5-2020

Use of Acetolactate Synthase-Inhibiting Herbicides in Inzen Grain Sorghum (Sorghum bicolor L. Moench ssp. bicolor)

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Use of Acetolactate Synthase-Inhibiting Herbicides in Inzen^{TM} Grain Sorghum
(*Sorghum bicolor* L. Moench ssp. *bicolor*)

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Crop, Soil, and Environmental Science

by

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University of Arkansas
Bachelor of Science in Agricultural Business, 2016

May 2020
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Abstract

Grain sorghum is typically grown as a rotational crop in Arkansas because of its many benefits, one being the effective control of Palmer amaranth through the use of atrazine. However, limited options exist for postemergence (POST) control of weedy grasses within the crop. Inzen™ grain sorghum is the result of a nicosulfuron resistant weedy sorghum biotype cross-bred with a commercial line of grain sorghum. Inzen™ allows for safe use of over-the-top applications of nicosulfuron within the crop. Nicosulfuron is an acetolactate synthase (ALS)-inhibiting herbicide, which has historically been used in corn for control of weedy grasses. Experiments were conducted in 2016 and 2017 to (1) evaluate the tolerance of Inzen™ grain sorghum to various herbicides in Weed Science Society of America Group 2 ALS-inhibiting herbicides, (2) evaluate weed control programs utilizing nicosulfuron, and (3) determine the sensitivity of conventional grain sorghum to low rates of nicosulfuron and glufosinate. Results indicate Inzen™ grain sorghum was tolerant to ALS-inhibiting herbicides evaluated when applied directly to the soil prior to crop emergence (PRE). When ALS-inhibiting herbicides were applied to Inzen™ grain sorghum at the V4 growth stage, a high level of resistance was observed to all herbicides, with the exception of bispyribac-Na, which resulted in 20% visible injury and a 35% yield reduction. Additionally, weed control programs utilizing *S*-metolachlor preemergence and nicosulfuron + atrazine applied POST resulted in a yield increase along with acceptable control of both Palmer amaranth and johnsongrass. Finally, conventional grain sorghum appeared to be most sensitive to low rates of nicosulfuron and glufosinate at V8, flagleaf, or heading growth stages. Yield reductions of up to 96% were observed from rates of nicosulfuron equivalent to 1/10X of a labeled use rate.

Nomenclature: Inzen; atrazine; byspyribac; glufosinate; nicosulfuron; *S*-metolachlor; johnsongrass, *Sorghum halepense* L. Pers.; Palmer amaranth, *Amaranthus palmeri* S. Wats.; corn, *Zea mays* L.; grain sorghum, *Sorghum bicolor* L. Moench *ssp. bicolor*

Keywords: Resistance, herbicides, grain sorghum, Inzen, nicosulfuron

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Chapter 1

Introduction

Worldwide, there are 259 weed species with confirmed resistance to one or more herbicide sites of action (SOA) (Heap 2019). Applying a herbicide repeatedly from the same SOA can lead to selection for herbicide-resistant (HR) biotypes of weeds (Riar et al. 2011). As resistance evolves, there are limited chemical options for controlling weeds in crop, which is partly due to the lack of discovery by the agricultural industry. Hence, weed resistance is a serious threat to modern agriculture.

Glyphosate-resistant (GR) soybean [*Glycine max* (L.) Merr.] was commercialized in 1996. Glyphosate is a herbicide that inhibits 5-enolpyruvyshikimate-3-phosphate (EPSPS), the enzyme which is involved in EPSPS conversion to aromatic amino acids, specifically tyrosine, tryptophan, and phenylalanine (Devine et al. 1993). By 2009, 91% of the soybean planted in the United States was glyphosate-resistant (Reddy and Norsworthy 2010). Large adoption of GR soybean, was fueled by excellent weed control, crop safety, simplicity of application, relatively low cost of weed control, and soil conservation (Feng et al. 2010). Prior to GR soybean, Palmer amaranth (*Amaranthus palmeri* S. Wats.) was one of the most troublesome weeds of soybean (Webster 2001). However, following commercialization of GR soybean, Palmer amaranth was effectively controlled in soybean with glyphosate (Scott et al. 2002). The high level of control of one of the most troublesome weeds led to an accelerated adoption rate and an over dependence on the herbicide. Continued use of glyphosate resulted in the confirmation of GR Palmer amaranth by 2006, in Arkansas (Norsworthy et al. 2008). Confirmation of GR Palmer amaranth came from an accession collected in 2005, reported to have survived at least two applications of glyphosate at 840 g ae ha^{-1} (Scott et al. 2007). Therefore, GR Palmer amaranth posed the end of an era.

In order to combat GR Palmer amaranth growers began to utilize more cultural practices, such as crop rotation. Liebman and Dyck (1993) showed that crop rotation can result in emerged weed densities in test crops that were lower in 21 cases, higher in 1 case, and equivalent in 5 cases compared to monoculture systems. Grain sorghum (*Sorghum bicolor* L. Moench ssp. *bicolor*) is a crop often used in these rotational programs because of the effective use of atrazine on Palmer amaranth (Owen et al. 2010). Recently, new HR technologies have been developed to utilize existing herbicide chemistry.

Grain sorghum was once the third most common cereal crop produced in the United States (DeFelice 2006). In 2015, Arkansas producers harvested 174,000 hectares of grain sorghum (Kelley and Lawson 2016), a vast increase from the 55,000 hectares harvested in 2014 (NASS 2014). The 2015 spike in production can be attributed to high commodity prices, benefits of grain sorghum in a crop rotation, and the increasing need for integrated weed management practices. Broadleaf weeds such as Palmer amaranth have numerous control options in grain sorghum, but there are few options for POST weedy grass control (Smith and Scott 2015). One of the most problematic grasses, johnsongrass [*Sorghum halepense* (L.) Pers.] possesses genetic similarities to grain sorghum (Bowers et al. 2003). Because of the similarity between grain sorghum and johnsongrass, there are no current options available to growers for removing johnsongrass chemically once the crop has emerged. Competition from weeds in grain sorghum cannot only reduce yield, if left uncontrolled, but also increase weed seed in the soil seedbank (Moore et al. 2004). Diversifying weed management programs is an important factor in minimizing weed seed production, which can end up in the soil seedbank (Norsworthy et al. 2012).

The genetic makeup of johnsongrass makes it a serious problem globally in grain sorghum. Johnsongrass reproduces through seed and rhizomes, which makes it more difficult to control than annual grasses (Horowitz 1973). Johnsongrass is distributed in warm regions and typically grows 50 to 150 cm tall, with culms arising from an extensively creeping and rooting rhizome. Panicles are mostly 10 to 35 cm long (Monaghan 1979). The weed was labeled in Arkansas as a noxious weed (Heap 2019) soon after resistance to glyphosate was confirmed in a field near West Memphis in 2007 (Riar et al. 2011). Selection pressure placed on weeds due to overuse of herbicide tactics can often result in resistance of a weed species to the herbicide (Owen and Zelaya 2005). The evolution of glyphosate resistance illustrates the importance for an integrated weed control approach including multiple SOA's to prevent further resistance.

HR crops account for greater than 90% of all crops planted in the United States (Owen and Zelaya 2005). With the development of a grain sorghum with resistance to an acetolactate synthase (ALS)-inhibiting herbicide, growers could be provided with a viable option for weedy grass control in grain sorghum, including johnsongrass. However, it is important to consider an integrated weed management approach because resistance to ALS-inhibiting herbicides occurs more frequently than resistance to any other SOA (Merotto et al. 2009). Therefore, utilization of the inzen™ trait will require crop and/or herbicide rotation to reduce selection for resistance in johnsongrass and other grass species.

ALS-inhibiting herbicides can be very effective at low rates due to their highly specific inhibition of the ALS enzyme (Ray 1984). ALS-inhibiting herbicides primarily starve the plant of branched chain amino acids, specifically isoleucine, valine, and leucine (WSSA 2017). The ALS SOA is made up of five herbicide families which include imidazolinone, pryrimidinylthiobenzoic, triazolinone, sulfonylurea, and triazolopyrimidine. Some overlapping of the binding site of ALS-inhibiting herbicides to branched chain amino acids between the families does occur, but there are often differing binding sites of branched chain amino acids per family (Subramanian et al. 1991). The repeated use of the ALS-inhibiting herbicide chlorosulfuron, a sulfonylurea, rapidly led to resistant weed populations in only 5 years after its introduction (Mallory-Smith et al. 1990). Sulfonylurea herbicides have been documented to confer frequent mutations causing resistance in plants with low to no cross-resistance to imidazolinone herbicides. Therefore, ALS-inhibiting herbicide resistance is generally grouped into one of three categories: sulfonylurea resistance, imidazolinone resistance, or a broad cross-resistance to all five families (Tranel and Wright 2002).

Currently, there are 160 confirmed weed species with resistance to the ALS herbicides (Heap 2019). In a survey of johnsongrass populations along roadsides in Arkansas, it was concluded that ALS-resistant populations are present within the state (Norsworthy, personal communication). Additionally, Indiana, Kentucky, Texas, and West Virginia have confirmed ALS-resistant johnsongrass (Heap 2019). Resistance in West Virginia was confirmed when a johnsongrass biotype was unable to be controlled after two postemergence (POST) applications of nicosulfuron in corn (*Zea mays* L.) (Chandran et al. 2004).

When selecting a rotational crop, it is important to note the potential for herbicide carryover from chemicals applied the prior growing season. Carryover injury occurs as a result of a herbicide's ability to persist in the soil beyond one growing season. Susceptibility to injury from herbicide carryover varies by crop (Barber et al. 2015). Imazaquin is a herbicide that has been documented to cause visible injury the subsequent year in grain sorghum as high as 35%, but has not reduced seed heads or yield of the crop (Johnson et al. 1995). In addition to imazaquin, two more ALS-inhibiting herbicides that are commonly used in Arkansas that pose a rotational risk of crop injury to grain sorghum are pyrithiobac and imazethapyr. Pyrithiobac is applied early-season in cotton (*Gossypium hirsutum* L.) for control of non ALS-resistant broadleaf weeds. The problem with using pyrithiobac in cotton is that its lengthy soil persistence causes subsequent grain sorghum injury up to 18 months after the initial application (Anonymous 2016). As with soybean, grain sorghum can be a useful weed control mechanism when used in rotation to cotton. With only 8 GR Palmer amaranth present per m row of cotton, yield can be reduced up to 92% (MacRae et al. 2013).

Clearfield® rice (*Oryza sativa* L.) confers resistance to ALS-inhibiting herbicides within the imidazolinone family. Imidazolinone-resistant rice was created through seed mutagenesis, the concept of exposing seed to chemicals or radiation and creating mutants (Croughan 1994). Failure to control barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] can result in a 30-100% reduction in rice yields (Johnson et al. 1998). Therefore, the Clearfield technology was commercialized for control of barnyardgrass, red rice, and broadleaf signalgrass (*Urochloa platyphylla* Nash) (Masson and Webster 2001; Masson et al 2001). Due to poor management of the clearfield® technology conformation of imazethapyr-resistant barnyardgrass was reported only six years after its release (Heap 2019; Wilson et al. 2011).

With the widespread evolution of resistance to multiple herbicide sites of action and lack of POST control for johnsongrass in grain sorghum, a new herbicide trait called Inzen™ has recently been commercialized. The inzen™ trait is labeled for use with the ALS-inhibiting herbicide nicosulfuron which was predominately used in corn, prior to the introduction of glyphosate-resistant corn for weedy grass control. A naturally occurring weedy sorghum exhibiting resistance to nicosulfuron was utilized for creation of the inzen™ trait (Currie and Geier 2015). Using traditional breeding techniques, ALS-inhibiting resistant shattercane (*Sorghum bicolor* L. Moench *ssp. drummondii*) was crossbred with grain sorghum to create the first herbicide resistant hybrid of the crop (Werle 2016). Development and design of HR crops has proven to provide safety from off-target movement as well as injury from herbicides present in the soil (Tranel and Wright 2002). The development of multiple HR crops also introduces an increasing diversity of herbicides applied in a given geography. With new diversity, it is important to note the potential of off-target movement of a herbicide. Rates ranging from 1/10X to 1/100X have been documented to be similar to a drift occurrence (Wolf et al. 1993). Al-Khatib et al. (2003) showed that imazethapyr exposure could cause visual injury to grain sorghum up 20% at these low rates. The studies also revealed that after the exposure to imazethapyr, death of the shoot halted further growth of grain sorghum.

Inzen™ will allow for the opportunity of POST weedy grass control in grain sorghum by utilizing nicosulfuron. It also has the potential to reduce the plant-back intervals of other ALS-inhibiting herbicides such as imazaquin, pyrithiobac, or imazethapyr to grain sorghum. Nicosulfuron is only effective as a POST herbicide, and has limited to no soil residual activity (Carey and Kells 1995). Therefore, the objective of this research was to evaluate the cross-resistance of Inzen™ grain sorghum to PRE and POST applications of various ALS-inhibiting herbicides, to determine if effective weed management programs exist in Inzen™ sorghum, and to determine the growth stages that are most sensitive to off-target movement of nicosulfuron.

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Chapter 2

Resistance of Inzen™ Grain Sorghum to Multiple Preemergence- and Postemergence-applied Acetolactate Synthase-inhibiting Herbicides

Abstract

Grass weeds have predominately been most troublesome in grain sorghum production. To combat weedy grass in grain sorghum, a new non-GMO trait called Inzen™ was commercialized allowing the use of nicosulfuron within the crop. Inzen™ grain sorghum carries a double mutation in the acetolactate synthase (ALS) gene Val_{560}Ile and Trp_{574}Leu, which results in cross-resistance to ALS-inhibiting herbicides. However, it is unknown if the Val_{560}Ile and Trp_{574}Leu mutation will provide cross-resistance to all five families of the ALS-inhibiting herbicide site of action (SOA) applied either preemergence (PRE) or postemergence (POST) to the crop. In order to evaluate the scope of cross-resistance to other Weed Science Society of America Group 2 herbicides, tests were conducted in 2016 and 2017 at the Lon Mann Cotton Research Station, near Marianna, AR, the Arkansas Agricultural Research & Extension Center, in Fayetteville, AR, and in 2016 at the Pine Tree Research Station, near Colt, AR. The test included ALS-inhibiting herbicides from all five families: sulfonylureas, imidazolinones, pyrimidinylthiobenzoics, triazolinones, and triazolopyrimidines. Treatments were made either PRE or POST to the grain sorghum crop at a 1X rate for crops in which each herbicide is labeled. Grain sorghum planted in the PRE trial included Inzen™ and a conventional hybrid. Visible estimates of injury and plant heights were taken at 2 and 4 weeks after herbicide application and yield data were collected at crop maturity. In the PRE trial, there was no visible injury, plant height reduction, or yield loss in plots containing the Inzen™ cultivar. Applications made POST to the Inzen™ grain sorghum caused visible injury, plant height reduction, and yield loss of 20%, 13%, and 35%, respectively, only in plots where bispyribac-Na was applied. There was no impact on the crop from other POST-applied ALS-inhibiting herbicides. These results demonstrate that the Inzen™ trait confers cross-resistance to most ALS-inhibiting herbicides and could offer promising new alternatives for weed control in grain sorghum.

Nomenclature: nicosulfuron; pyrithiobac; grain sorghum, *Sorghum bicolor* L. Moench *ssp. bicolor*

Keywords: Postemergence, preemergence, cross-resistance, acetolactate synthase

Introduction

Grain sorghum is a popular crop to implement into a crop rotation because of the effective use of atrazine for control of many weeds, particularly broadleaf weeds (Owen et al. 2010). However, there are still weed control issues producers face in grain sorghum due to the limited postemergence (POST) chemical options for weedy grass control once the crop has emerged (Smith and Scott 2015). More specifically, johnsongrass [*Sorghum halepense* (L.) Pers., fall panicum (*Panicum dichotomiflorum* Michx.), barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.], broadleaf signalgrass (*Urochloa platyphylla* Nash), and Texas panicum [*Panicum texanum* (Buckl.) R.Webster] can be the most troublesome grass species.

Because of the lack of new herbicide chemistry in grain sorghum and the difficulty with grass weed control in the crop, a new herbicide-resistant trait has been commercialized in the crop that allows over-the-top application of nicosulfuron (Anonymous 2016). The ALS chemistry has not been available in grain sorghum until now due to the strong genetic similarities between johnsongrass and grain sorghum (Bowers et al. 2003). However, with the discovery of nicosulfuron-resistant weedy sorghum, researchers were able to cross nicosulfuron-resistance into grain sorghum, allowing for safe use of nicosulfuron in crop (Tuinstra and Al-Khatib 2008).

Johnsongrass can reproduce through seed as a summer annual or through rhizomes as a perennial. Johnsongrass has the ability to produce up to 28,000 seed and up to 90 m of rhizomes in one season of grain sorghum growth (Horowitz 1973). Not only does johnsongrass reduce grain sorghum yield by competing with the crop, but it also produces allelopathic chemicals that inhibit grain sorghum growth (Mueller 1993). Johnsongrass alone can reduce corn yields 74 to 100% (Bendixen 1986). Control of the troublesome weed has been achieved in other crops such as soybean by using selective herbicides such as dinitroanilines, and acetyl-coenzymeA carboxylase (ACCase)-inhibiting herbicides (Langemeier and Witt 1986; McWhorter 1977; Riley and Shaw 1988). The evolution of resistance; however, has reduced these options (Heap 2017). With the introduction of glyphosate-resistant (GR) corn (*Zea mays* L.), growers were provided a more effective option with glyphosate. Prior to GR corn, nicosulfuron was the primary option for control of grasses in corn.

With the Inzen™ technology introduced to the market, questions have been proposed as to whether or not the herbicide-resistant trait will confer resistance to other Weed Science Society of America (WSSA) Group 2 ALS-inhibiting herbicides. WSSA Group 2, ALS-inhibiting herbicides, as defined by the WSSA, primarily starve the plant of branched chain amino acids, specifically leucine, isoleucine, and valine (WSSA 2017). The 5 families that comprise herbicides that inhibit the ALS include sulfonylureas, imidazolinones, pyrimidinylthiobenzoics, triazolinones, and triazolopyrimidines.

Cross resistance across a suite of ALS-inhibiting herbicide families has been documented by Tranel and Wright (2002). However, their research found that generally ALS-resistance is grouped into one of three categories: sulfonylurea resistant, imidazolinone resistant, or a broad cross resistance. Resistance to ALS herbicides was first discovered only five years after the introduction of the first sulfonylurea herbicide chlorosulfuron. The mechanism of resistance to chlorosulfuron was reduced sensitivity of the target ALS enzyme to inhibition by the herbicide (Mallory-Smith et al. 1990). Currently, there are nine confirmed ALS enzyme mutations which confer resistance to ALS-inhibiting herbicides: Ala_{122}, Pro_{197}, Ala_{205}, Asp_{376}, Arg_{377}, Trp_{574}, Ser_{653}, Val_{560}, and Gly_{654}. The Ala_{122} mutation is the most common one documented to confer cross-resistance (Tranel et al. 2016). Through PCR screening of Inzen™ sorghum a double mutation of Val_{560}Ile and Trp_{574}Leu was confirmed (Tuinstra and Al-Khatib 2008). The

$Trp_{574}Leu$ point mutation has been found in Palmer amaranth, which resulted in ALS cross-resistance to all five families in the species (Molin et al. 2016). The $Val_{560}Ile$ point mutation has also been documented conferring ALS cross-resistance to all five families in weedy sorghum species such as johnsongrass (Werle et al. 2016). Another mechanism of ALS-inhibiting herbicide resistance is increased herbicide metabolism resulting in detoxification of the herbicide. However, metabolism only results in cross-resistance of ALS herbicides less than 10 percent of the time (Hall et al. 1994). Therefore, the objective of this research was to evaluate the cross-resistance of Inzen™ grain sorghum to PRE and POST applications of various ALS-inhibiting herbicides in WSSA Group 2.

Materials and Methods

Field experiments were conducted in 2016 and 2017 at the Lon Mann Cotton Research Station (LMCRS), near Marianna, AR, the Arkansas Agricultural Research & Extension Center (AAREC), in Fayetteville, AR, and in 2016 at the Pine Tree Research Station (PTRS), near Colt, AR to determine grain sorghum sensitivity to multiple ALS-inhibiting herbicides applied PRE and POST. The soil texture at LMCRS was a Calloway silt loam (fine-silty, mixed, active, thermic Aquic Fraglossudalfs) with 2% sand, 82.3% silt, and 15.6% clay with a pH of 5.5, and contained 2.2% organic matter (OM). The AAREC soil texture was a Captina silt loam (fine-silty, siliceous, active, mesic Typic Fragiudults) with 22% sand, 64% silt, and 14% clay with a pH of 5.8, and contained 1.8% OM. Finally, the PTRS soil texture was a Calloway silt loam (fine-silty, mixed, active, thermic Aquic Fraglossudalfs) with 10.6% sand, 68.6% silt, and 20.8% clay with a pH of 7.5, and contained 1.3% OM.

For comparisons both years, Inzen™ (Pioneer, Johnston IA) and a Dekalb (Monsanto Company, St. Louis, MO) conventional grain sorghum hybrid was planted at all locations

(Figures 1-3). Both varieties were planted at 217,000 seeds ha^{-1} to a 2.5- to 3-cm depth. All plots consisted of 2 rows, 8.5 meters long. Plots to evaluate ALS resistance PRE were arranged as a split-plot design where the whole plot factor was ALS herbicide applied (22 herbicides), and the sub-plot factor was seed technology planted (Inzen™ or conventional). The purpose of the POST experiments was to determine Inzen™ sensitivity to multiple ALS-inhibiting herbicides applied POST. The experimental design was a randomized complete block with the fixed effect being herbicide treatment and random effects including site year and replication.

PRE herbicide applications were made immediately following planting and POST herbicide applications were made when sorghum reached the V4 growth stage using an air pressurized tractor mounted spray boom, at LMCRS, and a CO_2-pressurized backpack sprayer, at PTRS and AAREC. Both sprayers were equipped with TeeJet® Air Induction XR 110015 nozzles (TeeJet® Technologies, Glende Heights, IL). All treatments were applied at 4.8 km hr^{-1}. Herbicides were applied in a spray volume of 112.2 L ha^{-1} at LMCRS and 140 L ha^{-1} at PTRS and AAREC. Herbicide treatments and corresponding rates are listed in Table 1.

In order to maintain weed-free plots an application of atrazine (Aatrex, Syngenta Crop Protection, LLC, Greensboro, NC) plus *S*-metolachlor (Dual, Syngenta Crop Protection, LLC, Greensboro, NC) was applied at planting. Any escapes from the at planting application were controlled by a postemergence application of the same mixture. Further escapes were hand weeded. Recommendations from soil sample results analyzed at the soil testing and research laboratory in Marianna, AR were followed for fertility management. Pest management decisions were based on University of Arkansas extension recommendations (Espinoza 2015; McLeod and Greene 2015). Traditional furrow irrigation was used to provide soil moisture for all tests.

Visible ratings for crop injury and crop canopy heights were recorded at 2 and 4 weeks after planting (WAP) and POST application (WAA) in 2016 and 2017. Visible ratings of herbicide application were on a 0 to 100 scale, with 0 being no injury and 100 equaling plant death (Frans et al. 1986). Five random plants in each plot were measured to estimate an average of crop canopy height and then divided by the average crop height of nontreated plots in order to obtain relative heights. Due to an issue of sterile seed in 2016, yield data were only collected in 2017. Both rows of Inzen™ and conventional were harvested using a small-plot combine and moisture was then adjusted to 14%. Harvested plots were then recorded as kg ha^{-1} and converted to relative yield by dividing each plot by the average of the nontreated plots.

All data collected were subjected to analysis of variance (ANOVA using JMP (JMP Pro13, SAS Institute Inc., Cary, NC), with significant means separated using Fisher's protected LSD ($\alpha = 0.05$). Since the nontreated check in each replication was used to convert height and yield to a percent of the nontreated , data from these plots were not included in the analysis. All treatments containing Inzen™ technology and halosulfuron were also excluded from the statistical analysis of visible crop injury because no injury was observed. All data between site years were analyzed together, with locations considered random.

Results and Discussion

Sensitivity to Preemergence Applications of ALS-inhibiting Herbicides. Soil moisture is important for activation of soil-applied herbicides (Curran 2001). Herbicides when applied to the soil are taken up by the hypocotyl of plants as they germinate, so it is necessary that the soil receives adequate rainfall or irrigation within 7 days of application. All experiments received adequate rainfall within 7 days of application, with the exception of LMRCS in 2016 (Figures 1-3). To combat the lack of rainfall, an irrigation application was applied on May 18th at LMRCS in 2016. Activation of herbicides by irrigation did not result in a difference of visible injury 2 WAP (P = 0.1725) or 4 WAP (P = 0.3930) between locations.

For the response variable visible injury at 2 WAP a main effect of herbicide was found (P = 0.0309) (Table 2). At 2 WAP, flumetsulam-methyl and bispyribac-Na caused the least injury (6%) to grain sorghum and thiencarbazone-methyl caused the most injury (96%). Bispyribac-Na is used for POST control of weeds (Anonymous 2012) and does not have residual activity. Inzen™ grain sorghum at 2 WAP had a high degree of resistance to all herbicides applied, based on no more than 1% injury being observed (data not shown). Nicosulfuron which is labeled in Inzen™ grain sorghum caused 49% injury to conventional sorghum (Table 3). Consistent with previous studies of injury to conventional grain sorghum applied with nicosulfuron, which showed levels ranging from 19 to 67% dependent upon rate applied (Matocha and Jones 2015).

By 4 WAP a main effect of herbicide was found (P < 0.0001) for the response variable visible injury (Table 2). By 4 WAP, Inzen™ grain sorghum exhibited no injury from any of the herbicides (data not shown). The conventional grain sorghum seemed to recover from some of the injury at 4 WAP time, similar to results from experiments in 2010 (Matocha and Jones 2015). The conventional technology was injured only 1% by bispyribac-Na whereas 96% injury was caused by thiencarbazone-methyl (Table 3). Nicosulfuron at 4 WAP time resulted in 44% injury, which was similar to injury seen from trifloxysulfuron, diclosulam, and propoxycarbazone. Rimsulfuron and imazapic both caused 93% injury, which was not significantly different from thiencarbazone-methyl or pyrithiobac. It is important to note injury with imazethapyr (75%) and pyrithiobac (83%), due to the potential of herbicide carryover in a crop-rotation with rice or cotton (Table 3); however, no injury was observed on Inzen™ grain sorghum. These data prove that Inzen™ grain sorghum can be implemented as a safe option in a rotation, which follows ALS-inhibiting herbicide applications.

For the response variables height 2 WAP a two-way interaction between herbicide and technology was observed (P = 0.0071) (Table 2). A reduction in plant height was found in all plots containing the conventional 2 WAP, with the exception of imazosulfuron, imazamox, bispyribac-Na, penosulam-methyl, and flumetsulam-methyl. There was no reduction in Inzen™ grain sorghum height at 2 WAP for any of the herbicides applied. Comparing Inzen and conventional grain sorghum within each herbicide, a height reduction of the conventional was found with all herbicides, except imazosulfuron, imazamox, bispyribac-Na, penosulam-methyl, and flumetsulam-methyl (Table 4).

Crop canopy heights a 4 WAP were influenced by the interaction of herbicide applied and technology planted (P = 0.0035) (Table 2). Plots containing the conventional grain sorghum, 4 WAP, had a reduction in relative plot heights ranging from 1 to 68%. The greatest height reductions in the conventional sorghum were observed when pyrithiobac, imazapic, thiencarbazone-methyl, and rimsulfuron were applied (Table 4). No height reductions were seen in plots containing Inzen™ grain sorghum.

No reduction in relative yield was found in Inzen™ plots. Differences in relative yield were found in plots of the conventional grain sorghum ($P < 0.0001$) with all herbicides except trifloxysulfuron, imazosulfuron, propoxycarbazone, flucarbazone, penoxsulam-methyl, and flumetsulam-methyl (Tables 2 & 5). When comparing technologies, a difference between conventional and Inzen™ grain sorghum was found with 13 of the herbicides tested (Table 5). These results for Inzen™ grain sorghum are consistent with Werle et al. (2016) where the double point mutation of $Val_{560}Ile$ and $Trp_{574}Leu$ provide broad cross-resistance to ALS-inhibiting herbicides.

Postemergence Inzen™ Grain Sorghum Evaluation. Injury at 2 WAA and 4 WAA revealed no statistical analysis could be performed due to the high level of resistance Inzen™ exhibited to all ALS-inhibiting herbicides tested. At 2WAA, some visible injury was observed in plots where nicosulfuron, rimsulfuron, and bispyribac-Na were applied up to 20%, 20%, and 50% respectively. These results are consistent with the Zest™ (nicosulfuron) label, which states typical ALS injury may be observed as early as 7 days after application. Injury from nicosulfuron results in temporary yellowing and a reduction in grain sorghum height (Anonymous 2016). By 4WAA, no visible injury was observed in plots treated with nicosulfuron or rimsulfuron. However, plots treated with bispyribac-Na still had up to 15% visible injury (data not shown).

A main effect of herbicide was found with the response variable plant height at 2 and 4 weeks after application ($P < 0.0001$; Table 6). Grain sorghum heights were reduced 28% by bispyribac-Na 2WAA, which decreased to a 12% height reduction by 4 WAA. All other plots from the experiment produced heights similar to the nontreated (Table 7). Grain yield was not impacted by ALS-inhibiting herbicide when applied to Inzen grain sorghum ($P = 0.6118$) (Table 6). Resistance exhibited is consistent with previously performed greenhouse experiments, where not only sulfonylurea resistance was documented but also imidazolinone resistance when the double gene mutation of Val_{560}Ile and Trp_{574}Leu was present (Werle et al. 2017).

Lack of visible injury, reduction of plant height or yield with Inzen™ grain sorghum can be attributed to the ALS double gene mutation of Val_{560}Ile and Trp_{574}Leu. Trp_{574} is the second most documented ALS gene mutation and has been identified in 41 weed species. The specific substitution of Trp_{574}Leu is the most documented and accounts for 38 of the 41 Trp_{574} substitutions. In all 38 cases documented of Trp_{574}Leu, resistance to two or more of the five ALS families was confirmed (Heap 2019). Palmer amaranth, barnyardgrass, and johnsongrass account for 3 of these 38 cases and are among the top 5 most problematic weeds in many Arkansas row-crops (Hernandez et al. 2015, Molin et al. 2016, Panozzo et al. 2013, Singh et al. 2019).

Practical Implications. As many weed species can evolve resistance to a specific SOA, it is important to note that within WSSA Group 2, resistance to a specific acetolactate synthase-inhibiting herbicide does not necessarily constitute resistance to all ALS-inhibiting herbicides. The broad cross-resistance to multiple ALS-inhibiting herbicides seen in Inzen™ could potentially allow for these products to be used within the crop for weed control, along with reducing current plant-back intervals for grain sorghum to WSSA Group 2 herbicides.

When developing a herbicide program for grain sorghum, these results may prove beneficial as there is potential to incorporate other herbicides into the program, depending on weed species present. By enabling use of the ALS SOA, Inzen™ will allow more options for herbicide diversification, further delaying the development of weed resistance. However, Werle et al. (2016) confirmed cross-resistance to nicosulfuron and imazethapyr in populations of johnsongrass present in Kansas, Missouri, and Nebraska. The possibility of ALS-resistant johnsongrass spreading further emphasizes the necessity of proper stewardship of the Inzen™ technology.

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Table 1. Herbicides and rates applied for preemergence and postemergence acetolactate synthase-inhibiting herbicide sensitivity experiments in 2016 and 2017[abc].

Herbicide	Trade name	ALS herbicide family	Rate	Manufacturer	Address
			g ai or ae ha^{-1}		
Rimsulfuron	Resolve	Sulfonylurea	17.5	DuPont	Wilmington, DE
Primisulfuron	Beacon	Sulfonylurea	40.0	Syngenta	Greensboro, NC
Nicosulfuron	Accent	Sulfonylurea	35.2	DuPont	Wilmington, DE
Trifloxysulfuron	Envoke	Sulfonylurea	7.9	Syngenta	Greensboro, NC
Chlorosulfuron + metsulfuron	Finesse	Sulfonylurea	21.9 + 4.4	DuPont	Wilmington, DE
Chlorimuron	Classic	Sulfonylurea	8.8	DuPont	Wilmington, DE
Imazosulfuron	League	Sulfonylurea	336	Valent	Walnut Creek, CA
Imazapic	Cadre	Imidazolinone	70.1	BASF	Research Triangle Park, NC
Imazethapyr	Newpath	Imidazolinone	70.1	BASF	Research Triangle Park, NC
Imazamox	Beyond	Imidazolinone	43.8	BASF	Research Triangle Park, NC
Imazaquin	Scepter	Imidazolinone	17.2	BASF	Research Triangle Park, NC
Pyrithiobac	Staple	Pyrimidinylthiobenzioc acid	58.9	DuPont	Wilmington, DE
Bispyribac-Na	Regiment	Pyrimidinylthiobenzioc acid	35.3	Valent	Walnut Creek, CA
Diclosulam	Strongarm	Triazolopyrimidine	26.5	Dow	Indianapolis, IN
Cloransulam-methyl	First Rate	Triazolopyrimidine	17.7	Dow	Indianapolis, IN
Penoxsulam-methyl	Grasp	Triazolopyrimidine	40.3	Dow	Indianapolis, IN
Flumetsulam-methyl	Python	Triazolopyrimidine	7.2	Dow	Indianapolis, IN
Thiencarbazone-methyl	Varro	Sulfonylaminocarbonyl triazolinone	28.0	Bayer	Research Triangle Park, NC
Propoxycarbazone	Olympus	Sulfonylaminocarbonyl triazolinone	44.2	Bayer	Research Triangle Park, NC
Flucarbazone	Everest	Sulfonylaminocarbonyl triazolinone	15.3	Arysta	Cary, NC

[a]Sensitivity experiment conducted near Colt, AR in 2016.
[b]Sensitivity experiment conducted near Marianna, AR in 2016 and 2017.
[c]Sensitivity experiment conducted in Fayetteville, AR in 2016 and 2017.

Table 2. Analysis of variance for preemergence acetolactate synthase-inhibiting herbicides to grain sorghum from 2016 and 2017[abcd]

Variable	Source	DF	F Ratio	P value[e]
Visible injury 2 WAP (%)	Herbicide	20	2.3597	0.0309*
Canopy heights 2 WAP (cm)	Herbicide	20	2.3597	0.0309*
	Hybrid	1	4.1527	0.2904
	Herbicide*Hybrid	20	3.1191	0.0071*
Visible injury 4 WAP (%)	Herbicide	20	63.8662	<0.0001*
Canopy heights 4 WAP (cm)	Herbicide	20	4.7759	0.0005*
	Hybrid	1	5.2362	0.2623
	Herbicide*Hybrid	20	3.5162	0.0035*
Relative yield (%)	Herbicide	20	45.1931	<0.0001*
	Hybrid	1	871.9586	<0.0001*
	Herbicide*Hybrid	20	60.2547	<0.0001*

[a]Sensitivity experiment conducted near Colt, AR in 2016.
[b]Sensitivity experiment conducted near Marianna, AR in 2016 and 2017.
[c]Sensitivity experiment conducted in Fayetteville, AR in 2016 and 2017.
[d]Abbreviations: DF, degrees of freedom; WAP, weeks after planting; WAA, weeks after application
[e]*Denotes significance

Table 3. Visible injury (%) from preemergence applications of acetolactate synthase-inhibiting herbicides to conventional grain sorghum in 2016 and 2017.[abcd]

Herbicide	Rate	Injury[e]	
		2 WAP	4 WAP
	g ai or ae ha^{-1}	------------------%------------------	
Rimsulfuron	17.5	91	93
Primisulfuron	40.0	60	58
Nicosulfuron	35.2	49	44
Trifloxysulfuron	7.9	43	36
Chlorosulfuron + metsulfuron	21.9 + 4.4	37	29
Chlorimuron	8.8	32	22
Imazosulfuron	336	12	1
Imazapic	70.1	87	93
Imazethapyr	70.1	67	75
Imazamox	43.8	25	20
Imazaquin	17.2	21	13
Pyrithiobac	58.9	79	83
Bispyribac-Na	35.3	6	1
Diclosulam	26.5	48	42
Cloransulam-methyl	17.7	21	12
Penoxsulam-methyl	40.3	12	3
Flumetsulam-methyl	7.2	6	1
Thiencarbazone-methyl	28.0	96	99
Propoxycarbazone	44.2	46	38
Flucarbazone	15.3	20	10
LSD (0.05)[f]		10	12

[a]Sensitivity experiment conducted near Colt, AR in 2016.
[b]Sensitivity experiment conducted near Marianna, AR in 2016 and 2017.
[c]Sensitivity experiment conducted in Fayetteville, AR in 2016 and 2017.
[d]Abbreviations: WAP, weeks after planting; ai, active ingredient; ae, acid equivalent; LSD, least significant
[e]Visual injury ratings were conducted on a scale of 0 to 100%, with 0% being no injury and 100% equaling total plant death.
[f] Means within the LSD are not significantly different according to Fisher's protected LSD (α=0.05)

Table 4. Grain sorghum plant heights at 2 and 4 weeks following preemergence acetolactate synthase-inhibiting herbicide applications in Fayetteville, AR and near Marianna, AR in 2017[a]

		Height					
		2 WAP[bc]			4 WAP[de]		
Herbicide	Rate	Inzen		Conventional	Inzen		Conventional
	g ai or ae ha^{-1}	--------------- % of nontreated ---------------					
Rimsulfuron	17.5	87		1	98		1
Primisulfuron	40.0	106		51	109		53
Nicosulfuron	35.2	104		44	108		94
Trifloxysulfuron	7.9	102		61	111		68
Chlorosulfuron + metsulfuron	21.9 + 4.4	96		49	100		37
Chlorimuron	8.8	102		47	102		51
Imazosulfuron	336	103		94	109		95
Imazapic	70.1	91		25	95		16
Imazethapyr	70.1	100		36	104		27
Imazamox	43.8	102		80	107		85
Imazaquin	17.2	108		73	111		65
Pyrithiobac	58.9	92		26	99		16
Bispyribac-Na	35.3	106		95	104		81
Diclosulam	26.5	102		59	102		59
Cloransulam-methyl	17.7	102		52	105		85
Penoxsulam-methyl	40.3	100		89	103		89
Flumetsulam-methyl	7.2	102		98	104		98
Thiencarbazone-methyl	28.0	103		4	108		4
Propoxycarbazone	44.2	97		54	100		90
Flucarbazone	15.3	104		64	108		85
LSD (0.05)[f]		24	24	24	20	16	20

[a]Abbreviations: WAP, weeks after planting; LSD, least significant difference; ALS, acetolactate synthase

[b]Average plant height of Inzen grain sorghum in nontreated plots was 32 cm 2 WAP and 40 cm in nontreated conventional plots

[c]Whole plot LSD for herbicide 2 WAP 24 and sub-plot LSD for seed technololgy planted 24

[d]Average plant height of Inzen grain sorghum in nontreated plots was 45 cm 4 WAP and 54 cm in nontreated conventional plots

[e]Whole plot LSD for herbicide 4 WAP 16 and sub-plot LSD for seed technololgy planted 20

Table 5. Relative yield from 2017 of Inzen and conventional grain sorghum following preemergence acetolactate synthase-inhibiting herbicide applications in Fayetteville, AR and near Marianna, AR.

		Grain yield[a]		
Herbicide	Rate	Inzen[b]		Conventional[c]
	g ai or ae ha^{-1}	--------- % of nontreated ---------		
Rimsulfuron	17.5	109		1
Primisulfuron	40.0	104		45
Nicosulfuron	35.2	104		58
Trifloxysulfuron	7.9	98		69
Chlorosulfuron + metsulfuron	21.9 + 4.4	104		39
Chlorimuron	8.8	107		43
Imazosulfuron	336	97		81
Imazapic	70.1	106		0
Imazethapyr	70.1	96		12
Imazamox	43.8	101		52
Imazaquin	17.2	109		42
Pyrithiobac	58.9	93		40
Bispyribac-Na	35.3	95		50
Diclosulam	26.5	104		63
Cloransulam-methyl	17.7	100		71
Penoxsulam-methyl	40.3	95		93
Flumetsulam-methyl	7.2	105		100
Thiencarbazone-methyl	28.0	99		1
Propoxycarbazone	44.2	115		84
Flucarbazone	15.3	118		98
LSD (0.05)[d]		12	8	12

[a]Whole plot LSD for herbicide 8 and sub-plot LSD for seed technology planted 12

[b]Nontreated plots containing the Inzen™ technology yielded 8480 kg ha^{-1}

[c]Nontreated plots containing the conventional technology yielded 7828 kg ha^{-1}

[d]Means within the LSD are not significantly different according to Fisher's protected LSD (α=0.05)

Table 6. Analysis of variance of Inzen™ grain sorghum from postemergence-applied acetolactate synthase-inhibiting herbicides in 2016 and 2017.[abcd]

Variable	Source	DF	F Ratio	P value[e]
Canopy heights 2 WAA (cm)	Herbicide	20	72.6881	<0.0001*
Canopy heights 4 WAA (cm)	Herbicide	20	5.4095	<0.0001*
Relative yield (%)	Herbicide	20	0.8829	0.6118

[a]Sensitivity experiment conducted near Colt, AR in 2016.
[b]Sensitivity experiment conducted near Marianna, AR in 2016 and 2017.
[c]Sensitivity experiment conducted in Fayetteville, AR in 2016 and 2017.
[d]Abbreviations: DF, degrees of freedom; WAA, weeks after application
[e]*Denotes significance

Table 7. Relative plant heights and yield response of Inzen™ grain sorghum to postemergence-applied acetolactate synthase-inhibiting herbicides in 2017 at Fayetteville, AR and near Marianna, AR.[a]

		Relative heights	
Herbicide	Rate	2 WAA[b]	4 WAA[c]
	g ai or ae ha^{-1}	----------------% of nontreated---------------	
Rimsulfuron	17.5	103	99
Primisulfuron	40.0	103	99
Nicosulfuron	35.2	102	101
Trifloxysulfuron	7.9	102	99
Chlorosulfuron + metsulfuron	21.9 + 4.4	103	100
Chlorimuron	8.8	103	100
Imazosulfuron	336	103	99
Imazapic	70.1	103	99
Imazethapyr	70.1	104	101
Imazamox	43.8	103	100
Imazaquin	17.2	103	101
Pyrithiobac	58.9	103	100
Bispyribac-Na	35.3	72	88
Diclosulam	26.5	103	100
Cloransulam-methyl	17.7	103	101
Penoxsulam-methyl	40.3	102	100
Flumetsulam-methyl	7.2	102	100
Thiencarbazone-methyl	28.0	102	101
Propoxycarbazone	44.2	103	99
Flucarbazone	15.3	103	101
LSD (0.05)[d]		2	3

[a]Abbreviations: WAA, weeks after application; LSD, least significant difference;

[b]Average height of nontreated plots was 82 cm at 2 WAA

[c]Average height of nontreated plots was 93 cm at 4 WAA

[d]Means within the LSD are not significantly different according to Fisher's protected LSD (α=0.05)

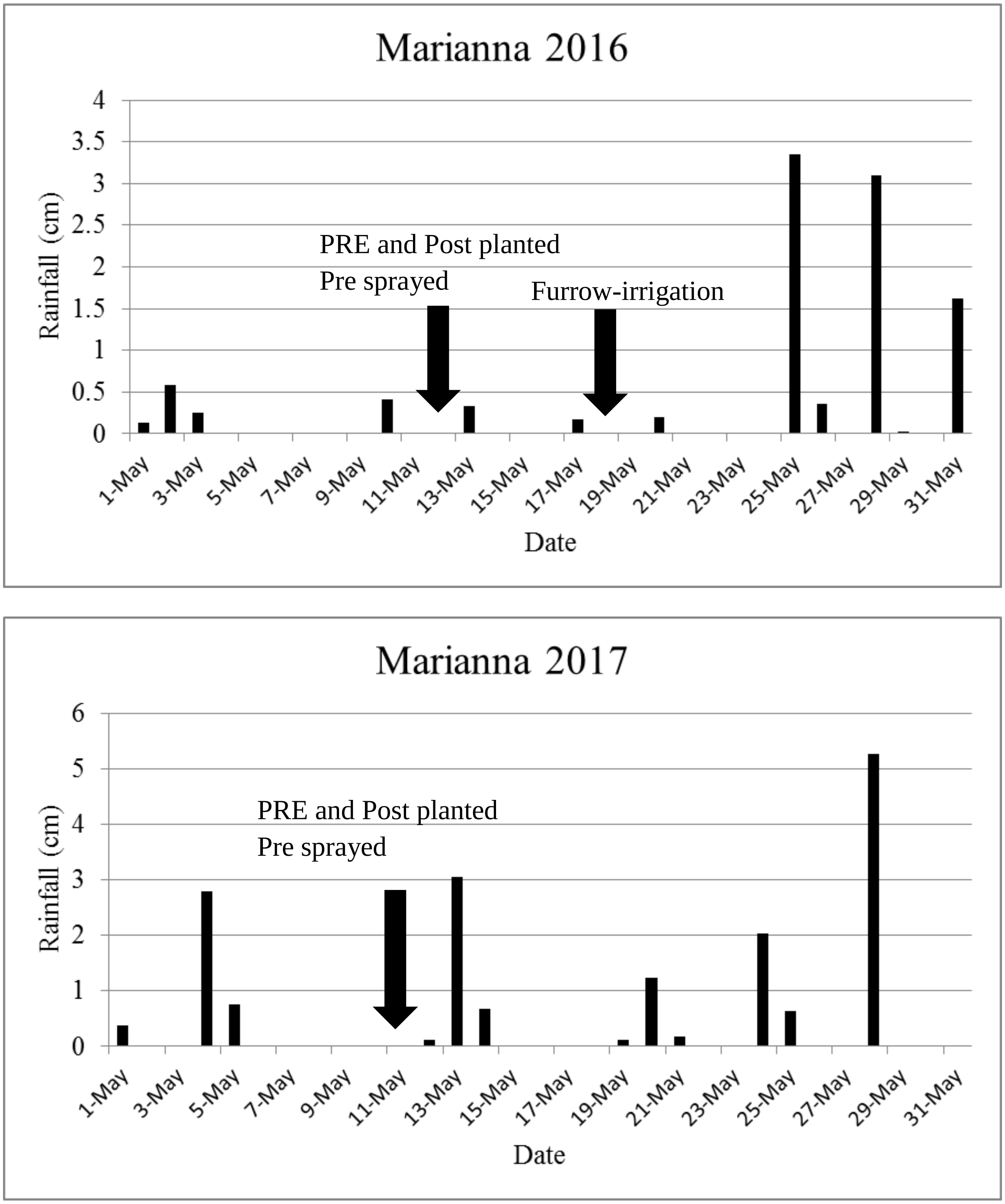

Figure 1. Planting/application timing and rainfall at Marianna, AR, in 2016 and 2017.

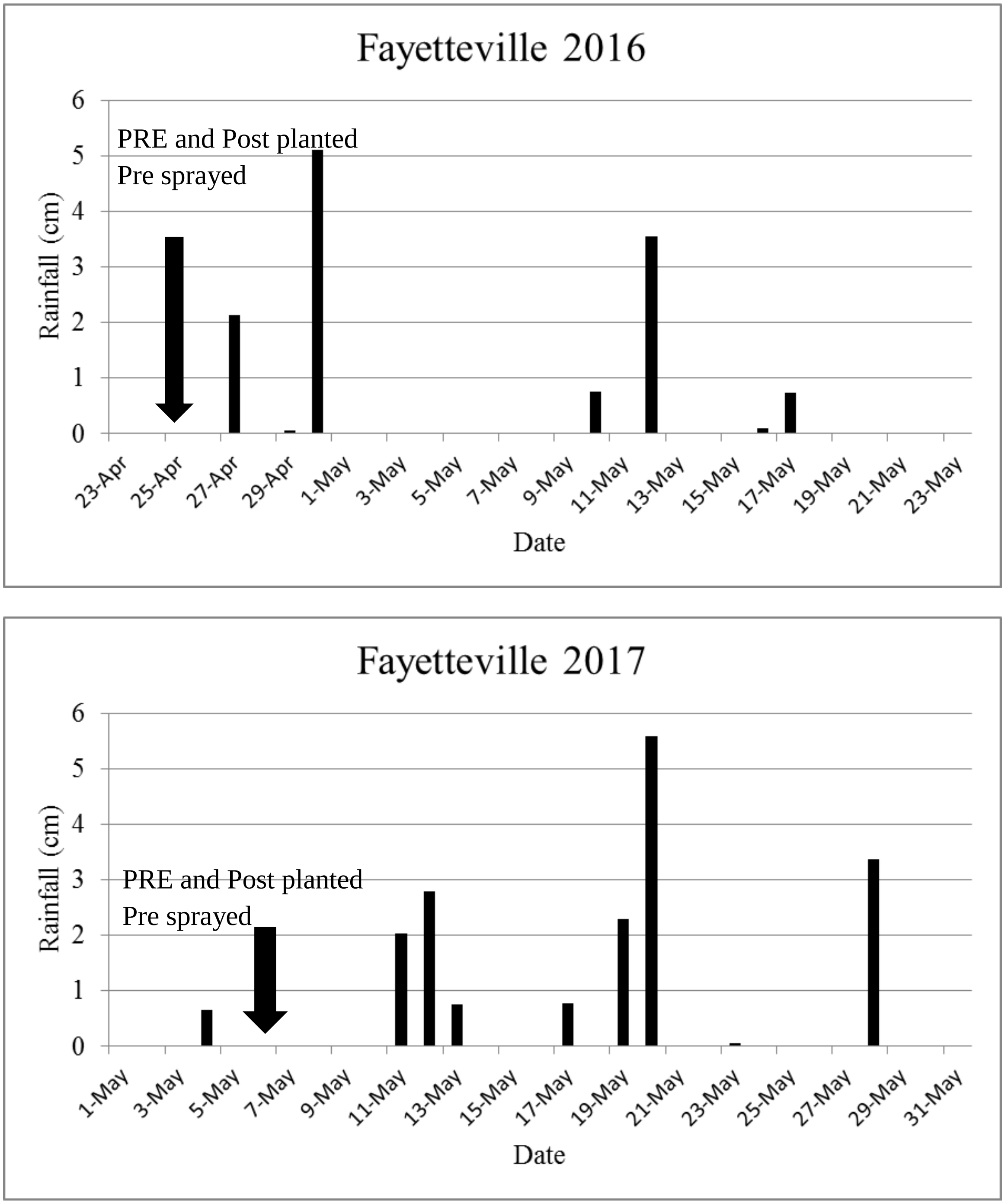

Figure 2. Planting/application timing and rainfall at Fayetteville, AR, in 2016 and 2017.

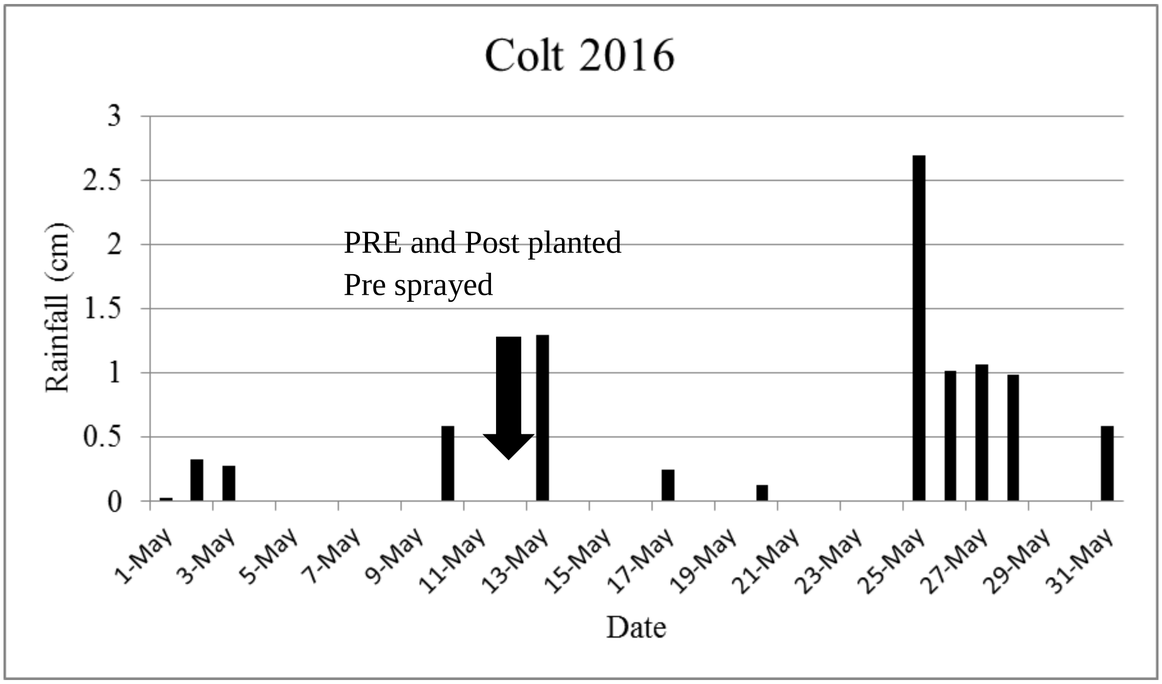

Figure 3. Planting/application timing and rainfall at Colt, AR, in 2016.

Chapter 3

Evaluation of Inzen™ Grain Sorghum Herbicide Programs

Abstract

Grain sorghum hectares significantly increased in Arkansas to 174,000 in 2015. With record increase in production, the lack of ability to control weedy grass was realized. Lack of weedy grass control along with commodity prices can be attributed as one of the reasons sorghum hectares are not constant. A new herbicide-resistant trait called Inzen™ was recently commercialized, which exhibits tolerance to acetolactate synthase-inhibiting herbicides. The primary herbicide introduced with this trait is nicosulfuron. Nicosulfuron has traditionally been used in corn production for control of annual and perennial weedy grass species, including difficult to control johnsongrass. The introduction of this new herbicide to grain sorghum has resulted in the need for research to assess the spectrum of weed control and determine best management practices. A field experiment was conducted in 2016 and 2017 at the Lon Mann Cotton Research Station, near Marianna, AR, the Arkansas Agricultural Research & Extension Center, in Fayetteville, AR, and in 2016 at the Pine Tree Research Station, near Colt, AR, to evaluate control of johnsongrass and other troublesome weeds in Inzen™ grain sorghum. Fourteen approaches were tested, including rimsulfuron + thifensulfuron, nicosulfuron, atrazine, *S*-metolachlor, and pyrsulfotole + bromoxynil applied as PRE and/or POST weed control programs. Data were analyzed as a randomized complete block with four replications. Results indicate that johnsongrass was controlled at 90% or higher when nicosulfuron was applied POST. Palmer amaranth was controlled at 94% or higher any time atrazine or *S*-metolachlor were utilized in the herbicide program. All applications of nicosulfuron displayed minimal injury

to grain sorghum, which subsided quickly, illustrating that the Inzen™ trait will be a safe alternative for long season grass control in grain sorghum.

Nomenclature: nicosulfuron; rimsulfuron; thifensulfuron; johnsongrass, *Sorghum halepense* L. Pers.; Palmer amaranth, *Amaranthus palmeri* S. Wats.; corn, *Zea mays* L.; grain sorghum, *Sorghum bicolor* L. Moench *ssp. bicolor*

Keywords: postemergence, preemergence, ALS, weed control

Introduction

The number one most troublesome weed in grain sorghum is johnsongrass. Johnsongrass, a weed in the Graminaceae family can act as both a summer annual that reproduces through seed and a perennial that reproduces through rhizomes. This species can produce up to 28,000 seed and up to 90 m of rhizomes in one cropping season (Horowitz 1973). Johnsongrass can greatly reduce grain sorghum yield, due to competition for light, soil nutrients, and moisture along with producing allelopathic chemicals (Mueller 1993). In grain sorghum, herbicides may be applied preemergence (PRE) or postemergence (POST). Currently, the only soil-applied herbicide registered for control of johnsongrass in U.S. grain sorghum is *S*-metolachlor, which only provides up to 60% control of seedling johnsongrass and provides no control of rhizome johnsongrass. No herbicides can currently be applied POST for johnsongrass control (Smith and Scott 2015).

Selective control of johnsongrass, has been achieved in soybean [*Glycine max* (L.) Merr.] with the introduction of glyphosate-resistant (GR) soybean, which provided growers with a new and more effective management option for the troublesome weed. Acetyl-CoA carboxylase (ACCase)-inhibiting herbicides have also been proven to provide adequate control of johnsongrass in soybean (Langemeier and Witt 1986; Riley and Shaw 1988). Unfortunately, grain sorghum varieties exhibiting tolerance to glyphosate or ACCase-inhibiting herbicides have not been commercialized.

Prior to the introduction of GR crops, nicosulfuron was widely used in corn (*Zea mays* L.) for control of grass weeds. Previous research found that 95% control of johnsongrass along with more than 80% control of other grasses, like barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] or broadleaf signalgrass (*Urochloa platyphylla* Nash), could be achieved through the utilization of ALS-inhibiting herbicides (Dobbles and Kapusta 1993; Schuster et al. 2008; Swanton et al. 1996). Johnsongrass which is problematic in the Midsouth can result in yield reductions of 74 to 100% when present in a corn crop (Bendixen 1986).

Recently, a new herbicide-resistant grain sorghum trait is expected to provide a viable option for POST control of johnsongrass in grain sorghum. Inzen™ grain sorghum exhibits resistance to acetolactate synthase (ALS)-inhibiting herbicides. ALS is a common enzyme in the production of branched chain amino acids valine, leucine, and isoleucine (Durner et al. 1990; Ray 1984). There are currently more than 50 herbicides, which make up this site of action (SOA) (Heap 2019).

The addition of nicosulfuron into grain sorghum herbicide programs could result in differing levels of control depending on the timing of the herbicide application (PRE or POST). Previous research has already shown the effectiveness of nicosulfuron in grain sorghum. In 2007 and 2008 trials conducted in Kansas found control of barnyardgrass was 99%, green foxtail [*Setaria viridis* (L.) Beauv.] control was 86%, and giant foxtail (*Setaria faberi* Herrm.) control was 91% for up to 6 weeks after application of nicosulfuron (Hennigh et al. 2010). Prior to nicosulfuron, quinclorac was utilized to provide up to 80% control of barnyardgrass and up to 70% control of the foxtail species when applied postemergence before weeds were greater than 5 cm in height (Scott et al. 2018). Barnyardgrass has been known to be one of the most troublesome weeds in Arkansas rice production. Barnyardgrass produces up to 31,500 seeds and has been shown to reduce yield in soybean up to 0.25% per plant in a m row and reduce rice yield 30 to 100% (Bagavathiannan et al. 2011; Johnson et al. 1998; Vail and Oliver 1993). Difficulty in barnyardgrass control can be attributed to its rapid growth, prolific seed production, photoperiodic insensitivity, and lengthy seed dormancy (Maun and Barret 1986). In only six years after the introduction of imidazolinone-resistant rice, imazethapyr-resistant barnyardgrass was confirmed in Arkansas (Heap 2019; Wilson et al. 2011). Such evolutions prove the importance of developing complete herbicide programs, which mitigate the evolution of resistant weeds to new technologies. Therefore, trials were conducted to develop effective herbicide control programs for control of the troublesome grasses and broadleaves in an Inzen™ grain sorghum production system.

Materials and Methods

Field experiments to evaluate weed control programs in Inzen™ grain sorghum were conducted in 2016 and 2017 at the Lon Mann Cotton Research Station (LMCRS), near Marianna, AR, the Arkansas Agricultural Research & Extension Center (AAREC), in Fayetteville, AR, and in 2016 at the Pine Tree Research Station (PTRS), near Colt, AR. Soil texture at LMCRS was a Calloway silt loam (fine-silty, mixed, active, thermic Aquic Fraglossudalfs) with 2% sand, 82.3% silt, and 15.6% clay with a pH of 5.5, and contained 2.2% organic matter (OM). The AAREC soil texture was a Captina silt loam (fine-silty, siliceous, active, mesic Typic Fragiudults) with 22% sand, 64% silt, and 14% clay with a pH of 5.8, and contained 1.8% OM. Finally, the PTRS soil texture was a Calloway silt loam (fine-silty, mixed, active, thermic Aquic Fraglossudalfs) with 10.6% sand, 68.6% silt, and 20.8% clay with a pH of 7.5, and contained 1.3% OM.

Inzen™ grain sorghum was planted at 217,000 seeds ha^{-1} (Figures 1-3). Plots were 3.8 m wide at all locations. Seven PRE applications consisting of rimsulfuron or nicosulfuron were made immediately following planting with the addition of *S*-metolachlor in certain treatments. Once 5 to 10 cm tall weeds were present, 14 differing herbicide combinations were applied including the addition of nicosulfuron in certain treatments (Table 1). At LMRCS, applications were made at 4.8 km hr^{-1} in a carrier volume of 112.2 L ha^{-1} to plots 9 m in length, using an air pressurized tractor mounted spray boom. Applications at AAREC and PTRS were made using a CO_2-pressurized backpack sprayer traveling at 4.8 km hr^{-1} in a carrier volume of 140 L ha^{-1} to plots 6 m in length. Both the air pressurized tractor mounted spray boom and the CO_2-pressurized backpack sprayer were equipped with TeeJet® Air Induction XR 110015 nozzles (TeeJet® Technologies, Glende Heights, IL). Treatments were arranged as a randomized complete block design with site year considered a random effect. There were 14 herbicide combinations and 1 nontreated. Fertilizer and pest management decisions were made by following University of Arkansas extensions recommendations (Espinoza 2015; McLeod and Greene 2015). Furrow irrigation was utilized at all locations to maintain adequate soil moisture.

In 2016 and 2017, visible ratings for weed control were made at 2 weeks after planting (WAP) and 4 weeks after POST application. The rating system for the visible weed control was conducted on a scale of 0 to 100%, with 0% being no control and 100% being complete weed control (Frans et al. 1986). Each weed present was evaluated separately using this scale. The nontreated in each replication served as the comparative basis for weed control. Relative yield reductions or increases were calculated by dividing yield of plots by the average yield of plots in which a standard herbicide program utilizing *S*-metolachlor PRE and atrazine + *S*-metolachlor POST was applied. At each location, the center two rows were harvested using a small-plot research combine and recorded as kg ha^{-1} after moistures were adjusted to 14%. All data collected were subjected to analysis of variance (ANOVA) using JMP (JMP Pro 13, SAS Institute Inc., Cary, NC), with significant means separated using Fisher's protected LSD (α= 0.05). The nontreated in each replication was not included in analysis since they are used only for relative comparisons of weed control (Table 2).

Results and Discussion

Soil-applied herbicide efficacy is affected by soil texture, organic matter, pH, and soil moisture (Hartzler 2002). At all locations adequate rainfall for herbicide activation was received within 7 days, with the exception of LMCRS in 2016 (Figures 1-3). Plants take up PRE-applied herbicides through the roots of young seedlings at the time of germination, therefore 1 to 2 cm of rainfall is necessary (Rao 2000). In order to combat the lack of rainfall at LMCRS in 2016, an irrigation application was made 7 days after planting.

Analysis of variance indicates significant differences were observed across weed control programs for all weed species at each evaluation timing resulting in a difference in realative grain sorghum yield (Table 2). PRE applications evaluated 2 WAP for Palmer amaranth ($P<0.0001$) indicated the best control (≥93%) any time *S*-metolachlor was applied, with the highest control (99%) being with treatments including atrazine + *S*-metolachlor (Table 3). Applications of nicosulfuron or rimsulfuluron plus thifensulfuron PRE resulted in poor control of Palmer amaranth (49-71%) 2 WAP. Johnsongrass control levels were lowest ($P<0.0001$) 2 WAP (74%) when only rimsulfuron + thifensulfuron were applied. Utilizing *S*-metolachlor alone or in combination with rimsulfuron + thifensulfuron + atrazine increased control of johnsongrass (≥88%). Goosegrass control levels ($P<0.0001$) were not increased by applications of *S*-metolachlor alone (Table 3). Highest goosegrass control (>91%) was only achieved with multiple herbicides such as rimsulfuron + thifensulfuron + atrazine + *S*-metolachlor applied PRE. Rimsulfuron has been shown to provide >80% control of annual grasses such as large crabgrass (Boydston 2007). *S*-metolachlor is often applied because of its effectiveness in controlling annual grasses along with small seeded broadleaf weeds (Grichar et al. 2004), which are also effectively controlled with atrazine (Smith and Scott 2015). These results prove the importance of utilizing more than one effective SOA for each weed species present (Table 3).

Programs that did not include a POST application resulted in poor results of johnsongrass (15-22%) and goosegrass (60-61%) control by 4 WAA (Table 4). Similarly, control of these weeds were reduced significantly when a total POST program was applied with no prior applied PRE. Johnsongrass, goosegrass and Palmer amaranth were only controlled 64, 69 and 87% respectively 4 WAA of nicosulfuron + atrazine + pyrasulfotole + bromoxynil which indicates the importance of residual herbicides for control of these weeds species in addition to the importance of timely applications POST (Table 4). The standard herbicide program (*S*-metolachlor PRE fb atrazine + *S*-metolachlor POST) provided 94 and 98% control of goosegrass and Palmer amaranth respectively, but only controlled johnsongrass 61%. The greatest control of johnsongrass was achieved by programs including applications of nicosulfuron POST (>90%). The increased control can be attributed to the effectiveness of nicosulfuron in controlling both annual and perennial grasses (Dobbles and Kapusta 1993). One weakness with nicosulfuron was observed with Palmer amaranth control. When atrazine was not included in the PRE or POST herbicide program, Palmer amaranth control only ranged from 3-70%, with the higher level resulting from *S*-metolachlor applied PRE. When only ALS chemistry was utilized PRE and POST, control was reduced to <17%, which indicates the importance of atrazine to Palmer amaranth control.

Yield was influenced by herbicide program utilized (P<0.0001) (Table 2). Relative yield increase was greatest (257%) when atrazine and *S*-metolachlor were applied PRE followed by nicosulfuron POST, which also provided the highest control of johnsongrass. Applying nicosulfuron PRE instead of POST, as labeled, resulted in yield decreases of up to 34% (Table 5).

Selection pressure from overreliance of a single herbicide could result in the spread of herbicide-resistant weeds, but outcrossing of Inzen™ grain sorghum and johnsongrass carries a low potential. The low potential of grain sorghum and johnsongrass outcrossing is influenced by the ploidy of the species. An infertile triploid is obtained when johnsongrass a tetraploid and grain sorghum a diploid cross (Hadley 1958).

Practical Implications. These results show the importance of a program approach for weed control in Inzen™ grain sorghum and highlight consistency of johnsongrass control when nicosulfuron is applied to 5 to 10 cm tall weeds. However, nicosulfuron should not be the only weed management tactic deployed in an Inzen™ grain sorghum weed management program. It should be an addition to a robust weed control system which utilizes multiple effective chemical and non-chemical methods.

With confirmed ALS-resistant Palmer amaranth in Arkansas (Heap 2019), ALS-inhibiting herbicides cannot be expected to provide broad-spectrum weed control in grain sorghum. Also, it is important to utilize more than one effective SOA (Norsworthy et al. 2012). When *S*-metolachlor + atrazine is applied PRE and followed by nicosulfuron POST the highest level of broad spectrum weed control was achieved, reducing competition for essential nutrients and producing significantly higher yields. Applying *S*-metolachlor PRE also leaves growers the option of planting other crops in the event that the grain sorghum crop fails. Whereas, applying an ALS-inhibiting herbicide or atrazine PRE can greatly reduce a grower’s option of planting alternate crops in a crop failure situation.

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Tables

Table 1. Inzen™ grain sorghum herbicide programs evaluated in 2016 and 2017[abcd]

Herbicide		Rate	
PRE	POST	PRE	POST
		g ha^{-1}	
NA	NA	-	-
Rimsulfuron +thifensulfuron	Atrazine + *S*-metolachlor	18 + 18	1830 + 1420
Nicosulfuron	Atrazine + *S*-metolachlor	35	1830 + 1420
S-metolachlor	Atrazine + *S*-metolachlor	1064	1830 + 1420
Nicosulfuron + *S*-metolachlor	Atrazine + *S*-metolachlor	35 + 1064	1830 + 1420
Rimsulfuron +thifensulfuron + atrazine + *S*-metolachlor	NA	18 + 18 + 1830 + 1420	-
Nicosulfuron + atrazine + *S*-metolachlor	NA	35 + 1830 + 1420	-
Rimsulfuron +thifensulfuron + atrazine + *S*-metolachlor	Nicosulfuron	18 + 18 + 1830 + 1420	35
Rimsulfuron + thifensulfuron	Nicosulfuron	18 + 18	35
NA	Nicosulfuron	-	35
NA	Nicosulfuron + atrazine	-	35 + 1120
S-metolachlor	Nicosulfuron	1064	35
S-metolachlor	Nicosulfuron + atrazine	1064	35 + 1120
NA	Nicosulfuron + atrazine + pyrasulfotole + bromoxynil	NA	35 + 1120 + 30 + 210

[a]Abbreviations: NA, no application; PRE, application made at planting prior to weed emergence; POST, application to emerged weeds 5-10 cm in height

[b]Weed control experiment conducted near Colt, AR in 2016.

[c]Weed control experiment conducted near Marianna, AR in 2016 and 2017.

[d]Weed control experiment conducted in Fayetteville, AR in 2016 and 2017.

Table 2. Analysis of variance for Inzen™ grain sorghum herbicide programs from 2016 and 2017.[abc]

Variable[d]	DF[a]	F Ratio	P value
Visible weed control of Palmer amaranth 2 WAP (%)	6	112.9654	<0.0001*
Visible weed control of goosegrass 2 WAP (%)	6	13.3982	<0.0001*
Visible weed control of johnsongrass 2 WAP (%)	6	4.2272	<0.0001*
Visible weed control of Palmer amaranth 4 WAA (%)	13	50.1830	<0.0001*
Visible weed control of goosegrass 4 WAA (%)	13	33.8231	<0.0001*
Visible weed control of johnsongrass 4 WAA (%)	13	61.2438	<0.0001*
Relative yield at crop maturity (%)[e]	13	312.3487	<0.0001*

[a]Weed control experiment conducted near Colt, AR in 2016.
[b]Weed control experiment conducted near Marianna, AR in 2016 and 2017.
[c]Weed control experiment conducted in Fayetteville, AR in 2016 and 2017.
[d]Abbreviations: DF, degrees of freedom; WAP, weeks after planting; WAA, weeks after POST application.
[e]Yield relative to plots following University of Arkansas herbicide recommendations.

Table 3. Evaluation of weed control from herbicide treatments in 2016 and 2017 applied preemergence at 2 WAP[abcd]

Herbicide	Weed control 2WAP					
PRE	Johnsongrass		Goosegrass		Palmer amaranth	
	---------------------% of nontreated------------					
Rimsulfuron +thifensulfuron	74	b	75	b	71	c
Nicosulfuron	77	b	76	b	49	d
S-metolachlor	88	a	81	b	93	b
Nicosulfuron + *S*-metolachlor	82	ab	91	a	94	b
Rimsulfuron +thifensulfuron + atrazine + *S*-metolachlor	88	a	96	a	99	a
Nicosulfuron + atrazine + *S*-metolachlor	89	a	95	a	96	ab
Rimsulfuron +thifensulfuron + atrazine + *S*-metolachlor	86	a	94	a	99	a

[a]Abbreviations: WAP, weeks after planting
[b]Weed control experiment conducted near Colt, AR in 2016
[c]Weed control experiment conducted near Marianna, AR in 2016 and 2017
[d]Weed control experiment conducted in Fayetteville, AR in 2016 and 2017
[e]Means followed by the same letter are not significantly different according to Fisher's protected LSD (α=0.05)

Table 4. Evaluation of weed control from herbicide treatments in 2016 and 2017 applied postemergence 4WAA.[abcd]

Herbicide		Weed control 4WAA[a]		
PRE	POST	Johnsongrass	Goosegrass	Palmer amaranth
		----------------------% of nontreated--------------------		
Rimsulfuron +thifensulfuron	Atrazine + *S*-metolachlor	16 c	81 c	95 a
Nicosulfuron	Atrazine + *S*-metolachlor	9 c	89 bc	97 a
S-metolachlor	Atrazine + *S*-metolachlor	61 b	70 d	98 a
Rimsulfuron +thifensulfuron	Atrazine + *S*-metolachlor	19 c	93 ab	97 a
Nicosulfuron + *S*-metolachlor	Atrazine + *S*-metolachlor	69 b	94 ab	98 a
Rimsulfuron +thifensulfuron + atrazine + *S*-metolachlor	NA	22 c	61 e	97 a
Nicosulfuron + atrazine + *S*-metolachlor	NA	15 c	60 e	98 a
Rimsulfuron +thifensulfuron + atrazine + *S*-metolachlor	Nicosulfuron	94 a	99 a	96 a
Rimsulfuron + thifensulfuron	Nicosulfuron	90 a	90 b	17 e
NA	Nicosulfuron	92 a	79 c	3 f
NA	Nicosulfuron + atrazine	91 a	78 c	84 c
S-metolachlor	Nicosulfuron	93 a	83 c	70 d
S-metolachlor	Nicosulfuron + atrazine	92 a	91 b	90 b
NA	Nicosulfuron + atrazine + pyrasulfotole + bromoxynil	64 b	69 d	87 bc

[a]Abbreviations: NA, no application; WAA, weeks after POST application; LSD, least significant difference
[b]Weed control experiment conducted near Colt, AR in 2016
[c]Weed control experiment conducted near Marianna, AR in 2016 and 2017
[d]Weed control experiment conducted in Fayetteville, AR in 2016 and 2017
[e]Means followed by the same letter are not significantly different according to Fisher's protected LSD (α=0.05)

Table 5. Relative yield of Inzen™ grain sorghum herbicide program experiment from 2017 in Fayetteville, AR and near Marianna, AR.

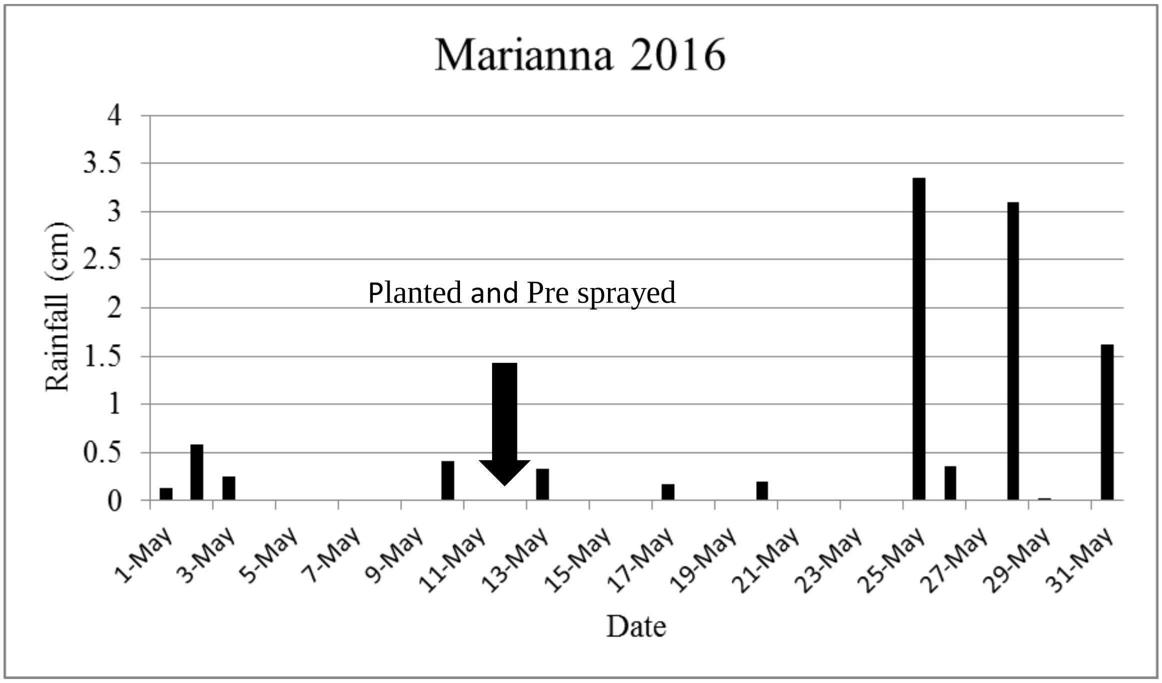
Herbicide[a]		Relative yield[bc]	
PRE	POST		
		%	
Rimsulfuron +thifensulfuron	Atrazine + *S*-metolachlor	102	c
Nicosulfuron	Atrazine + *S*-metolachlor	83	cd
S-metolachlor	Atrazine + *S*-metolachlor	97	c
Rimsulfuron +thifensulfuron	Atrazine + *S*-metolachlor	181	b
Nicosulfuron + *S*-metolachlor	Atrazine + *S*-metolachlor	66	d
Rimsulfuron +thifensulfuron + atrazine + *S*-metolachlor	NA	81	cd
Nicosulfuron + atrazine + *S*-metolachlor	NA	87	cd
Rimsulfuron +thifensulfuron + atrazine + S-metolachlor	Nicosulfuron	257	a
Rimsulfuron + thifensulfuron	Nicosulfuron	83	cd
NA	Nicosulfuron	80	d
NA	Nicosulfuron + atrazine	177	b
S-metolachlor	Nicosulfuron	103	c
S-metolachlor	Nicosulfuron + atrazine	192	b
NA	Nicosulfuron + atrazine + pyrasulfotole + bromoxynil	196	b

[a]Abbreviations: NA, no application; LSD, least significant difference

[b]Yield relative to plots following University of Arkansas Extension herbicide recommendations 4552 kg ha^{-1}

[c]Means followed by the same letter are not significantly different according to Fisher's protected LSD (α=0.05)

Figures



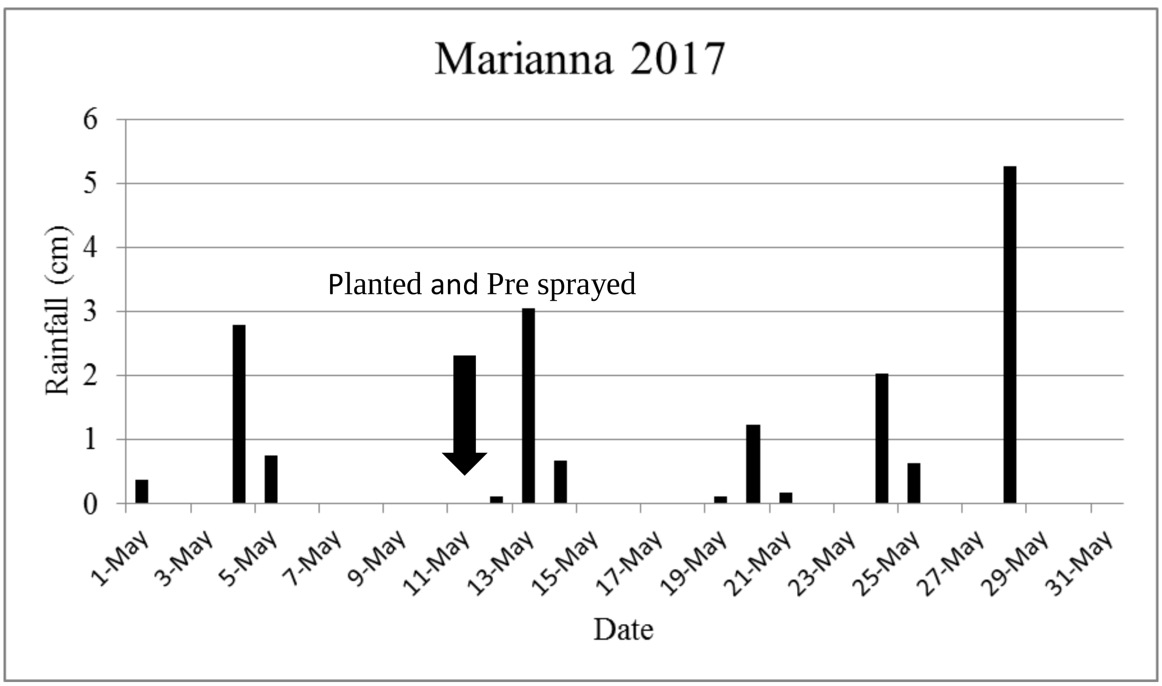


Figure 1. Planting dates and rainfall at Marianna, AR, in 2016 and 2017.

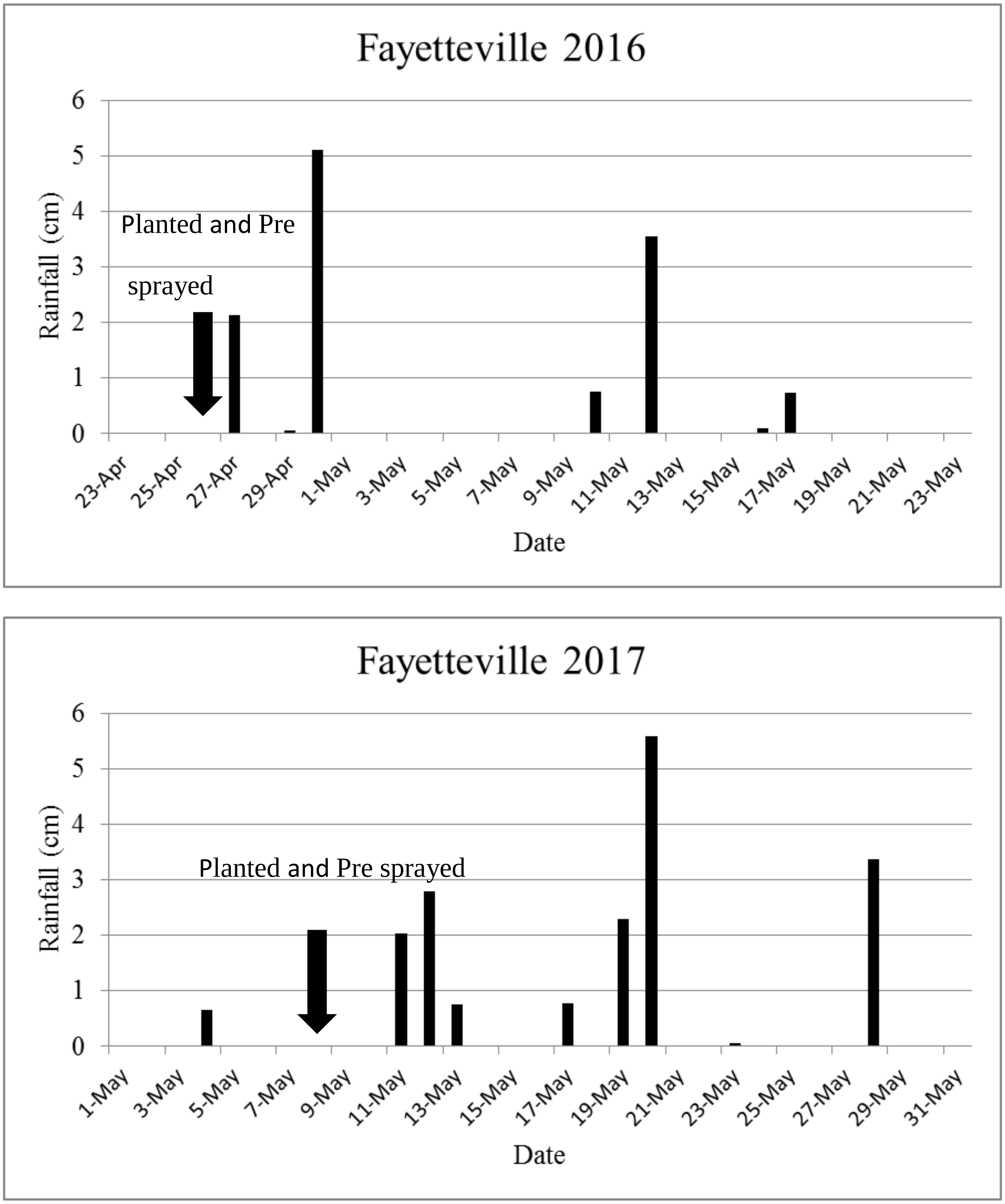


Figure 2. Planting dates and rainfall at Fayetteville, AR, in 2016 and 2017.

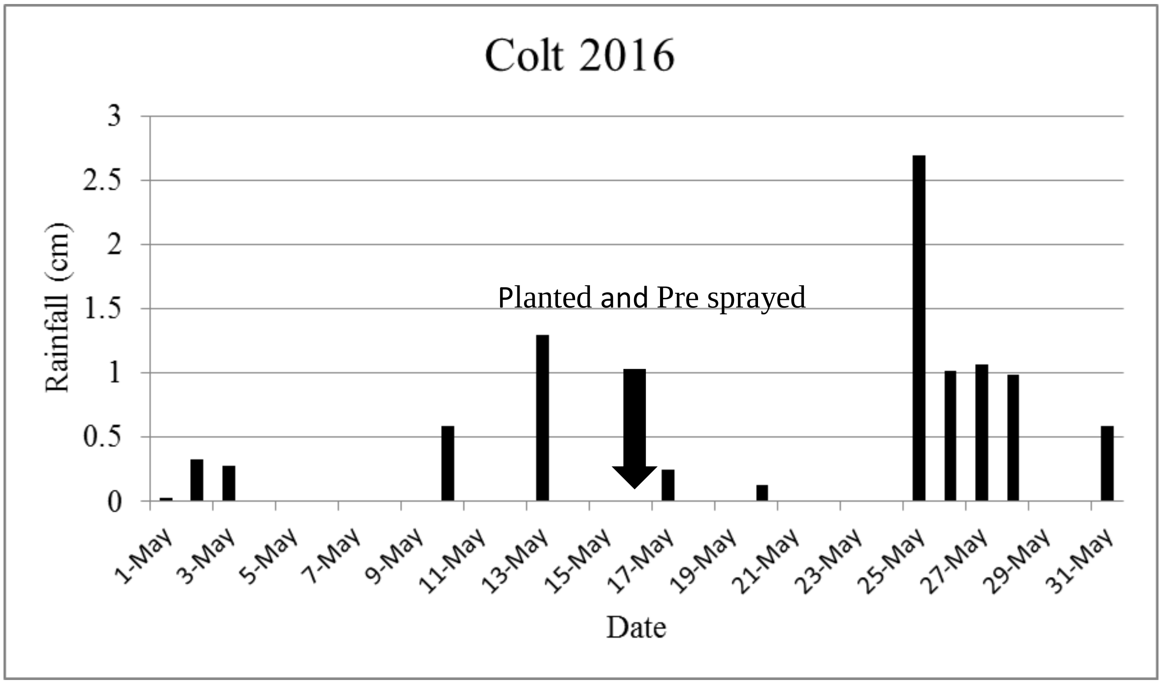


Figure 3. Planting dates and rainfall at Colt, AR, in 2016.

Chapter 4

Response of Grain Sorghum to Low Rates of Glufosinate and Nicosulfuron

Abstract

With the frequent use of aerial application for herbicides in Arkansas, off-target movement can be a common issue. Previous research has shown that glufosinate and nicosulfuron at low rates can cause yield loss to grain sorghum. However, research has not been conducted to pinpoint the growth stage at which these herbicides are most injurious to grain sorghum. Therefore, tests were conducted in 2016 and 2017 to determine the most sensitive growth stage for grain sorghum exposure to both glufosinate and nicosulfuron. Experiments were designed as a three-factor factorial with factor A being the herbicide applied (glufosinate or nicosulfuron) and, factor B consisted of timing of herbicide application including V3, V8, flagleaf, heading, and soft dough stages. Factor C was glufosinate or nicosulfuron rate where a proportional rate of 656 g ai ha^{-1} of glufosinate and 35 g ai ha^{-1} of nicosulfuron was applied at 1/10X, 1/50X, and 1/250X. At the V3 growth stage visible injury of 32% from the 1/10X rate of glufosinate and 51% from the 1/10X rate of nicosulfuron was observed. However, this injury was reduced by 4 WAA and no yield loss was observed from these early applications. Nicosulfuron was more injurious than glufosinate at a 1/10X and 1/50X rate when applied at the V8 and flagleaf growth stages resulting in death of the shoot, reduced heading and yield. Yield losses from the 1/10X rate of nicosulfuron were observed from V8 through early heading and ranged from 41-96%. Yield losses from the 1/50X rate of nicosulfuron were 14-16% at the flagleaf and V8 growth stages respectively. The 1/10X rate of glufosinate caused 36% visible injury 2 WAA when applied at the flagleaf stage, which resulted in a 16% yield reduction. No other appligations of glufosinate resulted in yield loss. By 4 WAA visible injury from either herbicide at less than 1/10X rates was not greater than 4%. Results indicate that injury can occur, but yield losses are more probable from low rates of nicosulfuron at V8 and flagleaf growth stages.

Nomenclature: glufosinate, nicosulfuron; grain sorghum, *Sorghum bicolor* L. Moench *ssp. bicolor*

Keywords: growth stage, off-target movement, visible injury

Introduction

In Arkansas, grain sorghum is often grown adjacent to rice (*Oryza sativa* L.*)*, corn (*Zea mays* L.), soybean [*Glycine max* (L.) Merr.], or cotton (*Gossypium hirsutum* L.). Due to its ability to perform well in hot dry climates it may even be planted in non-irrigated field corners of these other crops (Bennet et al. 1990). When environmental conditions are favorable, herbicides applied to these crops can move off-target resulting in injury to nearby grain sorghum (Al-Khatib and Peterson 1999). Since the evolution of glyphosate resistance, a majority of row crop hectares are treated with glufosinate in Arkansas, to control problematic weeds. Off-target movement of herbicides released from an unshielded sprayer can range from a 1/100 to 1/10X rate (Al-Khatib and Peterson 1999). The injury to non-tolerant crops from off-target movement can differ depending on the herbicide, sensitivity of crop, and growth stage of the plant (Hanks 1995; Miller 1993).

Glufosinate is a herbicide often used in cotton and soybean fields to control glyphosate-resistant Palmer amaranth (*Amaranthus palmeri* S. Wats.). Applications of glufosinate result in decreased production of glutamine synthetase in susceptible plants. Glutamine synthetase is an enzyme necessary in the conversion of glutamate and ammonia to the amino acid glutamine (Coetzer and Al-Khatib 2001; Devine et al. 1993). Glufosinate-resistant crop varieties were created using the gene bialophos (bar) from *Streptomyces hygroscopius*, a bacterium. Phosphinothricin acetyltransferase enzyme is expressed by the bar gene, conferring resistance to glufosinate (Culpepper et al. 2009). Currently, glufosinate-resistant grain sorghum varieties have not yet been developed, therefore all varieties are sensitive and due to the rotation with cotton, the probability of glufosinate being sprayed in the neighboring fields are high.

Nicosulfuron was applied in corn to control numerous weedy grass species, prior to the introduction of glyphosate-resistant (GR) corn. It is an acetolactate synthase (ALS)-inhibiting (Weed Science Society of America Group 2) herbicide. This site of action was arguably one of the most widely used in agriculture, prior to the introduction of GR crops. The ALS enzyme is the first in the biosynthetic pathway of branched chain amino acids leucine, isoleucine, and valine (Ray 1984). By inhibiting this pathway susceptible plants can be starved of branched chain amino acids, leading to mortality. In four site years, giant foxtail (*Setaria faberi* Herm.) was controlled >98% by nicosulfuron (Dobbels and Kapusta 1993). Shattercane (*Sorghum bicolor* (L.) Moench ssp. *drummondii*) a plant from the same genus as grain sorghum was controlled >90% when nicosulfuron at 30 g ha^{-1} was applied (Rosales-Robles 1993).

Since development of herbicide-resistant (HR) crops can provide growers with efficacious options to control problematic weeds, researchers have created grain sorghum varieties that confer resistance to the ALS-inhibiting herbicides. This resistance is conferred through a double mutation contained in the ALS gene of Val_{560}Ile and Trp_{574}Leu (Tuinstra and Al-Khatib 2008). This new HR grain sorghum will provide growers with the option of applying ALS-inhibiting herbicides, such as nicosulfuron, in areas where weedy grass species are problematic (Hennigh 2010).

Previous research has been conducted in corn and grain sorghum to show the effect of low rates of glufosinate, glyphosate, imazethapyr, and sethoxydim (Al-Khatib et al. 2003). However, this research only looked at these herbicides when applied to susceptible crops at the 3- to 4-leaf growth stage. During this research, it was observed that symptoms from imazethapyr were similar to these reported for nicosulfuron (Al-Khatib and Peterson 1999; Al-Khatib and Tamhane 1999). However, since both of these herbicides inhibit ALS, these results were not surprising (Beyer et al. 1988; Stidham and Singh 1991). The objective of this experiment was to evaluate the tolerance of grain sorghum to low rates of glufosinate and nicosulfuron at varying growth stages to determine the most sensitive period for severe injury and/or yield loss to occur.

Materials and Methods

Field experiments were conducted at the Rohwer Agricultural Research Station (RRS), near Rohwer, AR, the Northeast Research & Extension Center (NREC) in Keiser, AR, in 2016, the Lon Mann Cotton Research Station (LMCRS), near Marianna, AR, and the Arkansas Agricultural Research & Extension Center (AAREC), in Fayetteville, AR in 2016 and 2017. Soil texture at the RRS was a Herbert silt loam (fine-salty, mixed, active, thermic Aeric Epiaqualf) with 16% sand, 67% silt, 17% clay, a pH of 7.1 and 2.2% organic matter (OM). The NREC site was a Sharkey clay (very fine, montmorillonitic, nonacid, thermic, Vertic Haplaquept) with 22% sand, 25% silt, 53% clay, pH of 6.7, and 1.7% OM. At the LMCRS, the soil texture was a Calloway silt loam (fine-silty, mixed, active, thermic Aquic Fraglossudalfs) with 2% sand, 82.3% silt, and 15.6% clay with a pH of 5.5, and contained 2.2% OM. The AAREC soil texture was a Captina silt loam (fine-silty, siliceous, active, mesic Typic Fragiudults) with 22% sand, 64% silt, and 14% clay with a pH of 5.8, and contained 1.8% OM. A Dekalb® (Monsanto Company, St. Louis, MO) grain sorghum hybrid, DKS 53-67, was planted at 217,000 seeds ha^{-1} at all locations. DKS 56-67 did not provide resistance to either glufosinate or nicosulfuron. Plots were 4 rows wide at all locations. This experiment was arranged as a three-factor factorial including herbicide, rate, and timing of application. The herbicide factor was comprised of either glufosinate or nicosulfuron. A proportional rate of 656 g ai ha^{-1} of glufosinate and 35 g ai ha^{-1} of nicosulfuron at 1/10X, 1/50X, and 1/250X was applied. Growth stages of V3, V8, flagleaf, heading, and soft dough grain sorghum were chosen to determine which stage of growth the highest sensitivity exists. All applications were made using an air pressurized four-nozzle spray boom equipped with TeeJet® Air Induction XR 110015 nozzles, traveling at 4.8 km hr^{-1} and calibrated to deliver 140 L ha^{-1} (TeeJet® Technologies, Glende Heights, IL). Plots at RRS and LMCRS were 9 m long plots and 6 m long at NREC and AAREC. In order to maintain weed-free plots, an application of atrazine (Aatrex, Syngenta Crop Protection, LLC, Greensboro, NC) and *S*-metolachlor (Dual II Magnum, Syngenta Crop Protection, LLC, Greensboro, NC) were applied at planting. Any escapes from this application were controlled by a postemergence application of the same mix. Further escapes were removed by hand. Fertilizer and pest management decisions were based on University of Arkansas extension recommendations (Espinoza 2015; McLeod and Greene 2015).

In 2016, visible crop injury was rated at 2 and 4 weeks after application (WAA) and grain yield was collected at crop maturity. In 2017, crop injury was rated at 2 and 4 weeks after application WAA, along with crop canopy heights (cm), days to 50% heading and yield at crop maturity. Visible crop injury was rated on a scale of 0 to 100%, with 0% being no injury and 100% being complete plant mortality. In each plot, 5 random grain sorghum plants were measured in cm, then averaged together and divided by the average of the nontreated plots and recorded as relative crop canopy heights. The center two rows at each location were harvested using a small-plot research combine and recorded as kg ha^{-1}, after moistures were adjusted to 14%. Reductions or increases in relative yield were calculated by dividing yield of plots by the average yield of nontreated plots.

All data collected were subjected to analysis of variance (ANOVA) using JMP (JMP PRO 13, SAS Institute Inc., Cary, NC), with significant means separated using Fisher’s protected LSD ($P = 0.05$). Herbicide, rate, and timing of application were included as fixed effects, with location and year being random effects. The nontreated in each replication was excluded from the analysis since they were only included for relative comparisons.

Results and Discussion

A three-way interaction, for factors herbicide, rate, and timing, was observed for visible injury ($P<0.0001$) and canopy heights ($P=0.0002$) at 2 WAA (Table 1). Response of grain sorghum differed between herbicide, with nicosulfuron generally causing more visible injury than glufosinate. However, increasing herbicide rate resulted in an increase of visible injury for both herbicides (Table 2). At 2 WAA, injury from glufosinate at 1/10X ranged from 14-36% across all growth stages. The greatest visible injury (32-36%) from glufosinate was observed following applications to V3 and flagleaf stages (Table 2). Grain sorghum injury from glufosinate was <12% for 1/50 and 1/250X rates regardless of growth stage.

The greatest injury (65%) observed at 2 WAA resulted from applications of nicosulfuron at 1/10X rate applied to V8 sorghum. At this rate and crop stage, along with the flagleaf stage (21%), growth was halted, and death of the shoot occurred. Injury from glufosinate was high at this growth stage but did not result in death of the growing point. Results from nicosulfuron injury are similar to symptoms of imazethapyr seen in other research (Al-Khatib et. al 2003). Injury from nicosulfuron was greater than glufosinate at the 1/50X rate ranging from 14-36%, with the highest occurring from applications to theV8 growth stage. All other injury was ≤ 4% at this rate. Visible injury to grain sorghum caused by the 1/250X rate of glufosinate and nicosulfuron was minimal, not exceeding 6% no matter the growth stage of sorghum at the time of application (Table 2).

Glufosinate at the 1/10X rate resulted in height reductions at all growth stages 2 WAA, except for V8 (Table 3). Height reductions were found with the 1/10X rate of nicosulfuron at V3 (21%) and flagleaf (31%) growth stages. Generally, no reduction in height occurred with applications 1/50X or 1/250X rate of either herbicide, except for 1/50X rate of glufosinate applied at V3 (9%) and a 1/50X rate of nicosulfuron applied at flagleaf (8%) (Table 3).

Visible injury decreased by 4 WAA, so only plots where the 1/10X rate was applied were included in the statistical analysis. A two-way interaction of herbicide and timing ($P<0.0001$) was observed (Table 1). Glufosinate applied at the 1/10X rate to flagleaf sorghum resulted in 19% injury. At 4 WAA, the least amount of injury (3%) was observed when applications at the 1/10X rate of glufosinate were made to soft dough sorghum (Table 4). The greatest injury (78%) was recorded with the 1/10X rate of nicosulfuron applied to V8 sorghum, which was significantly higher than injury (22%) with the same rate at flagleaf. Injury from glufosinate or nicosulfuron at the 1/50X or 1/250X rate did not exceed 4% (Table 4). By 4 WAA there was a difference in canopy height found in the main effect of herbicide ($P= 0.0053$) (Table 1). Plots where glufosinate was applied were taller than plots applied with nicosulfuron (Data not shown).

No delay in heading was observed in plots applied with glufosinate. However, plots applied with the 1/10X rate of nicosulfuron to V8 and flagleaf sorghum often did not mature into a headed plant (Data not shown). Al-Khatib and others (2003) found similar effects from low rates of imazethapyr. The seeds head^{-1} of grain sorghum can be greatly impacted if plants are using sugars and energy towards the metabolism of herbicides (Saeed et al. 1986). A three-way interaction of herbicide, rate, and timing was found for the response variable relative yield ($P<0.0001$) (Table 1). Injury caused by glufosinate applications only resulted in a yield reduction of greater than 10% when applied at the 1/10X rate to flagleaf sorghum (Table 5). At the 1/10X rate of glufosinate on flagleaf grain sorghum, a 16% yield reduction occurred; however, this reduction did not differ from the 1/10X rate of glufosinate on heading and soft dough grain sorghum, which resulted in 6 and 8% reductions, respectively (Table 5). The greatest yield reduction of 96% was collected from plots where nicosulfuron was applied at a 1/10X rate to V8 and flagleaf grain sorghum. When nicosulfuron was applied to heading sorghum at the same 1/10X rate a 41% yield reduction was found. All other applications of nicosulfuron only resulted in a 16% or less reduction in yield (Table 5). Nicosulfuron at the 1/50X rate applied to V8 and flagleaf sorghum did cause a 14 and 16% yield reduction respectively (Table 5). These results show that the V8 and flagleaf growth stages appear to be the most sensitive stages for yield loss to occur from off target nicosulfuron or glufosinate herbicide movement and that grain sorghum is not sensitive to yield loss from low rates of glufosinate.

Practical Implications. With the diversity of herbicide SOAs currently in the agricultural market, it is important that we understand the risk of these herbicides to susceptible crops. Glufosinate herbicide is used on a high percentage of crop acres in the Midsouth and other areas for control of Palmer amaranth. Off-target movement of glufosinate has been observed previously in Arkansas on non-tolerant crops. These results indicate that a high rate of glufosinate (1/10X or greater) is required to cause significant injury to grain sorghum that ultimately results in any yield loss.

Nicosulfuron applications may increase in cropping areas where Inzen grain sorghum or conventional corn is produced. Results from the previous experiments indicate that yield can be reduced by low rates (1/50X) of nicosulfuron when applied to grain sorghum at sensitive stages (V8 through heading). However, most herbicides are often applied early in the year when crops are in vegetative stages. High rates of 1/10X or greater can cause significant yield reductions to grain sorghum cultivars that are not bred with a resistance to nicosulfuron, therefore tank cleanout and field identification practices become more important when traited or non-traited grain sorghum varieties are grown in the same area.

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Tables

Table 1. Analysis of variance for grain sorghum injury, canopy heights, and grain yield from low rates of postemergence-applied glufosinate and nicosulfuron applications from 2016 and 2017.[abcd]

Variable[e]	Source	DF[a]	F Ratio	P value[f]
Visible injury 2 WAA (%)	Herbicide	1	11.5877	0.0008*
	Rate	2	177.1027	<0.0001*
	Herbicide*Rate	2	5.9441	0.003*
	Timing	4	14.0215	<0.0001*
	Herbicide*Timing	4	18.802	<0.0001*
	Rate*Timing	8	6.0009	<0.0001*
	Herbicide*Rate*Timing	8	6.1219	<0.0001*
Canopy heights 2 WAA (cm)	Herbicide	1	1.3823	0.2411*
	Rate	2	61.5916	<0.0001*
	Herbicide*Rate	2	0.3755	0.6874
	Timing	4	13.2156	<0.0001*
	Herbicide*Timing	4	2.7439	0.0295*
	Rate*Timing	8	2.5698	0.0108*
	Herbicide*Rate*Timing	8	3.9713	0.0002*
Visible injury 4 WAA (%)	Herbicide	1	84.3457	<0.0001*
	Timing	4	50.4465	<0.0001*
	Herbicide*Timing	4	50.2725	<0.0001*
Canopy heights 4 WAA (cm)	Herbicide	1	7.9254	0.0053*
	Rate	2	1.5439	0.216
	Herbicide*Rate	2	0.4728	0.6239
	Timing	4	0.9848	0.4168
	Herbicide*Timing	4	0.9178	0.4545
	Rate*Timing	8	0.9592	0.469
	Herbicide*Rate*Timing	8	0.4273	0.9039
Relative yield (%)	Herbicide	1	18.8745	<0.0001*
	Rate	2	66.5464	<0.0001*
	Herbicide*Rate	2	33.7638	<0.0001*
	Timing	4	18.0419	<0.0001*
	Herbicide*Timing	4	19.1681	<0.0001*
	Rate*Timing	8	11.8419	<0.0001*
	Herbicide*Rate*Timing	8	10.0001	<0.0001*

[a]Injury experiment conducted near Colt, AR in 2016.
[b]Injury experiment conducted in Keiser, AR in 2016.
[b]Injury experiment conducted near Marianna, AR in 2016 and 2017.
[d]Injury experiment conducted in Fayetteville, AR in 2016 and 2017.
[e]Abbreviations: DF, degrees of freedom; WAA, weeks after application
[f]*Denotes significance

Table 2. Visible estimates of injury to grain sorghum at various growth stages and herbicide rates 2 WAA.[abcde]

		Injury 2 WAA[f]									
Herbicide	Rate	V3		V8		Flagleaf		Heading		Soft dough	
		----------------------------% of nontreated----------------------------									
Glufosinate[g]	1/10	32	C	22	de	36	c	23	d	14	fgh
	1/50	9	hij	5	jklm	12	ghi	8	ijk	8	ijk
	1/250	3	klm	1	m	2	lm	3	klm	1	m
Nicosulfuron[h]	1/10	51	B	65	a	21	de	17	efg	19	def
	1/50	14	fgh	36	c	4	klm	4	klm	4	klm
	1/250	3	klm	1	m	0	m	0	m	0	m

[a]Injury experiment conducted near Colt, AR in 2016.
[b]Injury experiment conducted in Keiser, AR in 2016.
[c]Injury experiment conducted near Marianna, AR in 2016 and 2017.
[d]Injury experiment conducted in Fayetteville, AR in 2016 and 2017.
[e]Abbreviations: WAA, weeks after application
[f]Means followed by the same letter are not different (P=0.05)
[g]Glufosinate rates are proportional to 656 g ai ha-1
[h]Nicosulfuron rates are proportional to 35 g ai ha-1

Table 3. Relative plant heights from grain sorghum in 2017 at various growth stages, 2 WAA of nicosulfuron and glufosinate at low rates in near Marianna, AR and Fayetteville, AR[a].

		Heights 2 WAA[b]				
Herbicide	Rate	V3	V8	Flagleaf	Heading	Soft dough
		-----------------% of nontreated-----------------				
Glufosinate[c]	1/10	80 J	93 efghi	86 ij	88 hi	85 ij
	1/50	91 ghi	99 bdefg	93 efghi	94 defghi	99 bcdefg
	1/250	97 bcdefg	101 abcd	94 defghi	103 abc	109 a
Nicosulfuron[d]	1/10	79 J	95 cdefgh	69 k	96 bcdefg	97 bcdefg
	1/50	100 bcde	99 bcdefg	92 fghi	101 abcd	104 ab
	1/250	102 abcd	102 abcd	99 bcdefg	104 ab	100 bcde

[a]Abbreviations: WAA, weeks after application

[b]Means followed by the same letter are not different (P=0.05)

[c]Glufosinate rates are proportional to 656 g ai ha^{-1}

[d]Nicosulfuron rates are proportional to 35 g ai ha^{-1}

Table 4. Visible injury to grain sorghum at various growth stages and herbicide rates 4 WAA.[abcde].

Herbicide	Rate	Injury 4 WAA[f]				
		V3	V8	Flagleaf	Heading	Soft dough
		---	---	% of nontreaed	---	---
Glufosinate[g]	1/10	12 c	10 cd	19 b	13 c	3 e
	1/50[e]	0	0	3	2	1
	1/250[e]	0	0	1	1	0
Nicosulfuron[h]	1/10	10 cd	78 a	22 b	10 cd	5 de
	1/50[e]	4	3	2	0	0
	1/250[e]	0	0	0	0	0

[a]Injury experiment conducted near Colt, AR in 2016.
[b]Injury experiment conducted in Keiser, AR in 2016.
[b]Injury experiment conducted near Marianna, AR in 2016 and 2017.
[d]Injury experiment conducted in Fayetteville, AR in 2016 and 2017.
[e]Abbreviations: WAA, weeks after application
[f]Means followed by the same letter are not different (P=0.05)
[g]Glufosinate rates are proportional to 656 g ai ha^{-1}
[h]Nicosulfuron rates are proportional to 35 g ai ha^{-1}

Table 5. Relative yield of grain sorghum near Marianna, AR and in Fayetteville, AR, collected in 2017 after applications of low rates of nicosulfuron and glufosinate

		Relative yield[ad]				
Herbicide	Rate	V3	V8	Flagleaf	Heading	Soft dough
		--------	--------	% of nontreated	--------	--------
Glufosinate[b]	1/10	94 bcdef	96 abcdef	81 g	91 cdefg	89 defg
	1/50	96 abcdef	96 abcdef	100 abcde	95 abcdef	95 abcdef
	1/250	103 ab	96 abcdef	103 ab	100 abc	92 cdef
Nicosulfuron[c]	1/10	97 abcdef	1 i	1 i	56 h	110 a
	1/50	106 abc	81 fg	83 efg	108 ab	97 abcdef
	1/250	95 abcdef	103 abcde	95 abcdef	110 a	105 abcd

[a]Means followed by the same letter are not different (α=0.05)

[b]Glufosinate rates are proportional to 656 g ai ha^{-1}

[c]Nicosulfuron rates are proportional to 35 g ai ha^{-1}

[d]Yield relative to the nontreated check average of 8174 kg ha^{-1}

General Conclusions

These results prove that a new herbicide-resistant grain sorghum technology (Inzen™) will provide growers an effective management option for johnsongrass. As demonstrated, nicosulfuron provides effective control of johnsongrass after it emerges, proving its ability to be implemented in grain sorghum for season-long control. As shown this new grain sorghum technology opens the door to utilizing another herbicide site of action in crop, which contains more than 50 herbicides that can be safely applied both PRE and POST. Proper utilization of this technology will result in a robust herbicide program that controls many weeds including not only johnsongrass, but also glyphosate-resistant Palmer amaranth, barnyardgrass, broadleaf signalgrass, and many other troublesome weeds in the Midsouth.

Management of off-target movement in the Midsouth is a pressing issue as commercialization of multiple herbicide-resistant technologies approaches. As shown, grain sorghum sensitivity to both nicosulfuron and glufosinate seems to be low, except V8 through flagleaf stage. In summary, these experiments show that weed control programs in grain sorghum can be optimized by integrating the Inzen™ technology and utilizing ALS-inhibiting herbicides such as nicosulfuron.