mesotrione, tembotrione, and propanil were applied at low rates to simulate drift events, in combination with the insecticide seed treatments thiamethoxam and clothianidin, at labeled rates. In grain sorghum, glyphosate, imazethapyr, and quizalofop were applied at low rates in combination with the insecticide seed treatments thiamethoxam, clothianidin, and imidacloprid, at labeled rates. In soybean, a safening effect was seen at one or more site years for all herbicides evaluated, except for propanil. Injury reduction was seen at one site year for 2,4-D, dicamba, mesotrione, and tembotrione, at two site years for both glyphosate and glufosinate, and at three of four site years for halosulfuron. At one site year, the successful safening in halosulfuron resulted in increases in both crop height and yield in plots with seed treatments. In some instances a main effect of seed treatment was observed, in which case the inclusion of an insecticide seed treatment reduced overall soybean injury across all herbicides evaluated. In grain sorghum, reducing injury via seed treatments was generally more successful. All three herbicides applied in sorghum displayed instances of injury reduction at one or more site years, including reducing injury upwards of 40% in the case of quizalofop + clothianidin at Marianna in 2016. For two site years, substantial injury reduction through the use of insecticides resulted in increases in crop height, as well as yield in sorghum compared to when no insecticide was used. Although degree of safening seen varied depending on site year in both crops, growers who use insecticide seed treatments on an annual basis may expect to see a safening effect from drift events of most herbicides evaluated in both soybean and grain sorghum.

Nomenclature: 2,4-D; clothianidin; dicamba; glyphosate; glufosinate; halosulfuron; imazethapyr; imidacloprid; mesotrione; propanil; quizalofop; tembotrione; thiamethoxam; soybean, *Glycine max* (L.) Merr; grain sorghum, *Sorghum bicolor* L.

Key words: Herbicide drift, herbicide safeners

Introduction

Herbicide-resistant weeds pose a significant threat to crop production in Arkansas and throughout the United States. Palmer amaranth (*Amaranthus palmeri* S. Wats.) and barnyardgrass (*Echinochloa crus-galli* L. Beauv.) are among the most troublesome weeds encountered in agricultural production in the midsouthern United States (Webster 2013). These two weeds are particularly difficult to control due to the existence of biotypes that are resistant to multiple herbicide sites of action (SOAs), including 5-enolpyruvylshikimate-3-phostphate (EPSPS)- and acetolactate synthase (ALS)-inhibitors (Heap 2017). In order to combat these herbicide-resistant weeds, diversifying management strategies to include multiple effective SOAs is recommended (Norsworthy et al. 2012). As part of this diversification, adoption of crops with resistance to a number of herbicides, including glufosinate, 2,4-D, dicamba, isoxafluotole, and mesotrione is expected to increase in the near future (Riar et al. 2013). With the expanding diversity of herbicides used, protecting sensitive crop species from off-target herbicide movement will become increasingly important. In Arkansas, both soybean and grain sorghum are important rotational crops and are often grown in close proximity to rice, cotton (*Gossypium hirsutum* L.), and crops with herbicide-resistance traits. As a result, the potential exists for both crops to be exposed to off-target applications of herbicide via both physical and vapor drift. For example, in 2016, over 120,000 ha of soybean across the Midsouth were damaged via dicamba drift (J.K. Norsworthy, personal communication).

Responses of both soybean and grain sorghum to herbicide drift events have been well documented, and vary greatly depending upon herbicide and rate. In order to study crop response to drift, applications ranging from 1/10 to 1/100x of labeled rates are often made (Al Katib et al. 2003; Roider et al. 2007; Webster et al. 2016). According to Wolf et al. (1993), applications within these ranges are consistent with in-crop exposure to a drift event, allowing for accurate estimations of crop response. Previous research has shown that grain sorghum exposure to 1/10x labeled rates of imazethapyr, glyphosate, and glufosinate can cause 20, 78, and 77% crop injury, respectively (Al Khatib et al. 2003). Additionally, Ellis and Griffin (2002) showed that similar drift rates of glyphosate and glufosinate resulted in 29 and 40% crop injury in soybean. Injury response can differ greatly depending upon type of herbicide and can manifest itself in a number of ways including stunting, chlorosis, and necrosis, among others. These symptoms are sometimes transient in nature, but can greatly impact yields if injury is severe. As demonstrated by Al-Khatib and Peterson (1999), soybean are capable of recovering from V2 to V3 applications of drift rates as high as 1/3x of labeled rates of both glyphosate and glufosinate by 30 days after application (DAA), but similar rates of dicamba, prosulfuron, rimsulfuron, and thifensulfuron caused prolonged injury, resulting in yield loss. In addition to type of herbicide and drift rate received, growth stage of crop during drift exposure can result in variations in yield response. Auch and Arnold (1978) showed that exposure of soybean at vegetative growth stages to dicamba at 5.6 g ae ha^{-1} caused no reduction in yield, but applications of the same rate to reproductive growth stages resulted in yield loss. Due to the damage associated with drift events, methods for reducing the risk of crop damage could provide great benefits for growers in situations where drift is a concern.

One area of interest that could significantly reduce the risk of off-target herbicide injury is the use of in-crop safeners. Safeners were discovered in the late 1940’s and allow for reduced crop injury from herbicide applications, without sacrificing control of target weeds (Davies and Caseley 1999). The use of safening compounds has proven to be effective in a number of monocotyledonous crops such as corn (*Zea mays* L.), rice (*Oryza sativa* L.), and sorghum

(Riechers et al. 2010). Safeners are commonly used in grain sorghum production and can effectively reduce injury from applications of both preemergence (PRE) and postemergence (POST) herbicides (Spotanski and Burnside 1973; Barrett 1989). In contrast, the lack of success of herbicide safeners in dicot crops, such as soybean, has been noted on numerous occasions (Hatzios 1989; Riechers et al. 2010). Continued research to expand the use of safeners may help broaden the number of herbicides available across crops, providing a valuable tool to help fight herbicide resistance and reduce economic losses associated with weed competition.

Recent research by Miller et al. (2016) showed evidence of a novel method of herbicide safening. Rice injury following applications of drift rates of both glyphosate and imazethapyr were reduced through the use of the neonicotinoid insecticide seed treatment thiamethoxam. Neonicotinoids are the most common class of insecticides used globally, and a vast majority of applications comes in the form of crop seed treatments (Douglas and Tooker 2015). Neonicotinoid seed treatments are most commonly used in corn, soybean, and cotton, but are also used in rice, wheat (*Triticum aestivum* L.), and other cereals to a lesser extent (Douglas and Tooker 2015). The positive impacts associated with these insecticide seed treatments, including improved early-season stand and protection against a wide range of insect pests, can often provide growers with economic benefits when compared to planting non-treated seed (North et al. 2016). Thanks in part to the agronomic and economic benefits of seed treatments, adoption in the state of Arkansas has also increased in recent years, with approximately 60 and 75% of grain sorghum and soybean, respectively, receiving insecticide seed treatments (G.M. Lorenz, personal communication). With the widespread popularity of insecticide seed treatments, a large number of growers stand to benefit from potential safening effects associated with neonicotinoids. Although research has shown the potential for insecticides to reduce herbicide injury in both cotton (York et al. 1991) and rice, research on safening effects conferred via insecticide seed treatments in soybean and grain sorghum are lacking. Thus, the objectives of this research were to determine (1) whether thiamethoxam or clothianidin seed treatments safen young soybean plants to low rates of dicamba, 2,4-D, glyphosate, glufosinate, halosulfuron, mesotrione, tembotrione, or propanil, and (2) whether thiamethoxam, clothianidin, or imidacloprid seed treatments safen young grain sorghum plants to low rates of glyphosate, imazethapyr, or quizalofop.

Materials and Methods

Soybean Field Study. A field study was conducted in 2015 at the Lon Mann Cotton Research Station (LMCRS) in Marianna, AR, to determine the feasibility of using insecticide seed treatments as herbicide safeners in soybean. Following the 2015 field trial, research was repeated in 2016 at the LMCRS, in addition to the Pine Tree Research Station (PTRS) near Colt, AR, and at the Northeast Research and Extension Center (NEREC) in Keiser, AR. According the USDA Web Soil Survey website (Anonymous 2016), the soil series at each location were: Convent silt loam (fine-silty, mixed, active thermic Typic Glossaqualf) at LMCRS, Calhoun silt loam (Coarse-silty, mixed, superactive, nonacid, thermic Fluvaquentic Endoaquepts) at PTRS, and Sharkey silty clay (very-fine, smectitic, thermic Chromic Epiaquerts) at NEREC. At each location, UA-5213C, a non-STS, non-herbicide-resistant soybean variety from the University of Arkansas, was planted at a seeding rate of 340,000 seeds ha^{-1} to a 2.5- to 3-cm depth. All plots consisted of four rows, 7.2 m in length. Row spacing was 96 cm at both LMCRS and NEREC, and 76 cm at PTRS. Experiments were established as randomized complete block factorials with four replications and two factors: insecticide seed treatment and herbicide applied. Plots were managed using agronomic recommendations provided in the University of Arkansas Soybean Production Handbook (Purcell et al. 2014).

Prior to planting, seeds received a seed treatment with either no insecticide, thiamethoxam (Cruiser 5FS, Syngenta Crop Protection, LLC, Greensboro, NC), or clothianidin (NipsIt Inside, Valent U.S.A. Corporation, Walnut Creek, CA), applied via a water-based slurry. Labeled use rates for soybean (0.5 g ai kg^{-1} seed) were used for both insecticides. Because insecticide seed treatments are rarely used without a co-application of fungicides, all treatments included the fungicide combination of mefenoxam, fludioxonil, and sedaxane (CruiserMaxx Vibrance, Syngenta Crop Protection, LLC, Greensboro, NC) to protect against early-season diseases. Labeled use rates of mefenoxam at 0.075 g kg^{-1} seed, fludioxonil at 0.025 g kg^{-1} seed, and sedaxane at 0.025 g kg^{-1} seed were applied using the same procedure as treating seeds with insecticide.

Herbicide drift events were simulated by applying low rates of eight herbicides, none of which are labeled in conventional soybean. Dicamba (9 g ae ha^{-1}), 2,4-D ester (84 g ae ha^{-1}), glyphosate (126 g ae ha^{-1}), glufosinate (61 g ai ha^{-1}), halosulfuron (4 g ai ha^{-1}), mesotrione (11 g ai ha^{-1}), tembotrione (9 g ai ha^{-1}), and propanil (560 g ai ha^{-1}) were applied using a CO_2-pressurized backpack sprayer calibrated to deliver a continuous carrier volume of 143 L ha^{-1} at 276 kPa. Applications were made 3 weeks after planting (WAP), corresponding to V2 or V3 soybean at all locations (Table 1). In order to maintain weed-free plots, a PRE application of flumioxazin at 71 g ai ha^{-1} (Valor SX, Valent U.S.A. Corporation, Walnut Creek, CA) was made, and late season escapes were controlled via hand removal.

Visual crop injury ratings were taken weekly following application. Injury ratings were on a scale of 0 to 100%, where 0% equals no injury and 100% equals plant death. In 2015, crop height (cm) was taken prior to harvest by measuring the average of five representative plants from each four-row plot. In 2016, in an attempt to see variations in crop height closer to herbicide application, height measurements were taken two to three weeks after application (WAA). Soybean yield data were collected by machine harvesting the two center rows of each plot and adjusting grain moisture to 13%.

Data collected were subjected to two-way analysis of variance (ANOVA) using JMP (JMP Pro 12, SAS Institute Inc., Cary, NC), with significant means separated using Fisher's protected LSD ($\alpha = 0.05$). Site-years were analyzed separately due to considerable variation in environmental conditions at each location (Tables 2-5). For responses that did not produce a significant herbicide by insecticide seed treatment interaction, seed treatment main effects were evaluated. At evaluation timings where no measurable injury was observed for one or more herbicide treatments, the assumptions for ANOVA were not met. When either no interaction was identified, or the response did not meet the assumptions for ANOVA, t-tests were conducted to compare treatments with no insecticide to each insecticide seed treatment within a herbicide.

Grain Sorghum Study. Similar to the soybean study, an experiment was conducted at the LMCRS in 2015 followed by additional studies at LMCRS, PTRS, and NEREC in 2016. DeKalb DK-54-00 grain sorghum (Monsanto Company, St. Louis, MO) was planted at a density of 222,000 seeds ha^{-1} at a 2.5-cm depth at all locations. All plots measured four rows by 7.2 m in length. Row spacing at both LMCRS and NEREC were 96 cm and row spacing at PTRS was 76 cm. Similarly to soybean trials, University of Arkansas agronomic recommendations for grain sorghum production were followed to maintain all plots (Espinosa and Kelley 2004).

Three insecticide seed treatments plus a nontreated check were included as part of a two factor factorial (insecticide seed treatment x herbicide). Thiamethoxam, clothianidin, and imidacloprid (Gaucho, Bayer CropScience, Research Triangle Park, NC) were applied via water-based slurry prior to planting at 2, 2, and 2.5 g ai kg^{-1} seed, respectively. Similarly to soybean trials, all treatments contained fungicides commonly co-applied with insecticides. Combinations of the fungicides mefenoxam (Apron XL, Syngenta Crop Protection, LLC, Greensboro, NC), azostrobin (Dyanasty, Syngenta Crop Protection, LLC, Greensboro, NC), and fludioxonil (Maxim 4FS, Syngenta Crop Protection, LLC, Greensboro, NC) were applied at 0.075, 0.02, and 0.05 g ai kg^{-1} seed, respectively.

Herbicides were applied identical to soybean trials using a backpack sprayer. Glyphosate (157 g ae ha^{-1}), imazethapyr (17.5 g ai ha^{-1}), and quizalofop (77 g ai ha^{-1}) were applied at sublethal rates 3 WAP, when sorghum plants were at three- to four-leaf growth stage. These rates are likely higher than what would typically occur in a drift event, but were chosen to more adequately determine whether safening would occur when using the insecticide seed treatments. A broadcast application of *S*-metolachlor plus atrazine, at 1.06 and 1.12 kg ai ha^{-1}, respectively, was made at planting to maintain weed-free conditions and late-season weed escapes were removed by hand as needed.

Data collection timings and analysis were the same as for the soybean experiment, with visual injury, crop height, and yield collected and subjected to ANOVA using JMP. For responses that did not produce a significant herbicide by insecticide seed treatment interaction, seed treatment main effects were evaluated. At evaluation timings where no measurable injury was observed for one or more herbicide treatments, the assumptions for ANOVA were not met. When either no interaction was identified or the response did not meet the assumptions for

ANOVA, t-tests were conducted to compare treatments with no insecticide to each insecticide seed treatment within an herbicide.

Results and Discussion

Soybean Study. Significant injury reduction through the use of insecticide seed treatments was seen in at least one site year for all herbicides evaluated, with the exception of propanil (Tables 6-9). Injury reduction from halosulfuron drift was the most successful, with safening effects seen at three of four site years evaluated, indicated by significant (α=0.05) seed treatment by herbicide interactions. At LMCRS (2015), injury from halosulfuron was reduced at all evaluation timings by both insecticides (Table 6). Maximum halosulfuron injury reduction was seen at 4 WAA, where injury was reduced from 43% to 13% and 3% using thiamethoxam and clothianidin, respectively, with similar levels of safening seen at both 1 and 2 WAA (Table 6). The safening seen at LMCRS in 2015 also caused a resultant increase in soybean height in both seed treatments, and increased grain yield from 3000 kg ha^{-1} in the nontreated plot to 3400 kg ha^{-1} in the clothianidin treatment. At LMCRS (2016), injury from halosulfuron was reduced from 16% with no insecticide seed treatment to 6% in both clothianidin and thiamethoxam treatments, with the thiamethoxam treatment also resulting in increased crop height of 7 cm and a 640 kg ha^{-1} increase in yield, compared to the nontreated (Table 7). Additional halosulfuron safening was seen at NEREC 1 WAA, where a thiamethoxam seed treatment reduced injury form 23% to 13%, but did not cause an increase in crop height or yield (Table 8).

Injury from both glyphosate and glufosinate was reduced via insecticide seed treatments at two of four site years evaluated. Following exposure to glyphosate, a significant herbicide by insecticide interaction was seen at NEREC, where clothianidin treatments reduced injury from

34% to 18% 1 WAA and from 30% to 23% 2 WAA (Table 8). At LMCRS (2016), no significant two-way interaction was seen. However, when comparing treatments with and without seed treatments via individual t-tests within herbicides, injury was reduced by using thiamethoxam at 2 and 4 WAA and by using clothianidin 4 WAA following an application of glyphosate (Table 7). Following a low rate of glufosinate, no significant two-way interaction was seen; however, when subjected to individual t-tests, injury was reduced at LMCRS (2016) at 2 and 4 WAA and at PTRS 1 WAA. At LMCRS (2016), injury 2 WAA was reduced from 13% to 7% using thiamethoxam, and injury 4 WAA was reduced from 12% to 4% and 6%, using thiamethoxam and clothianidin, respectively (Table 7). At PTRS, injury 1 WAA was reduced from 15% to 6% using thiamethoxam. For both glyphosate and glufosinate, height and yield were not improved as a result of the safening effects seen (Table 9).

In addition to herbicides that were safened at multiple locations, 2,4-D, dicamba, mesotrione, and tembotrione all saw significant injury reductions at one of the site years evaluated. With 2,4-D, injury 1 WAA at LMCRS (2015) was reduced from 23% to 9% when seed received a thiamethoxam treatment (Table 6). Following dicamba exposure, a significant reduction in injury both 2 and 4 WAA at LMCRS (2016) occurred. At 2 WAA, injury was reduced from 20% to 13% using thiamethoxam, and 4 WAA injury was reduced from 20% to 12% with the same seed treatment. In mesotrione treatments at PTRS, reduction in injury was seen at 1 and 2 WAA. Injury from mesotrione was reduced from 34% to 28% using both thiamethoxam and clothianidin at 1 WAA, and at 2 WAA, injury was reduced from 49% to 34% via a thiamethoxam seed treatment (Table 9). For tembotrione, injury 4 WAA at PTRS was reduced 8 percentage points by the thiamethoxam seed treatment (Table 9).

Overall, this research indicates that safening soybean to herbicide drift may be possible through the use of both thiamethoxam and clothianidin seed treatments. Although, with the exception of halosulfuron, degrees of safening seen were not comparable to commercially-available safeners in other crops, the possibility of successfully safening crop injury in soybean is a novel concept and would likely aid speed of recovery following a drift event. Likewise, any reduction in injury would also aid the ability of soybean to compete with weeds present within a field, a critical component of successful weed management. Examples of effective herbicide safening to sulfonylurea herbicides is documented in corn (*Zea mays* L.), rice, grain sorghum and wheat (*Triticum aestivum* L.), but not in any dicotyledonous species (Davies and Caseley 1999). More in-depth exploration of the safening effects seen in soybean in this study may prove that many potential safening options can exist in dicots.

Due to the wide variation in consistency and degree of injury reduction seen among site years, use of insecticide seed treatments solely as safeners in soybean is unlikely from a grower perspective. However, because insecticide seed treatments are used on a vast area, under differing environmental conditions, there is a high possibility that at least some of the producers who use them will see the benefits of potential safening. Injury reduction of 10 to 15% may seem negligible, but protecting seedling soybean is of vital importance. Reducing injury to seedlings decreases time to canopy closure, which in turn decreases weed interference, and can increase crop yields. Since insecticide seed treatments appear to be able to provide this benefit, in addition to protecting against early-season insect pests, adoption of insecticide seed treatments in the future is likely to increase among soybean growers.

Grain Sorghum Study. Compared to the soybean study, use of insecticide seed treatments as safeners appears to have even more potential in grain sorghum. Of the three herbicides evaluated, all were effectively safened in at least one site year. Injury from glyphosate, imazethapyr, and quizalofop were reduced at three, two, and one site year, respectively. Following glyphosate exposure, a significant two-way interaction was seen at both LMCRS (2016) and PTRS, and individual t-tests showed a reduction in injury at NEREC as well. Glyphosate injury to grain sorghum was reduced at all evaluation timings at PTRS through the use of clothianidin and imidacloprid. The most effective instance of safening could be seen at 4 WAA, where injury was reduced from 48% to 25%, 5%, and 6% through the use of thiamethoxam, clothianidin, and imidacloprid, respectively (Table 10). These safening effects from all three insecticide seed treatments provided an increase in yield compared to the treatments with no insecticide following glyphosate exposure at PTRS. Yield increases of 1710, 2000, and 1410 kg ha^{-1}were seen in the thiamethoxam, clothianidin, and imidacloprid plots, respectively (Table 10). At LMCRS (2016), injury was reduced at both the 2 and 4 WAA evaluation timing with thiamethoxam and imidacloprid. At 2 WAA, injury was reduced from 84% to 54% and 70% using thiamethoxam and imidacloprid, respectively, and similarly at 4 WAA, where injury was reduced from 86% to 48% and 65% (Table 11). Similarly to results at PTRS, the safening seen at LMCRS (2016) resulted in increases in both crop height and yield compared to treatments with no insecticide seed treatment. Plots with thiamethoxam and imidacloprid seed treatments were 18 and 6 cm taller, and had yields 2520 and 1330 kg ha^{-1} higher, respectively, compared to the nontreated (Table 11). At NEREC, while no significant insecticide by herbicide interaction was seen, when treatments with each insecticide were compared to those without insecticides, glyphosate injury was reduced 1 WAA by 7 percentage points, from 78% to 71% via clothianidin (Table 12).

Injury following exposure to imazethapyr was reduced at both LMCRS (2016) and PTRS via insecticide seed treatments. At LMCRS (2016), reduction in injury was seen at all rating timings using thiamethoxam, and at the 1 and 4 WAA timings using clothianidin. Injury 4 WAA was reduced from 33% to 19% and 7% via thiamethoxam and clothianidin, respectively (Table 11). An increase in height was seen as a result of this safening, where clothianidin treated plots were 5 cm taller compared to the nontreated plots; however, yield was not increased as a result of injury reduction. At PTRS, grain sorghum was safened against imazethapyr injury 1 WAA using clothianidin, where injury was reduced from 16% to 6% (Table 10). Unlike in instances of glyphosate safening, yields were not increased through the use of insecticide seed treatments following exposure to imazethapyr.

Of the three herbicides evaluated, quizalofop was the least successful in terms of safening seen with only one site year showing a reduction in injury through the use of insecticide seed treatments. Following exposure to drift rates of quizalofop, injury was reduced at 2 and 4 WAA at LMCRS (2016) using clothianidin. Maximum safening was seen at 4 WAA, where injury was reduced from 99% to 53% (Table 11). This drastic reduction in injury resulted in both increases in crop height (23 cm), as well as yield (4,020 kg ha^{-1}) compared to the treatment with no insecticide that also received a low rate of quizalofop.

Results from the grain sorghum studies are similar to those seen by Miller et al. (2016) for rice, where thiamethoxam seed treatments effectively reduced crop injury to reduced rates of glyphosate and imazethapyr. In addition to thiamethoxam, it appears that both clothianidin and imidacloprid provide similar safening benefits to seedling grass crops like grain sorghum. Since no herbicide-resistance traits are currently used in grain sorghum production, protecting seedlings from herbicide drift is particularly important. In the state of Arkansas, grain sorghum is often grown in close proximity to glyphosate-resistant soybean, corn, and cotton, and imazethapyr-resistant rice. In addition, in 2018, quizalofop-resistant rice will be grown for the first time on widespread acreage. Fortunately, for grain sorghum producers, incorporating seed treatments that include thiamethoxam, clothianidin, or imidacloprid may alleviate some of the concerns associated with drift of these herbicides.

Herbicide safening is a complex process and can occur through competitive inhibition of a target site, chemical antagonism, and increased herbicidal metabolism (Davies and Caseley 1999). Since the insecticides evaluated in this experiment were not analogous to herbicides applied, nor were they tank-mixed with herbicides, the most likely explanation is that herbicide metabolism was increased when safening effects were observed. Plant metabolism is a dynamic process primarily controlled by enzymatic function (Hatzios and Burgos 2004). The production of two of the most important enzymes involved in metabolism of xenobiotic compounds, cytochrome P450s (P450s) and glutathione S-transferases (GSTs), can be influenced by various environmental conditions (Droog 1997; Durst 1997; Marrs 1996). As such, temperature and rainfall likely played a significant role in variability of results from these studies. In this study, propanil was the only herbicide not safened in at least one location. Propanil is not metabolized via P450s or GSTs; rather it is metabolized by aryl acylamidase in tolerant plants (Hoagland 1987; Hoagland et al. 2004). The fact that it was not safened through the use of these insecticide seed treatments, while other herbicides were, lends more credibility to the assumption that the safening effect is a result of increased production of P450s and GSTs.

Aside from traditionally-understood herbicide safening, another possible explanation for injury reduction could be generalized increases in plant defense mechanisms caused by plant uptake of the insecticidal compounds. Research conducted by Ford et al. (2010) showed that plant uptake of clothianidin and imidacloprid (both neonicotinoids) leads to production of salicylic acid (SA), and SA mimics. Salicylic acid is an important activator molecule that triggers widespread plant defense mechanisms, which allow plants to cope with both biotic and abiotic stresses (Durrant and Dong 2004; Ryals et al. 1996; Vlot et al. 2009). These SA-triggered defense mechanisms are known to promote improved disease tolerance and increase vigor in plants, but the exact ways in which they could improve tolerance to herbicides is currently not well-understood (Yuan and Lin 2007). A more detailed investigation of these processes could, however, show more promise for the potential exploitation of neonicotinoid insecticides as herbicide safeners.

Instances where injury reduction was not seen may have been due to the fact that in-plant concentrations of insecticidal compounds were too low at the time of herbicide application to have an effect. According to Bailey et al. (2015), the concentration of neonicotinoid insecticides present in a plant is greatly diminished three weeks after planting. Some applications were made as late as 31 days after planting (both soybean and grain sorghum studies at NEREC), meaning any insecticide still remaining in the plant would have been present at very low levels. Because of the short-lived presence of insecticides in crops, safening effects can only be expected for early-season drift events. Aside from mitigating risks associated with herbicide drift, more research is needed to examine whether safening effects may be seen following applications of PRE herbicides where crop injury is a concern.

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Table 1. General description of experimental sites.[a]

Location	Year	Planting date	Application date	Sand	Silt	Clay	pH
				--------------%---------------			
LMCRS	2015	5/14/2015	6/8/2015	0.8	90.5	8.7	7.5
LMCRS	2016	5/5/2016	5/26/2016	0.8	90.5	8.7	7.5
NEREC	2016	4/19/2016	5/20/2016	22	25	53	6.7
PTRS	2016	5/19/2016	6/8/2016	0.4	78.1	21.5	7.8

[a] Abbreviations: LMCRS, Lon Mann Cotton Research Station in Marianna, AR; NEREC, Northeast Research and Extension Center in Keiser, AR; PTRS, Pine Tree Research Station near Colt, AR

Table 2. Environmental conditions at the Lon Mann Cotton Research Station in Marianna, AR in 2015 beginning at planting (May 14), with herbicide application date marked with an asterisk.

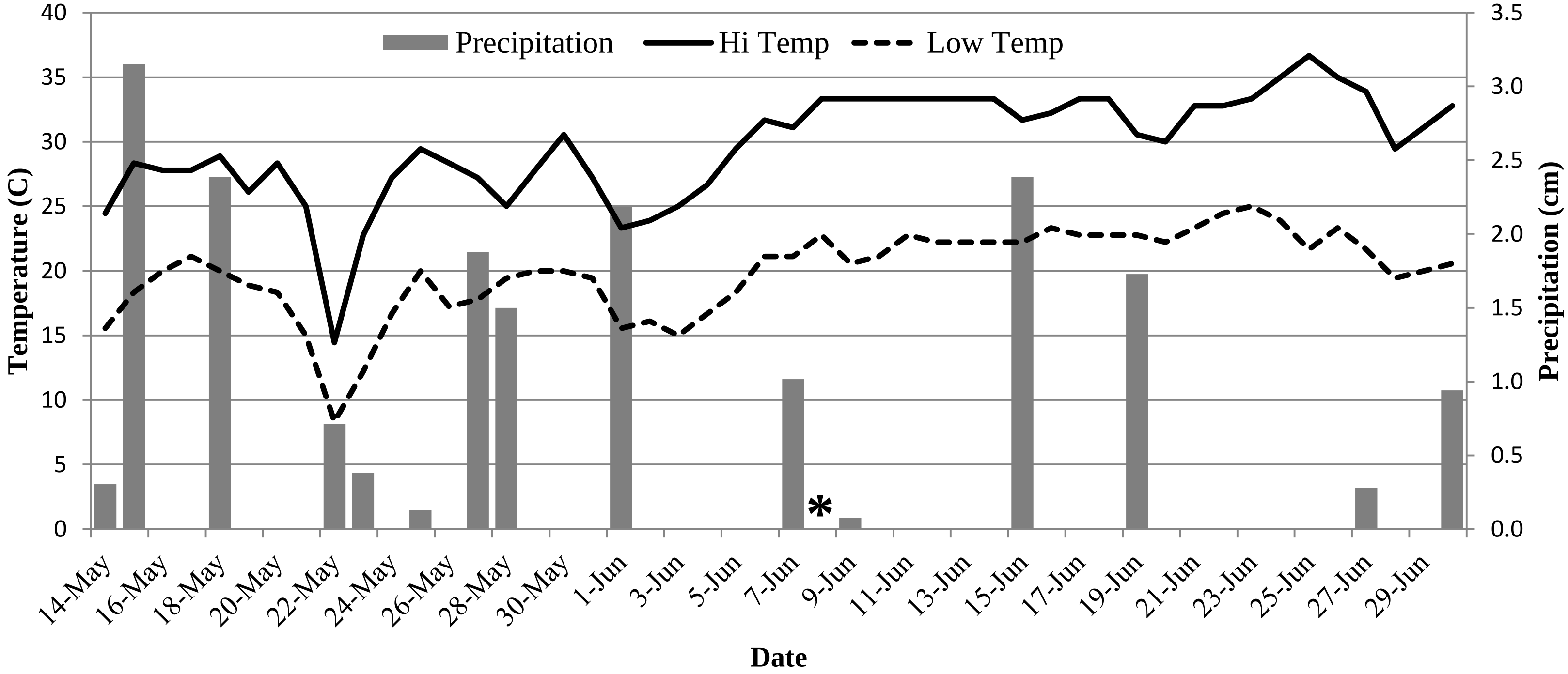

Table 3. Environmental conditions at the Lon Mann Cotton Research Station in Marianna, AR in 2016 beginning at planting (May 5), with herbicide application date marked with an asterisk.

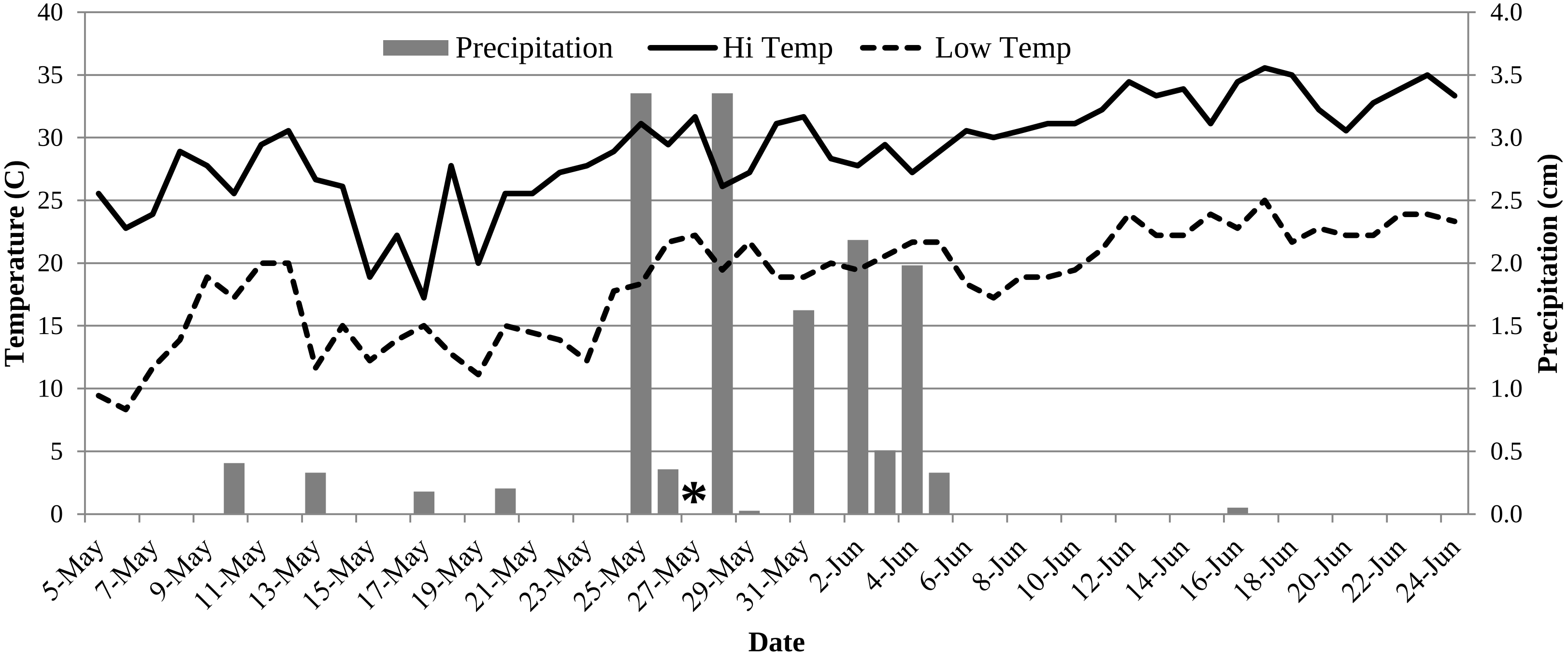

Table 4. Environmental conditions at the Northeast Research and Extension Center in Keiser, AR in 2016 beginning at planting date (April 19), with herbicide application date marked with an asterisk.

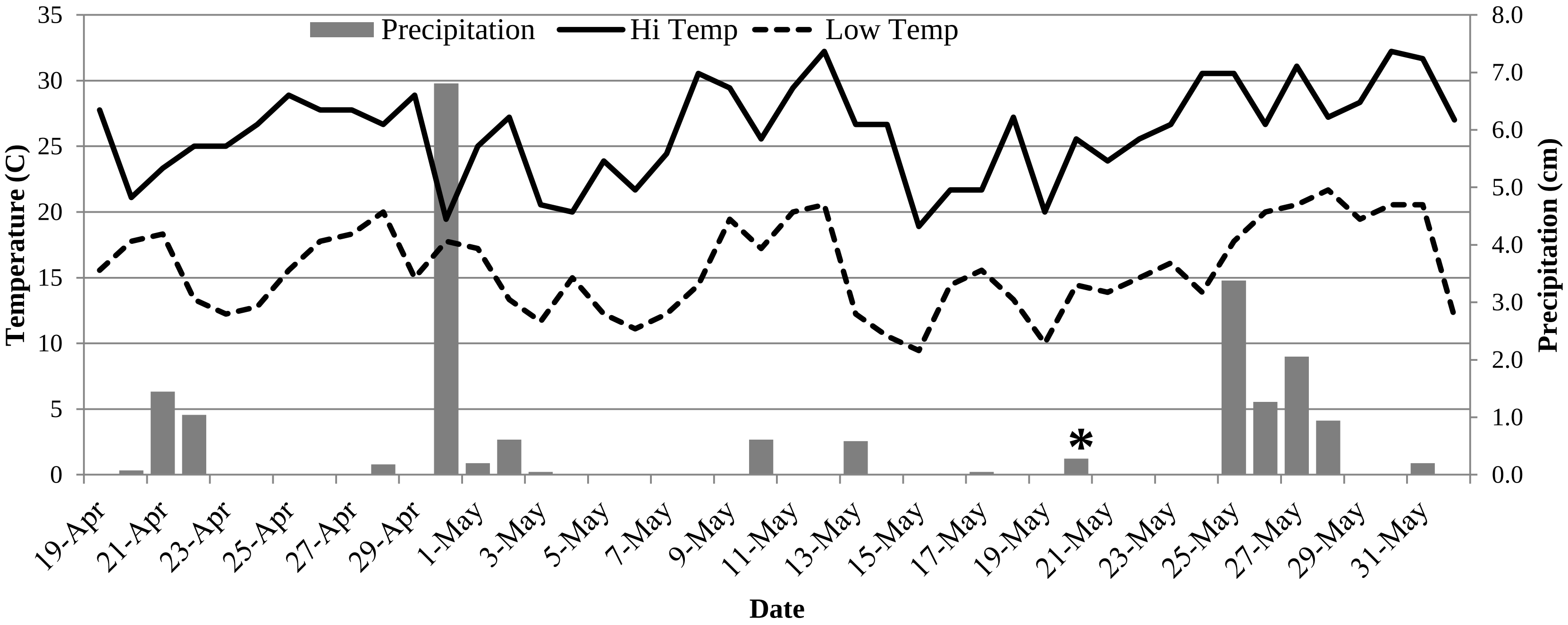

Table 5. Environmental conditions at the Pine Tree Research Station near Colt, AR in 2016 beginning at planting date (May 19), with herbicide application date marked with an asterisk.

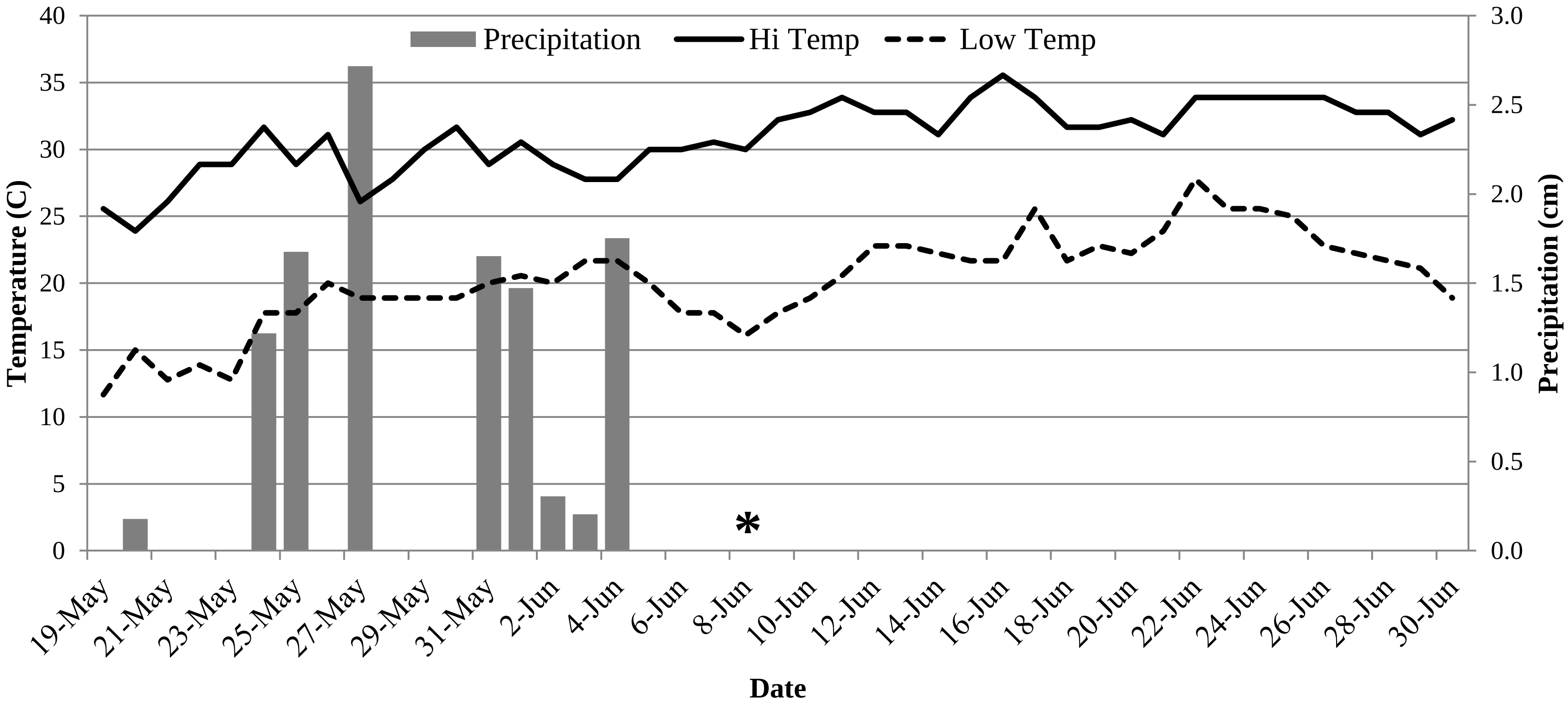

Table 6. Soybean injury, height, and yield at the Lon Mann Cotton Research Station in Marianna, AR in 2015.[a]

		Injury[b]				
Herbicide	Seed treatment	1 WAA	2 WAA	4 WAA	Height[c]	Yield[d]
		--------------------------- %	------------------------------		cm	kg ha^{-1}
None	None	0	0	0	71	3770
	Thiamethoxam	0	0	0	70	3170
	Clothianidin	0	0	0	71	3380
Dicamba	None	24	38	15	60	3360
	Thiamethoxam	21	46	16	54	3000
	Clothianidin	28	35	19	56	3340
2,4-D	None	23	9	1	68	3520
	Thiamethoxam	9*	9	2	72	3400
	Clothianidin	14	8	1	71	3390
Glyphosate	None	9	15	1	66	3570
	Thiamethoxam	6	10	2	67	3250
	Clothianidin	8	14	1	67	3540
Glufosinate	None	13	14	12	66	3300
	Thiamethoxam	13	9	6	65	3380
	Clothianidin	11	8	3	72	3360
Halosulfuron	None	40	46	41	58	3170
	Thiamethoxam	19*	16*	13*	67*	3000
	Clothianidin	10*	6*	3*	71*	3400*
Mesotrione	None	8	9	3	71	3460
	Thiamethoxam	11	4	1	71	3710
	Clothianidin	10	9	3	70	3580
Tembotrione	None	8	5	1	72	3360
	Thiamethoxam	10	8	1	70	3490
	Clothianidin	6	5	3	71	3580

Table 6 (Cont.) Soybean injury, height, and yield at LMCRS in Marianna, AR in 2015.[a]

		Injury[b]				
Herbicide	Seed treatment	1 WAA	2 WAA	4 WAA	Height[c]	Yield[d]
		--------------------------	%	-----------------------------	cm	kg ha^{-1}
Propanil	None	13	16	5	71	3310
	Thiamethoxam	18	16	8	67	3090
	Clothianidin	6	8	1	73	3260

[a] Abbreviations: LMCRS, Lon Mann Cotton Research Station; WAA, weeks after application; NS, non-significant

[b] Means followed by an asterisk indicate significant reduction in injury compared to no insecticide seed treatment within the same herbicide treatment according to Fisher's protected LSD (α=0.05).

[c] Means followed by an asterisk indicate significant increase in crop height compared to no insecticide seed treatment within the same herbicide treatment according to Fisher's protected LSD (α=0.05).

[d] No significant differences were seen among seed treatments within herbicide treatments according to Fisher's protected LSD (α=0.05).

Table 7. Soybean injury, height, and yield at LMCRS in Marianna, AR in 2016.[a]

Herbicide	Seed treatment	Injury[b]			Height[d]	Yield[e]
		1 WAA	2 WAA[c]	4 WAA		
		---------------------------- %	------------------------------		cm	kg ha^{-1}
None	None	0	0	0	42	2590
	Thiamethoxam	0	0	0	46	2460
	Clothianidin	0	0	0	46	2490
Dicamba	None	16	20	20	33	2700
	Thiamethoxam	13	13‡	12‡	36	2720
	Clothianidin	16	16	16	34	1910
2,4-D	None	2	3	5	40	2380
	Thiamethoxam	0	1	4	43	2530
	Clothianidin	0	0	3	42	2760
Glyphosate	None	23	13	12	36	2870
	Thiamethoxam	20	7‡	4‡	38	2450
	Clothianidin	18	11	6‡	36	2930
Glufosinate	None	33	14	13	33	2840
	Thiamethoxam	35	9‡	7‡	37	2660
	Clothianidin	28	11	9	36	2660
Halosulfuron	None	19	5	5	38	2010
	Thiamethoxam	6*	0	0	43‡	2650‡
	Clothianidin	6*	1	2	40	2600
Mesotrione	None	20	1	1	40	2730
	Thiamethoxam	25	2	1	41	2720
	Clothianidin	21	1	2	41	2940
Tembotrione	None	9	1	2	41	2760
	Thiamethoxam	9	0	0	45	2660
	Clothianidin	15	1	1	41	2690

Table 7 (Cont.) Soybean injury, height, and yield at LMCRS in Marianna, AR in 2016.[a]

		Injury[b]				
Herbicide	Seed treatment	1 WAA	2 WAA[c]	4 WAA	Height[d]	Yield[e]
		------------------------- %		-----------------------------	cm	kg ha^{-1}
Propanil	None	8	4	4	39	2890
	Thiamethoxam	9	2	1	40	2680
	Clothianidin	7	3	2	40	2730
Main effect	None		7	7	38	NS
	Thiamethoxam		6†	6†	39	NS
	Clothianidin		4†	4†	41†	NS

[a] Abbreviations: LMCRS, Lon Mann Cotton Research Station;WAA, weeks after application; NS, non-significant

[b] Means followed by an asterisk indicate significant reduction in injury, compared to no insecticide seed treatment, within the same herbicide treatment according to Fisher's protected LSD (α=0.05). Means followed by a single dagger indicate a significant seed treatment main effect using the same criteria.

[c,d,e] For responses that did not produce a herbicide by insecticide seed treatment interaction, a t-test was conducted to compare treatments with no insecticide to each insecticide seed treatment within an herbicide. Where use of an insecticide seed treatment reduced injury or increased height or yield compared to no insecticide, means are marked with a double dagger (‡)

Table 8. Soybean injury, height, and yield at NEREC in Keiser, AR in 2016.[a]

		Injury[b]				
Herbicide	Seed treatment	1 WAA	2 WAA	4 WAA	Height[c]	Yield
		----------------------------	%	-------------------------------	cm	kg ha^{-1}
None	None	0	0	0	31	3940
	Thiamethoxam	0	0	0	33	3800
	Clothianidin	0	0	0	33	3850
Dicamba	None	41	40	20	14	3500
	Thiamethoxam	38	36	14	28‡	3670
	Clothianidin	44	41	8	25‡	3920
2,4-D	None	11	4	0	26	3890
	Thiamethoxam	10	6	0	27	4030
	Clothianidin	10	4	0	28	3840
Glyphosate	None	34	36	5	25	3740
	Thiamethoxam	34	30	6	25	3560
	Clothianidin	18*	23*	5	24	3530
Glufosinate	None	15	15	8	29	3810
	Thiamethoxam	13	9	0	29	3710
	Clothianidin	13	9	0	31	3500
Halosulfuron	None	23	20	2	24	4120
	Thiamethoxam	13*	13	0	24	4230
	Clothianidin	24	27	0	25	3920
Mesotrione	None	16	13	0	27	3720
	Thiamethoxam	16	11	0	28	3700
	Clothianidin	13	9	0	29	3870
Tembotrione	None	18	16	0	28	3910
	Thiamethoxam	19	15	0	28	3650
	Clothianidin	19	17	0	28	3840

Table 8 (Cont.) Soybean injury, height, and yield at NEREC in Keiser, AR in 2016.[a]

		Injury[b]				
Herbicide	Seed treatment	1 WAA	2 WAA[c]	4 WAA	Height[c]	Yield
		--------------------------	%	-----------------------------	cm	kg ha^{-1}
Propanil	None	14	10	0	28	3860
	Thiamethoxam	6	4	0	29	3910
	Clothianidin	10	6	0	29	3930
Main effect	None			NS	27	NS
	Thiamethoxam			NS	28†	NS
	Clothianidin			NS	28†	NS

[a] Abbreviations: NEREC, Northeast Research and Extension Center; WAA, weeks after application; NS, non-significant

[b] Means followed by an asterisk indicate significant reduction in injury, compared to no insecticide seed treatment, within the same herbicide treatment according to Fisher's protected LSD (α=0.05). Means followed by a cross indicate a significant seed treatment main effect using the same criteria.

[c] For responses that did not produce a herbicide by insecticide seed treatment interaction, a t-test was conducted to compare treatments with no insecticide to each insecticide seed treatment within an herbicide. Where use of an insecticide seed treatment reduced injury or increased height or yield compared to no insecticide, means are marked with a double dagger (‡)

Table 9. Soybean injury, height, and yield at PTRS near Colt, AR in 2016.[a]

		Injury[b]				
Herbicide	Seed treatment	1 WAA[d]	2 WAA	4 WAA[e]	Height	Yield[c]
		---------------------------- %	----------------------------	----------------------------	cm	kg ha^{-1}
None	None	0	0	0	19	2500
	Thiamethoxam	0	0	0	19	2080
	Clothianidin	0	0	0	21	2670
Dicamba	None	17	45	36	16	2300
	Thiamethoxam	16	45	30	16	2230
	Clothianidin	18	46	30	16	2550
2,4-D	None	21	40	26	16	2160
	Thiamethoxam	18	46	31	14	2120
	Clothianidin	13	40	24	16	2610
Glyphosate	None	17	20	19	17	2550
	Thiamethoxam	18	28	25	17	2180
	Clothianidin	14	28	23	17	2780
Glufosinate	None	15	18	14	19	2360
	Thiamethoxam	6‡	21	24	20	2300
	Clothianidin	8	15	11	18	3000*
Halosulfuron	None	25	40	30	14	2350
	Thiamethoxam	26	48	42	16	1970
	Clothianidin	26	46	26	17	2420
Mesotrione	None	34	49	31	16	2520
	Thiamethoxam	28‡	34*	30	18	2640
	Clothianidin	28‡	51	34	17	2510
Tembotrione	None	26	35	26	18	2550
	Thiamethoxam	20	30	18‡	18	2290
	Clothianidin	33	32	31	16	2350

Table 9 (Cont.) Soybean injury, height, and yield at PTRS near Colt, AR in 2016.[a]

		Injury[b]				
Herbicide	Seed treatment	1 WAA[d]	2 WAA	4 WAA[c]	Height	Yield[e]
		----------------------------	%	------------------------------	cm	kg ha^{-1}
Propanil	None	18	44	23	17	2660
	Thiamethoxam	21	41	23	18	2420
	Clothianidin	17	39	30	18	2580
Main effect	None	22		NS	27	NS
	Thiamethoxam	21		NS	28†	NS
	Clothianidin	19†		NS	28†	NS

[a] Abbreviations: PTRS, Pine Tree Research Station; WAA, weeks after application; NS, non-significant

[b,c] Means followed by an asterisk indicate significant reduction in injury, compared to no insecticide seed treatment, within the same herbicide treatment according to Fisher's protected LSD (α=0.05). Means followed by a cross indicate a significant seed treatment main effect using the same criteria.

[d,e] For responses that did not produce a herbicide by insecticide seed treatment interaction, a t-test was conducted to compare treatments with no insecticide to each insecticide seed treatment within an herbicide. Where use of an insecticide seed treatment reduced injury or increased height or yield compared to no insecticide, means are marked with a double dagger (‡)

Table 10. Grain sorghum injury, height, and yield at PTRS near Colt, AR in 2016.[a]

		Injury[b]			
Herbicide	Seed treatment	1 WAA	2 WAA	4 WAA	Yield[c]
		---------------------------	%	------------------------------	kg ha^{-1}
None	None	0	0	0	4040
	Thiamethoxam	0	0	0	5750*
	Clothianidin	0	0	0	6040*
	Imidacloprid	0	0	0	5450*
Glyphosate	None	76	65	48	1760
	Thiamethoxam	76	59	28*	4380*
	Clothianidin	24*	9*	5*	4710*
	Imidacloprid	21*	11*	6*	5450*
Imazethapyr	None	16	5	0	4800
	Thiamethoxam	6*	2	0	4550
	Clothianidin	10	3	0	5130
	Imidacloprid	11	3	0	5310
Quizalofop	None	36	20	8	3320
	Thiamethoxam	28	15	5	3320
	Clothianidin	35	24	4	3980
	Imidacloprid	30	22	1	5420*

[a] Abbreviation: PTRS, Pine Tree Research Station; WAA, weeks after application

[b] Means followed by an asterisk indicate significant reduction in injury compared to no insecticide seed treatment within the same herbicide treatment according to Fisher's protected LSD (α=0.05).

[c] Means followed by an asterisk indicate increase in yield compared to no insecticide seed treatment within the same herbicide treatment according to Fisher's protected LSD (α=0.05).

Table 11. Grain sorghum injury, height, and yield at LMCRS at Marianna, AR in 2016[a].

		Injury[b]				
Herbicide	Seed treatment	1 WAA	2 WAA	4 WAA	Height[c]	Yield[e]
		------------------------------	%	-----------------------------	cm	kg ha^{-1}
None	None	0	0	0	27	4780
	Thiamethoxam	0	0	0	29	3920
	Clothianidin	0	0	0	29	4480
	Imidacloprid	0	0	0	28	4350
Glyphosate	None	60	84	86	10	1910
	Thiamethoxam	62	54*	48*	28*	4430*
	Clothianidin	59	83	85	11	1950
	Imidacloprid	51	70*	65*	16*	3240*
Imazethapyr	None	43	29	33	25	4060
	Thiamethoxam	31*	19*	19*	24	4570
	Clothianidin	28*	26	7*	30*	4010
	Imidacloprid	46	32	36	22	4440
Quizalofop	None	80	96	99	6	520
	Thiamethoxam	80	99	99	8	440
	Clothianidin	74	68*	53*	29*	4540*
	Imidacloprid	81	99	99	3	380

[a] Abbreviation: LMCRS, Lon Mann Cotton Research Station; WAA, weeks after application

[b] Means followed by an asterisk indicate significant reduction in injury compared to no insecticide seed treatment within the same herbicide treatment according to Fisher's protected LSD (α=0.05).

[c] Means followed by an asterisk indicate significant increase in height compared to no insecticide seed treatment within the same herbicide treatment according to Fisher's protected LSD (α=0.05).

[d] Means followed by an asterisk indicate increase in yield compared to no insecticide seed treatment within the same herbicide treatment according to Fisher's protected LSD (α=0.05).

Table 12. Grain sorghum injury, height, and yield at Northeast Research and Extension Center at Keiser, AR in 2016.[a]

		Injury			
Herbicide	Seed treatment	1 WAA[b]	2 WAA	Height[c]	Yield[b]
		------------------- %	------------------	cm	kg ha^{-1}
None	None	0	0	62	5590
	Thiamethoxam	0	0	61	6390
	Clothianidin	0	0	63	5460
	Imidacloprid	0	0	63	4670
Glyphosate	None	78	90	17	1090
	Thiamethoxam	74	90	17	100
	Clothianidin	71‡	88	18	280
	Imidacloprid	75	90	17	130
Imazethapyr	None	64	58	19	4480
	Thiamethoxam	61	60	21	3970
	Clothianidin	63	59	17	3360
	Imidacloprid	63	64	19	5240
Quizalofop	None	78	63	20	2530
	Thiamethoxam	81	68	17	1460
	Clothianidin	74	66	17	3360
	Imidacloprid	79	70	19	1900
Main effect	None		NS	NS	NS
	Thiamethoxam		NS	NS	NS
	Clothianidin		NS	NS	NS
	Imidacloprid		NS	NS	NS

[a] Abbreviation: WAA, weeks after application; NS, non-significant

[b,] For responses that did not produce a herbicide by insecticide seed treatment interaction (α=0.05), a t-test was conducted to compare treatments with no insecticide to each insecticide seed treatment within an herbicide. Where use of an insecticide seed treatment reduced injury or increased height or yield compared to no insecticide, means are marked with a double dagger (‡)

Table 13. Grain sorghum injury, height, and yield at the Lon Mann Cotton Research Station in Marianna, AR in 2015.[a,b]

		Injury[b]				
Herbicide	Seed treatment	1 WAA	2 WAA	4 WAA	Height	Yield[c]
		--------------------------- %	------------------------------		cm	kg ha^{-1}
None	None	0	0	0	139	6120
	Thiamethoxam	0	0	0	141	6850
	Clothianidin	0	0	0	140	7430
	Imidacloprid	0	0	0	146	6710
Glyphosate	None	60	49	9	133	4980
	Thiamethoxam	63	48	15	131	3790
	Clothianidin	65	49	7	125	5740
	Imidacloprid	66	48	15	121	3560
Imazethapyr	None	15	18	0	130	4730
	Thiamethoxam	13	14	0	142	5620
	Clothianidin	15	11	0	141	5720
	Imidacloprid	13	11	0	137	6010
Quizalofop	None	97	94	76	112	2440
	Thiamethoxam	97	98	83	101	1540
	Clothianidin	96	93	76	100	2170
	Imidacloprid	95	93	81	100	1490
Main effect	None	NS	NS	NS	NS	NS
	Thiamethoxam	NS	NS	NS	NS	NS
	Clothianidin	NS	NS	NS	NS	NS
	Imidacloprid	NS	NS	NS	NS	NS

[a] Abbreviation: WAA, weeks after application; NS, non-significant

[b] Mean separation showed no significant difference at the α = 0.05 level for injury, height, or yield among insecticide seed treatments, within herbicides.

Chapter 3

Use of Insecticide Seed Treatments as Safeners to Applications of Residual Herbicides in Soybean and Grain Sorghum

Abstract

With increased instances of weed resistance to applications of postemergence herbicides, applying soil-applied herbicides that offer residual activity is becoming popular. Unfortunately, under some conditions, the use of residual herbicides can result in unintentional injury to crops. However, there are a number of ways to reduce the risks associated with these herbicides, including the use of in-crop herbicide safeners. Based on previous research conducted in rice, the potential may exist for seeds treated with insecticides to be successfully safened to certain herbicides, including those applied to the soil. Field trials were conducted in Marianna, Arkansas in 2015 and 2016 and in Keiser and near Colt, Arkansas in 2016 to explore this possibility in soybean and grain sorghum. In soybean, seeds were treated with the insecticide thiamethoxam and subsequently the herbicides metribuzin, saflufenacil, pyroxasulfone, sulfentrazone, chlorimuron, flumioxazin, mesotrione, chlorsulfuron, and flumioxazin+pyroxasulfone+chlorimuron were applied immediately after planting. Of the nine herbicides evaluated, successful safening was observed in six, including saflufenacil, pyroxasulfone, sulfentrazone, flumioxazin, and flumioxazin+pyroxasulfone+chlorimuron. The highest degree of safening was seen 1 WAE at Keiser, where injury from flumioxazin+pyroxasulfone+chlorimuron was reduced from 44% to 29%. Results indicate that although a thiamethoxam seed treatment may reduce phytotoxicity from some herbicides in soybean, these benefits do not correspond to increased crop height, density, or yield compared to non-treated seed. In grain sorghum, seeds were treated with thiamethoxam and the herbicides fomesafen, imazethapyr, and quizalofop applied preemergence. Injury in grain sorghum was ≤4% in all site-years, except for one. This low injury may have been a function of the herbicides not being incorporated into the soil profile for uptake by the seedling grain sorghum. Based on the results from this study, not enough crop injury was produced in grain sorghum to detect a safening response; as a result, more research is needed to definitively prove or disprove the utility of insecticide seed treatments as effective safeners.

Nomenclature: chlorsulfuron; clomazone; fomesafen; fluxofenin; imazethapyr; mesotrione; metribuzin; metolachlor; pyroxasulfone; quizalofop; saflufenacil; sulfentrazone; thiamethoxam; soybean, *Glycine max* (L.) Merr; grain sorghum, *Sorghum bicolor* (L.) Moensch ssp. Bicolor.

Key words: Residual herbicides, herbicide safeners

Introduction

Herbicide use in the U.S. is a vital component of agriculture production. Gianessi and Reigner (2007) estimate that herbicide use provides a labor equivalent of 70 million hand laborers, and increases crop yields as much as 20%. The introduction of herbicide-resistant (HR) crops has also significantly improved the efficiency of crop production, both in the U.S. and globally (Brookes and Barfoot 2012). Beginning with the introduction of glyphosate-resistant soybean in 1996, the widespread adoption of HR crops provided growers with the ability to effectively control a broad spectrum of weeds by utilizing just one or two postemergence (POST) applications of a herbicide with a single mode of action (Young 2006). Unfortunately, this reduction in diversity of chemical weed control practices resulted in the evolution of weed populations with resistance to herbicides that were once highly effective (Vencill et al. 2012). For example, overreliance upon glyphosate has resulted in glyphosate-resistance in 37 individual weed species since 2000 (Heap 2017). In order to effectively combat herbicide resistance, the use of herbicides with residual activity is recommended (Norsworthy et al. 2012; Owen et al. 2011).

Residual herbicides are applied to the soil surface and their use offers a number of benefits to crop producers. They typically control a broad spectrum of weeds, including both grasses and broadleaves, and can offer several weeks of residual weed control (DeWerff et al. 2015; Meyer et al. 2016). Although the application of a residual herbicide alone is seldom adequate for season-long weed control, when used as a component of a sequential herbicide program, high levels of weed control can be achieved, resulting in increased crop yields compared to programs that do not include residual herbicides (Aulakh and Jhala 2015; Loux et al. 2011). The residual activity provided by these herbicides typically allows for later applications of POST-applied herbicides and, thus, improved flexibility for crop producers (Ellis and Griffin 2002). Apart from being applied by themselves, residual herbicides can be tank-mixed with a number of POST-applied herbicides. In these instances, the POST herbicide controls weeds that have already emerged, whereas the residual herbicide provides lasting control of weeds that have not yet germinated at the time of application. This approach results in high levels of weed control, which can consequently improve crop yield (Aulakh and Jhala 2015).

In addition to providing the obvious benefit of successfully controlling weeds, residual herbicides are also important herbicide-resistance management tools. Because residual herbicides greatly decrease the number of weeds present early in the season, there is decreased resistance selection on POST herbicides in subsequent applications. Reduced selection results in less likelihood for herbicide resistance, which in turn increases the potential lifespan of a given herbicide (Beckie 2006; Norsworthy et al. 2012). Including residual herbicides as part of a tank mixture with POST herbicides results in an increased number of herbicide modes of action (MOA) applied to weeds. Applications of multiple, effective herbicide MOAs is one of the most important methods for delaying the onset of herbicide resistance (Norsworthy et al. 2012).

Unfortunately, one main drawback associated with the use of residual herbicides is crop injury following application. In some cases, herbicides that are labeled for use in-crop can cause injury to young plants. Flumioxazin, sulfentrazone, chlorimuron, *S*-metolachlor, and pyroxasulfone are some examples in soybean production (McNaughton et al. 2014; Talyor-Lovell 2001; Whitaker et al. 2010). Crop response to these preemergence (PRE) herbicides can be greatly variable depending upon both soil and environmental conditions, with cool, wet, and low pH conditions causing the most crop injury in soybean following applications of flumioxazin and sulfentrazone (Taylor-Lovell et al. 2001). In addition to temperature, moisture, and pH, soil organic matter (SOM) and texture can impact the activity of herbicides to varying degrees, depending upon the herbicide (Eberlein 1984; Gannon 2014). Aside from environmental effects, varietal selection can cause substantial variation in response to soil-applied herbicides (Swantek et al. 1998). Early-season injury from herbicides typically dissipates quickly with no adverse effects on crop yield, but in some cases, more severe injury symptoms and stand loss can cause reduced yields (McNaughton et al. 2014; Taylor-Lovell et al. 2001).

Another concern with residual herbicides is injury to successive crops. Due to their relatively long half-lives, plant-back restrictions are needed for many soil-applied herbicides in order to protect crops in replant situations following crop failure, as well as crops grown the next season (Barber et al. 2014). These plant-back restrictions can greatly limit rotational options and can drive growers' decisions on what to plant the following year. One notable example of where crop rotation is directly influenced by herbicide use in the state of Arkansas can be seen in imidazolinone-resistant (Clearfield®, BASF Corporation, Research Triangle Park, NC) rice (*Oryza sativa* L.). Imidazolinone-resistant rice is tolerant to applications of the herbicide imazethapyr, an acetolactate synthase (ALS)-inhibiting imidazolinone. According to Renner et al. (1998), imidazolinones can persist in the soil as long as two years after their initial application. Grain sorghum, cotton, and conventional rice all have a rotational restriction of 18 months following imazethapyr applications, meaning rice producers in Arkansas are limited to planting soybean, corn (*Zea mays* L.), or imidazolinone-resistant rice the following season (Barber et al 2014).

A possible solution to preventing or reducing the effects of crop injury when using residual herbicides is the use of herbicide safeners. Safeners typically act by increasing a plants' ability to metabolize herbicides. Through the use of safeners, crop injury can be reduced such that a herbicide can be used in crops where it causes unacceptable levels of injury when applied without a safener (Davies and Caseley 1999). The herbicide safener fluxofenin (Concep III, Syngenta Crop Protection, LLC, Greensboro, NC) is already used extensively in grain sorghum production to prevent injury from PRE herbicides. Without a fluxofenin seed treatment, chloroacetamide herbicides such as *S*-metolachlor and alachlor cannot be applied in sorghum production (Espinosa and Kelley 2004).

The benefits of applying herbicide safeners as seed treatments are twofold: injury from herbicides is greatly decreased, and the safener is selectively applied to the crop (Davies and Caseley 1999). Applying the safener only to the crop ensures that safening effects are not conferred to the weeds present in a field, maintaining herbicidal efficacy. This property is highly desirable, and thus, seed-applied safeners have great value. Recently, Miller et al. (2016) reported that the insecticide seed treatment thiamethoxam (Cruiser 5S, Syngenta Crop Protection, LLC, Greensboro, NC), in addition to protecting seedling rice from early-season insect damage, also provided a reduction in crop injury following application of some POST herbicides. Although in-plant concentrations of insecticides decrease substantially 3 to 4 weeks after planting (Bailey et al. 2015), enough insecticidal material was still present in the rice at this time to produce a safening effect. Since safening effects were seen even in the case of low thiamethoxam presence, it was hypothesized that similar effects may be seen at crop emergence, when thiamethoxam concentration is much higher in the plant. Thus, research was conducted to determine whether thiamethoxam could be used to reduce crop injury from select soil-residual herbicides in soybean and grain sorghum.

Materials and Methods

Soybean Study. A soybean experiment was conducted at the Lon Mann Cotton Research Station (LMCRS) in Marianna, AR, in 2015 to assess potential safening effects to residual herbicides conferred via insecticide seed treatments. In 2016, experiments were repeated at LMCRS, in addition to at the Northeast Research and Extension Center (NEREC) in Keiser, AR, and at the Pine Tree Research Station (PTRS) near Colt, AR. DG5067LL (Delta Grow Seed Company Inc., England, AR), a glufosinate-resistant, non-STS, maturity group 5.2 soybean, was planted at a seeding rate of 340,000 seeds ha^{-1} to an approximate 2.5-cm depth. Four-row plots were established utilizing a randomized complete block design with four replications. Row spacings were 96 cm at LMCRS and NEREC, and 76 cm at PTRS, with plot length at all locations of 7.2 m. Plots were managed using agronomic recommendations provided in the University of Arkansas Soybean Production Handbook (Purcell et al. 2014).The soils at LMCRS, NEREC, and PTRS were a Convent silt loam (fine-silty, mixed, active thermic Typic Glossaqualf), Sharkey silty clay (very-fine, smectitic, thermic Chromic Epiaquert), and Calhoun silt loam (coarse-silty, mixed, superactive, nonacid, thermic, Fluvaquentic Endaquept), respectively (Anonymous 2016). Prior to planting, all seeds received a fungicide seed treatment of mefenoxam+fludioxonil+sedaxane (Cruiser plus Vibrance, Syngenta Crop Protection, LLC, Greensboro, NC) at a rate of 0.075+0.025+0.025 g ai kg^{-1} seed. In addition to fungicides, seeds were treated with either no insecticide or thiamethoxam (Cruiser 5S, Syngenta Crop Protection, LLC, Greensboro, NC) at 0.5 g ai kg^{-1} seed. Both fungicide and insecticide seed treatments were made using a water-based slurry. Herbicide applications were made at planting, using a CO_2-pressurized backpack sprayer calibrated to deliver 143 L ha^{-1} at 276 kPa (Table 1). Seven herbicides that are labeled for use in soybean were applied at, or slightly above, their recommended PRE rates to encourage injurious symptomology. These herbicides included:

metribuzin (841 g ha^{-1}), saflufenacil (75 g ha^{-1}), pyroxasulfone (268 g ha^{-1}), sulfentrazone (533 g ha^{-1}), chlorimuron (79 g ha^{-1}), flumioxazin (107 g ha^{-1}), and chlorimuron+flumioxazin+pyroxasulfone (29+108+136 g ha^{-1}). In addition, two herbicides that commonly cause injury to soybean via carryover - mesotrione (42 g ha^{-1}) and chlorsulfuron (1.8 g ha^{-1}) - were applied at reduced rates to simulate amounts that may be present following applications the previous growing season.

Following application, visual injury ratings were collected weekly on a 0 to 100% scale, where 0% = no injury and 100% = soybean death. In addition, crop density and height measurements were made three weeks after application to allow for adequate germination across the test. Yield data were collected by harvesting the center two rows of each plot and correcting seed moisture to 13%. Data were subjected to analysis of variance, and significant means were separated using Fisher's protected LSD (α=0.05). Site-years were analyzed separately due to considerable variation in environmental conditions at each location (Tables 2-5) and differing responses at each of the sites. For responses that did not produce a significant herbicide by insecticide seed treatment interaction, seed treatment main effects were evaluated. At evaluation timings where no measurable injury was observed for one or more herbicide treatments, the assumptions for ANOVA were not met. When either no interaction was identified or the response did not meet the assumptions for ANOVA, t-tests were conducted to compare treatments with no insecticide to each insecticide seed treatment within a herbicide.

Grain Sorghum Study. Trials were initiated at LMCRS in 2015, and repeated in 2016 at LMCRS, NEREC, and PTRS. Plots established were four rows wide by 7.2 m in length. Row spacing at both LMCRS and NEREC was 96 cm, and row spacing at PTRS was 76 cm. DK-54-00 (Monsanto Company, St. Louis, MO) grain sorghum was planted to a 2.5-cm depth at a density of 222,000 seeds ha^{-1}. Seeds were treated with either no insecticide or thiamethoxam at 2 g ai kg^{-1} seed to produce a two-factor factorial (insecticide by herbicide). In addition to insecticide seed treatments, all seeds included a fungicide seed treatment of mefenoxam (Apron XL, Syngenta Crop Protection, LLC, Greensboro, NC) at 0.075 g ai kg^{-1} seed , plus azostrobin (Dyanasty, Syngenta Crop Protection, LLC, Greensboro, NC) at 0.02 g ai kg^{-1} seed, plus fludioxonil (Maxim 4FS, Syngenta Crop Protection, LLC, Greensboro, NC) at 0.05 g ai kg^{-1} seed. Three herbicides that commonly cause carryover injury in the state of Arkansas were applied PRE to assess whether a safening effect would occur. Fomesafen was applied at 18 and 70 g ai ha^{-1}, in addition to imazethapyr at 13 g ai ha^{-1}. Quizalafop, which is labeled for application in quizalofop-resistant Provisia rice (BASF Corporation, Research Triangle Park, NC), was also applied at 159 g ai ha^{-1} to simulate planting grain sorghum following a rice crop failure. Plots were managed consistently with University of Arkansas agronomic recommendations for grain sorghum production in the state (Espinosa and Kelley 2004).

All herbicides were applied using a CO_2-pressurized backpack sprayer calibrated to deliver 143 L ha^{-1} at 276 kPa. Following application, visual crop injury ratings were made on a 0 to 100% scale, with 0 corresponding to no crop injury and 100 corresponding to sorghum death. Sorghum height and stand density were collected three weeks after application. Plant height was measured by randomly selecting five plants within the two center rows of each plot and measuring distance from soil surface to apical meristem and averaging the five heights. Stand was collected by measuring emerged plants in a meter-long section of each of the two center rows for each plot, with the average of the two numbers representing plot density. Yield was collected by machine harvesting the two center rows from each plot and correcting grain moisture to 13%. Data were subjected to analysis of variance, and means separated using

Fisher’s protected LSD (α=0.05). Site-years were analyzed separately due to considerable variation in environmental conditions at each location (Tables 2-5). For responses that did not produce a significant herbicide by insecticide seed treatment interaction, seed treatment main effects were evaluated. At evaluation timings where no measurable injury was observed for one or more herbicide treatments, the assumptions for ANOVA were not met. When either no interaction was identified or the response did not meet the assumptions for ANOVA, t-tests were conducted to compare treatments with no insecticide to each insecticide seed treatment within a herbicide.

Results and Discussion

Soybean Study. Of the nine herbicides evaluated, six showed reductions in injury in at least one site year. Injury reduction was seen at two site years for saflufenacil, flumioxazin, chlorsulfuron, and flumioxazin+pyroxasulfone+chlorimuron, and at one site year in pyroxasulfone and sulfentrazone. The highest level of safening was for flumioxazin+pyroxasulfone+chlorimuron at NEREC where injury 1 WAE was reduced from 44% to 29% via thiamethoxam seed treatment (Table 6). Similar safening occurred at LMCRS (2016), where injury was reduced 1 WAE from 15% to 5% when treated with thiamethoxam (Table 7). Additionally, injury from flumioxazin+pyroxasulfone+chlorimuron at NEREC 4 WAE was reduced via clothianidin when compared to no insecticide treatment (Table 6). At this particular timing, injury was reduced from 24% to 13%. Injury from saflufenacil was reduced at NEREC 1 and 4 WAE where injury decreased from 56% to 45% and from 67% to 55%, respectively. Soybean was also safened to saflufenacil at PTRS 2 WAE, where injury was reduced from 22% to 15%. Injury from flumioxazin was reduced at LMCRS (2016) at 1 and 2 WAE, where thiamethoxam reduced injury from 13% at both evaluation timings to 8% and 5% at 1 and 2 WAE, respectively (Table 7). Additionally, at PTRS, injury caused by flumioxazin at 2 WAE was reduced from 15% to 8% (Table 4). Chlorsulfuron injury was reduced 1 WAE at LMCRS (2016) from 7% to 3%, and at NEREC 1 WAE from 61% to 53% via thiamethoxam seed treatment (Tables 6 and 7).

Both pyroxasulfone and sulfentrazone were safened at only one of four locations evaluated. Injury from pyroxasulfone was reduced at PTRS 1 and 2 WAE, where injury was reduced from 13% to 4% and from 14% to 5%, respectively. Following applications of sulfentrazone, safening was seen at PTRS 1 WAA, where injury was reduced from 8% to 2% via thiamethoxam seed treatment.

Injury from metribuzin, chlorimuron, and mesotrione was not reduced at any evaluation timing at each of the four locations (Tables 6-9). Similar to studies by McNaughton et al. (2014), soybean injury from chlorimuron, flumioxazin, or pyroxasulfone alone was less than injury seen when the three were combined. Aside from a significant seed treatment main effect at LMCRS (2016), where crop height was increased from 47 cm to 50 cm when treated with thiamethoxam, plant height was not affected by seed treatment (Tables 6-9). Additionally, while safening effects were seen in a number of herbicide-insecticide combinations, crop yield relative to a nontreated check was not increased in these situations (Tables 6-9).

All herbicides evaluated, except for chlorsulfuron and mesotrione, are labeled for use in soybean. As a result, overall soybean injury was low in many cases. Additionally, based on the low levels of injury following application of both metribuzin and sulfentrazone, it is likely that the variety chosen for these studies was tolerant to these herbicides. Choosing a susceptible variety would likely increase crop injury response to these herbicides, which may make the safening benefits associated with insecticide seed treatments more obvious than in this study. In future research, variety selection should be heavily scrutinized in order to select crops that will exhibit high levels of injury.

Grain Sorghum Study. Overall, crop injury in grain sorghum trials was low compared to the soybean trials, with the exception of LMCRS in 2015 (Table 10). In 2016 at LMCRS and NEREC, injury was ≤4% for all herbicide-insecticide combinations at all evaluation timings (Tables 11 and 12). Sorghum injury generally peaked 2 WAE, and dissipated by 4 WAE. With the exception of imazethapyr (20%), injury at PTRS at all evaluation timings was ≤7% (Table 13). Peak injury from quizalofop was similar to that seen by Lancaster et al. (2014), except at LMCRS in 2015. Research by Lancaster et al. (2014) showed an increase in sorghum injury following applications of select graminicides, such as quizalofop, to the soil following activation by a rainfall event. In 2015, LMCRS received 3.1 cm of precipitation the day after herbicide application (Table 2). Similar rainfall events did not occur at any of the other locations, and this difference may help to explain at least some of the increase in injury seen at LMCRS in 2015 compared to the other locations. In future research attempting to simulate herbicide carryover, incorporating the herbicides into the soil profile via a rainfall or irrigation event is recommended in order to produce a crop response.

Similar to findings by Walsh et al. (1993), sorghum injury increased as rate of fomesafen increased. Injury from both rates of fomesafen was lower than that of imazethapyr, indicating sorghum is more sensitive to imazethapyr, consistent with 10- and 18-month plant-back restrictions following applications of fomesafen and imazethapyr, respectively. Regardless of injury, no safening effects were seen through the use of a thiamethoxam seed treatment at any location or evaluation timing in grain sorghum, nor were there any statistical differences in crop density, height, or yield.

Practical Implications. Although injury from saflufenacil, flumioxazin, chlorsulfuron, pyroxasulfone, sulfentrazone, and flumioxazin+pyroxasulfone+chlorimuron was reduced at some locations/evaluation timings in soybean, most combinations did not provide a safening effect. Additionally, even in cases where injury was reduced, yield did not differ among seed treatments within a herbicide. This is consistent with research by Johnson et al. (2002) that determined early-season crop injury is not always a good indicator of end-of-season crop yield. Rather, according to Hagood et al. (1980) and Geier et al. (2009), stand loss is a more predictive measure. Since no significant differences in stand in either crop were seen, it is logical that yield would not differ as well.

In these experiments, overall injury was too low to easily distinguish a safening effect associated with insecticide seed treatments in most cases. However, the fact that insecticide seed treatments caused significant safening, in some cases, under these circumstances may indicate that safening effects will be more discernable in instances of high levels of injury. Future research examining safening effects under cases of higher levels of injury may prove more definitively that these insecticides can provide a safening effect in both soybean and grain sorghum following applications of some soil-applied herbicides.

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Table 1. General description of experimental sites.[a]

Location	Year	Planting date	Application date	Sand	Silt	Clay	pH
					---------------%---------------		
LMCRS	2015	5/14/2015	5/14//2015	0.8	90.5	8.7	7.5
LMCRS	2016	5/5/2016	5/5/2016	0.8	90.5	8.7	7.5
NEREC	2016	4/19/2016	4/19/2016	22	25	53	6.7
PTRS	2016	5/19/2016	5/19/2016	0.4	78.1	21.5	7.8

[a] Abbreviations: LMCRS, Lon Mann Cotton Research Station in Marianna, AR; NEREC, Northeast Research and Extension Center in Keiser, AR; PTRS, Pine Tree Research Station near Colt, AR

Table 2. Environmental conditions at the Lon Mann Cotton Research Station in Marianna, AR in 2015 beginning at planting (May 14).

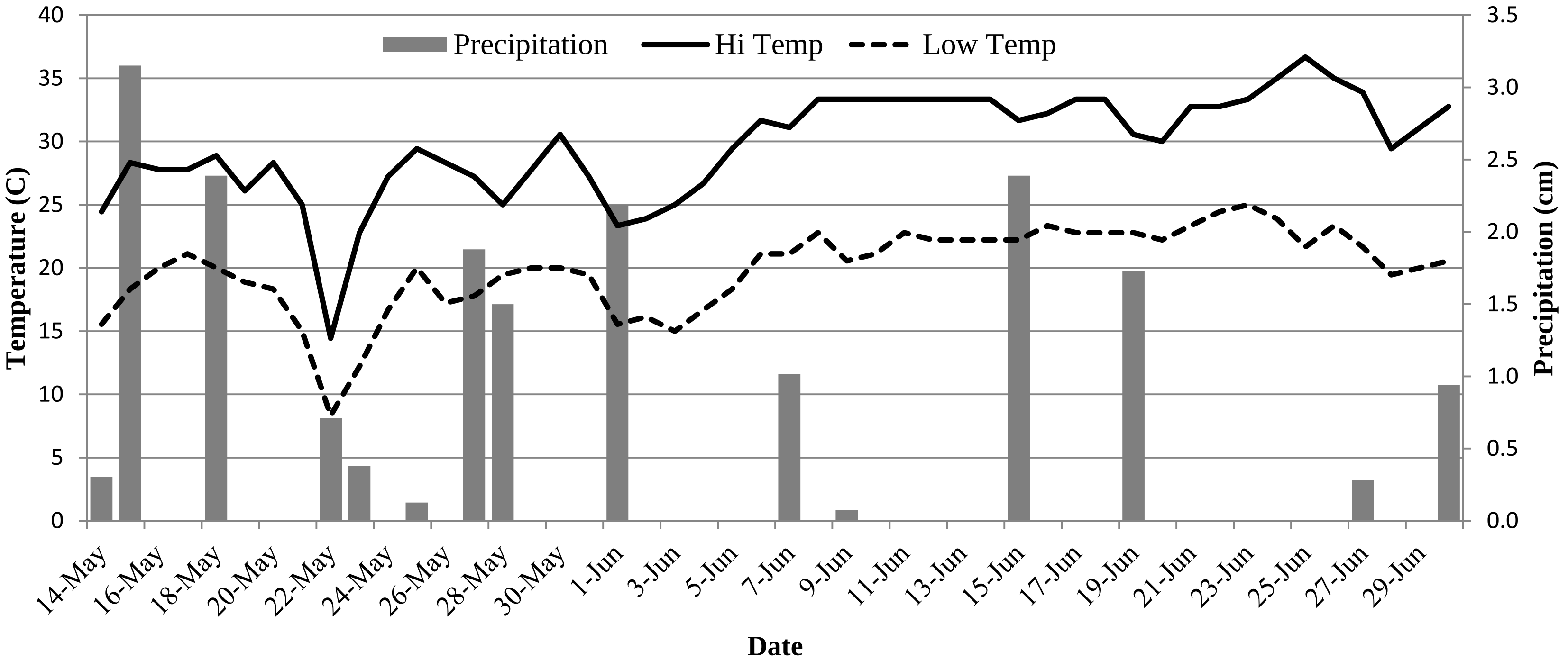

Table 3. Environmental conditions at the Lon Mann Cotton Research Station in Marianna, AR in 2016 beginning at planting (May 5).

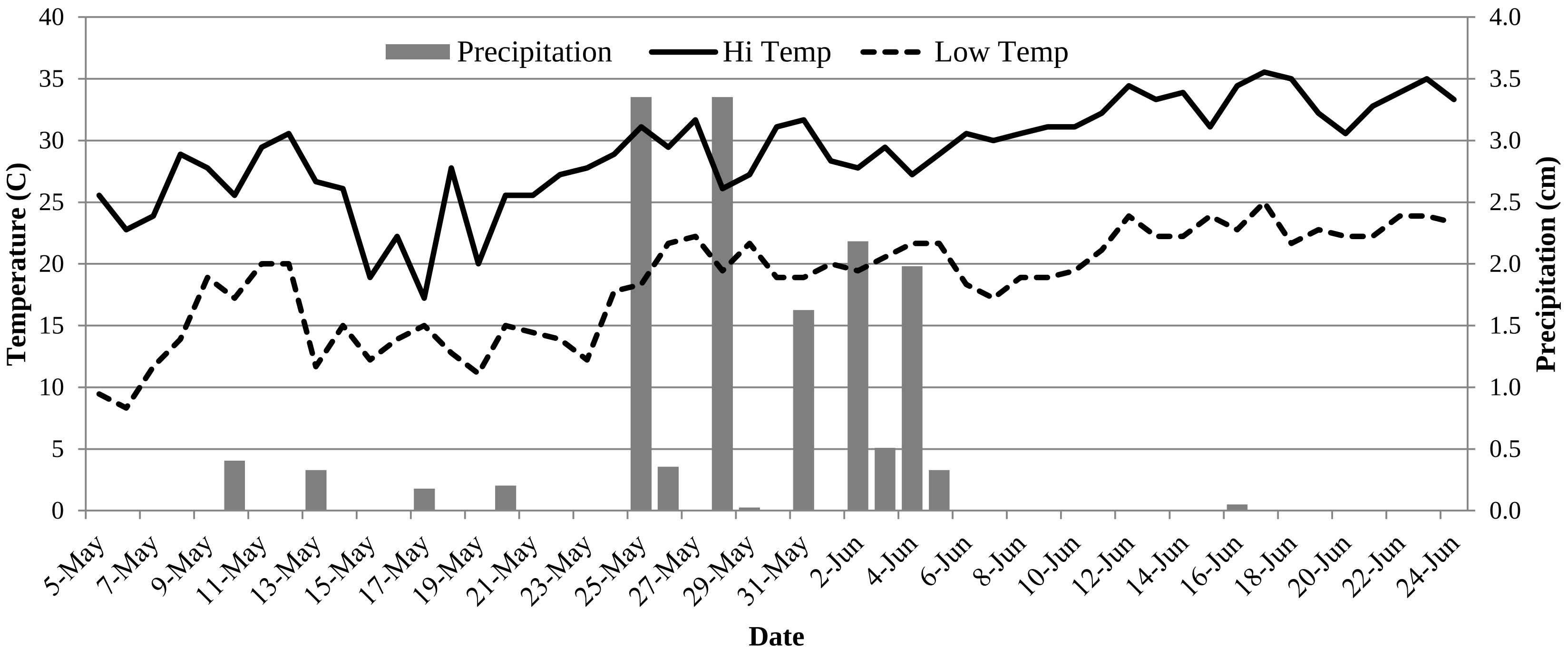

Table 4. Environmental conditions at the Northeast Research and Extension Center in Keiser, AR in 2016 beginning at planting (April 19).

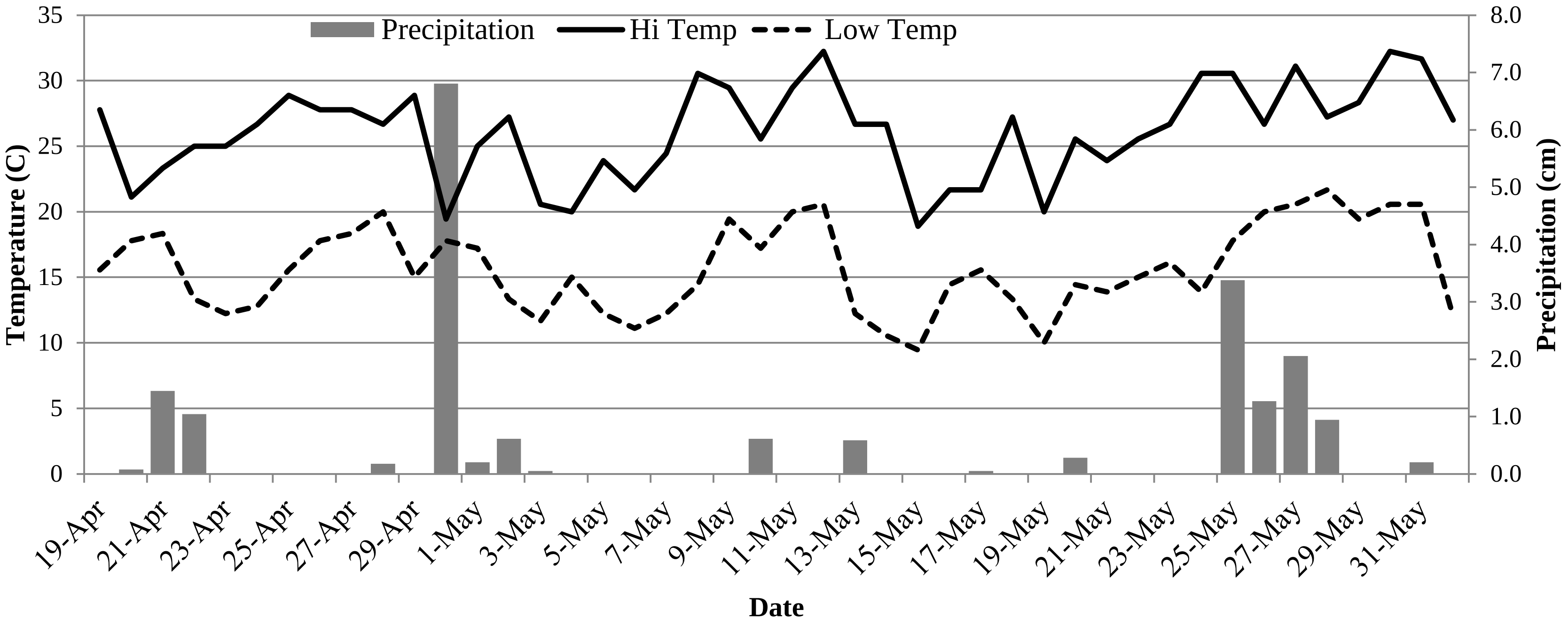

Table 5. Environmental conditions at the Pine Tree Research Station near Colt, AR in 2016 beginning at planting date (May 19).

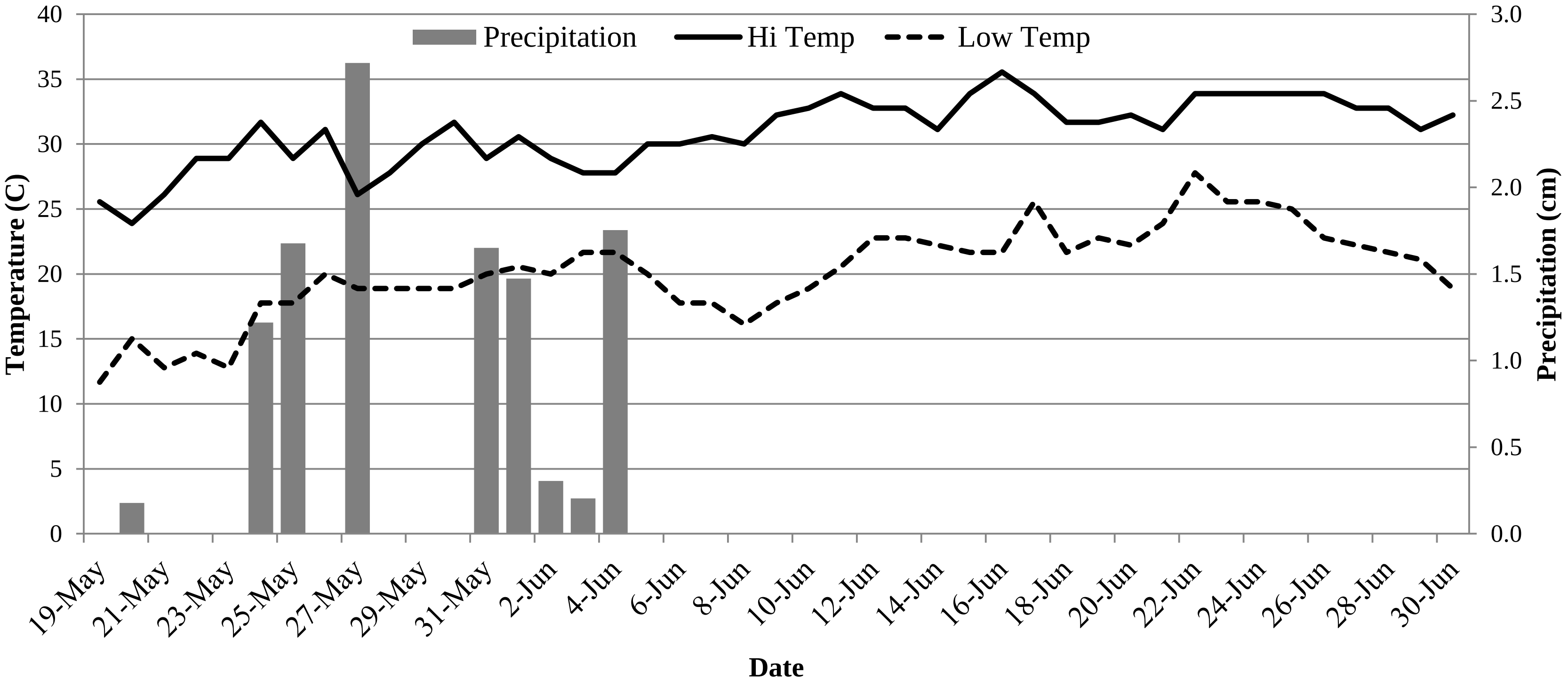

Table 6. Visible soybean injury, height, and yield at NEREC at Keiser, AR in 2016.[a,b]

Herbicide	Seed treatment	Injury 1 WAE	 2 WAE	 4 WAE	Height	Yield
		------------------------- %	-------------------------	-------------------------	cm	kg ha^{-1}
None	None	0	0	0	15	2930
	Thiamethoxam	0	0	0	16	3110
Metribuzin	None	9	5	2	16	3330
	Thiamethoxam	5	6	1	15	3170
Saflufenacil	None	56	67	61	8	2830
	Thiamethoxam	45*	55‡	55	10	2700
Pyroxasulfone	None	5	6	1	16	2930
	Thiamethoxam	3	2	0	16	2660
Sulfentrazone	None	13	6	3	16	2870
	Thiamethoxam	11	5	4	16	3230
Chlorimuron	None	4	6	7	15	2770
	Thiamethoxam	1	6	7	15	2820
Flumioxazin	None	25	15	5	16	2820
	Thiamethoxam	23	8	0	16	2650
Chl+Flu+Pyr	None	44	35	24	14	3790
	Thiamethoxam	29*	25	13‡	15	2640
Mesotrione	None	14	11	6	16	2770
	Thiamethoxam	12	9	3	15	3180
Chlorsulfuron	None	61	91	95	3	280
	Thiamethoxam	53*	87	90	4	100
Main effect[c]	None		26	23	NS	NS
	Thiamethoxam		22†	19†	NS	NS

[a] Abbreviations: NEREC, Northeast Research and Extension Center; WAE, weeks after emergence; NS, non-significant; Chl+Flu+Pyr, Chlorimuron + Flumioxazin + Pyroxasulfone

[b] Means followed by an asterisk indicate a significant herbicide by insecticide interaction ($\alpha=0.05$) or a significant injury reduction via insecticide seed treatment, within the same herbicide, compared to no insecticide. Where no significant interaction is present, insecticide seed treatment main effect is given below. For responses that did not produce a herbicide by insecticide seed treatment interaction, a t-test was conducted to compare treatments with no insecticide to each insecticide seed treatment within an herbicide. Where use of an insecticide seed treatment reduced injury or increased height or yield compared to no insecticide, means are marked with a double dagger (‡)

Table 7. Visible soybean injury, density, height, and yield at the Lon Mann Cotton Research Station in Marianna, AR in 2016.[a,b]

Herbicide	Seed treatment	Injury 1 WAE	 2 WAE	 4 WAE	Density	Height	Yield
		-------------------	%	----------------	plants m^{-1} row	cm	kg ha^{-1}
None	None	0	0	0	27	8	2520
	Thiamethoxam	0	0	0	27	9	2550
Metribuzin	None	1	2	1	25	8	2540
	Thiamethoxam	1	0	0	26	9	2460
Saflufenacil	None	3	14	6	24	8	2400
	Thiamethoxam	1	14	1	26	8	2730
Pyroxasulfone	None	4	2	0	27	9	2630
	Thiamethoxam	1	3	0	26	8	2610
Sulfentrazone	None	1	14	4	25	8	2650
	Thiamethoxam	1	14	3	25	9	2460
Chlorimuron	None	4	5	6	27	8	2380
	Thiamethoxam	3	4	1	25	9	2540
Flumioxazin	None	13	13	5	24	8	2600
	Thiamethoxam	5*	8*	1	24	8	2480
Chl+Flu+Pyr	None	15	17	6	27	8	2620
	Thiamethoxam	5*	12*	1	25	8	2710
Mesotrione	None	10	4	3	25	8	2740
	Thiamethoxam	7	4	0	26	8	2470
Chlorsulfuron	None	5	7	8	27	8	2690
	Thiamethoxam	2	3*	4	27	8	2480
Main effect[e]	None			4	NS	NS	NS
	Thiamethoxam			1†	NS	NS	NS

[a] Abbreviations: WAE, weeks after emergence; NS, non-significant; Chl+Flu+Pyr, Chlorimuron + Flumioxazin + Pyroxasulfone

[b] Means followed by an asterisk indicate a significant herbicide by insecticide interaction ($\alpha=0.05$) or a significant injury reduction via insecticide seed treatment, within the same herbicide, compared to no insecticide. Where no significant interaction is present, insecticide seed treatment main effect is given below. For responses that did not produce a herbicide by insecticide seed treatment interaction, a t-test was conducted to compare treatments with no insecticide to each insecticide seed treatment within an herbicide. Where use of an insecticide seed treatment reduced injury or increased height or yield compared to no insecticide, means are marked with a double dagger (‡)

Table 8. Visible soybean injury, density, and yield at the Pine Tree Research Station near Colt, AR in 2016[a].

Herbicide	Seed treatment	Injury[b]			Density	Yield
		1 WAE	2 WAE	4 WAE		
		----------------------------	%	----------------------------	plants m^{-1} row	kg ha^{-1}
None	None	0	0	0	16	2770
	Thiamethoxam	0	0	0	20	2930
Metribuzin	None	6	9	7	19	2700
	Thiamethoxam	0	6	0	18	3130
Saflufenacil	None	12	22	5	17	2950
	Thiamethoxam	9	15‡	6	18	2780
Pyroxasulfone	None	13	14	6	18	3000
	Thiamethoxam	4‡	5‡	5	17	3210
Sulfentrazone	None	8	13	0	18	3180
	Thiamethoxam	2‡	8	3	19	3040
Chlorimuron	None	8	10	1	17	2300
	Thiamethoxam	8	7	3	15	2810
Flumioxazin	None	9	15	10	19	3090
	Thiamethoxam	5	8‡	5	19	3170
Chl+Flu+Pyr	None	18	19	6	19	2850
	Thiamethoxam	15	15	5	19	2930
Mesotrione	None	9	9	5	20	2970
	Thiamethoxam	8	5	6	19	3050
Chlorsulfuron	None	3	10	8	20	2860
	Thiamethoxam	6	5	5	19	2730
Main effect	None	9	13	NS	NS	NS
	Thiamethoxam	6†	8†	NS	NS	NS

[a] Abbreviations: WAE, weeks after emergence; NS, non-significant; Chl+Flu+Pyr, Chlorimuron + Flumioxazin + Pyroxasulfone

[b] Where no significant interaction ($\alpha=0.05$) is present, insecticide seed treatment main effect is given below. For responses that did not produce a herbicide by insecticide seed treatment interaction, a t-test was conducted to compare treatments with no insecticide to each insecticide seed treatment within an herbicide. Where use of an insecticide seed treatment reduced injury or increased height or yield compared to no insecticide, means are marked with a double dagger (‡)

Table 9. Visible soybean injury, density, height, and yield at the Lon Mann Cotton Research Station in Marianna, AR in 2015.[a]

		Injury[b]					
Herbicide	Seed treatment	1 WAE	2 WAE	4 WAE	Density	Height[c]	Yield
		------------------ % ----------------			plants m^{-1} row	cm	kg ha^{-1}
None	None	0	0	0	21	57	3890
	Thiamethoxam	0	0	0	23	58	3740
Metribuzin	None	2	5	3	19	59	3900
	Thiamethoxam	0	6	3	22	62	3720
Saflufenacil	None	15	29	13	17	51	4040
	Thiamethoxam	14	24	15	18	57	3640
Pyroxasulfone	None	14	24	14	19	53	3650
	Thiamethoxam	10	25	11	22	53	3800
Sulfentrazone	None	24	43	24	15	47	3740
	Thiamethoxam	21	40	21	17	48	3500
Chlorimuron	None	3	6	4	20	38	3790
	Thiamethoxam	1	4	3	23	45	3820
Flumioxazin	None	2	1	3	21	57	3700
	Thiamethoxam	1	0	1	22	61	3770
Chl+Flu+Pyr	None	28	49	39	15	39	3250
	Thiamethoxam	26	48	41	13	41	3290
Mesotrione	None	1	13	3	20	57	3880
	Thiamethoxam	1	9	3	20	58	4050
Chlorsulfuron	None	18	53	83	21	12	1870
	Thiamethoxam	13	51	81	21	12	1350
Main effect	None	12	NS	NS	NS	47	NS
	Thiamethoxam	10†	NS	NS	NS	50†	NS

[a] Abbreviation: WAE, weeks after emergence; NS, non-significant; Chl+Flu+Pyr, Chlorimuron + Flumioxazin + Pyroxasulfone

[b] Where no significant interaction ($\alpha=0.05$) is present, insecticide seed treatment main effect is given below and a significant main effect is denoted with a cross (†)

Table 10. Grain sorghum injury, density, height, and yield at the Lon Mann Cotton Research Station in Marianna, AR in 2015.[a]

		Injury					
Herbicide	Seed treatment	1 WAE	2 WAE	4 WAE	Density	Height	Yield
		--------------------%	--------------------		plants m^{-1} row	cm	kg ha^{-1}
None	None	0	0	0	20	144	6720
	Thiamethoxam	0	0	0	21	144	6000
Fomesafen Lo	None	8	18	16	20	144	6500
	Thiamethoxam	6	15	8	20	145	6120
Fomesafen Hi	None	20	29	21	18	144	5540
	Thiamethoxam	20	24	16	19	148	5800
Imazethapyr	None	10	30	21	21	143	5810
	Thiamethoxam	10	28	20	22	137	5450
Quizalofop	None	95	92	92	2	127	2180
	Thiamethoxam	92	90	87	2	120	1800
Main effect	None	NS	NS	NS	NS	NS	NS
	Thiamethoxam	NS	NS	NS	NS	NS	NS

[a] Abbreviations: WAE, weeks after emergence; NS, non-significant; Lo, low; Hi, High

[b] Mean separation showed no significant difference at the α = 0.05 level for injury, height, or yield among insecticide seed treatments, within herbicides.

Table 11. Grain sorghum injury, density, height, and yield at the Lon Mann Cotton Research Station in Marianna, AR in 2016.[a,b]

Herbicide	Seed treatment	Injury[a]			Density	Height	Yield
		1 WAE	2 WAE	4 WAE			
		--------------------% -------------------			plants m^{-1} row	cm	kg ha^{-1}
None	None	0	0	0	14	9	4270
	Thiamethoxam	0	0	0	17	9	4590
Fomesafen Lo	None	0	0	0	14	9	4210
	Thiamethoxam	0	0	0	17	9	4190
Fomesafen Hi	None	4	0	1	16	9	3350
	Thiamethoxam	0	0	0	15	9	4000
Imazethapyr	None	14	4	1	14	8	4200
	Thiamethoxam	0	0	0	17	9	3780
Quizalofop	None	0	0	0	17	9	4130
	Thiamethoxam	0	0	0	19	9	4350
Main effect	None	NS	NS	NS	NS	NS	NS
	Thiamethoxam	NS	NS	NS	NS	NS	NS

[a] Abbreviations: WAE, weeks after emergence; NS, non-significant; Lo, low; Hi, High

[b] Mean separation showed no significant difference at the α = 0.05 level for injury, height, or yield among insecticide seed treatments, within herbicides.

Table 12. Grain sorghum injury, density, height, and yield at the Northeast Research and Extension Center in Keiser, AR in 2016.[a,b]

		Injury[a]					
Herbicide	Seed treatment	1 WAE	2 WAE	4 WAE	Density	Height	Yield
		-----------------------------	%	----------------------	plants m^{-1} row	cm	kg ha^{-1}
None	None	0	0	0	24	10	4670
	Thiamethoxam	0	0	0	24	10	4970
Fomesafen Lo	None	0	0	0	23	10	4670
	Thiamethoxam	1	0	0	25	11	3820
Fomesafen Hi	None	1	0	0	25	10	5480
	Thiamethoxam	0	1	0	25	10	5130
Imazethapyr	None	2	2	1	25	10	5310
	Thiamethoxam	0	0	0	25	10	5520
Quizalofop	None	2	1	1	25	10	5710
	Thiamethoxam	0	0	0	24	9	5790
Main effect	None	NS	NS	NS	NS	NS	NS
	Thiamethoxam	NS	NS	NS	NS	NS	NS

[a] Abbreviations: WAE, weeks after emergence; NS, non-significant; Lo, low; Hi, High

[b] Mean separation showed no significant difference at the $\alpha = 0.05$ level for injury, height, or yield among insecticide seed treatments, within herbicides.

Table 13. Grain sorghum injury, density, height, and yield at the Pine Tree Research Station near Colt, AR in 2016.[a,b]

Herbicide	Seed treatment	Injury[a] 1 WAE	2 WAE	4 WAE	Density	Height	Yield
		---------------------------%		-----------------------	plants m^{-1} row	cm	kg ha^{-1}
None	None	0	0	0	22	8	5710
	Thiamethoxam	0	0	0	22	10	5890
Fomesafen Lo	None	0	3	0	19	8	4120
	Thiamethoxam	0	3	0	23	8	5050
Fomesafen Hi	None	0	6	1	18	8	5310
	Thiamethoxam	0	1	0	20	8	4830
Imazethapyr	None	10	20	13	18	7	4870
	Thiamethoxam	3	11	6	20	9	4860
Quizalofop	None	5	7	3	18	7	5080
	Thiamethoxam	3	6	0	21	8	4760
Main effect	None	NS	NS	NS	NS	NS	NS
	Thiamethoxam	NS	NS	NS	NS	NS	NS

[a] Abbreviations: WAE, weeks after emergence; NS, non-significant; Lo, low; Hi, High

[b] Mean separation showed no significant difference at the α = 0.05 level for injury, height, or yield among insecticide seed treatments, within herbicides.

Chapter 4

Use of Insecticide Seed Treatments as Safeners to Injurious Postemergence Herbicides in Soybean

Abstract

Applications of postemergence (POST) herbicides are an important part of weed control in soybean; however, some crop injury can accompany these applications. While uncommon, this injury can result in yield loss depending upon the severity and timing of application. Herbicide safeners may offer a potential solution to this issue, but currently no effective safeners exist for use in soybean production. Field trials were conducted in Arkansas in 2015 and 2016 to determine whether a safening effect could be seen using thiamethoxam in soybean following applications of POST herbicides that commonly cause crop injury. Chlorimuron, fomesafen, and 2,4-DB were applied 21 days after planting. In Marianna in 2016, injury from chlorimuron and fomesafen was reduced 8 and 7 percentage points, respectively, in thiamethoxam-treated seed. At the same location and evaluation timing, clothianidin-treated seed reduced injury from 2,4-DB by 8 percentage points 1 week after application (WAA). Additionally, a seed treatment main effect was seen at Marianna in 2016 both 2 WAA and 4 WAA, where a thiamethoxam seed treatment reduced injury 5 and 6 percentage points, respectively, averaged across all herbicides. Aside from Marianna in 2016, visible injury was not reduced at any location or evaluation timing. Height was not affected by seed treatment at any location, nor was yield. Based on the results from these experiments, the insecticides clothianidin and thiamethoxam, applied as a seed treatment, are unlikely to successfully safen the evaluated POST-applied herbicides that are often injurious to soybean.

Nomenclature: 2,4-DB; clothianidin; chlorimuron; fomesafen; imidacloprid; thiamethoxam; soybean, *Glycine max* (L.) Merr.

Key words: Postemergence herbicides, herbicide safeners

Introduction

Weed control in soybean production is a vital part of producing a high-yielding crop. Krausz et al. (2001) found that season-long competition from weeds reduced grain yield 68% in a glyphosate-resistant soybean cultivar. Since 94% of soybean hectares in the U.S. contain a herbicide resistance trait, and the vast majority of that percentage is resistant to glyphosate, the need for effective weed management strategies is clear (USDA-NASS 2016). A survey of crop consultants across the Midsouth showed that production practices in the state of Arkansas follow these national trends, with 95% of soybean planted in the state containing a herbicide resistance trait, 88% of which is resistant to glyphosate (Riar et al. 2013). The same study indicates that Palmer amaranth (*Amaranthus palmeri* S. Wats.) and morningglories (*Ipomoea* spp) are the two most problematic weeds for soybean producers in the state. Historically, one or two POST glyphosate applications have been sufficient for control of most weeds in glyphosate-resistant cropping systems; however, these two weeds present unique challenges to growers wishing to continue this practice (Bradshaw et al. 2007; Culpepper and York 1998). Repeated applications of glyphosate in Midsouthern states have resulted in widespread resistance to the herbicide in Palmer amaranth populations, rendering it ineffective (Nichols et al. 2009). In addition, glyphosate efficacy on morningglories is generally marginal, and can differ based on species and population (Jordan et al. 1997; Scott et al. 2017; Stephenson 2007). Due to this limited control of the two most problematic weeds offered via POST applications of glyphosate, more effective herbicide options are needed for soybean production.

Fomesafen, chlorimuron, and 2,4-DB are herbicides that are labeled for use in soybean, and have the potential to control some problematic weeds, including Palmer amaranth and morningglories. Fomesafen (Reflex, Flexstar, Syngenta Crop Protection, Greensboro, NC) is a protoporphyrinogen oxidase (PPO)-inhibiting, diphenylether herbicide labeled for both preemergence (PRE) and POST applications in soybean (Anonymous 2016). Fomesafen offers excellent control of both Palmer amaranth and morningglories, in addition to a number of other broadleaf weeds (Scott et al. 2017). Chlorimuron (Classic, DuPont Crop Protection, Wilmington, DE) is an acetolactate synthase (ALS)-inhibiting, sulfonylurea herbicide that is also labeled for PRE and POST applications in soybean (Anonymous 2012). Although acetolactate synthase (ALS)-resistant Palmer amaranth populations exist in much of the Midsouth, chlorimuron does provide some control of susceptible populations (Heap 2017; Scott et al. 2017). However, chlorimuron is most notably known for its efficacy on morningglory populations, often providing greater than 90% control (Stephenson et al. 2007). 2,4-DB (Butyrac 200, Albaugh, LLC, Ankeny, IA) is a phenoxy herbicide labeled for POST application in soybean that also provides excellent control of morningglories (Anonymous 2016; Scott et al. 2017).

While these three herbicides are labeled for use in soybean, crop injury can result following application. Vidrine et al. (2002) reported soybean injury as high as 30% in herbicide mixtures containing chlorimuron. Johnson et al. (2002) and Harris et al. (1991) reported that 15% soybean injury from fomesafen is common. Soybean injury potential is relatively high following applications of 2,4-DB, and Culpepper et al. (2001) found that injury ranged from 7% to 27% following POST applications. Although injury from early-season herbicide applications may not be an entirely dependable predictor, yield loss can be expected from all three herbicides if symptomology persists (Hagood et al. 1980).

One possible method for reducing herbicide injury and subsequent yield loss is the use of herbicide safeners. Safeners typically increase a plant’s ability to metabolize a herbicide, and their utility can be maximized when applied as treatments to seeds prior to planting (Abu-Qare and Duncan; Davies and Caseley 1999 Hatzios and Burgos 2004). Miller et al. (2016) showed that neonicotinoid insecticide seed treatments provided a safening effect from drift of some POST herbicides in rice. Exploiting a similar effect in soybean could provide an added benefit to soybean producers with concerns for injury following POST applications. Hence, the objective of this research was to determine whether soybean injury from chlorimuron, fomesafen, and 2,4-DB could be reduced through the use of clothianidin and thiamethoxam seed treatments.

Materials and Methods

A field trial was conducted at the Lon Mann Cotton Research Station (LMCRS) in Marianna, Arkansas, in 2015. The experiment was repeated in 2016 at LMCRS, in addition to the Northeast Research and Extension Center (NEREC) in Keiser, Arkansas, and the Pine Tree Research Station (PTRS) near Colt, Arkansas. The soil was a Convent silt loam (fine-silty, mixed, active thermic Typic Glossaqualf) at LMCRS, Calhoun silt loam (Coarse-silty, mixed, superactive, nonacid, thermic Fluvaquentic Endoaquepts) at PTRS, and Sharkey silty clay (very-fine, smectitic, thermic Chromic Epiaquerts) at NEREC (Anonymous 2016). Plots were established at each location as two-factor (herbicide x insecticide), randomized complete block designs with four replications. Plots were four rows wide and measured 11 m in length. Row width at both LMCRS and NEREC were 96 cm, and 78 cm at PTRS, consistent with cultural production practices at each location. DG5067LL (Delta Grow Seed Company Inc., England, AR), a maturity group 5.2, glufosinate-resistant, non-STS soybean, was planted at a seeding rate of 340,000 seeds ha^{-1} at a 2.5-cm depth. Plots were maintained weed-free by making an application of flumioxazin at 71 g ai ha^{-1} (Valor SX, Valent U.S.A. Corporation, Walnut Creek, CA), with later escapes controlled with glufosinate at 0.53 g ai ha^{-1} and via hand removal.

Prior to planting, all soybean seeds were treated with a fungicide combination of mefenoxam+fludioxonil+sedaxane (0.075+0.025+0.025 g ai kg^{-1} seed) via a water-based slurry. In addition, seeds were either treated with thiamethoxam (0.5 g ai kg^{-1} seed), clothianidin (0.5 g ai kg^{-1} seed), or no insecticide. Herbicide applications were made 21 days after planting (DAP) using a CO_2-pressurized backpack sprayer calibrated to deliver 143 L ha^{-1} at 276 kPa (Table 1). Three herbicides that commonly cause phytotoxicity in soybean following application, chlorimuron, fomesafen, and 2,4-DB, were applied. In an effort to promote soybean injury, herbicides were applied at 13 g ai ha^{-1}, 1186 g ai ha^{-1}, and 505 g ae ha^{-1} for chlorimuron, fomesafen, and 2,4-DB, respectively. These application rates correspond with the highest recommended use rate in soybean for chlorimuron, and approximately 1.5x and 2x rates for fomesafen and 2,4-DB, respectively.

Following application, visible injury ratings were collected weekly for four weeks. Injury was rated on a 0 to 100% scale, where 0% equals no injury and 100% equals crop death. Distance from soil surface to the apical meristem of five randomly selected soybean plants was measured from the center rows of each plot at harvest in 2015 and at 2 WAA in 2016. Grain yield was collected at the end of the growing season by harvesting the middle two rows and correcting moisture to 13%. Data collected were subjected to analysis of variance (ANOVA) using JMP 12.1 (SAS Institute, Cary, NC) with site-years analyzed separately due to variation in environmental conditions at each location (Tables 2-5). Means were separated using Fisher's protected LSD (α=0.05).

Results and Discussion

A significant (p=0.029) seed treatment by herbicide interaction was seen 1 WAA at LMCRS in 2016. Soybean injury from chlorimuron and fomesafen was reduced 8 and 7 percentage points, respectively, when seeds were treated with thiamethoxam, and 8 percentage points when treated with clothianidin at this evaluation timing (Table 6). At 2 and 4 WAA, no interaction was present, but an insecticide main effect did occur. Injury, averaged across all herbicides, was reduced 5 percentage points 2 WAA and 6 percentage points 4 WAA in thiamethoxam-treated seed. With the exception of injury reduction at LMCRS in 2016, no other safening effects were seen (Tables 7, 8, 9).

Neither crop height nor yield was affected by seed treatment at any location (Tables 2-5). However, a significant (p<0.001) herbicide main effect was seen at LMCRS in 2015 and at PTRS in 2016. At these locations, applications of 2,4-DB resulted in lower yields compared to the no herbicide treatments, averaged across all seed treatments. In contrast, yields following applications of fomesafen and chlorimuron were not reduced. This can likely be attributed to the numerically higher injury ratings following 2,4-DB applications, which generally persisted at higher levels, even at the 4 WAA evaluation timing.

Based on the fact that injury reduction, although statistically significant, was minimal, and that neither crop height nor yield was increased when seed treatments were included, it is unlikely that the insecticide seed treatments thiamethoxam or clothianidin can be used as effective safeners in soybean for POST application of the three herbicides evaluated. The lack of safening may be partly attributed to the fact that in-plant concentrations of insecticides are greatly diminished at the time of typical POST applications. However, in general, the lack of utility of herbicide safeners in dicots has been noted previously (Bailey et al. 2015; Davies and

Caseley 1999; Hatzios 1989; Hatzios and Burgos 2004; Hatzios and Wu 1996; Riechers 2010). The majority of literature available indicates that the effective use of safeners is primarily limited to monocotyledonous crops, and that the use of safeners in soybean is unlikely. Findings from this experiment supports an overall lack of safening in soybean.

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Table 1. General description of experimental sites.[a]

Location	Year	Planting date	Application date	Sand	Silt	Clay	pH
					---------------%---------------		
LMCRS	2015	5/14/2015	6/8/2015	0.8	90.5	8.7	7.5
LMCRS	2016	5/5/2016	5/26/2016	0.8	90.5	8.7	7.5
NEREC	2016	4/19/2016	5/20/2016	22	25	53	6.7
PTRS	2016	5/19/2016	6/8/2016	0.4	78.1	21.5	7.8

[a] Abbreviations: LMCRS, Lon Mann Cotton Research Station in Marianna, AR; NEREC, Northeast Research and Extension Center in Keiser, AR; PTRS, Pine Tree Research Station near Colt, AR

Table 2. Environmental conditions at the Lon Mann Cotton Research Station in Marianna, AR in 2015 beginning at planting (May 14), with herbicide application date marked with an asterisk.

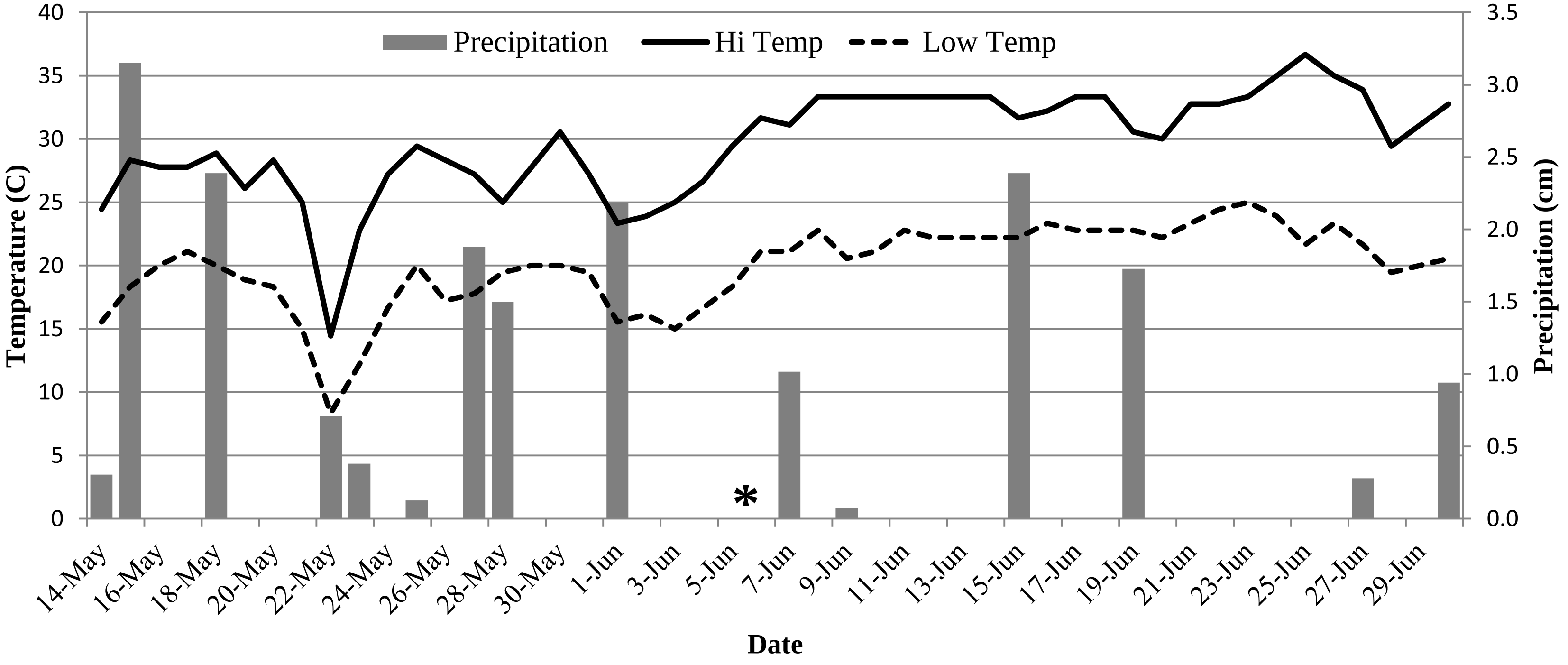

Table 3. Environmental conditions at Lon Mann Cotton research Station in Marianna, AR in 2016 beginning at planting (May, 5), with herbicide application date marked with an asterisk.

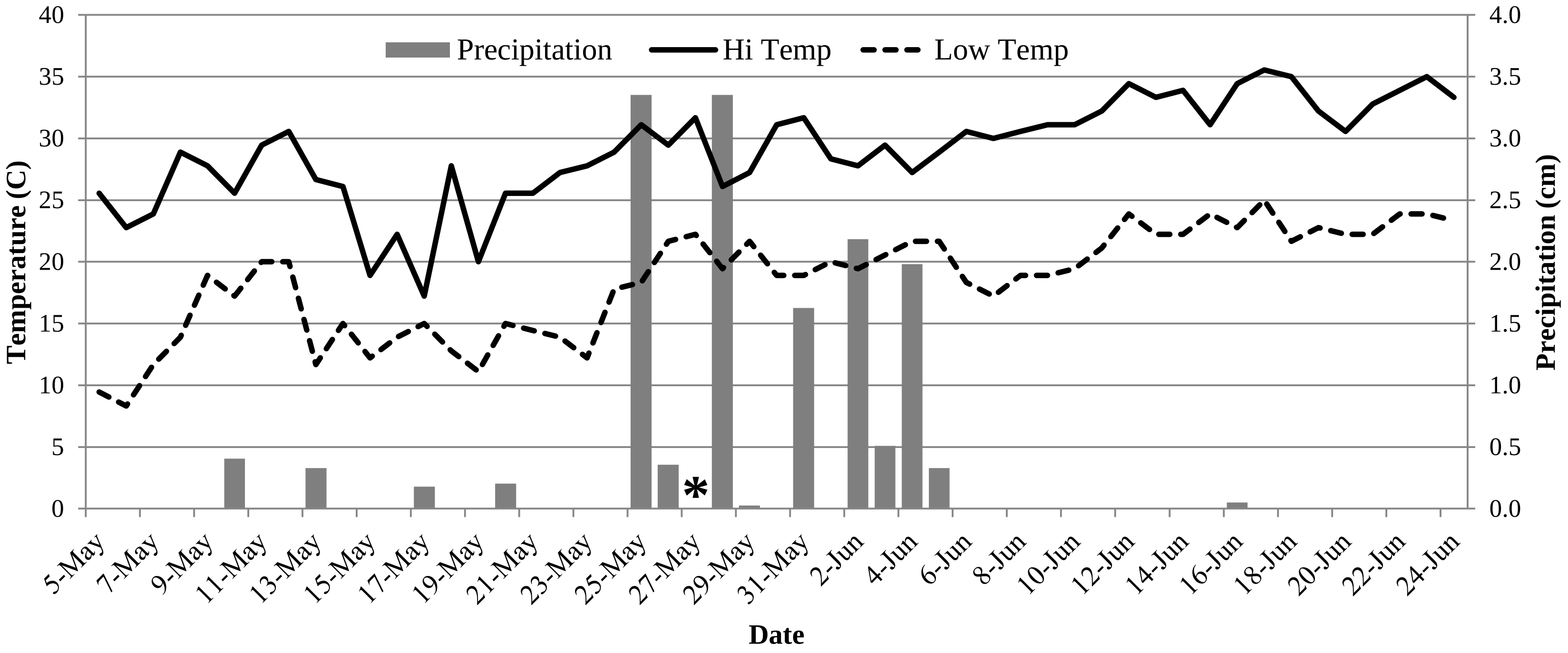

Table 4. Environmental conditions at the Northeast Research and Extension Center in Keiser, AR in 2016 beginning at planting (April 19), with herbicide application date marked with an asterisk.

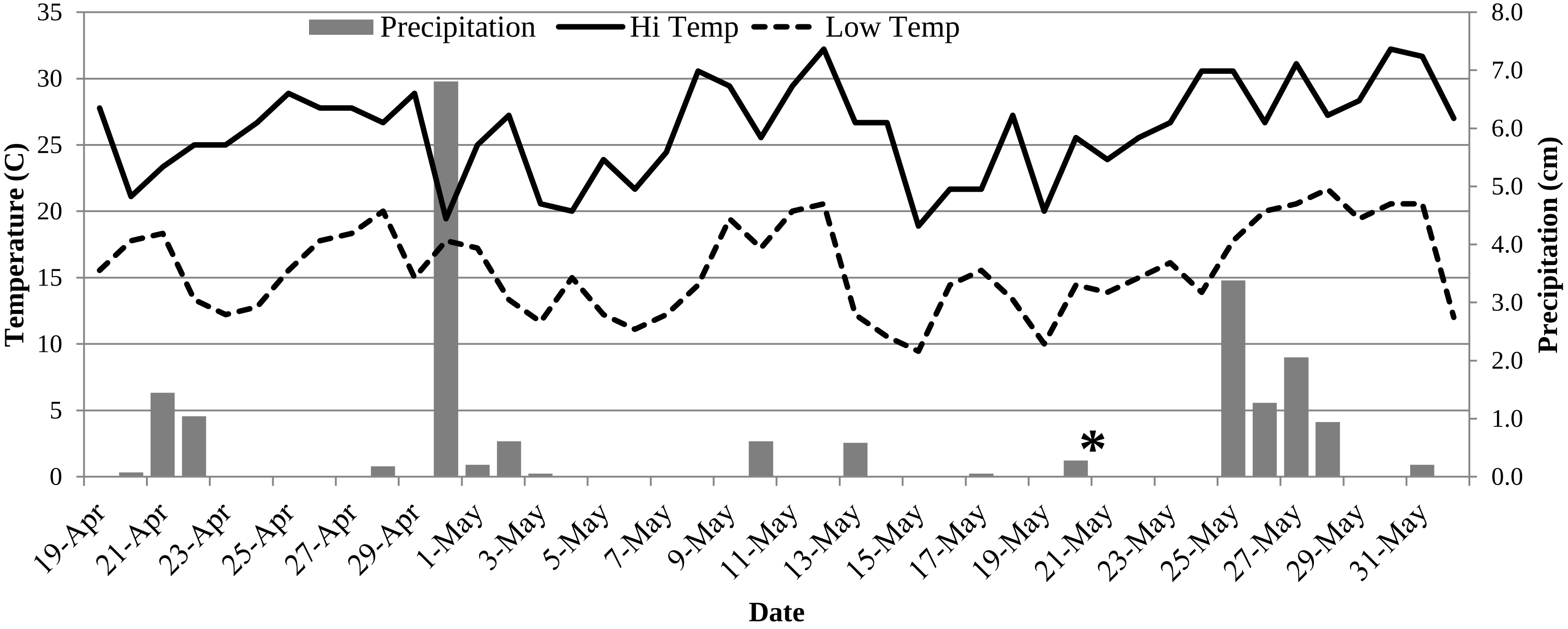

Table 5. Environmental conditions at the Pine Tree Research Station near Colt, AR in 2016 beginning at planting (May 19), with herbicide application date marked with an asterisk.

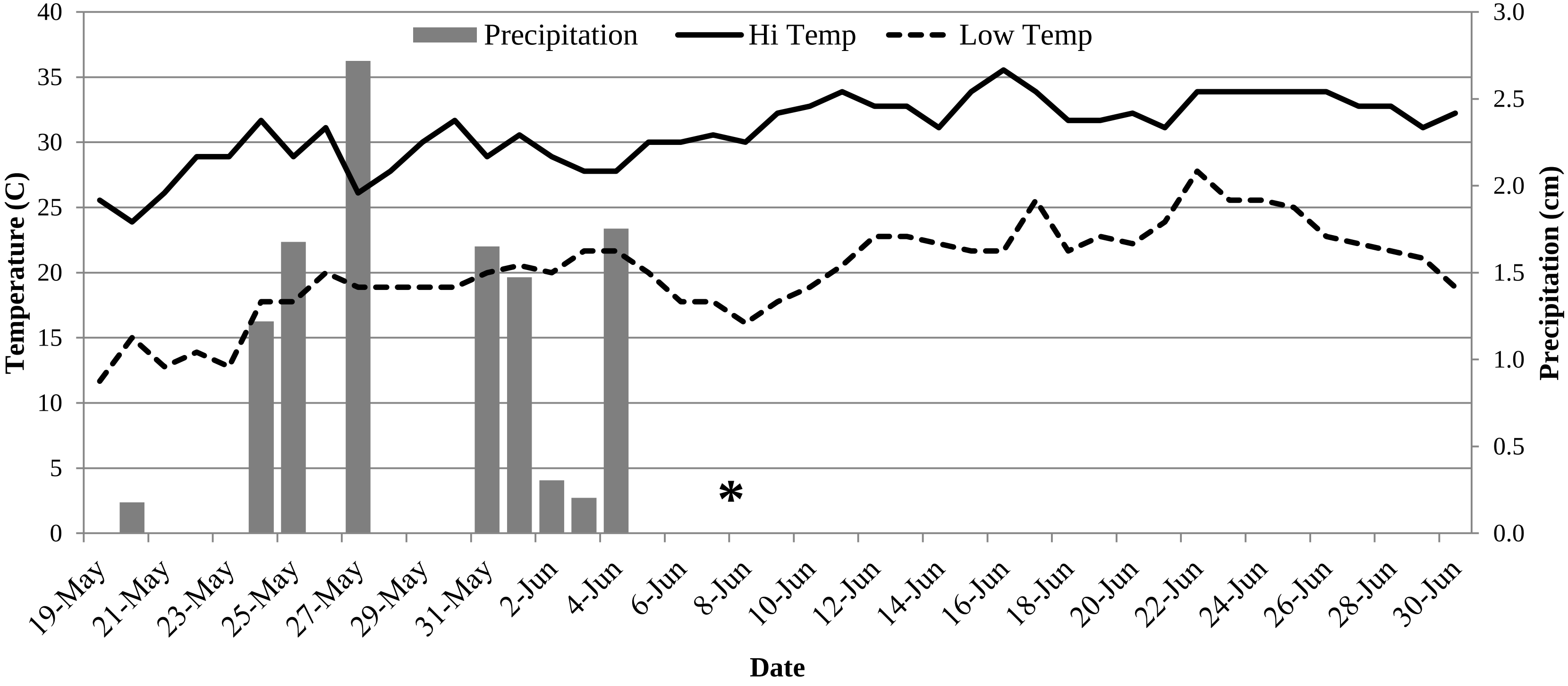

Table 6. Soybean injury, height, and yield at the Lon Mann Cotton Research Station in Marianna, AR in 2016.[a,b]

		Injury				
Herbicide	Seed treatment	1 WAA	2 WAA	4 WAA	Height	Yield
		---------------------------- %	----------------------------	----------------------------	cm	kg ha^{-1}
None	None	0	0	0	15	2310
	Thiamethoxam	0	0	0	14	2550
	Clothianidin	0	0	0	15	2530
Chlorimuron	None	18	13	8	13	2310
	Thiamethoxam	10*	5	2	13	2590
	Clothianidin	16	8	4	13	2610
Fomesafen	None	39	15	11	12	2420
	Thiamethoxam	38	10	6	13	2490
	Clothianidin	31*	15	10	13	2430
2,4-DB	None	31	24	20	13	2480
	Thiamethoxam	23*	20	13	12	2480
	Clothianidin	34	24	21	12	2610
Main effect	None		17	13	NS	NS
	Thiamethoxam		12†	7†	NS	NS
	Clothianidin		15	12	NS	NS

[a] Abbreviations: WAA, weeks after application; NS, non-significant

[b] Means followed by an asterisk indicate a significant herbicide by insecticide interaction or a significant injury reduction via insecticide seed treatment, within the same herbicide, compared to no insecticide according to Fisher's protected LSD ($\alpha = 0.05$). Where no significant interaction is present, insecticide seed treatment main effect is given below, with a cross indicating a significant main effect using the same criteria.

Table 7. Soybean injury, height, and yield at the Lon Mann Cotton Research Station in Marianna, AR in 2015.[a,b]

		Injury				
Herbicide	Seed treatment	1 WAA	2 WAA	4 WAA	Height	Yield
		--------------------------	%	----------------------------	cm	kg ha^{-1}
None	None	0	0	0	51	3700
	Thiamethoxam	0	0	0	53	4060
	Clothianidin	0	0	0	52	3690
Chlorimuron	None	21	11	6	47	3710
	Thiamethoxam	26	15	10	49	3750
	Clothianidin	27	11	3	47	3820
Fomesafen	None	16	4	0	55	3750
	Thiamethoxam	20	6	0	55	3580
	Clothianidin	18	7	3	55	3670
2,4-DB	None	34	48	35	38	2530
	Thiamethoxam	33	48	35	36	2660
	Clothianidin	32	47	34	41	2400
Main effect	None	NS	NS	NS	NS	NS
	Thiamethoxam	NS	NS	NS	NS	NS
	Clothianidin	NS	NS	NS	NS	NS

[a] Abbreviations: WAA, weeks after application; NS, non-significant

[b] Mean separation showed no significant difference at the $\alpha = 0.05$ level for injury, height, or yield among insecticide seed treatments, within herbicides.

Table 8. Soybean injury, height, and yield at the Northeast Research and Extension Center in Keiser, AR in 2016.[a,b]

		Injury			
Herbicide	Seed treatment	1 WAA	2 WAA	Height	Yield
		--- % ---		cm	kg ha^{-1}
None	None	0	0	30	2650
	Thiamethoxam	0	0	31	2950
	Clothianidin	0	0	32	2970
Chlorimuron	None	14	9	31	2710
	Thiamethoxam	13	8	31	2820
	Clothianidin	14	8	30	3080
Fomesafen	None	15	9	29	2610
	Thiamethoxam	15	8	31	2820
	Clothianidin	20	10	29	3000
2,4-DB	None	41	55	24	3100
	Thiamethoxam	40	58	23	2970
	Clothianidin	40	53	22	2880
Main effect	None	NS	NS	NS	NS
	Thiamethoxam	NS	NS	NS	NS
	Clothianidin	NS	NS	NS	NS

[a] Abbreviations: WAA, weeks after application; NS, non-significant

[b] Mean separation showed no significant difference at the $\alpha = 0.05$ level for injury, height, or yield among insecticide seed treatments, within herbicides.

Table 9. Soybean injury and yield at the Pine Tree Research Station near Colt, AR in 2016.[a,b]

		Injury			
Herbicide	Seed treatment	1 WAA	2 WAA	4 WAA	Yield
		------------------------- % -------------------------			kg ha^{-1}
None	None	0	0	0	3180
	Thiamethoxam	0	0	0	3620
	Clothianidin	0	0	0	3300
Chlorimuron	None	26	18	9	3360
	Thiamethoxam	21	15	13	2890
	Clothianidin	21	16	9	2620
Fomesafen	None	13	1	0	3650
	Thiamethoxam	13	3	0	3530
	Clothianidin	13	5	1	3380
2,4-DB	None	28	30	30	2820
	Thiamethoxam	27	29	30	2640
	Clothianidin	30	30	35	1960
Main effect	None	NS	NS	NS	NS
	Thiamethoxam	NS	NS	NS	NS
	Clothianidin	NS	NS	NS	NS

[a] Abbreviations: WAA, weeks after application; NS, non-significant

[b] Mean separation showed no significant difference at the α = 0.05 level for injury, height, or yield among insecticide seed treatments, within herbicides.

General Conclusions

Insect pest management early in the growing season is vital to producing a successful crop in the Midsouth region. Neonicotinoid seed treatments play an important role in controlling insect pests, and their continued use is inevitable in future years. Based on the evaluation of thiamethoxam, clothianidin, and imidacloprid, neonicotinoid seed treatments appear to also have a limited fit as herbicide safeners in soybean and grain sorghum. Although the insecticides evaluated in this study did not appear to adequately safen soybean or grain sorghum against soil-applied and postemergence herbicides, or to injurious POST-applied herbicides in soybean, significant reductions in injury from low rates of herbicides were seen in both crops. This finding is of particular interest due to recent concerns of herbicide drift in the Midsouth, where, in 2016, over 100,000 ha of soybean were damage by off-target herbicide movement.

Compared to commercially available safeners, the degree of safening, as well as consistency of injury reduction provided via insecticide seed treatments is relatively low. However, based on the widespread use of insecticide seed treatment, the potential for growers who use these insecticide seed treatments to see some of the safening effects may be relatively high. This safening may be viewed as a low-cost form of insurance to growers who already incorporate insecticide seed treatments and have concerns of herbicide drift. As a result, the adoption of neonicotinoid insecticide seed treatments, including thiamethoxam, clothianidin, and imidacloprid, is likely to increase in coming years, especially if off-target herbicide movement continues to be an issue for crop producers across the Midsouth.