Understanding the Potential Utility of TamArkTM Grain Sorghum

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Understanding the Potential Utility of TamArk^{TM} Grain Sorghum

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Abstract

Herbicide-resistant crops have been commonly used in corn, cotton, and soybean since the 1990s to control numerous different grass and broadleaf weeds. However, this technology has not been available for grain sorghum producers whom have faced challenges controlling grass weeds. Recently a collaboration between the University of Arkansas and Texas A&M University has resulted in a new bred line of grain sorghum, TamArk™, which is resistant to ACCase inhibitor herbicides which have been previously used to control grass weeds in broadleaf crops. Multiple studies were conducted to determine the sensitivity of TamArk™ and problematic grass weeds to ACCase inhibitor herbicides, and to determine if johnsongrass in major sorghum producing states is resist to new herbicides that could be used in herbicide resistant grain sorghum. It was determined that TamArk™ grain sorghum was not sensitive to ACCase inhibitors from the aryloxyphenoxypropionate and phenylpyrozolin families. These herbicides also resulted in greater than 90% control of problematic grass weeds in grain sorghum. Johnsongrass resistance was found with fluazifop, nicosulfuron, imazamox, and glyphosate but was not deemed widespread.

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Chapter 1

General Introduction and Review of Literature

Introduction

Grain sorghum [*Sorghum bicolor* (L.) Moench] has been a staple crop in the United States (US) since its introduction from Africa and Asia in the late 1850s. Initially brought over as a forage crop, grain sorghum has been making an impact as a food crop, being milled into flour for use of its antioxidant properties and more recent use in the ethanol market. In 2020, the US planted 2.26 million hectares of grain sorghum, with the top producing states being Kansas and Texas, with a combined 1.78 million hectares of grain sorghum, making grain sorghum the 5th most-produced cereal crop in the US. Arkansas is the 6th largest producer of grain sorghum in the US, with 5,000 hectares planted mainly in the Mississippi River delta in the eastern part of the state (NASS 2020). What makes grain sorghum so appealing to producers, especially in the Midwest, is its ability to withstand high-temperature stress and low water situations and still successfully produce a crop (Prasad et al. 2008).

Though the practices used to control weeds in grain sorghum have changed over the decades, a problem that persists is grass control. Since grain sorghum is part of the Gramineae family and is even in the same genus as weeds such as johnsongrass [*Sorghum halapense* (L.) *Pers.*] and shattercane [*Sorghum bicolor (L.) Moench ssp.*], there are very few options when it comes to chemical control of annual and perennial grasses.

This study will evaluate the use of acetyl CoA carboxylase (ACCase) inhibitors on TamArk^{TM} grain sorghum. Examining crop sensitivity and weed control when applying ACCase inhibitors postemergence (POST). These data will help determine the best ACCase inhibitors available to control problem grasses in grain sorghum while also determining which herbicides cause the least injury to TamArk^{TM} grain sorghum, allowing us to better understand potential of TamArk^{TM} grain sorghum and determine the benefit for producers in the future.

<u>Herbicide-Resistant Grain Sorghum</u>

In-season postemergence grass control has always been limited in grain sorghum, with producers having the ability to use the herbicide paraquat, which must be sprayed under hoods, for johnsongrass control, and quinclorac for control of other grass species (Barber et al. 2020). Sorghum producers are currently applying preemergence (PRE) herbicides such as metolachlor or dimethenamid for grass control. To safely use these herbicides growers must rely on a fluxofenim-based seed treatment that safens grain sorghum to these herbicides (Al-Khatib et al. 2004). However, since grain sorghum is a crop typically grown in hot and dry conditions, decreased efficacy can occur if soil moisture is inadequate for herbicide activation (Brown et al. 1988; Regehr et al. 2008). Because of the limited grass control options, there has been a focus within the industry to develop grain sorghum germplasm that allows typically non-labeled herbicides to be safely applied.

<u>*ALS-inhibitor-resistant Grain Sorghum*</u>

Initially launched in 1982, Group 2 herbicides inhibit the critical enzyme acetolactate synthase (ALS), the main pathway for multiple branched chain amino acids (Al-Khatib 2020). These herbicides were extensively used because of the low amount of active ingredient (ai) needed for proper weed control (Tranel and Wright 2002). Unfortunately, ALS inhibitors have been plagued by the rapid selection for herbicide-resistant weed biotypes. In 1998, ALS inhibitors became the herbicide group with the largest number of resistant weed species because of overuse and ease of selecting for resistance (Tranel and Wright 2002).

The Inzen^{TM} technology is the first approved herbicide-resistant grain sorghum. Initially developed by Dow AgroScience, now Corteva Agriscience (Indianapolis, IN) and approved for global use in 2016, this grain sorghum is resistant to the ALS inhibitor herbicide nicosulfuron known by the trade name Zest (Pinkerton 2020) as well as many other ALS-inhibiting herbicides (Bowman et al. 2021). Nicosulfuron was a commonly used herbicide to control johnsongrass in corn (*Zea mays* L.) throughout the 1980s and early 1990s before the introduction of glyphosate-resistant crops. This technology allows for both PRE and POST applications of the ALS inhibitor nicosulfuron. Moreover, it can be mixed with other commonly applied broadleaf herbicides, such as dicamba and 2,4-D, with no increased injury, which results in an effective herbicide program for postemergence control of annual grasses (Pinkerton 2020). One problem this technology presents is the potential for rapid selection for herbicide resistance (Tranel and Wright 2002), and the fact that resistance to ALS herbicides is already common in some geographies where johnsongrass is problematic (Bagavathiannan and Norsworthy 2014; Heap 2015; Johnson et al. 2014b). Even with these concerns, the Inzen^{TM} technology is expected to be commercially launched in 2021.

Another grain sorghum technology, developed through a collaboration between UPL (King of Prussia, Pennsylvania) and Alta Seeds (Amarillo, Texas), has resulted in the Igrowth^{TM} technology. The Igrowth^{TM} technology will allow producers to utilize the ALS inhibitor imazamox, which will be marketed under the name Imiflex^{TM}. Imazamox is a herbicide that has been effective for grass control in rice production since the introduction of the Clearfield system (Fish et al. 2017). The concerns noted for the Inzen^{TM} technology are similar issues with the Igrowth^{TM} technology, and commercialization is expected in 2021.

ACCase-Resistant Grain Sorghum

Group 1 herbicides have been commercialized for over 40 years and act by inhibiting acetyl Coenzyme A carboxylase (ACCase), an enzyme vital for fatty acid biosynthesis in the chloroplast of the cell (Dekker 1999). There are currently two lines of grain sorghum being developed for commercial use that are resistant to the herbicides quizalofop and fluazifop in the aryloxyphenoxypropionate (FOP) family of ACCase-inhibiting herbicides.

Quizalofop-resistant grain sorghum, known as Double Team, is being developed through a collaboration between S&W Seed Company (Longmont, Colorado) and ADAMA (Raleigh, North Carolina) and is being marketed as resistant to the herbicide quizalofop. This mutation is due to a single point mutation at the 2027 location that results in a cystine replacing a tryptophan (Kershner et al. 2011). Quizalofop-resistant grain sorghum will allow for over-the-top applications of the corresponding herbicide to control annual and perennial grass weeds (Pinkerton 2020).

TamArk^{TM} grain sorghum, which will be a focus in this study, is being developed through a collaboration between the University of Arkansas and Texas A&M University. This line was bred for resistance to fluazifop by successfully crossing TamArk^{TM} johnsongrass with grain sorghum (Bagavathiannan et al. 2018). This resistance is due to a target site mutation at position 2248 on the ACCase gene which causes the plant to act like the quizalofop-resistant cultivar previously discussed (Norsworthy et al. 2020). The location of this mutation is different than that of the quizalofop resistant varieties coming to market which could result in different tolerance levels to ACCase-inhibitors. TamArk™ grain sorghum is believed to have commercial tolerance to four times an anticipated labeled rate of fluazifop (Piveta et al. 2020). TamArk™ grain sorghum unlikely to show resistance to the cyclohexanedione (DIM) family of ACCase inhibitors, which includes the herbicides clethodim and sethoxydim, leaving options for control of volunteer plants within the family of ACCase-inhibiting herbicides.

Johnsongrass

Johnsongrass was brought to South Carolina from Turkey in the 1800s, where it was used as a forage crop due to its ability to produce large quantities of biomass. It was not until the 1840s when johnsongrass was introduced to fertile river bottoms in Alabama, that the spreading capabilities and the inability to contain it were seen (Miller 2014). Johnsongrass is a spreading perennial grass that can grow more than two meters tall and can be spread through both seed dispersal and rhizomes, which are horizontally growing underground stems from which new plants can reproduce. Johnsongrass can produce upwards of 5,000 rhizomes per plant in a single growing season, which is why it is difficult to contain and control (McWhorter 1971). Rhizomes are another reason why herbicide control is not always effective. If a johnsongrass plant begins to produce rhizomes before it is sprayed, a lack of control may occur. Even though the aboveground plant may be controlled, the underground rhizomes may still be active and produce new plants after application. Due to the devastating effects of rhizome production, it is crucial to control johnsongrass before reaching the rhizome production stage (Horowitz 1972). Johnsongrass is also very adaptive to its climate and can quickly acclimate to new environments when introduced. This is another reason for its rapid spread. When first introduced, johnsongrass was a warm-season grass acclimated to weather conditions like that in the southeastern US. While johnsongrass infestation initially began in the southeast US, it can now be found in every US state and many foreign countries with less-than-ideal growing conditions (Burt 1974).

Gene Flow

With grass weeds such as johnsongrass and shattercane having the ability to hybridize with grain sorghum, the potential for herbicide resistance is present. Crossing weedy relatives with their cropped family members has been utilized many times to improve genetics and develop herbicide-resistant crops (Ohadi et al. 2017). Though herbicide-resistant traits in grain sorghum present many benefits, there can be adverse effects. For example, weedy rice (*Oryza sativa* L.) can hybridize with ALS-resistant rice cultivars producing herbicide-resistant weedy offspring. Evolution of ALS-resistant weedy rice can occur in as few as three years (Burgos et al. 2008). In this manner, the longevity of a potentially helpful new technology can be dramatically reduced. This same scenario with weedy rice could be prevalent in grain sorghum if proper rotational methods and the weedy relatives of grain sorghum are not adequately maintained and eradicated from the field and surrounding areas. The amount of gene flow between cultivated sorghum and weedy relatives. is not currently well understood, but it has been found that both cultivated sorghum and its weedy relatives have a 90% overlap in pollination timings (Tesso et al. 2008). Even though there is not a large difference between pollination timing, cultivated sorghum varieties are typically hybrid cultivars with male sterility which greatly reduces the potential for outcrossing of herbicide resistance genes (Ohadi et al. 2017). To help better understand the geneflow of herbicide resistance traits from grain sorghum to its weedy relatives, the university of Nebraska has been working to develop a genetic marking system which would allow for them to trace the movement of the ALS-resistance gene within Inzen™ sorghum to determine if resistant johnsongrass species are developing natural resistance or are hybridizing with cultivated sorghum (Zigafoos et al. 2017). Although the spreading of herbicide resistance genes into weeds could be extremely detrimental to grain sorghum, it could also lead to problems in many other crops such as soybean [*Glycine max* (L.) Merr.], cotton (*Gossypium hirsutum* L.), and rice where both ACCase and ALS inhibitor herbicides are used to control weeds such as johnsongrass. The addition of these herbicide-resistant genes to already problematic johnsongrass could leave us with very few options for control in other crops and lead to having the same limited control options in grain sorghum that were available prior to the introduction of herbicide-resistance technology.

<u>Herbicide-Resistant Johnsongrass</u>

Although mitigation of the potential creation of herbicide-resistant johnsongrass should be at the forefront of thinking, it is also important to note that herbicide-resistant johnsongrass biotypes have already been documented in many places around the world, including in Arkansas (Aiub et al. 2007; Bagavathiannan and Norsworthy 2014; Riar et al. 2011; Smeda et al. 1997; Werle et al. 2016). One of the first herbicide-resistant johnsongrass biotypes was discovered in the 1980s in Mississippi. The first recorded biotypes were resistant to the ACCase inhibitors fluazifop, quizalofop, and sethoxydim. In multiple field and greenhouse trials, these herbicides showed less than 35% control of both seedling and rhizome johnsongrass when applied at labeled rates (Smeda et al. 1997). In 2007, the first johnsongrass resistant to glyphosate was confirmed in Argentina (Vila-Aiub et al. 2007). Erratic control of johnsongrass was seen across a field of glyphosate-resistant soybean. This discovery led to dose-response testing of the johnsongrass biotype alongside biotypes with known susceptibility. In this study, researchers discovered a johnsongrass population that could withstand up to ten times the labeled glyphosate rate (Vila-Aiub et al. 2007). In 2016, a study was conducted in Nebraska and Kansas to identify johnsongrass biotypes resistant to ALS inhibitors. Johnsongrass was selected from 59 random locations across southern Nebraska and northern Kansas. These accessions were then planted in a field where they were treated with nicosulfuron and imazethapyr. After determining which accessions survived the initial herbicide treatment, a dose-response was conducted. The results confirmed that five johnsongrass populations were resistant to imazethapyr, and three johnsongrass populations were resistant to nicosulfuron (Werle et al. 2016).

There have also been populations of herbicide-resistant johnsongrass found in Arkansas (Bagavathiannan and Norsworthy 2014; Johnson et al. 2014; Riar et al. 2011). In the fall of 2007, erratic control of johnsongrass was seen in a field of glyphosate-resistant soybean in West Memphis, AR. This johnsongrass population was screened for resistance, and it was determined that the population required a rate equal to or greater than two times the labeled field rate of glyphosate for 50% control (Riar et al. 2011). Roadside biotypes could also contribute to the spread of herbicide resistant johnsongrass in Arkansas. In a study conducted by Bagavathiannan and Norsworthy (2014) 36-fold more fluazifop, 2.8-fold more glyphosate, and 4.1-fold more nicosulfuron were needed to achieve only 50% control. While this does not accurately describe the biotypes currently present in Arkansas production fields, it does present the risk for herbicide resistance. While herbicide resistance in johnsongrass could be a significant problem, it is crucial to understand that these are just instances where resistance has been recorded in one specific area, and these are not yet found to be widespread. From 2008 to 2010, 141 johnsongrass samples were collected from crop fields in 14 counties in eastern Arkansas, and screened for sensitivity to imazethapyr, glyphosate, fluazifop, and clethodim. All johnsongrass populations were controlled at least 95% with each herbicide (Johnson et al. 2014).

Integrated Weed Management

Combining mechanical, chemical, and cultural practices to develop a systems approach to better manage and control weeds while taking cultural, environmental, and social factors into account is known as integrated weed management. As discussed, one of the main focuses for weed control in grain sorghum is control of perennial grasses such as johnsongrass and annual grasses such as shattercane, broadleaf signalgrass [*Urochloa platyphylla* (Munro ex C. Wright) *R.D. Webster*], barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.], and Texas panicum [*Panicum texana* (Buckley) R. Webster]. A combination of mechanical, chemical, and cultural weed control practices will not only reduce or eliminate weed pressure for one specific year but could potentially reduce seed banks and prolong the efficacy of chemicals that are currently effective by reducing weed exposure to those chemicals and helping mitigate the potential for resistance.

Mechanical Control

Mechanical methods of weed control consist of tillage, mowing, hoeing, and hand weeding to remove or kill weeds actively growing in the field (Schwartz et al. 2016). These methods of control are non-selective and remove growing weeds from the area that is being treated. While these methods are not effective or economical if used alone, they have a lot to offer when integrated into a system. For perennial weeds such as johnsongrass, an intensive tillage program to help reduce stands in the subsequent crop is beneficial. This can be achieved through deep tillage that brings rhizomes of johnsongrass to the surface. If this can be done late in the fall, the rhizomes will be exposed to low winter temperatures reducing viability (Johnson et al. 1997). By reducing the population in the field, the use of herbicides that may lead to weed resistance may be reduced. Conversely, no-till or minimal tillage is another option that could benefit sorghum farmers. These programs show an increased control of broadleaf weeds and most annual grasses. One problem that persists with these programs is that no-till has no observable effect on johnsongrass control, and minimal tillage in some instances is observed to increase johnsongrass pressure (Phillips 1969: Brown et al. 1988). Though all weeds are not eradicated using tillage as a means of control, the number of broadleaf weeds and annual grasses is minimized, potentially reducing the use of herbicides such as atrazine.

Hand weeding is another form of non-chemical control that can be utilized in grain sorghum. Although this form when used alone is an inefficient and not cost-effective, it can be beneficial when integrated with other strategies. By hand weeding, a zero-tolerance for weeds can be implemented, which is especially important to reduce potential gene flow with johnsongrass. By hand weeding before anthesis, the seed bank is reduced the next year and those plants that may have been developing resistance are removed before depositing more seed into the seed bank (Norsworthy et al. 2018). While most weeds being hand weeded can be removed at the soil surface, it is essential to remember that johnsongrass must be uprooted and removed from the field due to presence of rhizomes. Although the seed is still being removed from the seed bank, johnsongrass populations will see minimal reduction since the majority of the year-to-year reproduction is from plants originating from rhizomes (McWhorter 1971).

Chemical Control

Although new trade names of herbicides are introduced on nearly a yearly basis, the rate of commercialization of new active ingredients has decreased significantly over the last two to three decades. Because new sites of action (SOAs) are not being developed the preservation of current herbicide technology is a vital part of any current weed control program. If a herbicide is lost due to overuse, it typically cannot be used again (Bagavathiannan et al. 2013).

The availability of a Concep seed treatment has greatly increased the number of available herbicides that can be utilized PRE in grain sorghum. For example, *S*-metolachlor can be used as a PRE option in Concep-treated grain sorghum to control grass weeds such as johnsongrass and barnyardgrass and help with problem broadleaf weeds such as Palmer amaranth in Arkansas. Concep seed treatment also allows for the use of acetochlor and dimethenamid-P, two active ingredients that were not previously available, allowing for a broader spectrum of herbicides to be used to help mitigate resistance (Bagavathiannan et al. 2013; Barber et al. 2020; Brabham 2019).

With the growing problem of ALS-resistant johnsongrass, overuse of the Inzen™ or Igrowth™ systems could limit the ability to use sulfonylurea or imidazalinone herbicides (Green 2007). The addition of a new ACCase-resistant variety such as TamArk™ grain sorghum will not only add another SOA to help mitigate resistance but will also offer herbicide options that can control johnsongrass better than other options currently available.

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Chapter 2

Sensitivity of Johnsongrass Accessions to Herbicides

Abstract

New technologies in grain sorghum allow the use of multiple acetyl CoA carboxylase (ACCase) or acetolactate synthase (ALS) inhibitors for johnsongrass control. With the growing issue of herbicide resistance, producers need to understand which herbicides will successfully control johnsongrass accessions. To determine the efficacy of herbicides recently registered or potentially can become available for use in grain sorghum for johnsongrass control, a study was conducted in Fayetteville, AR, where johnsongrass seeds collected in 2020 and 2021 in Arkansas, Kansas, Texas, and Oklahoma were screened for sensitivity to fluazifop, quizalofop, nicosulfuron, and imazamox. Additionally, glyphosate was evaluated because of its use prior to planting or postharvest. The ACCase inhibitors, fluazifop and quizalofop, resulted in greater than 90% mortality on all johnsongrass accessions other than two from Arkansas, which showed reduced sensitivity to fluazifop. There was 100% mortality of all johnsongrass accessions with glyphosate, except 7 of 63 from Arkansas. The ALS inhibitors, nicosulfuron and imazamox, resulted in 100% mortality of all Oklahoma accessions, but failures occurred on samples from other states. In both Kansas and Texas, one accession was found to have reduced sensitivity to both nicosulfuron and imazamox, and Arkansas had eight accessions with reduced sensitivity to nicosulfuron and imazamox. If producers plan to plant grain sorghum in areas with johnsongrass populations, an ACCase herbicide is most likely to provide effective control. Imazamox and nicosulfuron in conjunction with the appropriate trait can be utilized in areas with sensitive johnsongrass populations or where other sensitive grass species are present.

Keywords: Fluazifop; glyphosate; imazamox; nicosulfuron; quizalofop; johnsongrass, *Sorghum halepense* (L.) Pers*;* grain sorghum, *Sorghum bicolor* (L.) Moench

Introduction

Johnsongrass is one of the most problematic weeds in the world, causing up to 90 percent yield loss in crops such as cotton (*Gossypium hirsutum* L.), soybean [*Glycine max* (L.) Merr.], corn (*Zea mays* L.), and grain sorghum (Klein and Smith 2020). While the introduction of glyphosate in the 1970s and glyphosate-resistant crops in the 1990s significantly decreased johnsongrass presence in cotton, corn, and soybean, for crops like grain sorghum, johnsongrass is still one of the most troublesome weeds today. The genetic similarity between grain sorghum and johnsongrass, both being of the *Sorghum* genus, makes chemical removal in the absence of a herbicide-resistant trait challenging (Smith and Scott 2010).

Johnsongrass is a spreading perennial grass native to Asia but brought to the southern United States (US) in the 1800s to be utilized as a forage crop (Mitch 1987). The ability of johnsongrass to grow greater than two meters tall and create large quantities of biomass was optimal for forage producers but made it detrimental as a weed. Containment of johnsongrass was quickly lost because it has two processes of reproduction, seed dispersal and rhizomes. Rhizomes are horizontally growing underground stems from which new plants can develop, and one single johnsongrass plant can produce up to 5000 rhizomes in one growing season (McWhorter 1971). Rhizomes are often responsible for escapes or herbicide failures. Herbicides that control aboveground growth must also be able to translocate and control rhizomes below ground, or new johnsongrass plants could emerge. Therefore, producers must successfully control johnsongrass before rhizome development (Horowitz 1972).

To help grain sorghum producers better control johnsongrass, new herbicide resistance technologies are being researched, allowing producers to utilize either ACCase or ALS inhibitors for grass control in grain sorghum (Pinkerton 2020). The ACCase inhibitor technology in grain sorghum is known as Double Team^{TM} with resistance to quizalofop and is being developed by S&W seed company (Longmont, CO) and Adama (Raleigh, NC). The University of Arkansas System Division of Agriculture and Texas A&M jointly created TamArk^{TM} grain sorghum from a known johnsongrass population with resistance to fluazifop and a mutation different from that in Double Team^{TM}. The two ALS inhibitor technologies in grain sorghum include a genetic line developed by Corteva (Indianapolis, IN) known as Inzen^{TM} with resistance to nicosulfuron and a line developed by Alta seeds (Amarillo, TX) and UPL (King of Prussia, PA) known as Igrowth^{TM} with resistance to imazamox (Pinkerton 2020). While lines resistant to glyphosate are not available, the herbicide is important for johnsongrass control across the US in fallow areas, prior to crop planting, and in glyphosate-resistant crops (Brown et al. 1988; Smith and Scott 2010).

Both fluazifop and quizalofop control grasses but not broadleaf plants because the ACCase enzyme is sensitive to these herbicides only in grasses (Focke and Lichtenthaler 1987; Burton et al. 1989; Stoltenberg et al. 1989). Fluazifop and quizalofop have been used for grass removal from broadleaf crops such as cotton, soybean, and tobacco (*Nicotiana tabacum* L.). Fluazifop has been shown to control broadleaf signalgrass [*Urochloa platyphylla* (Nash) R.D. Webster], large crabgrass [*Digitaria sanguinalis* (L.) Scop.], goosegrass [*Eleusine indica* (L.) Gaertn], and johnsongrass greater than 90 percent (Byrd and York 1987; Clegg 1987). Quizalofop also effectively controlled similar grasses in broadleaf crops (Brewster and Spinney 1989; Sanders et al. 2020). Recently quizalofop has been utilized for grass control in rice (*Oryza sativa* L.) through the Provisia technology developed by BASF (Beaumont, TX) and Max-Ace technology from Adama (Raleigh, NC) (Lancaster et al. 2018; Tarundeep et al. 2019; Sanders et al. 2021).

Nicosulfuron and imazamox can successfully control grasses in both broadleaf and grass crops (Dobbels and Kapusta 1993; Gubbiga et al. 1995; Nelson et al. 1998; Geier et al. 2004). Nicosulfuron was used primarily for grass and broadleaf control in corn prior to the introduction of glyphosate-resistant crops in the mid- to late 1990s. Nicosulfuron was able to control johnsongrass at greater than 90 percent in production situations and was also desirable to producers because minimal amounts of active ingredient were needed (Camacho et al. 1991; Dobbels and Kaptusa 1993). Imazamox became popular through the Clearfield® (BASF, Triangle Park, NC) production system, which has allowed for the use of imazamox and imazethapyr for preemergence (PRE) and postemergence (POST) applications primarily in wheat (*Triticum aestivum* L.), corn, and rice but also other broadleaf and grass crops (Larson et al. 2000; Bond and Walker 2011; Jimenez et al. 2015). Although imazamox has not previously been used specifically for johnsongrass control, it has been successful for controlling annual grasses such as barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.], foxtails (*Setaria* spp.), and red rice (*Oryza sativa* L.) (Fish et al. 2016).

While ACCase and ALS inhibitors as well as glyphosate have been successful at controlling johnsongrass and other grasses, cases of resistance have been confirmed threatening the sustainability of these herbicides (Riar et al. 2011; Scarabel et al. 2014; Werle et al. 2016). Johnsongrass with resistance to ACCase-inhibiting herbicides was first documented in Mississippi in the 1980s with biotypes showing less than 35% control when treated with either fluazifop, quizalofop, or sethoxydim (Smeda et al. 1997). In 2007, erratic johnsongrass control was seen in a field of glyphosate-resistant soybean in Arkansas. After further evaluation, the accession was determined to be glyphosate-resistant, with greater than twice the labeled rate of glyphosate required to reach 50% control (Riar et al. 2011). In 2016, a study was conducted across Nebraska and Kansas to document ALS-resistant johnsongrass accessions. A total of 8 resistant accessions were found out of 59 johnsongrass accessions evaluated, 3 being resistant to nicosulfuron and 5 being resistant to imazethapyr, an imidazolinone herbicide (Werle et al. 2016). A survey of roadside johnsongrass accessions was conducted in Arkansas in 2014, and accessions resistant to glyphosate, fluazifop, and nicosulfuron were reported (Bagavathiannan and Norsworthy 2014). Considering that glyphosate is widely used to control johnsongrass in glyphosate-resistant crops and prior to crop planting and the use of ACCase- and ALS-inhibiting herbicides will likely increase in grain sorghum as new trait technologies are commercialized, a survey to determine the response of johnsongrass accessions collected from Arkansas, Texas, Oklahoma, and Kansas to glyphosate, quizalofop, fluazifop, nicosulfuron, and imazamox was conducted.

Materials and Methods

A greenhouse study was conducted at the Arkansas Agricultural Research and Extension Center in Fayetteville, AR. This was a completely randomized design with five herbicides evaluated (fluazifop, quizalofop, nicosulfuron, imazamox, and glyphosate on johnsongrass samples collected from Arkansas, Kansas, Oklahoma, and Texas. In the fall of 2020 and 2021, johnsongrass panicles from 117 different crop production fields were collected (Table 1; Figure 1). A minimum of 10 johnsongrass panicles with mature seeds were collected for each accession, and GPS coordinates were recorded using a handheld GPS, except in Kansas. These samples were then hand threshed, cleaned, and bagged. Samples were placed in cold storage (10 C) for two weeks before transferring to a cold room at 0 C for two days to attempt to break dormancy. Johnsongrass seeds from each accession were planted into individual 45 cm by 30 cm by 3 cm plastic trays (Greenhouse Megastore, Danville, IL) filled with Premier Tech (Quakertown, PA) Pro-Mix with a composition of 90% sphagnum peat moss and 10% perlite. These trays were then placed in a greenhouse temperature controlled at 25 +/- 8 C, with 16 hours of light and watered twice daily. Once the johnsongrass plants emerged, they were transplanted into 50 cell trays (Greenhouse Megastore, Danville, IL) filled with Premier Tech (Quakertown, PA) Pro-Mix at one plant per cell and returned to the greenhouse. Once johnsongrass plants reached the 2- to 3-leaf stage, applications were made using a spray chamber with TeeJet (TeeJet, Springfield, IL) 1100067 flat fan nozzles at 1.6 kph calibrated to deliver 187 L ha^{-1} (Table 2). Due to the low seed germination percentage of some johnsongrass accessions, not all herbicides were evaluated on all accessions collected.

Prior to application, the initial number of plants per tray was recorded if less than 50. Then, 28 days after application (DAA), the final number of living plants was recorded per tray, and data were used to calculate percent mortality using the equation $\frac{Initial\ johnsongrass - Final\ johsongrass}{Initial\ Johsongrass} \times 100.$ For each accession tested, there were at least two runs of the experiments in the greenhouse. Percent mortality of each accession was calculated to obtain descriptive statistics for each herbicide using JMP Pro 16.1 (SAS Institute Inc., Cary, NC).

Results and Discussion

Fluazifop. There were 111 of the 117 johnsongrass accessions evaluated for sensitivity to fluazifop. The mean mortality of the 111 johnsongrass accessions screened to fluazifop was 98% based on the descriptive statistics (Table 3). Only 4% of the accessions evaluated had less than complete mortality. These four accessions were from Arkansas, yet two still had 95% mortality. Mortality of the other two accessions, AR8 and AR9, was 6 to 20%, which is a good indication of the presence of fluazifop resistance. The surviving plants of the 4 accessions with reduced mortality all appeared unharmed and exhibited no symptoms following the fluazifop application. AR8 and AR9 are putative-resistant accessions but require dose response evaluations to determine the resistance level (Table 4). While johnsongrass resistant to fluazifop has been found previously in Arkansas, likely due to use in broadleaf crops such as cotton and soybean, it has not been widespread (Norsworthy et al. 2007; Johnson et al. 2014; Schwartz-Lazzaro et al. 2017). Because so few accessions were found to have reduced sensitivity, fluazifop remains an effective herbicide for johnsongrass control in most fields; however, overuse and heavy reliance on fluazifop could lead to an increase in the number of resistant populations in the future.

Quizalofop. A total of 96 of 117 johnsongrass accessions were evaluated for sensitivity to quizalofop, with the not tested a result of limited seed supply or lack of germination. Quizalofop treatments resulted in 100% mortality of the 96 johnsongrass accessions evaluated (Table 3). Documentation of quizalofop-resistant johnsongrass has not previously been documented in any state where johnsongrass was collected (Heap 2022). It was interesting that AR8 and AR9, both less sensitive to fluazifop, were controlled successfully by quizalofop even though both herbicides are from the aryloxyphenoxypropionate family of ACCase inhibitors. Similarly in other research, Tardiff and Powles (1994) and Leach et al. (1995) reported grasses that were resistant to fluazifop and had no resistance to other ACCase-inhibiting herbicides. Hence, quizalofop would be a highly effective option for johnsongrass control in grain sorghum technologies such as TamArk™ or Double Team™, both of which will allow postemergence application of the herbicide. Since these technologies are new for grain sorghum producers and offer increased johnsongrass control compared to previously available options, it will be important to utilize quizalofop in a systems approach with other effective herbicide sites of action, such as burndown applications of glyphosate or rotation to other crops, to mitigate the risk for resistance in the future.

Nicosulfuron. Johnsongrass resistant to nicosulfuron has been found in Arkansas, Texas, and Kansas, but nicosulfuron resistance has not been widespread (Bagavathiannan and Norsworthy 2014; Werle et al. 2016; Heap 2022). Nicosulfuron resulted in 100% and >90% mortality of 82 and 86%, respectively, of the 89 johnsongrass accessions evaluated. The 14% of johnsongrass accessions that did not result in >90% mortality ranged from 6 to 83% mortality (Table 4). Johnsongrass accessions resulting in less than acceptable mortality levels (<80%) were found in Arkansas, Texas, and Kansas, all states with previous documentation of nicosulfuron-resistant johnsongrass (Table 4). These 7 johnsongrass accessions with <80% mortality are worrisome with the new Inzen™ sorghum technology being released that allows producers to use nicosulfuron for postemergence johnsongrass control in grain sorghum. Similarly, nicosulfuron is also one of the few effective ALS-inhibiting herbicide options available for johnsongrass control in corn, specifically in the absence of glyphosate and glufosinate. Therefore, it will be important for producers to monitor johnsongrass control levels in fields when using nicosulfuron and to develop a crop rotation program that incorporates different effective herbicide sites of action in the following crop to control any potential johnsongrass escapes.

Imazamox. Of the 79 johnsongrass accessions evaluated for sensitivity to imazamox, 90% resulted in mortality >90%. The other 10% of johnsongrass accessions evaluated resulted in mortality ranging from 60% to 83% (Table 3). Accessions with <80% mortality were found in Arkansas, Texas, and Kansas (Table 4). One interesting observation was that 6 of the accessions with reduced sensitivity to imazamox also exhibited reduced sensitivity to nicosulfuron. Trends of ALS resistance similar to this have been observed where weed species resistant to a herbicide within the sulfonylurea family of ALS inhibitors, such as rice flatsedge (*Cyperus iria* L.), smallflower umbrella sedge (*Cyperus difformis* L.), barnyardgrass, and even johnsongrass, are also resistant to herbicides within the imidazolinone family like imazamox (Merotto et al. 2009; Riar et al. 2015; Heap 2022). Because of this cross-resistance trend, it is difficult to determine whether the reduced sensitivity is due to exposure to imazamox or only due to the cross-resistance trend with nicosulfuron.

Glyphosate. Glyphosate resulted in mortality all johnsongrass plants in 91% of the 73 accessions evaluated. Johnsongrass mortality >95% was observed in 93% of the johnsongrass accessions evaluated. The other 7% of accessions evaluated resulted in mortality ranging from 14 to 82% (Table 4). Six accessions evaluated, all from Arkansas, were considered likely resistant to glyphosate based on <80% mortality (Table 4). The number of glyphosate-resistant johnsongrass populations has been increasing since the mid-2000s due to frequent use of the herbicide in crops like corn, cotton, and soybean where weeds such as johnsongrass were prevalent (Heap 2022). Although glyphosate is not available for postemergence in-crop use in grain sorghum, many producers use it for fall and spring burndown of johnsongrass and as an effective postemergence option in the following crop (Smith and Scott 2010). While glyphosate is still going to be considered an effective option in most situations, based on these data, it will be important for producers to understand the effectiveness of the herbicide in particular fields and use alternative options when available to help preserve the herbicide for the future.

Practical Implications. Resistant johnsongrass accessions are becoming more and more prominent each growing season as the reliance on the same herbicides continues due to the lack of herbicide options for successful johnsongrass control. Based on this screening, ACCase inhibitors, specifically fluazifop and quizalofop, are the best option for producers to use for postemergence johnsongrass control in Double Team™ grain sorghum or eventually TamArk™ grain sorghum. However, other effective control options should be utilized in conjunction with these ACCase inhibitors to ensure maximum control and reduce the risk for herbicide resistance.

Although johnsongrass resistant to both ALS inhibitors was found, these two technologies can still be utilized in areas with known susceptible johnsongrass accessions in a rotation with other crops that can utilize different herbicide SOAs. However, lower levels of control would be expected under dryland conditions compared to the greenhouse. Overall, producers must be aware of which herbicides are effective in specific fields and utilize integrated weed management strategies to mitigate further resistance.

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Tables

Table 1. Location, year, and crop present for each johnsongrass sample collected for the screening

Accession	Year collected	Latitude	Longitude	Crop present[a]
AR1	2020	35.215933	-90.196417	Soybean
AR2	2020	35.251267	-90.166	Soybean
AR3	2020	35.24755	-90.148217	Soybean
AR4	2020	35.120717	-90.18975	Soybean
AR5	2020	35.092217	-90.215767	Soybean
AR6	2020	35.0909	-90.2153	Soybean
AR7	2020	35.090883	-90.216433	Soybean
AR8	2020	35.086417	-90.3058	Soybean
AR9	2020	34.962083	-90.30235	Corn
AR10	2020	35.962083	-90.643367	Soybean
AR11	2020	35.733817	-90.640667	Soybean
AR12	2020	35.733827	-90.640698	Soybean
AR13	2020	35.718067	-90.588883	Soybean
AR14	2020	35.79645	-90.4655	Soybean
AR15	2020	35.876383	-90.535517	Soybean
AR16	2020	35.836783	-90.55535	Soybean
AR17	2020	35.521317	-90.604	Soybean
AR18	2020	35.514633	-90.644817	Rice
AR19	2020	35.514583	-90.6448	Soybean
AR20	2020	35.464117	-90.663783	Soybean
AR21	2020	35.507367	-90.646683	Soybean
AR22	2020	35.507392	-90.646724	Soybean
AR23	2020	35.507357	-90.646854	Soybean
AR24	2020	35.56995	-90.6432	Soybean
AR25	2020	35.570233	-90.638783	Soybean
AR26	2020	35.570833	-90.63855	Rice
AR27	2020	35.569217	-90.638717	Soybean
AR28	2020	35.566533	-90.625267	Soybean
AR29	2020	35.566453	-90.625289	Soybean
AR30	2020	35.566723	-90.625326	Soybean
AR31	2020	35.734167	-90.652817	Soybean
AR32	2020	35.73335	-90.616367	Soybean
AR33	2020	35.227683	-90.346333	Soybean
AR34	2020	35.22775	-90.345517	Soybean
AR35	2020	35.2277	-90.345533	Soybean
AR36	2020	35.180933	-90.453667	Soybean

AR37	2020	35.224433	-90.399133	Sorghum
AR38	2020	35.22475	-90.398767	Soybean
AR39	2020	35.257683	-90.445017	Soybean
AR40	2020	35.3661	-90.329917	Soybean
AR41	2020	35.3651	-90.329823	Soybean
AR42	2020	35.365667	-90.292667	Soybean
AR43	2020	35.411717	-90.260967	Soybean
AR44	2020	35.327267	-90.18255	Soybean
AR45	2020	35.8976	-90.159133	Cotton
AR46	2020	35.90175	-90.149617	Soybean
AR47	2020	35.931167	-90.190317	Soybean
AR48	2020	35.968117	-90.275267	Soybean
AR49	2020	35.931967	-90.288017	Soybean
AR50	2020	35.932083	-90.28805	Soybean
AR51	2020	35.756883	-90.98205	Soybean
AR52	2020	35.756417	-90.0739	Cotton
AR53	2020	35.75685	-90.1736	Soybean
AR54	2020	35.769117	-90.17815	Soybean
AR55	2020	35.902067	-90.176817	Corn
AR56	2020	35.901917	-90.16665	Soybean
AR57	2020	36.187407	-90.369087	Soybean
AR58	2020	36.053002	-90.38693	Cotton
AR59	2020	36.18501	-90.663495	Soybean
AR60	2020	36.080292	-90.743387	Soybean
AR61	2020	35.667131	-90.074214	Soybean
AR62	2020	35.969103	-94.341383	Soybean
AR63	2020	35.931253	-90.190418	Soybean
TX1	2021	32.08097	-96.8172	Sorghum
TX2	2021	32.05273	-96.93	Sorghum
TX3	2021	31.97032	-97.1126	Corn
TX4	2021	32.11856	-97.2494	Corn
TX5	2021	31.85304	-96.9323	Sorghum
TX6	2021	30.99349	-97.1089	Corn
TX7	2021	30.9787	-96.753	Corn
TX8	2021	29.12965	-96.2478	Corn
TX9	2021	29.26327	-95.9469	Corn
TX10	2021	29.39906	-96.14	Soybean
TX11	2021	28.56942	-97.1948	Pasture
TX12	2021	28.51308	-96.7818	Corn
TX13	2021	27.99947	-97.5067	Cotton
TX14	2021	23.23466	-97.847	Corn
TX15	2021	29.791414	-94.472913	Rice
TX16	2021	29.858128	-94.531693	Rice
TX17	2021	26.26606	-98.1429	Corn

TX18	2021	26.28085	-98.0813	Pasture
TX19	2021	27.88906	-97.4385	Cotton
TX20	2021	28.6676	-96.7941	Corn
TX21	2021	28.38435	-96.8922	Cotton
TX22	2021	28.81035	-97.0512	Cotton
TX23	2021	29.46468	-96.3741	Corn
TX24	2021	29.02976	-96.2562	Sorghum
TX25	2021	30.92465	-97.0033	corn
TX26	2021	31.05275	-97.3378	Corn
TX27	2021	32.30514	-96.9969	Sorghum
TX28	2021	32.10851	-96.6322	Corn
TX29	2021	31.68768	-97.1772	Corn
TX30	2021	32.08495	-97.3593	Sorghum
TX31	2021	29.13119	-96.3702	Cotton
TX32	2021	28.96991	-96.3393	cotton
TX33	2021	29.48498	-96.3304	Soybean
TX34	2021	28.55589	-97.0123	Cotton
TX35	2021	28.58187	-96.7143	Cotton
TX36	2021	28.69579	-96.6976	Sorghum
TX37	2021	28.08474	-97.5495	Sorghum
TX38	2021	26.18314	-97.8631	Sorghum
TX39	2021	26.36166	98.0103	Sorghum
TX40	2021	29.788287	-94.580276	Rice
OK1	2021	36.131028	-97.104583	Corn
OK2	2021	35.9867665	-97.0452132	Unknown
OK3	2021	36.1086268	-97.3893772	Corn
OK4	2021	36.260972	-97.722667	Soybean
OK5	2021	35.852198	-97.6457511	Wheat
OK6	2021	36.1150718	-98.1092228	Wheat
OK7	2021	36.4050325	-98.2466897	Wheat
KS1	2020	N/A[b]	N/A	Corn
KS2	2020	N/A	N/A	Corn
KS3	2020	N/A	N/A	Soybean
KS4	2020	N/A	N/A	Corn
KS5	2020	N/A	N/A	Sorghum
KS6	2020	N/A	N/A	Soybean
KS7	2020	N/A	N/A	Corn
KS8	2020	N/A	N/A	Corn
KS9	2020	N/A	N/A	Corn
KS10	2020	N/A	N/A	Sorghum

[a] Crop present or last crop grown prior to seed collection
[b] Location data not available

Table 2. Herbicides and rates applied to johnsongrass accessions from Arkansas, Kansas, Texas, and Oklahoma in 2021 and 2022.

Trade name	Common name	Rate	Manufacturer	Location
		g ai ha^{-1}		
Fusilade DX	Fluazifop	105	Syngenta Crop Protection, LLC	Greensboro, NC
Assure II	Quizalofop	46	Corteva Agriscience	Indianapolis, IN
Imiflex	Imazamox	53	UPL	King of Prussia, PA
Zest	Nicosulfuron	47	Corteva Agriscience	Indianapolis, NC
Roundup Powermax	Glyphosate	962[a]	Bayer CropScience	Research Triangle Park, NC

[a] g ae ha^{-1}

Table 3. Susceptibility of johnsongrass accessions from Arkansas, Texas, Oklahoma, and Kansas to different herbicides.[a]

Herbicide	Rate	Accessions screened	Mortality			
			Minimum	Median	Mean	Maximum
	g ai ha^{-1}		-----------------------	%	-----------------------	
Fluazifop	105	111	6	100	98	100
Quizalofop	46	97	100	100	100	100
Imazamox	53	78	60	100	96	100
Nicosulfuron	47	89	6	100	95	100
Glyphosate	867[b]	73	14	100	94	100

[a] Descriptive statistics were generated from mortality rates.

[b] g ae ha^{-1}

Table 4. Johnsongrass accessions with a mortality percentage of 80% or less.

Herbicide	Accession	Mortality
		%
Fluazifop	AR8	6
	AR9	20
Nicosulfuron	AR2	60
	AR7	74
	AR22	66
	AR45	76
	AR47	78
	TX12	80
	KS7	6
Imazamox	AR1	64
	AR2	60
	AR5	68
	AR7	80
	AR9	70
	AR45	76
	AR47	70
	TX12	68
	KS7	80
Glyphosate	AR2	56
	AR3	16
	AR5	20
	AR7	80
	AR39	30
	AR40	14

Figures

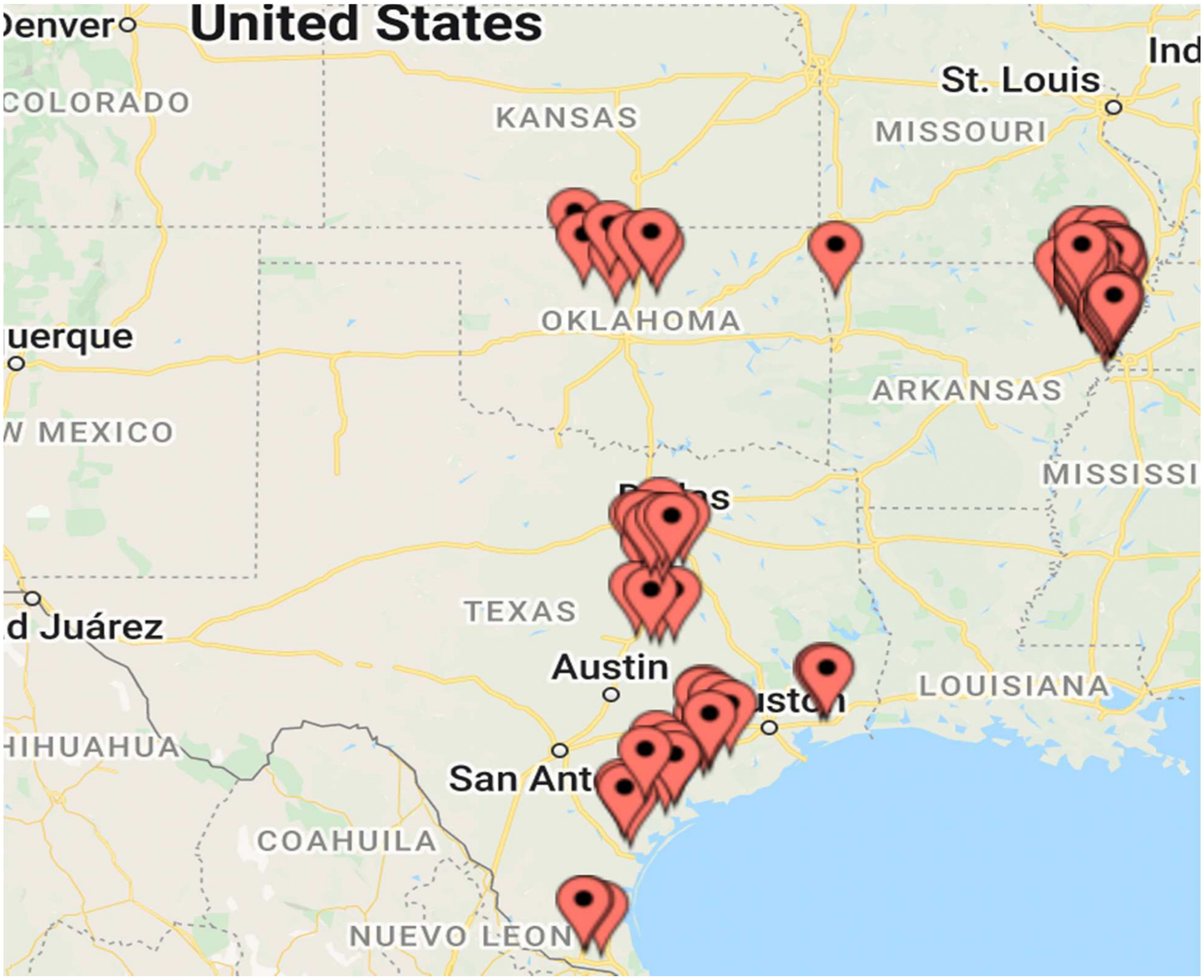

Figure 1. Johnsongrass sampling locations in Arkansas, Oklahoma, and Texas. Samples were also collected from sites in Kansas, but GPS coordinates were not provided.

Chapter 3

Sensitivity of TamArk^{TM} Grain Sorghum to ACCase-Inhibiting Herbicides

Abstract

A collaboration between the University of Arkansas System Division of Agriculture and Texas A&M University has resulted in a new grain sorghum trait with known resistance to the acetyl CoA carboxylase (ACCase) inhibitor fluazifop-p-butyl and is believed to have resistance to other herbicides within the ACCase inhibitor group. To assess the sensitivity of TamArk^{TM} grain sorghum to fluazifop-p-butyl and two additional ACCase-inhibiting herbicides, fenoxaprop-p-ethyl and quizalofop-p-ethyl, at multiple crop growth stages, greenhouse dose-response studies were conducted at Fayetteville, AR, and College Station, TX. In addition, studies were conducted to determine the sensitivity of TamArk^{TM} grain sorghum to ACCase inhibitors from the aryloxyphenoxypropionate, phenylpyrazolin, and cyclohexanedione families under field conditions in Fayetteville, AR, in 2020 and 2021. TamArk^{TM} grain sorghum was highly resistant to fluazifop-p-butyl applied to plants at 5- to 7- and 20- to 25-cm tall compared to a susceptible hybrid, with a resistant/susceptible (R/S) ratio of 460 and 167, respectively, based on 50% biomass reduction (GR_{50}). TamArk^{TM} grain sorghum exhibited a 27.2- and 394-fold increase in resistance to quizalofop-p-ethyl and fenoxaprop-p-ethyl based on GR_{50} comparison to a susceptible hybrid. A low level of sensitivity was observed in the field when TamArk^{TM} grain sorghum was treated with ACCase inhibitors from the aryloxyphenoxypropionate and phenylpyrazolin families, with <10% injury present from all herbicides within these families, outside of the combination of fluazifop-p-butyl + fenoxaprop-p-ethyl. Conversely, a high level of sensitivity was observed with the two herbicides from the cyclohexanedione family, which

resulted in greater than 90% injury. ACCase inhibitors from the aryloxyphenoxypropionate and phenylpyrazolin families could be utilized for postemergence grass control in TamArk^{TM} grain sorghum if labeled.

Nomenclature: fenoxaprop-p-ethyl; fluazifop-p-butyl; quizalofop-p-ethyl; grain sorghum, S*orghum bicolor* (L.) Moench

Introduction

Grass control has always been one of the significant challenges facing grain sorghum producers across the Midsouth. Since grain sorghum is a grass, many herbicides available to control problematic grasses will also lead to significant crop injury or crop mortality. Herbicide-resistant crops such as cotton (*Gossypium hirsutum* L.), corn (*Zea mays* L.), soybean [*Glycine max* (L.) Merr.], and rice (*Oryza sativa* L.) have been utilized by producers across the Midsouth for many years, allowing successful removal of weeds that would have been challenging to control in the absence of the resistance trait. However, herbicide resistance technology has not been available for grain sorghum producers in the past. Without herbicide resistance technologies, grain sorghum producers have been left with a limited number of herbicides that can be used to control problematic grass species postemergence.

Acetyl coenzyme A carboxylase (ACCase)-inhibiting herbicides were commercialized more than 40 years ago for control of many problematic grass species in broadleaf crops. These herbicides inhibit the ACCase enzyme, vital for fatty acid biosynthesis in the cell, of grasses whereas the ACCase enzyme of broadleaf plants is not sensitive to these herbicides (Dekker 1999). By inhibiting the ACCase enzyme, grasses cannot perform the first step of fatty acid biosynthesis, stopping the production of bicarbonate and adenosine triphosphate (ATP). Cessation of ATP and bicarbonate production leads to prevention of acyl lipid biosynthesis and ultimately plant death (Shulka and Devine 2000; Focke et al. 2003). The first known resistance to ACCase-inhibiting herbicides was in 1982 when blackgrass (*Alopecurus myosuroides* L.) was found to be resistant to the aryloxyphenoxypropionate (AOPP) family of ACCase inhibitors (Heap 2022). Since 1982, many cases of resistance to ACCase inhibitors have been reported throughout the United States and the world, including multiple cases reporting ACCase-resistant johnsongrass [*Sorghum halepense* (L.) Pers] (Heap 2022).

Through a collaboration between the University of Arkansas System Division of Agriculture and Texas A&M University, a johnsongrass biotype was used to breed for resistance to ACCase-inhibiting herbicides in grain sorghum (Bagavathiannan et al. 2018). The cross resulted in a TamArk™ grain sorghum line with a target-site mutation at position 2031 on the ACCase gene, where a cystine replaces a tryptophan, corresponding to the mutation conferring fluazifop-p-butyl resistance found in blackgrass (Norsworthy et al. 2020). A second mutation to the ACCase gene was also identified in the grain sorghum line at position 2248, which changed an alanine to a threonine, but this mutation is different from any previously reported mutations (Norsworthy et al. 2020). Further investigation showed that this allele was specific to the ACCase gene in johnsongrass, meaning the mutation serves as the conformation of fluazifop-p-butyl resistance.

The fluazifop-p-butyl-resistance mutation is a target-site mutation, meaning that resistance is achieved in the plant by altering the target enzyme's binding site, making it less sensitive to the herbicide (Shulka and Devine 2000). A varying range of resistance levels can be observed with target-site mutations, like in TamArk™ grain sorghum. For example, in a study of green foxtail [*Setaria viridis* (L.) Beauv.], R/S ratios ranged from an I_{50} of 31 for clethodim and 60 for quizalofop-p-ethyl (Marles et al. 1993). Other mutations have shown R/S ratios of 420 for cyclohexanedione (CHD) herbicides such as sethoxydim in weeds such as green foxtail and giant foxtail (*Setaria faberi* Herrm.) and a sethoxydim-resistant corn (*Zea mays* L.) line (Parker et al. 1990; Shulka et al. 1997a). Two other resistance patterns concerning fluazifop-p-butyl and other AOPP herbicides are of interest when evaluating the potential for TamArk™ grain sorghum. First is a pattern that has been found in goosegrass [*Eleusine indica* (L.) Gaertn.] and rigid ryegrass (*Lolium rigidum* L.), where the biotypes had high levels of resistance to fluazifop-p-butyl but showed little to no resistance to other AOPP or CHD herbicides (Leach et al. 1995; Tardif & Powles 1994). Second is a pattern in rigid ryegrass, Italian ryegrass (*Lolium perenne* L. ssp*. multiflorum*), and wild oat (*Avena fatua* L.), where a high level of resistance to AOPP herbicides was observed, but biotypes showed no resistance to CHD herbicides (Gronwald et al. 1992; Preston et al. 1996; Shulka et al. 1997b).

Preliminary research by Piveta et al. (2020) concluded that TamArk™ grain sorghum is believed to have commercial resistance to fluazifop-p-butyl. It is also thought that TamArk™ grain sorghum has resistance to other AOPP herbicides and potentially to the sole herbicide within the phenylpyrazolin (PPN) family, but no resistance to herbicides within the CHD family. This resistance pattern would be like the pattern seen by Shulka et al. (1997b), Gronwald et al. (1992), and Preston et al. (1996). Therefore, it is essential to determine the resistance pattern of TamArk™ grain sorghum to understand better which herbicides could be commercialized for use in this line, as well as to determine if some ACCase inhibitors commonly used on other production crops can be utilized for control of volunteer plants in subsequent growing seasons.

Materials and Methods

Greenhouse Trials.

General Setup for Sensitivity to Fluazifop-p-butyl. Greenhouse trials were conducted in Fayetteville, AR, in 2020 and College Station, TX, in 2021 to determine the sensitivity of TamArk^{TM} grain sorghum to fluazifop-p-butyl-butyl. The study was a two-factor, completely randomized design with five replications where the factors were fluazifop-p-butyl rate and application timing (5- to 7-cm or 20- to 25-cm). The susceptible line was treated with one of 7 fluazifop-p-butyl rates of 0, 3, 6, 23, 26, 53, or 106 g ai ha^{-1}. The TamArk^{TM} line was treated with one of eight rates of fluazifop-p-butyl of 0, 106, 211, 422, 844, 1686, 3373, or 6746 g ai ha^{-1}. Pots 10 cm in diameter were filled with standard potting soil, and five seeds were planted per pot of either TamArk^{TM} grain sorghum (resistant) or Pioneer 84P80 (susceptible) at a 1.3-cm depth. Grain sorghum plants in each pot were thinned to a single plant after emergence. Fluazifop-p-butyl applications were made when grain sorghum reached the proper height, based on treatment, using a spray chamber equipped with a two-nozzle boom having TeeJet (Glendale Heights, IL) 1100067 flat fan nozzles traveling at 1.67 kph at a spray volume of 187 L ha^{-1} in Fayetteville. Similar methods were used in College Station, except the sprayer had air induction extended range (AIXR) 110015 nozzles delivering 140 L ha^{-1}. After application, visible injury ratings were taken 28 days after application (DAA) on a scale of 0 to 100, where 0 represented no visible crop injury and 100 represented complete crop death (Frans and Talbert 1986). In Fayetteville at 28 DAA, all living aboveground plant material was placed in a dryer at 60 C for two weeks, then removed and weighed.

General Setup for Sensitivity to Fenoxaprop-p-ethyl and Quizalofop-p-ethyl. To determine TamArk™ grain sorghum resistance levels to fenoxaprop-p-ethyl and quizalofop-p-ethyl, greenhouse studies were conducted in Fayetteville, Arkansas, in the winter of 2020 and 2021 and College Station, TX, in 2021 in a similar manner to that described for the fluazifop-p-butyl trials. This study was a single-factor, completely randomized design with five replications. Applications were made when grain sorghum reached the 2- to 3-leaf stage using a spray chamber equipped with a two-nozzle boom fitted with TeeJet (Glendale Heights, IL) 1100067 flat fan nozzles at 1.6 kph and 187 L ha $^{-1}$ and using air induction extended range (AIXR) 110015 nozzles delivering 140 L ha^{-1} in College Station. Seven rates of each fenoxaprop-p-ethyl (0, 3, 6, 12, 24, 48, or 87 g ai ha^{-1}) and quizalofop-p-ethyl (0, 3.5, 7, 14, 28, 56, or 112 g ai ha^{-1}) were evaluated on the susceptible grain sorghum and eight rates of fenoxaprop-p-ethyl (0, 87, 174, 348, 699, 1396, 2792, or 5587 g ai ha^{-1}) and quizalofop-p-ethyl (0, 112, 224, 448, 896, 1792, 3584, or 7168 g ai ha^{-1}) on TamArk™ grain sorghum. Crop oil concentrate at 1% v/v was added to all treatments. After application, visible injury ratings were taken 28 DAA like the rating system described earlier. Aboveground biomass was harvested 28 DAA and dried before weighing.

Statistical Analysis for Greenhouse Trials. Percent injury and biomass data were analyzed using the Fit Curve platform in JMP PRO 16 (SAS Institute Inc., Cary, NC). Two different models were required to represent the data. TamArk™ treated with fluazifop-p-butyl and quizalofop-p-ethyl data were best fit with a Logistic 3P model ($y = c/(1 + \mathrm{Exp}(-a*(\text{g ai ha}^{-1} – b)))$, a = growth rate, b = inflection point, c = asymptote) when compared to other models using AICc, BIC, SSE, MSE, and R^2 values. The susceptible cultivar treated with fluazifop-p-butyl, fenoxaprop-p-ethyl growth reduction data were best fit with an Exponential 3P model (y = a + b * Exp(c*g ai ha^{-1}), a = asymptote, b = scale, c = growth rate) when compared to other models using AICc, BIC, SSE, MSE, and R^2 values. Data were pooled over location, and individual models were fit by herbicide and grain sorghum line. Inverse predictions were utilized to determine the fluazifop-p-butyl rate necessary to reach 50% injury (I_{50}) and 50% growth reduction (GR_{50}) and lower and upper 95% confidence intervals for each cultivar by herbicide. Values were then used to calculate resistance-fold levels for TamArk™ grain sorghum by herbicide rate by taking the value for the resistant line and dividing it by the value of the susceptible line.

TamArk™ Grain sorghum Response to ACCase Herbicides in the Field.

Field setup. To determine the sensitivity of TamArk™ grain sorghum to ACCase inhibitors under field conditions, experiments were conducted in 2020 and 2021 at the Milo J. Shult Arkansas Agricultural Research and Extension Center in Fayetteville, AR, on a leaf silt loam (fine, mixed, active, thermic Typic Albaquults). The soil consisted of 18.4% sand, 71.5% silt, 10.1% clay, and a pH of 6.7. TamArk™ grain sorghum was planted at a 1.3-cm depth into conventionally tilled, raised beds at 154,000 seed ha^{-1}. Plots consisted of four rows 4.8 m long and 3.6 m wide with a 91 cm row spacing. Plots were maintained weed-free using labeled applications of atrazine, 2,4-D, quinclorac, dicamba, and hand-weeding if necessary. The trial received two nitrogen applications of 84 kg ha^{-1}, one before planting and a second at 5- to 6-leaf. Overhead irrigation was provided on an as-need basis, but typically totaled at least 2.5 cm per week if rainfall did not occur. Other management practices, including preplant fertilizer rates, followed the Arkansas grain sorghum production handbook (Espinoza and Ross 2015).

The experiment was set up as a single-factor, randomized complete block design with 22 treatments, including the nontreated (Table 1). Each treatment was replicated four times. All applications were made when TamArk™ grain sorghum reached the 2- to 3-leaf stage utilizing a CO_2-pressured backpack sprayer that delivered 140 L ha^{-1} through TeeJet (Glendale Heights, IL) AIXR 110015 nozzles. The center two rows of each plot were sprayed with blockers on either side to ensure a two-row, non-treated buffer between treated plots.

Visible crop injury was assessed weekly after the initial herbicide application and continued for four weeks. Evaluations were made on a scale of 0 to 100, where 0 represented no visible crop injury and 100 represented complete crop mortality. At 28 DAA, the height of five random plants in each plot was recorded. The date when 50% of plants in a plot headed was recorded and made relative to the nontreated check in the respective replication. Grain from the center two rows of each plot was harvested using a small-plot combine, and yield was reported in kg ha^{-1} after adjusting to 14% moisture.

Statistical Analysis. Since nontreated plots were rated as having no injury, these plots were excluded from the visible injury analysis. Distributions were checked using the distribution function in JMP Pro 16.1 (SAS Institute Inc., Cary, NC) and all distributions were determined to be gamma based on AICc and BIC values. Data were then subject to analysis of variance using a single-factor PROC GLIMMIX statement in SAS 9.4 (SAS Institute Inc., Cary, NC). Year and replication were random effects. Visible crop injury, height, relative heading, and yield were subjected to mean separations using Tukey's HSD (p=0.05). Clethodim treatments were excluded from height, relative heading, and grain yield analysis because no data were present for these variables.

Results and Discussion

Sensitivity to Fluazifop-p-butyl in the Greenhouse.

Visible injury. The susceptible grain sorghum was highly sensitive to fluazifop-p-butyl at both application timings. The I_{50} values for the susceptible hybrid were 2.73 and 9.88 g ai ha^{-1} when fluazifop-p-butyl was applied at heights of 5 to 7 and 20 to 25 cm, respectively (Figure 1). The increase in I_{50} values is expected with an increase in grain sorghum size at the time of application. When I_{50} values of TamArk™ were compared to the susceptible hybrid, a 460- and 167-fold increase in fluazifop-p-butyl was necessary to achieve 50% injury when applied at 5 to 7 and 20 to 25 cm, respectively (Table 2). The increased rate needed to reach 50% injury compared to the susceptible hybrid confirms resistance to fluazifop-p-butyl in TamArk™. There was a decrease in resistance level between the 5 to 7 cm and 20 to 25 cm applications, which is due to more g ai ha^{-1} required to reach 50% injury of the susceptible hybrid when applications were made at 20 to 25 cm, while TamArk™ had very little increase (Figures 1 and 2). The lack of increase in the resistant line is due to the mutations being target-site; therefore, changes in sensitivity due to size at application will not be as apparent as if this were a non-target-site mutation. Similar increases in injury levels due to grain sorghum size at application timing were seen by Lancaster et al. (2018), where applications of a 1/10X drift rate of quizalofop-p-ethyl to a susceptible grain sorghum at the 2- to 3-leaf stage resulted in 29 percentage points more injury than applications made at the boot growth stage.

Growth reduction. A similar trend to injury was observed with a reduction in growth based on the weight of living plant material. Applications made to the susceptible grain sorghum resulted in GR_{50} values of 5.42 g ai ha^{-1} and 10.48 g ai ha^{-1} when applications were made at the 2- to 3-leaf and 5- to 6-leaf stage, respectively (Figure 3). Applications to the resistant line resulted in GR_{50} values of 1348 g ai ha^{-1} and 1674 g ai ha^{-1} at the 2- to 3-leaf and 5- to 6-leaf stage, respectively (Figure 4). This results in a R/S ratio of 248 at the 2- to 3-leaf stage and 159 at the 5- to 6-leaf stage (Table 2). Based on these data we can further conclude that a high level of resistance is present within TamArk™ when compared to the susceptible grain sorghum.

Sensitivity to Fenoxaprop-p-ethyl and Quizalofop-p-ethyl in the Greenhouse.

Visible injury. Both fenoxaprop-p-ethyl and quizalofop-p-ethyl were able to control the susceptible grain sorghum at rates standard with prior research with I_{50} values of 9.89 and 6.58, respectively (Lancaster et al. 2018). When comparing the I_{50} values of the resistant line to the susceptible line, a 394-fold increase in fenoxaprop-p-ethyl and a 27.2-fold increase of quizalofop-p-ethyl are required to reach 50% visible injury (Table 3; Figures 5 and 6). TamArk™ grain sorghum was more sensitive to quizalofop-p-ethyl than fenoxaprop-p-ethyl or fluazifop-p-butyl; however, a labeled 1X rate of quizalofop-p-ethyl is 110 g ai ha^{-1} and 179 g ai ha^{-1} was required to reach 50% injury. A 1X rate of fenoxaprop-p-ethyl is 87 g ai ha^{-1} and 3895 g ai ha^{-1} was required to cause 50% injury on TamArk™ grain sorghum. The fenoxaprop-p-ethyl rate required to reach 50% injury confirms resistance to fenoxaprop-p-ethyl.

Growth reduction. Low rates of either fenoxaprop-p-ethyl or quizalofop-p-ethyl were required to reach 50% growth reduction with applications to the susceptible grain sorghum (Table 3).

Like injury, a very high level of resistance to fenoxaprop-p-ethyl was observed with the resistant line. A R/S ratio of 727 was calculated when the GR_{50} values of the resistant and susceptible cultivars were compared further confirming resistance of the TamArk™ cultivar to fenoxaprop-p-ethyl. A GR_{50} of 132 g ai ha^{-1} was observed with quizalofop-p-ethyl application made to the resistant cultivar resulting in a R/S ratio of 33.6 (Table 3; Figures 7 and 8). However, the amount of quizalofop-p-ethyl required to reach 50% growth reduction was only 22 g ai ha^{-1} more than the 1X rate applied. While TamArk™ is still considered to be resistant compared to the susceptible grain sorghum, applications of quizalofop-p-ethyl may not be possible at the full labeled rate without experiencing significant injury and field testing would be required to determine if similar levels were present. Ironically, TamArk contains the same 2031 mutation in the ACCase gene that is present in grain sorghum patented by Kansas State University and shown to exhibit resistance to quizalofop (Nandakumar et al. 2018).

TamArk™ Grain sorghum Response to ACCase Herbicides in the Field.

Visible injury. TamArk™ grain sorghum exhibited a low level of sensitivity to postemergence applications of ACCase inhibitors from the AOPP and PPN families (Table 4). Only one herbicide from the AOP family, fluazifop-p-butyl + fenoxaprop-p-ethyl, resulted in greater than 10% injury at all evaluation timings. At 14 days after treatment (DAT), the PPN herbicide evaluated, pinoxaden, caused greater than 10% injury to TamArk™ grain sorghum, but at 21 and 28 DAT injury was 9% at the 60 g ai ha^{-1} or 1x rate of pinoxaden (Table 4).

A high level of sensitivity was observed when the two herbicides from the CHD family, clethodim and sethoxydim, were applied to TamArk™ grain sorghum. At 14 DAT, clethodim at both rates caused complete crop mortality. Similarly, sethoxydim resulted in greater than 90% injury at all application timings (Table 4). The resistance trend observed with TamArk^{TM} grain sorghum follows ACCase resistance trends previously documented by Leach et al. (1995) and Tardif and Powles (1994), where tolerance to the AOPP family but not the CHD family was observed. One interesting observation between the field and greenhouse studies was the injury levels of quizalofop-p-ethyl. In the greenhouse, a rate of 179 g ai ha^{-1} corresponded to 50% injury of TamArk^{TM} grain sorghum, while in the field, the injury did not exceed 10% from applications of 220 g ai ha^{-1}. The difference in injury is probably due to the growing condition differences between the greenhouse and field environments. Greenhouse conditions tend to be set for a perfect growing environment with few limiting factors. However, a field setting can often limit the plant through nutrient and water availability and temperature extremes. In addition, factors causing growth changes in the plant can also affect herbicide uptake, translocation, and detoxification, resulting in variations in injury levels.

Height, Heading, and Yield. Overall, a similar trend to injury was observed among plant height, relative heading date, and grain yield. The two rates of sethoxydim were the only two treatments different from the nontreated for each of the three evaluations (Table 5). At 35 DAT, all AOPP and PPN treatments resulted in grain sorghum heights ranging from 28 to 32 cm, with the nontreated being 31 cm. Sethoxydim reduced plant height to 16 and 13 cm at 210 and 420 g ai ha^{-1}, respectively. Similarly, sethoxydim was the only herbicide that caused a delay in heading compared to the nontreated, with this herbicide causing a heading delay up to 23 days relative to the nontreated. When treatments included herbicides from the AOPP or PPN family, heading date only fluctuated +/- 4 days from the nontreated. Finally, treatments from herbicides in the AOPP or PPN families did not negatively affect grain yield. In contrast, TamArk™ grain sorghum treated with sethoxydim experienced a reduction in grain yield (2,500 kg ha^{-1} or 47% yield loss) (Table 5). Grain yields of TamArk™ grain sorghum are consistent with and often exceeded the national average grain sorghum yield in 2021 of 4,600 kg ha^{-1} (National Grain sorghum Producers 2022); albeit strong efforts should be made to further enhance yield potential.

Practical Implications. Overall, TamArk™ grain sorghum exhibited commercially acceptable resistance to applications of multiple ACCase-inhibiting herbicides in the greenhouse and field. While none of the herbicides, except quizalofop, are currently labeled for use in grain sorghum, ACCase inhibitors from the AOPP and PPN families could potentially be labeled for use in TamArk™ grain sorghum. Using ACCase inhibitors from the AOPP and PPN families would give grain sorghum producers new, effective options for postemergence grass control while reducing the risk for resistance to herbicides such as quinclorac that are currently used to control grasses (Malik et al. 2010; Heap 2022). Although ACCase inhibitors from the CHD family cannot be utilized for postemergence grass control in TamArk™ grain sorghum, these herbicides are still important for TamArk™ grain sorghum because they offer an option to control volunteer plants in subsequent crops like soybean or cotton among others. While the potential for crossing between johnsongrass and grain sorghum is high, less than 25 percent of the crosses are reproductive in the next season (Arriola and Ellstrand 1996; Hodnett et al. 2019: Tesso et al. 2008; Ohadi et al. 2017). Therefore, the risk of successful fluazifop-p-butyl-resistance gene to johnsongrass from TamArk™ grain sorghum is low. If the resistance gene were to outcross to johnsongrass, the lack of resistance to the CHD family would still give soybean and cotton producers ACCase inhibitor herbicide options for control in the future.

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Tables

Table 1. Acetyl CoA carboxylase-inhibiting herbicide trade names, common names, and rates applied to TamArk™ at the 2-to 3-leaf stage under field conditions in Fayetteville, AR, in 2020 and 2021.

Trade name	Common name	Rate[a]
		g ai ha^{-1}
Assure II	Quizalofop-p-ethyl	110, 220
Axial XL	Pinoxaden	60, 120
Clincher	Cyhalofop	312, 625
Discover NG	Clodinafop-propargyl	70, 140
Fusilade DX	Fluazifop-p-butyl	210, 420, 840
Fusion	Fluazifop-p-butyl + fenoxaprop-p-ethyl	269, 538
Hoelon	Diclofop-methyl	1120, 2240
Poast Plus	Sethoxydim	210, 420
Ricestar	Fenoxaprop-p-ethyl	86, 172
Select Max	Clethodim	135, 271

[a] 1x rate followed by 2x rate for crops in which these herbicides are labeled

Table 2. Predictions from fluazifop-p-butyl dose response experiments conducted in Fayetteville, AR, and College Station, TX in 2020 and 2021 by Grain sorghum line and size at time of application; resistant is the TamArk™ line, and susceptible is Pioneer 84P80.

Herbicide		Grain sorghum line	Height	Predicted rate	Confidence interval (95%) Lower	Confidence interval (95%) Upper	Level of resistance
			cm	-------------- g ai ha^{-1} --------			R/S ratio[a]
Fluazifop-p-butyl	I_{50}	Resistant	5-7[b]	1258	1213	1302	460
		Susceptible		2.73	2.42	3.05	
	GR_{50}	Resistant		1348	1270	1426	248
		Susceptible		5.42	3.97	6.87	
	I_{50}	Resistant	20-25[c]	1649	1562	1735	167
		Susceptible		9.88	9.22	10.54	
	GR_{50}	Resistant		1674	1565	1783	159
		Susceptible		10.48	9.19	11.78	

[a] R/S ratio is determined by dividing the predicted value of the resistant (R) line by the predicted value of the susceptible (S) line.

[b] 2- to 3-leaf

[c] 5- to 6-leaf

Table 3. I_{50} predictions from fenoxaprop-p-ethyl and quizalofop-p-ethyl dose-dose response experiments conducted in 2020 and 2021 in Fayetteville, AR, and 2021 in College Station, TX; resistant is the TamArk™ line, and susceptible is Pioneer 84P80. Applications were made to both Grain sorghum lines at 2- to 3- leaf stage.

Herbicide		Grain sorghum trait	Confidence interval (95%) Predicted rate	Lower	Upper	Level of resistance
			--------- g ai ha^{-1} --------			R/S ratio[a]
Fenoxaprop-p-ethyl	I_{50}	Resistant	3895	3761	4028	394
		Susceptible	9.89	9.24	10.55	
	GR_{50}	Resistant	5957	5141	6774	727
		Susceptible	8.19	7.2	9.18	
Quizalofop-p-ethyl	I_{50}	Resistant	179	175	183	27.2
		Susceptible	6.58	6.40	6.76	
	GR_{50}	Resistant	132	120.4	143.8	33.6
		Susceptible	3.92	3.3	4.5	

[a] R/S ratio is determined by dividing the predicted value of the resistant (R) by the predicted value of the susceptible (S) grain sorghum.

Table 4. Visible injury levels of TamArkTM grain sorghum by herbicide and rate averaged over 2020 and 2021 at Fayetteville, AR.[a]

Herbicide	Rate	Visible crop injury					
		14 DAA		21 DAA		28 DAA	
	g ai ha^{-1}	---------------------------%-------------------------					
Sethoxydim	210	96	A	93	B	93	B
	420	98	A	98	A	98	A
Clethodim	135	100	A	100	A	100	A
	270	100	A	100	A	100	A
Clodinafop-propargyl	70	3	EF	4	GH	4	FG
	140	4	EF	5	EFGH	5	EFG
Cyhalofop	310	7	CDE	7	DEFGH	7	DEFG
	620	5	DEF	4	GH	4	FG
Diclofop-methyl	1120	4	EF	5	GH	5	EFG
	2240	2	F	3	H	3	G
Fenoxaprop-p-ethyl	80	3	EF	3	H	3	G
	160	6	DEF	4	GH	4	FG
Fluazifop-p-butyl	210	4	EF	4	GH	4	FG
	420	5	EF	4	GH	4	FG
	840	4	EF	4	GH	4	FG
Fluazifop-p-butyl + Fenoxaprop-p-ethyl	270	12	B	11	C	11	C
	540	11	BC	12	CD	12	CD
Quizalofop-p-ethyl	110	3	EF	3	H	3	G
	220	10	BC	7	DEFG	7	DEFG
Pinoxaden	60	9	BCD	9	CDEF	9	CDE
	120	12	B	9	CDE	8	CDEF

[a] Means within a column followed by the same letter are not significantly different based on Tukey's HSD (0.05)

Table 5. Relative height, heading date, and yield of TamArk™ grain sorghum at Fayetteville, AR, presented by the interaction of herbicide and rate averaged over 2020 and 2021.[a]

Herbicide	Rate	Height		Heading delay[b]		Yield	
	g ai ha^{-1}	cm		days		kg ha^{-1}	
Nontreated	--	31	A	0	B	5270	A
Sethoxydim	210	16	B	17	A	2770	B
	420	13	B	23	A	1725	C
Clodinafop-propargyl	70	31	A	1	B	5140	A
	140	30	A	-1	B	4660	AB
Cyhalofop	310	29	A	1	B	4250	AB
	620	30	A	4	B	4400	AB
Diclofop-methyl	1120	32	A	2	B	4845	A
	2240	30	A	-1	B	5155	A
Fenoxaprop-p-ethyl	80	32	A	-4	B	5540	A
	160	31	A	-2	B	4290	AB
Fluazifop-p-butyl	210	32	A	-2	B	4960	A
	420	30	A	1	B	4320	AB
	840	30	A	-2	B	4460	AB
Fluazifop-p-butyl+Fenoxaprop-p-ethyl	270	30	A	-4	B	5130	A
	540	28	A	-3	B	4960	A
Quizalofop-p-ethyl	110	30	A	-1	B	4530	AB
	220	31	A	-3	B	4615	AB
Pinoxaden	60	30	A	-4	B	5145	A
	120	30	A	-3	B	5215	A

[a] Means within a column followed by the same letter are not significantly different based on Tukey's HSD (0.05)

[b] Negative numbers represent heading prior to the nontreat

Figures

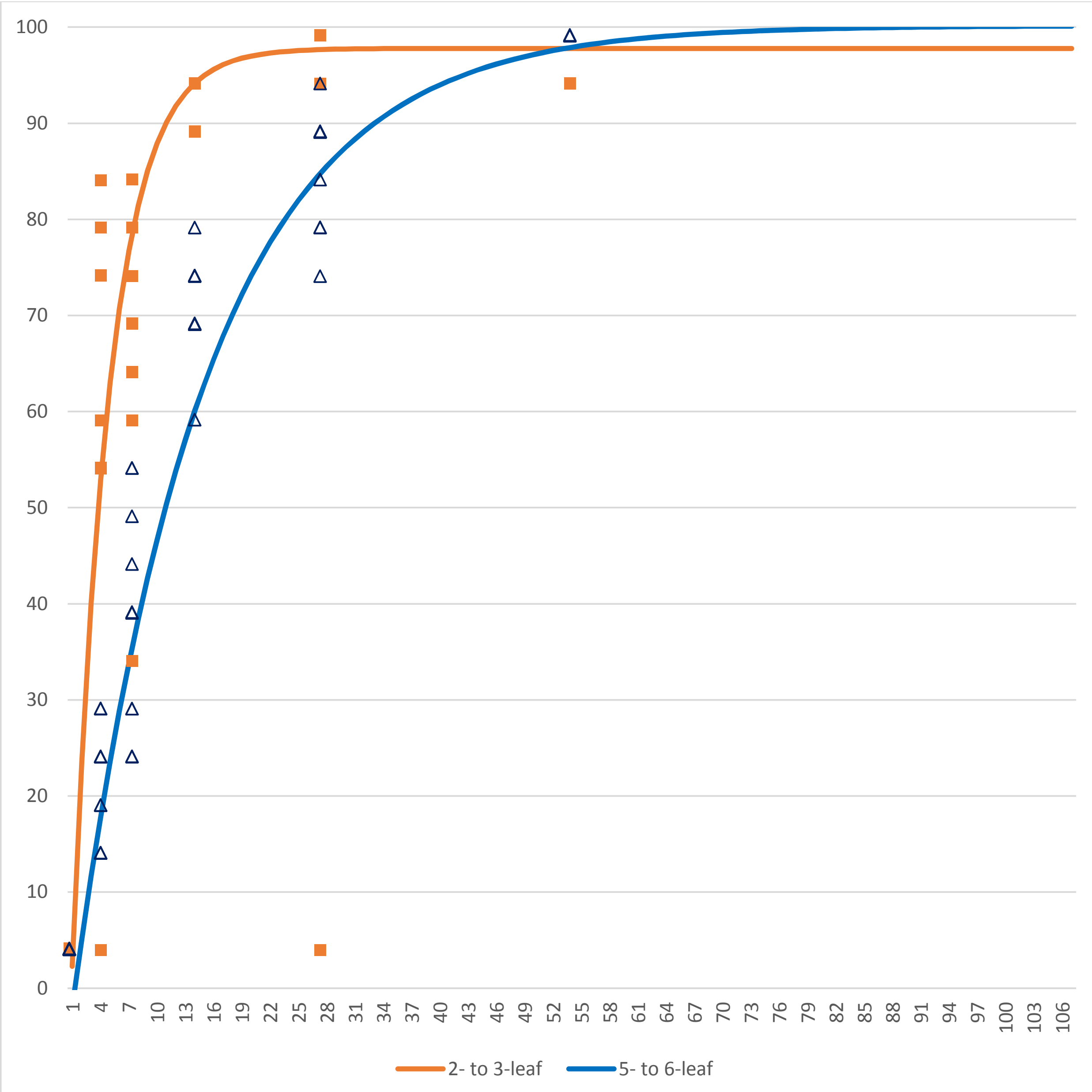

Figure 1. Exponential 3P curve to ($y = a + b * Exp(c*g$ ai $ha^{-1})$, a = asymptote, b = scale, c = growth rate) fit fluazifop-p-butyl dose-response injury data from Fayetteville, AR, in 2020 and 2021 and College Station, TX, in 2021 (n = 15 observations per rate) for the susceptible grain sorghum (Pioneer 84P80). a=97.79, b=-95.51, and c=-0.252 (2- to 3-leaf); a=100.16, b=-102.19, and c=-0.072 (5- to 6-leaf)

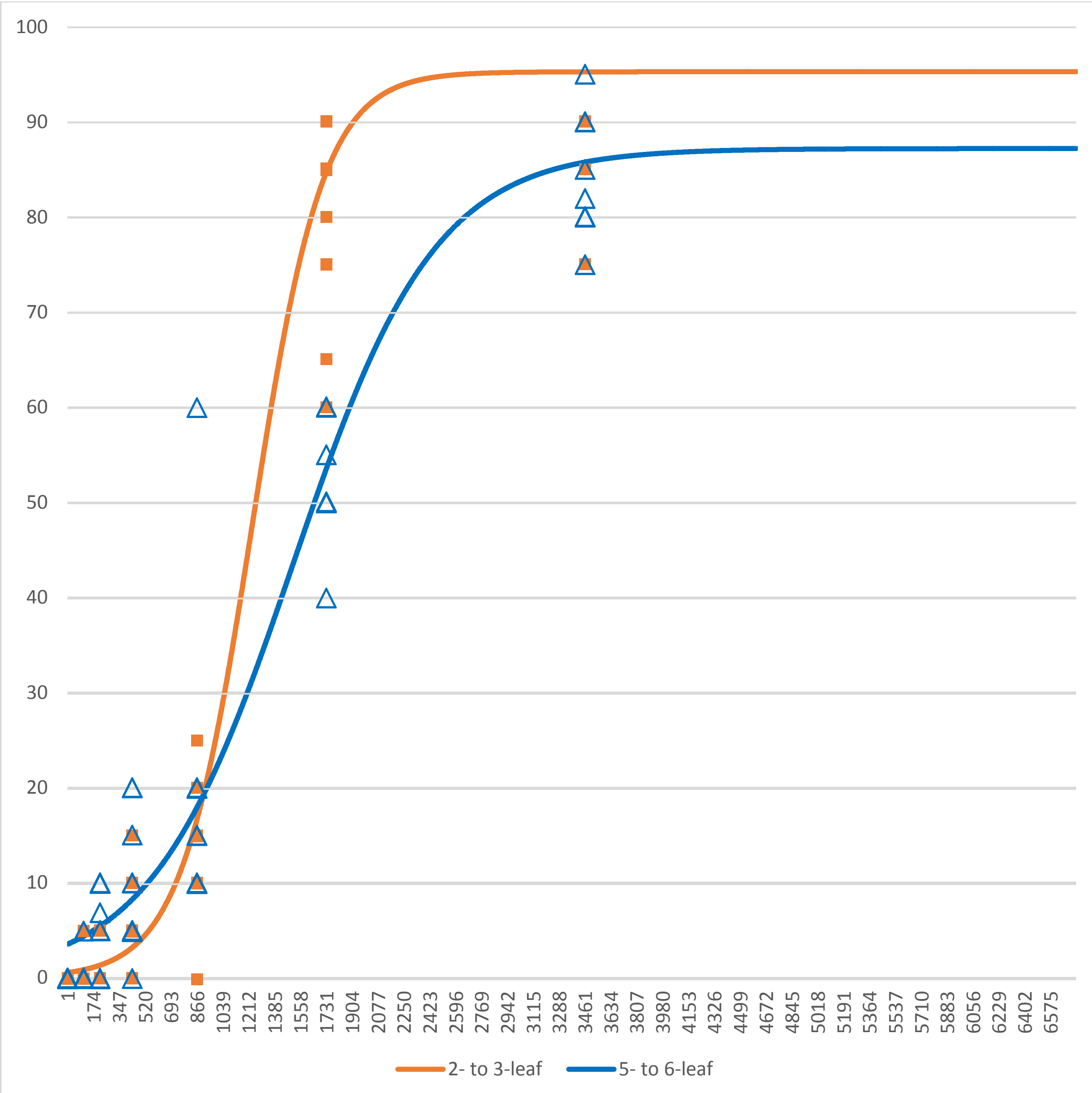

Figure 2. Logistic 3P curve to (y = c/(1 + Exp(-a*(g ai ha^{-1} – b))), a = growth rate, b = inflection point, c = asymptote) fit fluazifop-p-butyl dose-response injury data from Fayetteville, AR in 2020 and 2021 and College Station, TX, in 2021 (n = 15 observations per rate) for the resistant grain sorghum (TamArk^{TM}). a=0.0028, b=1308.82, and c=94.62 (2- to 3-leaf); a=0.0022, b=1517.75, and c=85.48 (5- to 6-leaf).

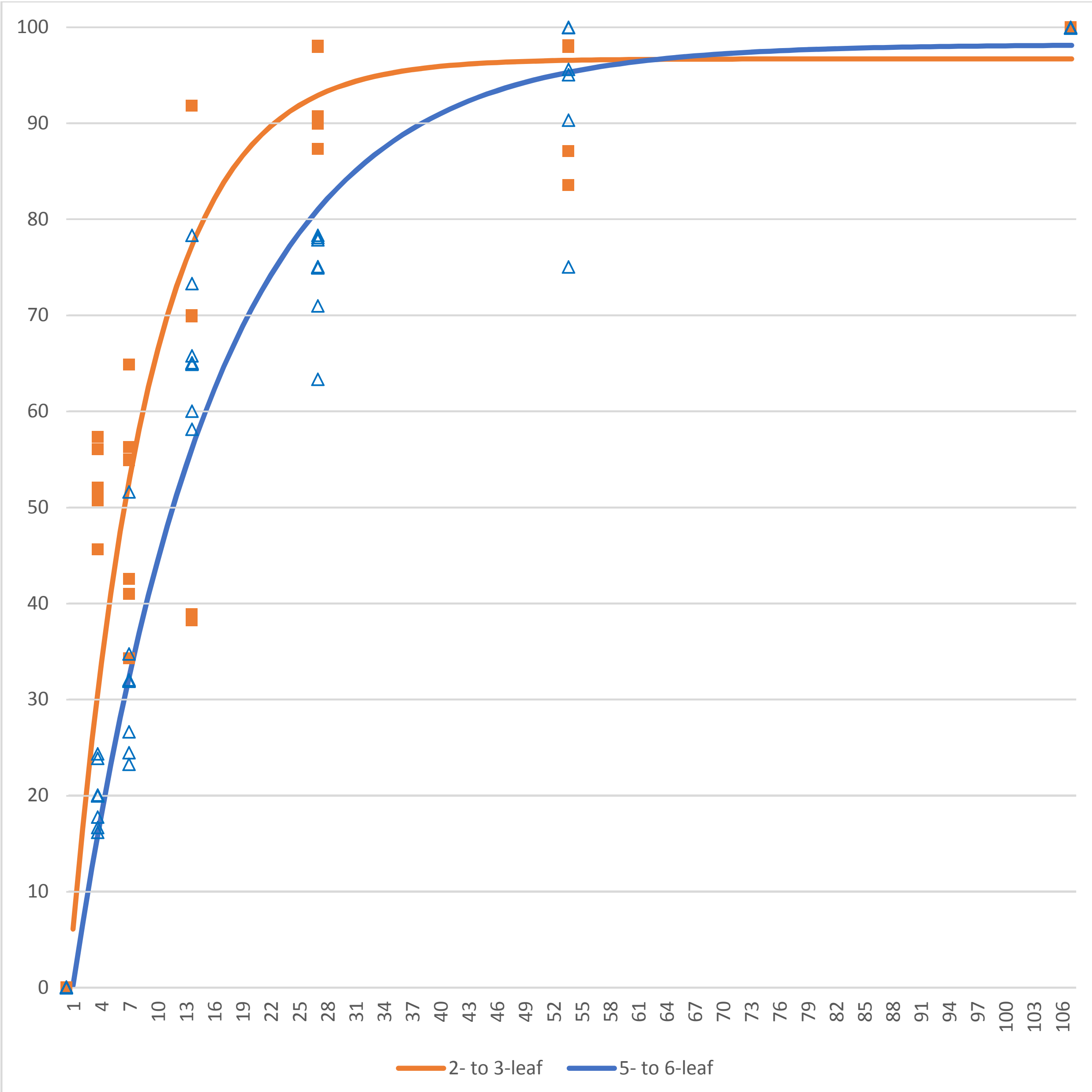

Figure 3. Exponential 3P curve to ($y = a + b * \mathrm{Exp}(c*\text{g ai ha}^{-1})$, a = asymptote, b = scale, c = growth rate) fit fluazifop-p-butyl dose-response growth reduction data from Fayetteville, AR, in 2020 and 2021 and College Station, TX, in 2021 (n = 15 observations per rate) for the susceptible grain sorghum (Pioneer 84P80). a=96.7, b=-90.64, and c=-0.122 (2- to 3-leaf); a=98.19, b=-98.004, and c=-0.067 (5- to 6-leaf).

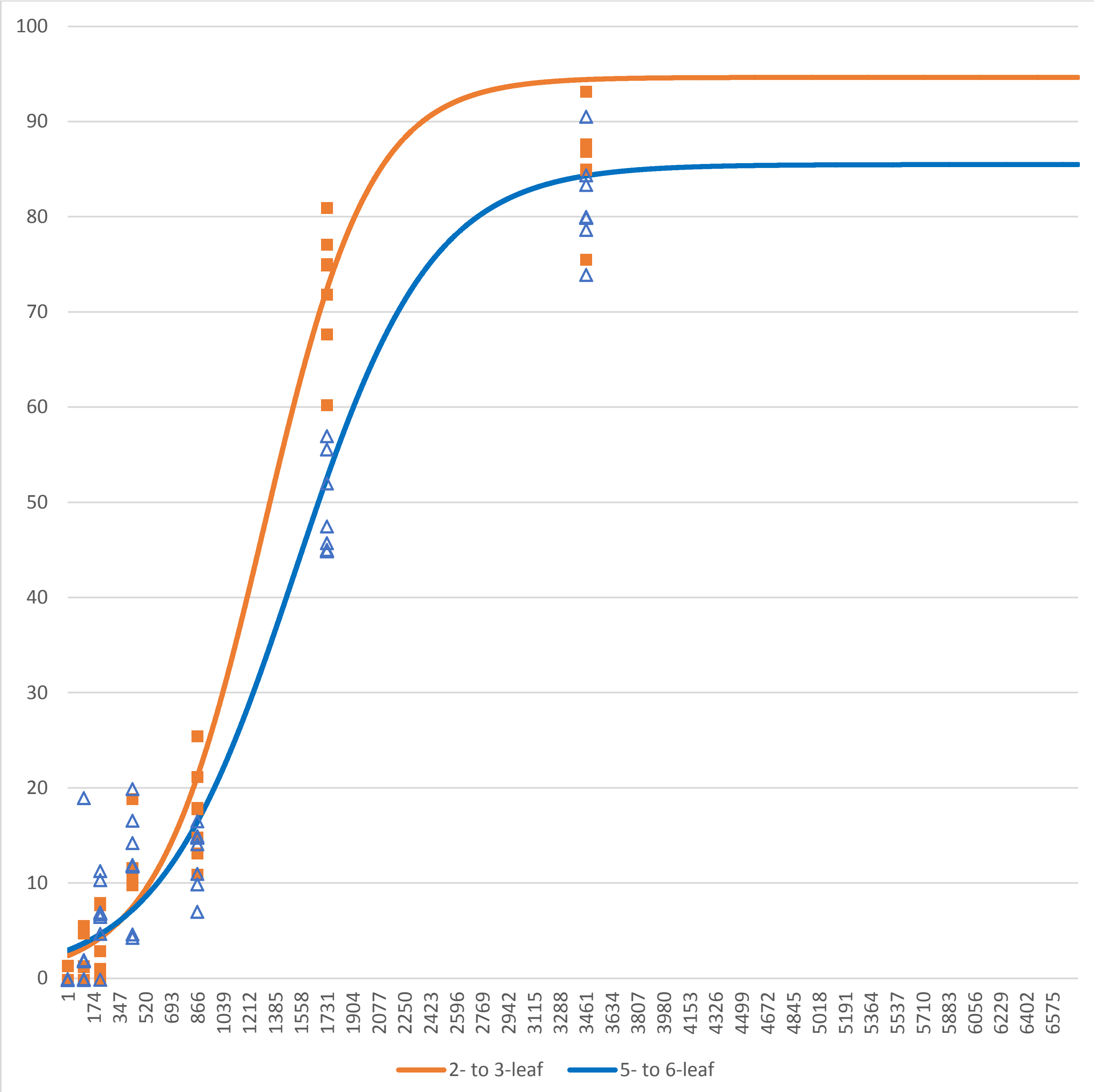

Figure 4. Logistic 3P curve to ($y = c/(1 + Exp(-a*(g\ ai\ ha^{-1} - b)))$, a = growth rate, b = inflection point, c = asymptote) fit fluazifop-p-butyl dose-response growth reduction data from Fayetteville, AR in 2020 and 2021 and College Station, TX in 2021 (n = 15 observations per rate) by size at time of application to TamArk™ grain sorghum. a=0.0042, b=1234.84, and c=95.31 (2- to 3-leaf); a=0.0021, b=1509.67, and c=87.23 (5- to 6-leaf).

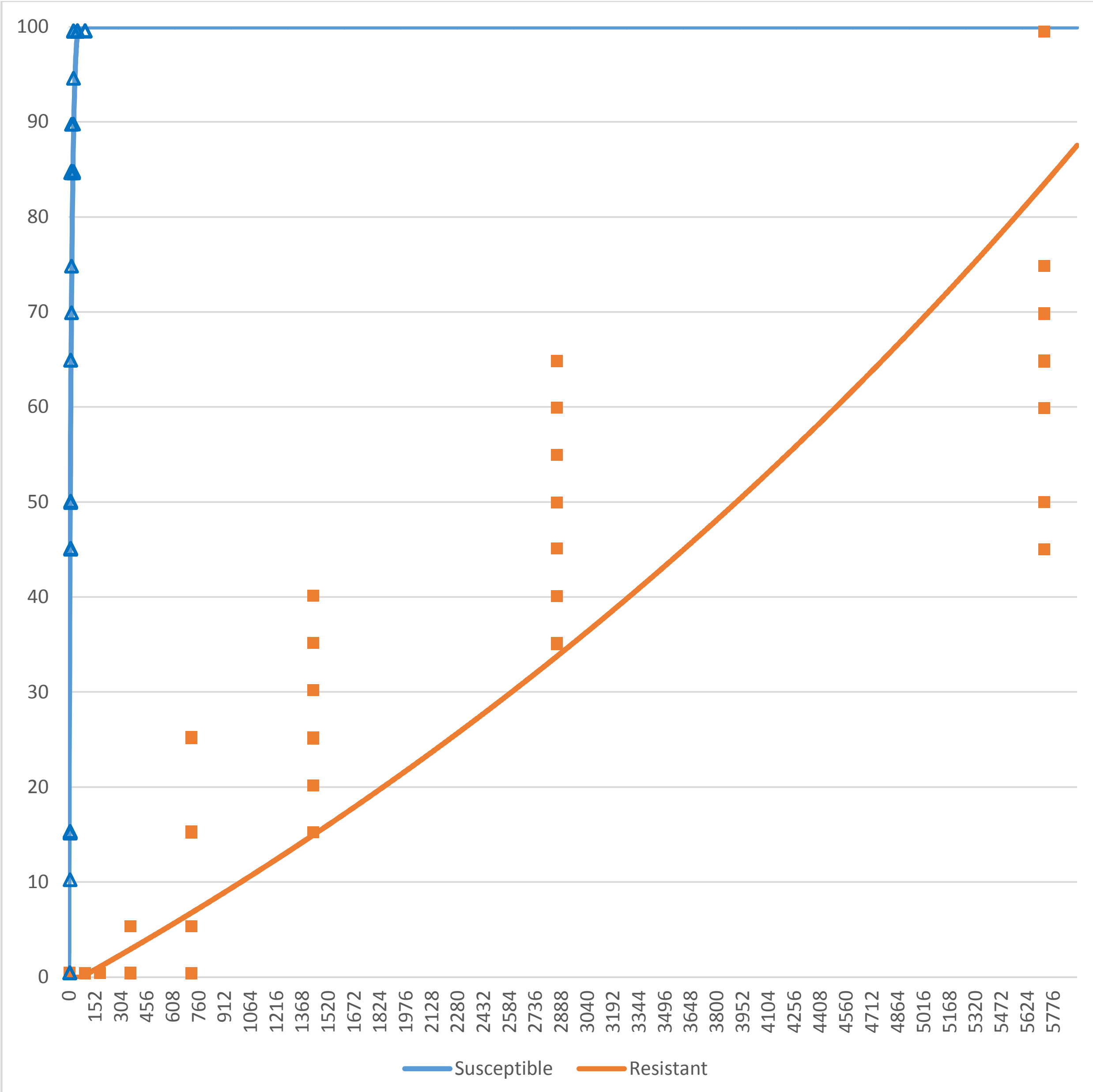

Figure 5. Exponential 3P curve to (y = a + b * Exp(c*g ai ha-1), a = asymptote, b = scale, c = growth rate) fit fenoxaprop-p-ethyl dose-response injury data from Fayetteville, AR at 2020 and 2021 and College Station, TX in 2021 (n = 15 observations per rate) by susceptible (Pioneer 84P80) and resistant (TamArk^{TM}) grain sorghum. a=-78.669, b=77.9, and c=0.000128 (resistant); a=101.81, b=-106.406, and c=-0.072 (susceptible).

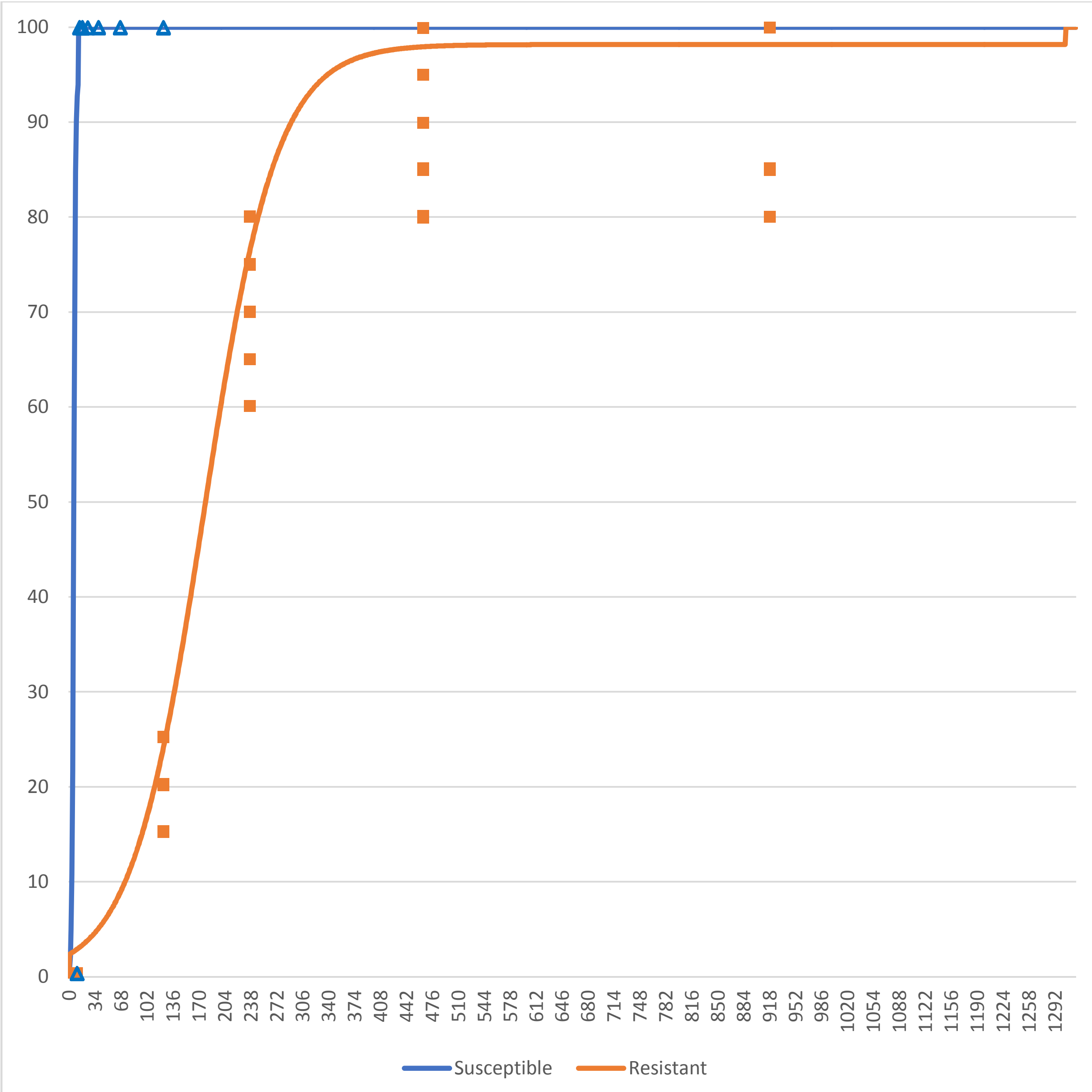

Figure 6. Logistic 3P curve to ($y = c/(1 + \mathrm{Exp}(-a*(\text{g ai ha}^{-1} – b)))$, a = growth rate, b = inflection point, c = asymptote) fit quizalofop-p-ethyl dose-response injury data from Fayetteville, AR in 2020 and 2021 and College Station, TX in 2021 (n = 15 observations per rate) by susceptible (Pioneer 84P80) and resistant (TamArk™) grain sorghum. a=0.021, b=177.402, and c=98.174 (resistant); a=0.837, b=6.456, and c=94.826 (susceptible)

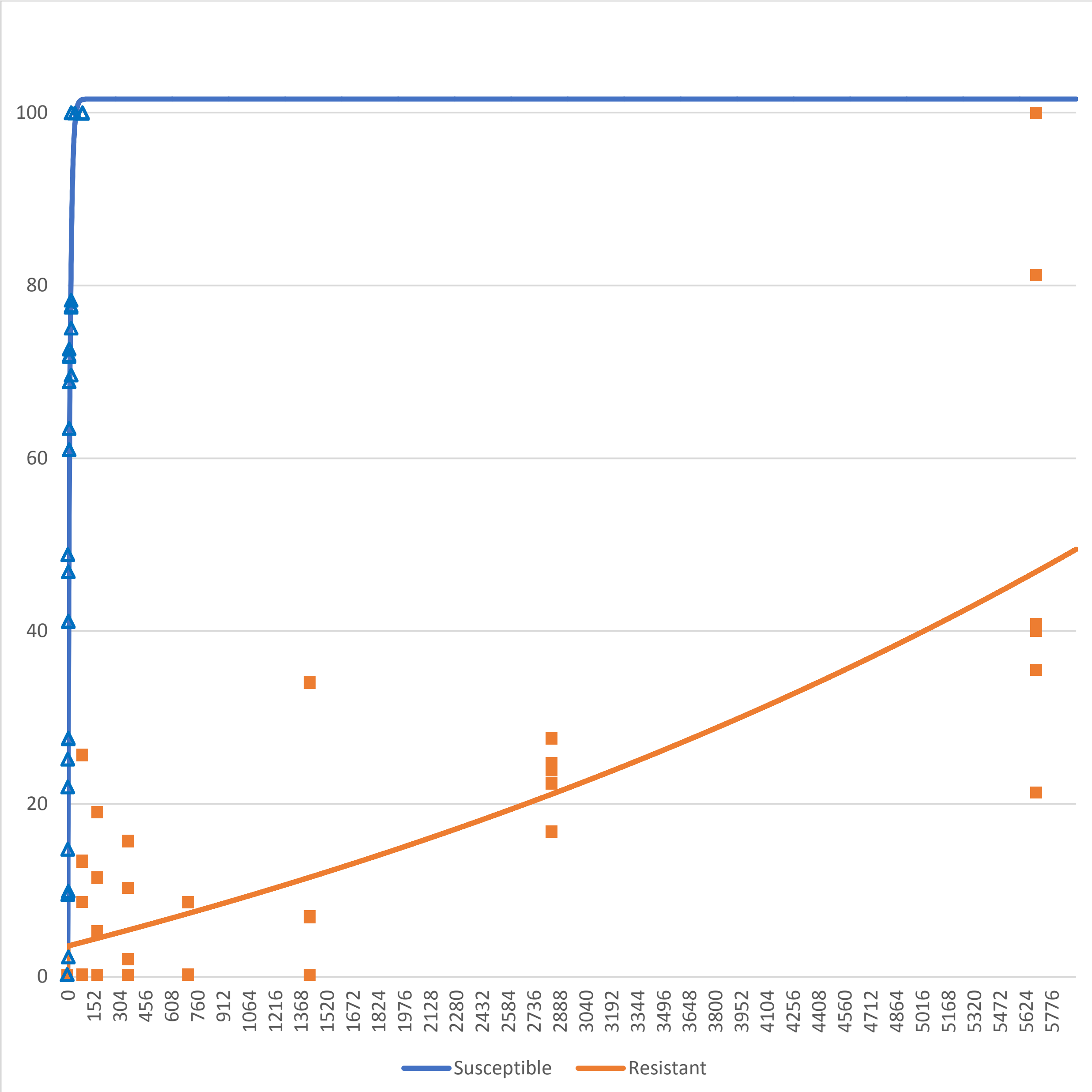

Figure 7. Exponential 3P curve to (y = a + b * Exp(c*g ai ha^{-1}), a = asymptote, b = scale, c = growth rate) fit fenoxaprop-p-ethyl dose-response growth reduction data from Fayetteville, AR in 2020 and 2021 and College Station, TX in 2021 (n = 15 observations per rate) by susceptible (Pioneer 84P80) and resistant (TamArk™) grain sorghum. a=-34.40, b=37.938, and c=0.000134 (resistant); a=101.59, b=-102.7392, and c=-0.0840 (susceptible).

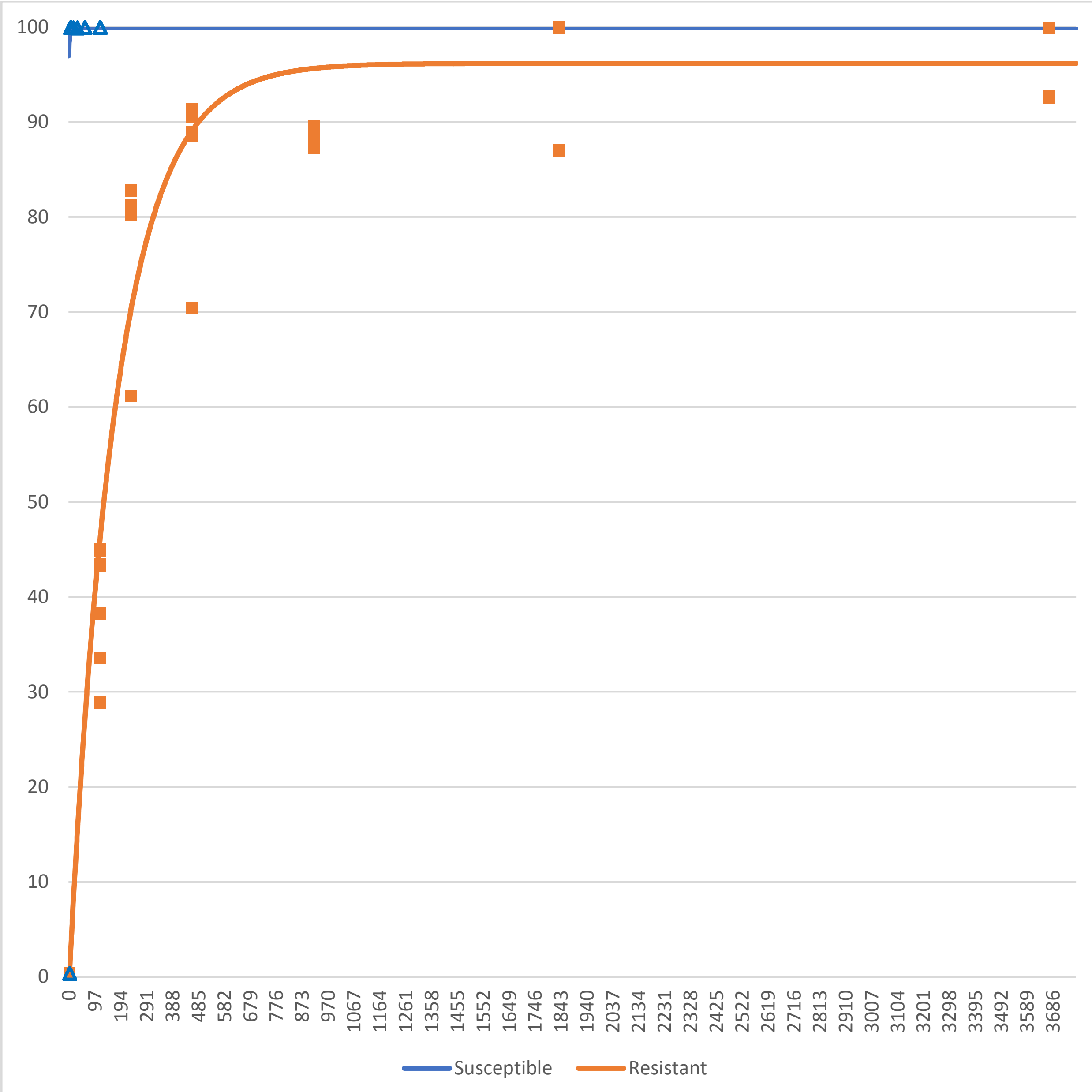

Figure 8. Exponential 3P curve to ($y = a + b * Exp(c*g \text{ ai ha}^{-1})$, a = asymptote, b = scale, c = growth rate) fit quizalofop-p-ethyl dose-response growth reduction data from Fayetteville, AR in 2020 and 2021 and College Station TX in 2021 (n = 15 observations per rate) by grain sorghum line (susceptible – Pioneer 84P80 and resistant - TamArk™). a=96.192, b=-97.6563, and c=-0.005667 (resistant); a=100, b=-100, and c=-4.487 (susceptible).

Chapter 4

Sensitivity of TamArk™ Grain Sorghum and Other Monocot Species to ACCase- and ALS-Inhibiting Herbicides

Abstract

Postemergence grass control in grain sorghum has been a significant issue due to the limited number of herbicides available. The herbicides currently labeled in grain sorghum either have strict use restrictions, low efficacy on johnsongrass, or resistance issues. To introduce new effective herbicide sites of action for grass control, multiple companies and universities have been developing herbicide-resistant grain sorghum that would allow producers to utilize either acetolactate synthase (ALS) or acetyl coenzyme A carboxylase (ACCase) inhibitors for postemergence grass control. To determine the effectiveness of these herbicides on TamArk™ grain sorghum, conventional grain sorghum, and problematic grass weed species, an experiment was conducted in Fayetteville, AR, in 2020 and 2021, evaluating two ALS-inhibiting herbicides and nine ACCase-inhibiting herbicides. Grain sorghum and grass weeds were sprayed when TamArk™ grain sorghum reached the 2- to 3-leaf stage. TamArk™ grain sorghum was resistant to all ACCase-inhibiting herbicides tested (≤10% injury), other than clethodim and sethoxydim, and showed no resistance to the ALS-inhibiting herbicides evaluated. Additionally, all ACCase inhibitors other than diclofop and pinoxaden resulted in ≥92% control of johnsongrass, broadleaf signalgrass, barnyardgrass, and Texas panicum by 28 DAA. Conversely, the two ALS inhibitors, imazamox and nicosulfuron, resulted in ≤81% control of broadleaf signalgrass 28 DAA but still offered ≥95% control of all other grasses. TamArk™ grain sorghum

appears to have low sensitivity to multiple ACCase-inhibiting herbicides and provides an effective option for postemergence grass control, with clethodim, sethoxydim, nicosulfuron, and imazamox as options for controlling volunteer plants. Imazamox and nicosulfuron, both ALS-inhibiting herbicides, while not useful on TamArk™ grain sorghum, are effective options for grass control in Igrowth™ and Inzen™ grain sorghum, respectfully.

Nomenclature: Fluazifop; barnyardgrass, *Echinochloa crus-galli* (L.) Beauv.; broadleaf signalgrass, *Urochloa platyphylla* (Nash) R.D. Webster; johnsongrass, *Sorghum halapense* (L.) Pers; Texas panicum, *Urochloa texana* (Buckl.) R. Webster; grain sorghum, *Sorghum bicolor* (L.) Moench

Introduction

The lack of postemergence (POST) herbicide options for late-season grass control is an issue for many grain sorghum producers across the United States (US) (Smith et al. 2010). With grain sorghum being a member of the Gramineae family, POST herbicides that control grass weeds have high risk of severely injuring the crop. Only three herbicides are available for POST grass control in conventional grain sorghum: atrazine, quinclorac, and paraquat (Barber et al. 2020). These herbicides present challenges, including paraquat requiring post-directed applications under hoods to mitigate significant crop injury, quinclorac resistance in multiple annual grasses, and atrazine only providing partial grass control (Fromme et al. 2012; Heap 2022).

One significant development for grass control in grain sorghum was introduction of fluxofenim-based seed treatments that allow producers to use chloroacetamide herbicides such as *S*-metolachlor and dimethenamid-p preemergence (PRE) for both grass and small-seeded broadleaf control without injuring grain sorghum (Al-Khatib et al. 2004). However, relying on chloroacetamide herbicides for grass control does present some issues in grain sorghum. Since grain sorghum is a crop commonly grown in hot and dry conditions without irrigation, decreased efficacy of chloroacetamide herbicides can be observed (Prasad et al. 2008). Chloroacetamide herbicides require adequate moisture for proper activation, which is not always present in grain sorghum production (Brown et al. 1988; Regher et al. 2008). When rainfall is less than 14mm within the first two weeks of application, a reduction in chloroacetamide efficacy can be observed on barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] (Jursik et al. 2013). Furthermore, chloroacetamide herbicides effectively control seedling johnsongrass greater than 95%, but do not control johnsongrass plants that emerge from rhizomes (Scarabel et al. 2014). Since a johnsongrass plant can produce 5000 or more rhizomes in a single growing season, other control options are necessary (McWhorter 1971).

Options for POST grass control in grain sorghum are needed. Four companies or universities have focused on development of herbicide-resistant grain sorghum to introduce new herbicides for POST grass control. Two would allow for the use of WSSA group 1 acetyl CoA carboxylase (ACCase) inhibitors, and two would allow the use of WSSA group 2 acetolactate synthase (ALS) inhibitors.

Corteva (Indianapolis, IN) has developed Inzen™ grain sorghum, which is resistant to the ALS inhibitor nicosulfuron, currently marketed under the tradename Accent® Q in corn (*Zea mays* L.) but labeled for grain sorghum as Zest™. Nicosulfuron is a sulfonylurea herbicide that has been used to control problematic grasses in corn, specifically johnsongrass (Camacho et al. 1991; Dobbles and Kapusta 1993). A collaboration between UPL (King of Prussia, PA) and Alta seeds (Amarillo, TX) led to the commercialization and release of grain sorghum resistant to the ALS inhibitor imazamox in 2021, known as Igrowth™. Imazamox, an imidazolinone herbicide, is commonly known by the tradenames Raptor or Beyond® (BASF, Triangle Park, NC) and utilized for grass control in Clearfield® production systems or soybean [*Glycine max* (L.) Merr.]. While imazamox is proven to control annual grasses such as barnyardgrass and goosegrass [*Eleusine indica* (L.) Gaertn] (Fish et al. 2016; Yadav et al. 2017), little data are available on the control of perennial grasses like johnsongrass.

S&W Seeds (Longmont, CO) collaborated with Adama (Raleigh, NC) resulting in development of grain sorghum resistant to the ACCase inhibitor quizalofop, known as Double

Team^{TM}. Quizalofop is an aryloxyphenoxypropionate (AOP) herbicide sold under many tradenames, but most recently integrated into rice production through the Provisia® system commercialized by BASF (Triangle Park, NC). Quizalofop has successfully controlled both problematic annual and perennial grass weeds (Brewster and Spinney 1989; Sanders et al. 2020). The University of Arkansas System Division of Agriculture and Texas A&M University collaboratively developed grain sorghum known as TamArk^{TM}, which has two mutations in the ACCase gene (Norsworthy et al. 2020). Preliminary data show these two mutations in grain sorghum confer resistance to other ACCase inhibitors within the AOP and phenylpyrazolin (PPN) families (Piveta et al. 2020).

The addition of new herbicide resistance technologies could significantly improve grass control in grain sorghum. The use of effective SOAs not previously labeled in grain sorghum could allow producers to better control problematic grasses while helping mitigate resistance (Norsworthy et al. 2012). While herbicides planning to be labeled for use in grain sorghum have demonstrated grass control in crops such as rice (*Oryza sativa* L.), corn, and soybean, it is essential to understand the control levels of grasses specific to grain sorghum under typical growing conditions. By understanding which herbicides are most effective on certain problematic grasses, a better decision can be made on which technologies should be utilized based on specific weed species present in an area. Therefore, research was conducted to determine the effectiveness of two ALS and nine ACCase-inhibiting herbicides on common grasses of grain sorghum along with sensitivity of conventional and TamArk^{TM} grain sorghum to these herbicides.

Materials and Methods

Field experiments were conducted in 2020 and 2021 at the Milo J. Shult Arkansas Agricultural Research and Extension Center in Fayetteville, AR, on a Leaf silt loam (fine, mixed, active, thermic Typic Albaquults) with 19.6% sand, 57.8% silt, 22.6% clay, and a pH of 6.2. Experiments were a single-factor randomized complete block design with 4 replications. Ten ACCase inhibitors and 2 ALS inhibitors were evaluated at various rates based on label suggestions in crops other than grain sorghum (Table 1). All treatments were mixed with crop oil concentrate at 1% v/v. A nontreated check was included for comparison purposes. The conventional grain sorghum hybrid DK553-67 and TamArk™ were planted at 18 seed m^{-1} row. Initial plans were for Inzen™ grain sorghum to be included in this study, but due to research restrictions on the technology it had to be removed. Common grass weeds were also included in the study, with johnsongrass, broadleaf signalgrass, barnyardgrass, and Texas panicum seeded in individual rows at approximately 40 seed m^{-1}. All grass weeds were obtained from Azlin Seed Service (Leland, MS). All species, including grain sorghum, were planted into a conventionally tilled area using a Hege (Hege Company, Waldenburg, Germany) drill with individual seed boxes for each row with 38 cm between rows. The plot size was 2 m by 3 m, and herbicide applications were made perpendicular to the direction planted. Weeds and crops were not grown past 28 days after application (DAA); hence, only preplant nitrogen applications were made based on the Arkansas grain sorghum production handbook (Espinoza 2015). Broadleaf weeds were removed from all plots using a single application of 2,4-D at 950 g ae ha^{-1} when grain sorghum was 25-cm tall. No herbicides were sprayed to control natural grass populations to ensure the planted grasses were not injured or controlled before treatment applications. Treatment applications were made when grain sorghum reached the 2- to 3-leaf stage (Table 2) using a CO_2-pressurized backpack sprayer and a 6-nozzle boom with air induction extended range (AIXR) 110015 nozzles (TeeJet, Springfield, IL) spaced 50 cm apart at 4.8 kph delivering 140 L ha^{-1}. Boom height was 46 cm above the tallest plant present in the plot to achieve proper coverage.

Grain sorghum was evaluated for visible injury at 14, 21, and 28 days after application (DAA) since both ACCase and ALS inhibitors typically elicit minimal symptoms in plants the first seven days after treatment. Injury was rated on a 0 to 100 scale, where 0 was equal to no visible injury, and 100 was equal to complete crop mortality (Frans and Talbert 1986). Similarly, visible grass control was rated the same days on a scale of 0 to 100, where 0 was equivalent to no grass control, and 100 was equal to no living tissue present (Frans and Talbert 1986). At 28 DAA, aboveground living tissue was collected by species or grain sorghum type. All living plants within 1 m of row of each species by plot were collected and air dried at 60 C for 2 weeks, then removed and weighed individually. Data were used to calculate percent biomass reduction by species using the following equation: $\frac{Nontreated(g) - treated(g)}{nontreated(g)}\ x\ 100$.

Data Analysis. All nontreated plots were rated as 0 at all evaluation timings across all species, hence, they were excluded from the statistical analysis. The distribution function in JMP 16.1 Pro (SAS Institute Inc. Cary, NC) was utilized to determine the correct distribution for analysis of each variable based on AICc and BIC values. Visible control ratings of all grass species and conventional grain sorghum injury at 14, 21, and 28 DAA were determined to follow a beta distribution. Visible sensitivity of TamArk^{TM} grain sorghum to the herbicides followed a gamma distribution. Biomass reduction for each grass species and grain sorghum type followed a beta distribution. A single-factor statement was developed with the main effect of herbicide treatment for grain sorghum and all grass weeds at each evaluation timing and biomass reduction using the PROC GLIMMIX model in SAS 9.4 (SAS Institute Inc. Cary, NC). Block and year were considered random effects in all statements. When herbicide treatment was significant, visible control and biomass reduction were subjected to means separation using Tukey's HSD at an alpha value of 0.05.

Results and Discussion

Conventional grain sorghum. High injury and biomass reduction levels occurred, with injury ranging from 94% to 100% across all herbicides and evaluation timings other than pinoxaden and diclofop (Table 3). Pinoxaden and diclofop caused lower levels of injury than all other herbicide treatments in each respective evaluation timing; albeit the injury level was ≥67% by 28 DAA for both herbicides, which would be deemed unacceptable. Like the injury evaluations, all treatments resulted in greater than 99% biomass reduction other than pinoxaden and diclofop, which caused 81% and 83% reduction in biomass, respectively. None of the evaluated herbicides are labeled for use in conventional grain sorghum, and it is known that grain sorghum is highly sensitive to ACCase inhibitors (Lancaster et al. 2018); hence, high levels of injury were expected.

TamArk™ grain sorghum. Differences in injury and biomass reduction to TamArk™ grain sorghum occurred among the herbicides tested at all evaluation timings (Table 4). The two ALS inhibitors, nicosulfuron and imazamox, completely controlled TamArk™ grain sorghum by 28 DAA, resulting in 100% biomass reduction. Since no known mutations to the ALS gene are present in TamArk™ grain sorghum, the high sensitivity to these herbicides was expected.

Among ACCase inhibitors, the highest level of injury resulted from the CHD family, where complete control was achieved with clethodim and sethoxydim by 21 DAA (Table 4). Conversely, the ACCase inhibitors from the AOP and PPN families, specifically clodinafop, cyhalofop, diclofop, fenoxaprop, fluazifop, quizalofop, and pinoxaden, resulted in relatively low injury levels, with the highest being 10% caused by quizalofop at 92 g ha^{-1} at 28 DAA. Similarly, Piveta et al (2020) observed high levels of resistance to fluazifop, fenoxaprop, and quizalofop when conducting dose responses experiments on TamArk™ grain sorghum. Therefore, herbicides from the AOP and PPN families could be safely utilized for grass control in TamArk™ grain sorghum, if labeled.

Johnsongrass. Like conventional grain sorghum, johnsongrass control by treatment varied 14 DAA, ranging from 80% to 100% control across herbicide treatments, excluding the pinoxaden and diclofop treatments (Table 5). Diclofop at 1,120 g ha^{-1} and pinoxaden at 60 g ha^{-1} provided only 32% and 59% johnsongrass control, respectfully, at 14 DAA. Johnsongrass control increased over time with pinoxaden, resulting in 92% control by 28 DAA; however, diclofop control at 28 DAA was only 38%, a level deemed unacceptable. Like the levels of johnsongrass control at 28 DAA, all ACCase-inhibiting herbicide treatments, except diclofop and pinoxaden, resulted in ≥93% johnsongrass biomass reduction. While multiple herbicide treatments resulted in high levels of control, any treatment that did not provide 100% control is concerning. Since live johnsongrass plants were still present within the field, there is potential for seed or rhizome production from these surviving plants. Those herbicides that provided complete johnsongrass control and biomass reduction by 28 DAA included clethodim, sethoxydim, fenoxaprop, fluazifop, and quizalofop. Of these, only fluazifop, fenoxaprop, and quizalofop would be viable options for johnsongrass control in TamArk™ grain sorghum based on the low levels of injury caused by these herbicides (Table 4). Prior to 2022, no POST herbicide was available for johnsongrass control in grain sorghum; therefore, the addition of multiple ACCase-inhibiting herbicides, such as those evaluated here, would provide much needed johnsongrass control options in grain sorghum production (Smith et al. 2010).

Broadleaf signalgrass. Control of broadleaf signalgrass varied among herbicide treatments at 14 DAA, with the highest control (≥90%) achieved with clethodim, sethoxydim, the two highest rates of fluazifop, both rates of fenoxaprop, pinoxaden, and all three rates of quizalofop; albeit none provided complete control (Table 6). By 21 DAA, clethodim, fenoxaprop (120 g ha^{-1}), and quizalofop (92 g ha^{-1}) resulted in 100% control of broadleaf signalgrass. At 28 DAA, a more apparent separation in treatments could be observed, specifically between the ALS and ACCase inhibitors. Both rates of imazamox and nicosulfuron at 28 DAA resulted in lower levels of broadleaf signalgrass control than all but one ACCase inhibitor treatment (diclofop). Like control levels, imazamox and nicosulfuron generally caused less broadleaf signalgrass biomass reduction than the ACCase-inhibiting herbicides, other than diclofop. Diclofop resulted in only 27% control of broadleaf signalgrass and 45% biomass reduction, which was not surprising considering it is listed as suppressed by the herbicide at the 3-leaf growth stage or smaller according to the label (Anonymous 2003). Broadleaf signalgrass in this trial was 4- to 6-leaf both years, which explains the low levels of control observed (Table 2). Similarly, imazamox is reported to only achieve suppression of 2- to 5-leaf broadleaf signalgrass unless sequential applications are applied (Anonymous 2019), and nicosulfuron is labeled for control of broadleaf signalgrass only when plants are no larger than 5-cm in height (Anonymous 2009). Because of the low levels of control achieved with the two ALS inhibitors or diclofop, these herbicides would not be recommended for broadleaf signalgrass control. Since TamArk™ grain sorghum is also sensitive to clethodim, one of the AOP family herbicides such as fenoxaprop, quizalofop, or fluazifop would be recommended.

Barnyardgrass. All treatments resulted in complete barnyardgrass control (100%) across all application timings, except diclofop which provided 91% control (Table 7). Similarly, all treatments resulted in 100% biomass reduction, except diclofop. Based on the diclofop label (Anonymous 2003), the herbicide is not recommended for control of larger than 4-leaf barnyardgrass, which was present in plots (Table 2). Overall, the ACCase and ALS inhibitors resulted in high barnyardgrass control levels, exceeding the effectiveness of traditional herbicides used for POST barnyardgrass control in grain sorghum (Grichar et al. 2005).

Texas panicum. Complete control of Texas panicum was obtained with all treatments evaluated at 14 DAA, except diclofop (Table 8). By 28 DAA, Texas panicum control with diclofop improved, with all herbicide treatments providing complete control. The high level of control was reflected in the absence of Texas panicum by 28 DAA for all herbicide treatments. Texas panicum is a common problematic weed of grain sorghum (Van Wychen 2020), and high levels of control are seldom achieved in the crop (Grichar et al. 2004). One of the most effective means of controlling Texas panicum in grain sorghum has been the use of dimethenamid-p and atrazine, and this combination generally provides <80% control (Grichar et al. 2004). Another herbicide that has been evaluated on Texas panicum in grain sorghum is quinclorac, but control is <40% (Kering et al. 2013), a level much lower than that achieved here with both ALS and ACCase inhibitors.

Practical implications. With commercial tolerance to the AOP and PPN herbicides within the ACCase-inhibitor group, TamArk^{TM} grain sorghum offers the ability to control the problematic grass weeds within grain sorghum using multiple POST options if labeled. Both fenoxaprop (120 g ai ha^{-1}) and quizalofop (96 g ai ha^{-1}) provided complete control of all grass weeds tested, making them ideal options for grass control in TamArk^{TM} grain sorghum. Neither of these herbicides at the rates tested caused more than 10% injury or biomass reduction to TamArk^{TM} grain sorghum.

While TamArk^{TM} grain sorghum did not show tolerance to the ALS inhibitors evaluated, these herbicides could be used in the proper technology platform, Inzen^{TM} or Igrowth^{TM}, for grass control. These herbicides were not as effective as fenoxaprop and quizalofop at controlling broadleaf signalgrass. Imazamox and nicosulfuron could also be used to remove volunteer TamArk^{TM} grain sorghum from fields of the crop planted to the Inzen^{TM} or Igrowth^{TM} traits. The addition of ACCase and ALS inhibitors to grain sorghum offers producers SOAs that are also effective for johnsongrass control POST, an option that has not been available previously (Smith et al. 2010).

The addition of ACCase and ALS inhibitors also offers a way to help mitigate herbicide resistance by adding two effective SOAs for grass control in grain sorghum (Norsworthy et al. 2012). By utilizing either ACCase or ALS inhibitors in a program for grass control in grain sorghum, producers can reduce the pressure currently on quinclorac, which has been heavily used for grass control in both rice (*Oryza sativa* L.) and grain sorghum, leading to an increased number of quinclorac-resistant grass populations (Talbert and Burgos 2007; Heap 2022). It is also important to note that either ALS- or ACCase-resistant populations of all the grasses evaluated in this study have been documented in the US and other countries worldwide (Heap 2022). While these resistant grass populations are not widespread, it will be important not to overuse ACCase or ALS herbicides for grass control in grain sorghum to mitigate future resistance. Therefore, these technologies should be used in a program approach along with proper cultural and mechanical weed control methods to reduce the risk of herbicide resistance.

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Tables

Table 1. Herbicides and rates applied for monocot tolerance studies in 2020 and 2021.

Common name	Trade name	WSSA group	Rate
			g ai ha^{-1}
Clethodim	Select Max	Group 1 ACCase inhibitor	135
Clodinafop	Discover NG	Group 1 ACCase inhibitor	70
Cyhalofop	Clincher	Group 1 ACCase inhibitor	312
Diclofop	Hoelon	Group 1 ACCase inhibitor	1120
Fenoxaprop	Ricestar	Group 1 ACCase inhibitor	86
			120
Fluazifop	Fusilade DX	Group 1 ACCase inhibitor	210
			280
			420
Imazamox	Beyond	Group 2 ALS inhibitor	52
			78
Nicosulfuron	Accent Q	Group 2 ALS inhibitor	35
			51
Pinoxaden	Axial XL	Group 1 ACCase inhibitor	60
Quizalofop	Assure II	Group 1 ACCase inhibitor	46
			77
			92
Sethoxydim	Poast Plus	Group 1 ACCase inhibitor	210

Table 2. Average density and size of grain sorghum and grasses at the time of herbicide application in Fayetteville, AR in 2020 and 2021.

		2020		2021	
Common name	Scientific name	Density[a]	Size[b]	Density	Size
TamArk™ grain sorghum	*Sorghum bicolor* (L.) Moench	13	2-3	14	2-3
Conventional grain sorghum	*Sorghum bicolor* (L.) Moench	16	2-3	15	2-3
Johnsongrass	*Sorghum halapense* (L.) Pers	6	3-4	10	3-4
Barnyardgrass	*Echinochloa crus-galli* (L.) Beauv.	8	2-3	7	3-4
Broadleaf signalgrass	*Urochloa platyphylla* (Nash) R.D. Webster	20	4-6	15	4-5
Texas panicum	*Urochloa texana* (Buckl.) R. Webster	5	4-6	6	3-4

[a] Density recorded as plants per meter of row

[b] Size recorded as number of true leaves present

Table 3. Percent visible injury and biomass reduction of conventional grain sorghum by herbicide and rate in Fayetteville, AR in 2020 and 2021, averaged over year.

		Injury			Biomass reduction[a]
Herbicide	Rate	14 DAA[b]	21 DAA	28 DAA	28 DAA
	g ai ha^{-1}	----------	----------	% ----------	----------
Clethodim	135	100 A[c]	100 A	100 A	100 A
Clodinafop	70	100 A	100 A	100 A	100 A
Cyhalofop	312	100 A	100 A	100 A	100 A
Diclofop	1120	77 B	72 B	75 B	83 C
Fenoxaprop	86	96 A	98 A	100 A	100 A
Fenoxaprop	120	97 A	99 A	100 A	100 A
Fluazifop	210	98 A	97 A	99 A	99 B
Fluazifop	280	98 A	100 A	100 A	100 A
Fluazifop	420	100 A	100 A	100 A	100 A
Imazamox	52	100 A	100 A	100 A	100 A
Imazamox	78	100 A	100 A	100 A	100 A
Nicosulfuron	35	94 A	97 A	99 A	99 B
Nicosulfuron	51	100 A	100 A	100 A	100 A
Pinoxaden	60	19 C	51 C	67 C	81 C
Quizalofop	46	94 A	100 A	100 A	100 A
Quizalofop	77	100 A	100 A	100 A	100 A
Quizalofop	92	100 A	100 A	100 A	100 A
Sethoxydim	210	97 A	99 A	100 A	100 A
P-value		<0.0001	<0.0001	<0.0001	<0.0001

[a] Percent reduction is relative to the nontreated plot within each replication

[b] Abbreviation: DAA, days after application

[c] Means within in a column followed by the same letter are not significantly different based on Tukey's HSD (0.05)

Table 4. Percent visible injury and biomass reduction of TamArk™ grain sorghum by herbicide rate in Fayetteville, AR, in 2020 and 2021, averaged over year.

		Injury			Biomass reduction[a]
Herbicide	Rate	14 DAA[b]	21 DAA	28 DAA	28 DAA
	g ai ha^{-1}	----------	---------- % ----------	----------	----------
Clethodim	135	95 A[c]	100 A	100 A	100 A
Clodinafop	70	5 D	5 E	5 C	6 B
Cyhalofop	312	7 D	7 DE	7 BC	6 B
Diclofop	1120	6 D	5 E	5 C	0 B
Fenoxaprop	86	4 D	4 F	5 C	2 B
Fenoxaprop	120	5 D	6 DEF	6 BC	2 B
Fluazifop	210	4 D	5 EF	5 C	2 B
Fluazifop	280	5 D	5 EF	5 C	4 B
Fluazifop	420	5 D	7 DE	7 BC	5 B
Imazamox	52	55 C	92 C	100 A	100 A
Imazamox	78	56 C	92 C	100 A	100 A
Nicosulfuron	35	70 B	95 B	100 A	100 A
Nicosulfuron	51	90 A	100 A	100 A	100 A
Pinoxaden	60	8 D	6 DEF	6 BC	8 B
Quizalofop	46	4 D	4 F	6 BC	7 B
Quizalofop	77	4 D	6 DEF	7 BC	7 B
Quizalofop	92	7 D	8 D	10 B	10 B
Sethoxydim	210	73 B	100 A	100 A	100 A
P-value		<0.0001	<0.0001	<0.0001	<0.0001

[a] Percent reduction is relative to the nontreated plot within each replication

[b] Abbreviation: DAA, days after application

[c] Means within in a column followed by the same letter are not significantly different based on Tukey's HSD (0.05)

Table 5. Percent visible control and biomass reduction of johnsongrass by herbicide rate in Fayetteville, AR in 2020 and 2021, averaged over year.

		Control			Biomass reduction[a]
Herbicide	Rate	14 DAA[b]	21 DAA	28 DAA	28 DAA
	g ai ha^{-1}	----------------	---------------- %	----------------	----------------
Clethodim	135	100 A[c]	100 A	100 A	100 A
Clodinafop	70	95 A	96 B	98 AB	97 AB
Cyhalofop	312	92 A	96 B	97 AB	96 ABC
Diclofop	1120	32 D	37 D	38 C	45 D
Fenoxaprop	86	96 A	100 A	100 A	100 A
Fenoxaprop	120	98 A	100 A	100 A	100 A
Fluazifop	210	80 B	92 BC	100 A	100 A
Fluazifop	280	92 A	100 A	100 A	100 A
Fluazifop	420	96 A	100 A	100 A	100 A
Imazamox	52	86 A	94 B	97 AB	85 C
Imazamox	78	93 A	97 B	98 AB	89 BC
Nicosulfuron	35	87 A	92 BC	95 AB	93 ABC
Nicosulfuron	51	91 A	96 B	97 AB	93 ABC
Pinoxaden	60	59 C	87 C	92 B	89 BC
Quizalofop	46	83 AB	97 A	97 AB	98 A
Quizalofop	77	87 A	100 A	100 A	100 A
Quizalofop	92	96 A	100 A	100 A	100 A
Sethoxydim	210	98 A	100 A	100 A	100 A
P-value		<0.0001	<0.0001	<0.0001	<0.0001

[a] Percent reduction is relative to the nontreated plot within each replication

[b] Abbreviation: DAA, days after application

[c] Means within in a column followed by the same letter are not significantly different based on Tukey's HSD (0.05)

Table 6. Percent visible control and biomass reduction of broadleaf signalgrass by herbicide rate in Fayetteville, AR, in 2020 and 2021, averaged over year.

		Control			Biomass reduction[a]
Herbicide	Rate	14 DAA[b]	21 DAA	28 DAA	28 DAA
	g ai ha^{-1}	%			
Clethodim	135	95 AB[c]	100 A	100 A	100 A
Clodinafop	70	86 BC	95 AB	97 AB	98 A
Cyhalofop	312	86 BC	88 C	95 AB	92 AB
Diclofop	1120	27 G	27 F	27 E	45 D
Fenoxaprop	86	94 AB	98 AB	96 AB	98 A
Fenoxaprop	120	99 A	100 A	100 A	100 A
Fluazifop	210	89 B	91 BC	92 B	93 A
Fluazifop	280	94 AB	95 AB	95 AB	94 A
Fluazifop	420	94 AB	95 AB	97 AB	95 A
Imazamox	52	56 E	72 E	68 D	65 DC
Imazamox	78	70 D	80 D	81 C	83 ABC
Nicosulfuron	35	36 F	71 E	68 D	62 CD
Nicosulfuron	51	40 F	72 E	70 D	68 BCD
Pinoxaden	60	90 AB	96 AB	95 AB	95 A
Quizalofop	46	92 ABC	95 AB	96 AB	93 A
Quizalofop	77	96 AB	97 AB	97 AB	95 A
Quizalofop	92	97 A	100 AB	100 A	100 A
Sethoxydim	210	98 A	98 AB	98 A	98 A
P-value		<0.0001	<0.0001	<0.0001	<0.0001

[a] Percent reduction is relative to the nontreated plot within each replication

[b] Abbreviation: DAA, days after application

[c] Means within in a column followed by the same letter are not significantly different based on Tukey’s HSD (0.05)

Table 7. Percent visible control and biomass reduction of barnyardgrass by herbicide rate in Fayetteville, AR in 2020 and 2021, averaged over year.

Herbicide	Rate	Control			Biomass reduction[a]
		14 DAA[b]	21 DAA	28 DAA	28 DAA
	g ai ha^{-1}	---------------------------------------%	----------------------------------		
Clethodim	135	100 A[c]	100 A	100 A	100 A
Clodinafop	70	100 A	100 A	100 A	100 A
Cyhalofop	312	100 A	100 A	100 A	100 A
Diclofop	1120	91 B	91 B	91 B	92 B
Fenoxaprop	86	100 A	100 A	100 A	100 A
Fenoxaprop	120	100 A	100 A	100 A	100 A
Fluazifop	210	100 A	100 A	100 A	100 A
Fluazifop	280	100 A	100 A	100 A	100 A
Fluazifop	420	100 A	100 A	100 A	100 A
Imazamox	52	100 A	100 A	100 A	100 A
Imazamox	78	100 A	100 A	100 A	100 A
Nicosulfuron	35	100 A	100 A	100 A	100 A
Nicosulfuron	51	100 A	100 A	100 A	100 A
Pinoxaden	60	100 A	100 A	100 A	100 A
Quizalofop	46	100 A	100 A	100 A	100 A
Quizalofop	77	100 A	100 A	100 A	100 A
Quizalofop	92	100 A	100 A	100 A	100 A
Sethoxydim	210	100 A	100 A	100 A	100 A
P-value		<0.0001	<0.0001	<0.0001	<0.0001

[a] Percent reduction is relative to the nontreated plot within each replication

[b] Abbreviation: DAA, days after application

[c] Means within in a column followed by the same letter are not significantly different based on Tukey's HSD (0.05)

Table 8. Percent visible control and biomass reduction of Texas panicum by herbicide rate in Fayetteville, AR in 2020 and 2021, averaged over year.

		Control					Biomass reduction[a]
Herbicide	Rate	14 DAA[b]		21 DAA		28 DAA	28 DAA
	g ai ha^{-1}	---------------------------------------%---------------------------------------					
Clethodim	135	100	A	100	A	100	100
Clodinafop	70	100	A	100	A	100	100
Cyhalofop	312	100	A	100	A	100	100
Diclofop	1120	94	B	96	B	100	100
Fenoxaprop	86	100	A	100	A	100	100
Fenoxaprop	120	100	A	100	A	100	100
Fluazifop	210	100	A	100	A	100	100
Fluazifop	280	100	A	100	A	100	100
Fluazifop	420	100	A	100	A	100	100
Imazamox	52	100	A	100	A	100	100
Imazamox	78	100	A	100	A	100	100
Nicosulfuron	35	100	A	100	A	100	100
Nicosulfuron	51	100	A	100	A	100	100
Pinoxaden	60	100	A	100	A	100	100
Quizalofop	46	100	A	100	A	100	100
Quizalofop	77	100	A	100	A	100	100
Quizalofop	92	100	A	100	A	100	100
Sethoxydim	210	100	A	100	A	100	100
P-value		<0.0001		<0.0001		-	-

[a] Percent reduction is relative to the nontreated plot within each replication

[b] Abbreviation: DAA, days after application

[c] Means within in a column followed by the same letter are not significantly different based on Tukey's HSD (0.05)

Chapter 5

Influence of Fluazifop Timing and Rate on Johnsongrass Control in TamArk^{TM} Grain Sorghum

Abstract

Genetic similarities between johnsongrass and grain sorghum leave producers limited options for postemergence johnsongrass control. TamArk^{TM} grain sorghum with resistance to acetyl CoA carboxylase-inhibiting herbicides was developed through a collaboration between the University of Arkansas System Division of Agriculture and Texas A&M University. Two experiments were conducted at two locations. The objective of the first was to determine the optimal rate and application timing of fluazifop-butyl for control of natural johnsongrass populations in a non-crop setting and the second was to evaluate johnsongrass control and TamArk^{TM} grain sorghum tolerance in response to fluazifop-butyl applied at different timings and rates based on crop stage. The highest levels of johnsongrass control occurred when sequential applications of fluazifop-butyl were utilized. All sequential treatments provided at least 80% johnsongrass control at any rate or application timing tested. A single application of fluazifop-butyl provided greater than 90% johnsongrass control when applied at 210 g ai ha^{-1}. Weed size played a role in achieving high levels of johnsongrass control. Greater than 90% control was achieved when johnsongrass had 6-leaves or less at the initial application for the sequential application treatments. A single application of fluazifop-butyl at 105 g ai ha^{-1} resulted in no more than 82% johnsongrass mortality at any application timing. Grain sorghum injury did not exceed 6% at any application timing or rate. It was, therefore, safe if the initial application was made before the 6-leaf crop stage. Since no unacceptable levels of injury were

observed with TamArk™ grain sorghum, johnsongrass size at the time of application should be the most critical aspect for control with fluazifop-butyl.

Nomenclature: Fluazifop-butyl; johnsongrass, *Sorghum halapense* (L.) Pers.; grain sorghum, *Sorghum bicolor* (L.) Moench

Introduction

Johnsongrass was first utilized in the United States as a forage crop throughout the southeast in the 1800s. While the ability of johnsongrass to produce large quantities of biomass made it great for forage, it also made it detrimental as a weed (Mitch 1987). The inability to contain johnsongrass as a forage crop was first documented during the 1840s in the fertile river bottoms of Alabama (Miller 2014; Mitch 1987). Johnsongrass is a spreading perennial grass known to produce large quantities of biomass and spread rapidly through both rhizome and seed production (McWhorter 1971). Rhizome production is one of the main reasons johnsongrass is challenging to control. One johnsongrass plant can produce up to 5,000 rhizomes, potentially leading to new plants, making control of johnsongrass before rhizome production necessary (McWhorter 1971; Horowitz 1972). The adaptability of johnsongrass also makes it difficult to control. Johnsongrass can currently be found in every state in the United States and many foreign countries, even though the climate does not fit the warm, dry conditions that johnsongrass originated from (Burt 1974).

Since its introduction as a forage crop, johnsongrass control has been a significant issue for row crop producers across the Mid-south. Historically, johnsongrass control was achieved by soil incorporating dinitroaniline herbicides in-row cultivation, physical removal, and spot treatments with non-selective postemergence herbicides (McWhorter 1989). In the 1980s, control methods were improved by commercializing multiple postemergence herbicides targeting acetyl-CoA carboxylase (ACCase) and acetolactate synthase (ALS) (Bridges 1989; Camacho et al. 1991; Foy and Witt 1990; McWhorter 1989; Obrigawitch et al. 1990). While these herbicides successfully controlled johnsongrass in corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.], neither could be used in grain sorghum.

Until recently, grain sorghum producers relied on methodologies that are over 30 years old to control johnsongrass in grain sorghum (Brown et al. 1988; McWhorter and Hartwig 1965; Smith and Scott 2010). The two primary johnsongrass control methods were tillage and fall-applied glyphosate. Tillage for johnsongrass control in grain sorghum is one of the first control methods utilized by producers. Fall tillage brings rhizomes to the surface and exposes them to harsh winter weather, reducing germination the following year and resulting in 75 to 85% johnsongrass control the subsequent year (McWhorter and Hardwig 1965). In the 1970s, the introduction of glyphosate improved johnsongrass control for grain sorghum producers. While glyphosate could not be applied postemergence in grain sorghum, producers could utilize the non-selective herbicide prior to crop emergence. The addition of glyphosate as a fall burndown paired with a preplant burndown increased johnsongrass control in grain sorghum to greater than 90% (Brown et al. 1988). In more recent years, some producers still utilize a glyphosate burndown for johnsongrass control in grain sorghum (Smith and Scott 2010).

The introduction of a fluxofenim seed treatment known as Concep® allowed chloroacetamide herbicides such as *S*-metolachlor to be applied preemergence for grass control, significantly advancing weed control in grain sorghum. *S*-metolachlor provides greater than 90% control of seedling johnsongrass while causing less than 5% injury to grain sorghum hybrids treated with fluxofenim (Ghosheh and Chandler 1998; Wright et al. 1992).

While glyphosate and *S*-metolachlor have been successful for johnsongrass control in grain sorghum for many years, current herbicide resistance trends threaten the sustainability of these herbicides (Brabham et al. 2019; Johnson et al. 2014a; Meyer et al. 2015; Johnson et al. 2014b). Quinclorac and bromoxynil are postemergence herbicides labeled for postemergence grass control in grain sorghum, but neither provide effective johnsongrass control (Kering et al. 2013; Corbett et al. 2004). Paraquat is also labeled for in-season grass control in grain sorghum, but must be applied post-directed, under hoods to prevent significant crop injury. With the increasing number of herbicide-resistant weed populations and a lack of effective options for johnsongrass and other grasses, grain sorghum producers need new tools that aid weed control.

Herbicide-resistant lines of grain sorghum have been researched and commercialized, adding new options for grass control in grain sorghum (Pinkerton 2020). Specifically, the University of Arkansas Systems Division of Agriculture and Texas A&M University have worked collaboratively to develop a new line of grain sorghum, TamArk™, with known resistance to the ACCase inhibitor fluazifop. TamArk™ grain sorghum is also potentially resistant to other herbicides within the aryloxyphenoxypropionate (AOPP) family of ACCase inhibitors (Piveta et al. 2020). ACCase inhibitors have been utilized for over 30 years to successfully control grass weeds in crops such as cotton (*Gossypium hirsutum* L.) and soybean (Camacho et al. 1991; Meyer et al. 2015; Minton et al. 1989). Grain sorghum producers will benefit from the addition of the TamArk™ grain sorghum line, along with other new ACCase-resistant grain sorghum lines, by adding new effective modes of action to control problematic grasses (Norsworthy et al. 2012). Therefore, research was conducted to determine the application number, rate, and timings necessary to effectively control johnsongrass using fluazifop-butyl and to determine the effect of application timing and rate on johnsongrass control in TamArk™ grain sorghum.

Materials and Methods

Effect of fluazifop-butyl rate, timing, and application number on johnsongrass.

Experimental setup. A trial was conducted in 2021 at the Lon Mann Cotton Research Station in Marianna, AR, on a Convent silt loam (Coarse-silty, mixed, superactive, thermic Fluvaquentic Endoaquepts) consisting of 9% sand, 11% clay, 80% silt, with an organic matter content of 1.9%, and a pH of 6.3 and at the Northeast Research and Extension Center in Keiser, AR, on a Sharkey silty clay (Very fine, smectitic, thermic Chromic Epiaquerts) consisting of 31% sand, 26% silt, and 43% clay, with an organic matter content of 1.9% and a pH of 6.7. These fields contained a natural infestation of johnsongrass, comprised of both seedling and rhizome plants. These trials were conducted in the absence of a crop in plots 1.9-m wide by 4.8-m long. A single application of dicamba ay 560 g ae ha^{-1} and hand weeding were used to control broadleaf weeds in the test. The trial did not receive any fertilization since no crop was present but did receive furrow irrigation when 2.5 cm of rainfall did not occur for a period of 7 days.

The experimental was setup as a three-factor, randomized complete block design with 13 treatments, including a nontreated control. Each treatment was replicated four times. Factors included three johnsongrass sizes at the time of application (2- to 3-leaf, 5- to 6-leaf, and 8- to 9-leaf or heading), two fluazifop-butyl rates (105 and 210 g ai ha^{-1}), and single and sequential applications. Plots receiving sequential applications were treated with the same rate with 21 days between applications. Herbicide were applied using a CO_2-pressurized backpack sprayer and a 4-nozzle boom calibrated to deliver 140 L ha^{-1} at 6.4 kph. Air induction extended range (AIXR) 110015 nozzles (TeeJet, Springfield, IL) were used for all applications. Boom height was 46-cm above the johnsongrass canopy.

Visible johnsongrass control was evaluated weekly after the initial herbicide application and continued for four weeks after the final application. Evaluations were made on a scale of 0 to 100, where 0 represented no johnsongrass control and 100 represented complete johnsongrass control (Frans and Talbert 1986). Two 0.5-m^2 quadrants were established in each plot, and initial johnsongrass densities were recorded. Twenty-eight days after final application (DAFA), the total number of live johnsongrass plants in each quadrant was recorded, and percent mortality was calculated using the equation:

$$\frac{Initial\ johnsongrass - Final\ johsongrass}{Initial\ Johsongrass} \times 100$$

Data analysis. Data were analyzed using JMP 16.1 Pro (SAS Institute Inc., Cary, NC). A general regression with factorial to degree was utilized to determine the level of significance, with fixed factors being rate, timing, and application for 21 and 28 DAFA and percent mortality. A factorial to degree was used to allow two-way interactions to be evaluated and to determine if the initial count as a covariate as a was significant. A covariate of initial count with the variable of percent mortality was not significant (P = 0.79) and therefore was not included within the analysis. Block was considered random to account for variance amongst replications. Visible control and percent mortality were assumed to follow a beta distribution (Gbur et al. 2012). A three-factor factorial was constructed with the main effects of rate, timing, and application with their respective interactions in the PROC GLIMMIX model in SAS 9.4 (SAS Institute Inc., Cary, NC). Location and block were considered random effects. Means were separated using Fisher's protected LSD at α = 0.05 when four or less treatments were compared. When comparing treatments resulting from a three-way interaction, a Tukey's HSD was used to separate means at α = 0.05.

Johnsongrass control in TamArk™ grain sorghum using fluazifop-p-butyl.

Experimental setup. Field trials were also conducted in 2021 at the Lon Mann Cotton Research Station in Marianna, AR, on a Convent silt loam (Coarse-silty, mixed, superactive, thermic Fluvaquentic Endoaquepts) consisting of 9% sand, 11% clay, 80% silt, with an organic matter content of 1.9%, and a pH of 6.3 and the Arkansas Agricultural Research and Extension Center in Fayetteville, AR, on a Leaf silt loam (fine, mixed, active, thermic Typic Albaquults) with 19.6% sand, 57.8% silt, 22.6% clay, and a pH of 6.2. Both locations consisted of a mixture of natural seedling and rhizomatous johnsongrass populations.

TamArk™ grain sorghum was planted at both locations using a conventional John Deere planter with Almaco cone attachments, 1.2-cm deep in conventionally tilled, and raised beds at 154,000 seed ha^{-1}. Plots were 4.8-m long by 3.8-m wide with row spacing of 91-cm in Fayetteville and 4.8-m long by 3.9-m wide with a row spacing of 96-cm in Marianna. A single application of dicamba ay 560 g ae ha^{-1} and hand weeding were used to control broadleaf weeds in the test. In addition, the trial received split nitrogen applications, one incorporated before planting and a second at the boot stage. In-furrow irrigation was provided on an as-need basis. All other management practices, including fertilizer rates, followed the Arkansas grain sorghum production handbook (Espinoza 2015).

The experimental design was a two-factor, randomized complete block design with 13 treatments, including a nontreated and a weed-free check for comparison, each replicated 4 times. The factors consisted of TamArk™ grain sorghum size at time of application (2- to 3- leaf or 5- to 6- leaf) and fluazifop-butyl rate (140 g ai ha^{-1}, 210 g ai ha^{-1}, and 140 g ai ha^{-1} followed by (fb) 140 g ai ha^{-1} 21 days later). Fluazifop-butyl was applied using CO_2-pressurized backpack sprayers and a 4-nozzle boom calibrated to deliver 140 L ha^{-1} at 6.4 kph. Air induction extended range (AIXR) 110015 nozzles (TeeJet, Springfield, IL) were used for all applications. Boom height was 46-cm above the largest plant in the canopy. Each application was blocked on either side, and only the center two rows of each plot were treated to eliminate overlap and create a running check throughout the trial.

Two 0.5-m^2 quadrants were established in each plot. The number of johnsongrass plants in each was recorded before initial application. At 28 DAFA, the total number of alive johnsongrass plants was counted and used to calculate percent mortality. In addition, the total number of johnsongrass panicles per quadrant was recorded, and panicles were removed before harvest. The seed was then harvested and counted to determine the treatment's percentage of seed reduction. Visible crop injury was assessed weekly until 28 DAFA on a scale of 0 to 100, where 0 represented no visible crop injury and 100 represented complete crop death. Visible johnsongrass control was also evaluated on a scale of 0 to 100, where 0 represented no visible johnsongrass control and 100 was equal to no live johnsongrass present (Frans and Talbert 1986). The date of 50% heading was recorded by plot and made relative to the nontreated within the replication. Yield data could not be collected due to significant yield loss caused by bird injury after seed development.

Data analysis. Since nontreated plots were rated as 0 on visible injury and control, data were made relative, and nontreated plots were excluded from the analysis. Visible johnsongrass control, percent mortality, and percent johnsongrass seed reduction were assumed to follow a beta distribution, and grain sorghum injury was assumed to follow a gamma distribution by assessing the AICc values in the distribution function of JMP Pro 16.1 (SAS Institute Inc., Cary, NC) (Gbur et al. 2012). The relative heading date was assumed to follow a normal distribution. A two-factor factorial statement was developed with the main effects of application rate and timing, including interactions using the PROC GLIMMIX model in SAS 9.4 (SAS Institute Inc., Cary, NC). Block and location were considered random effects. Visible crop injury, johnsongrass control, percent mortality, percent seed reduction, and relative heading were subjected to means separations using Fisher's protected LSD ($\alpha = 0.05$).

Results and Discussion

Effect of fluazifop-butyl rate, timing, and application number on johnsongrass.

Control. Overall, no interactions were observed among rate, timing, and application number when visible johnsongrass control was evaluated at 14, 21, and 28 DAFA (Table 1). Johnsongrass control increased 5 to 7 percentage points when fluazifop-butyl was applied at 210 g ai ha^{-1} compared to 105 g ai ha^{-1}, resulting in at least 94% control at each rating averaged over timing and application number (Table 2). Even with an increase in control at the higher rate, it is important to recognize that at 21 and 28 DAFA fluazifop-butyl at 105 g ai ha^{-1} resulted in greater than 90% johnsongrass control (Table 2). These findings are like those of Rosales-Robles et al. (1999), where approximately 90% johnsongrass control was achieved with fluazifop-butyl at 105 g ai ha^{-1}. For >95% johnsongrass control, a rate of 210 g ai ha^{-1} was needed (Table 2).

Johnsongrass control differed based on the growth stage at the initial application. Johnsongrass control was lower when the initial application was made to plants at the 8- to 9-leaf stage than the 5- to 6-leaf stage, with a 9-percentage point difference in control between the smallest and largest plants at 28 DAFA (Table 3). Initial applications to 2- to 3-leaf johnsongrass resulted in control levels similar those of 5- to 6-leaf plants at all evaluation timings with greater than 90% control achieved. Likewise, Rosales-Robles et al. (1999) observed that fluazifop-butyl applications to johnsongrass at the 5- to 7-leaf stage resulted in greater than 90% control.

Sequential applications of fluazifop-butyl, regardless of fluazifop-butyl rate and johnsongrass size at the initial application, resulted in increased control compared to a single application at all three evaluations. Sequential applications resulted in a 5, 4, and 10-percentage point increase in johnsongrass control at 14, 21, and 28 DAA, respectively (Table 4). Winton-Daniels (1990) reported that sequential applications of fluazifop-butyl at 140 g ai ha^{-1} resulted in greater than 85% johnsongrass control over a three-year period, which was higher than a single application of 280 g ai ha^{-1}.

Mortality. A significant three-way interaction of fluazifop-butyl rate by application number by johnsongrass size at initial application was observed for johnsongrass mortality 28 DAFA (P = 0.029). Three treatment combinations resulted in 99% johnsongrass mortality, with those being fluazifop-butyl at 105 g ai ha^{-1} applied sequentially beginning on 5- to 6-leaf johnsongrass and fluazifop-butyl at 210 g ai ha^{-1} applied sequentially beginning on 2- to 3-leaf or 5- to 6-leaf johnsongrass. Single applications did provide greater than 95% johnsongrass mortality, but fluazifop-butyl at 210 g ai ha^{-1} applied once to 2- to 3-leaf or 5- to 6-leaf johnsongrass was not different from the three sequential treatments that reached 99% mortality (Table 5). The lowest levels of johnsongrass mortality resulted when a single application of fluazifop-butyl at 105 or 210 g ai ha^{-1} was made to 8- to 9-leaf johnsongrass, which did not result in greater than

66% mortality. Likewise, Bridges and Chandler (1987) observed reductions in fluazifop-butyl efficacy when applied to johnsongrass greater than 6-leaf. Bridges and Chandler also evaluated sequential applications of fluazifop-butyl at 140 g ai ha^{-1} and reported 93 to 95% johnsongrass control when applications were made to plants having fewer than 6 leaves.

Johnsongrass control programs in TamArk™ grain sorghum.

Control. No significant interactions between rate and application timing across all evaluation timings were observed. The main effect of timing was significant across all visible johnsongrass control timings but was not significant for johnsongrass mortality (P = 0.1922). Fluazifop-butyl rate was significant across all visible johnsongrass control evaluations and johnsongrass mortality (Table 6).

The application timings of 2- to 3-leaf and 5- to 6-leaf TamArk™ grain sorghum resulted in johnsongrass control and mortality greater than 90% when averaged across rate and location. A 5 to 7-percentage point increase in johnsongrass mortality occurred when fluazifop-butyl applications were made at the 2- to 3-leaf stage of grain sorghum compared to applications made at the 5- to 6-leaf stage (Table 7). Johnsongrass size at the time of application led to increased control at the earlier application timing since application timings were based on grain sorghum stage. At the 2- to 3-leaf applications, johnsongrass plants within the treated plots ranged from 5 to 20 cm and had 2 to 5 leaves. Conversely, at the 5- to 6-leaf stage of grain sorghum, johnsongrass within the treated plots ranged from 10 to 70 cm with 4 to 9 leaves, which is above the size recommended for effective control (Anonymous 2019).

The main effect of fluazifop-butyl rate was significant across all control ratings as well as mortality. A similar trend was seen in the non-crop study, where sequential applications of a lower fluazifop-butyl rate provided similar control levels as using a single application of a higher rate. For the in-crop study, fluazifop-butyl 210 g ai ha^{-1} provided control levels not different from sequential applications of 140 g ai ha^{-1} fb 140 g ai ha^{-1}, except for the 21 DAFA evaluation. Furthermore, both rates controlled johnsongrass greater than 90% across all evaluation timings and mortality (Table 8). Single applications of fluazifop-butyl at 140 g ai ha^{-1} resulted in lower johnsongrass control and mortality percentages than 210 g ai ha^{-1} and 140 g ai ha^{-1} fb 140 g ai ha^{-1} across all evaluation timings and did not result in greater than 84% johnsongrass mortality, averaged over timing and location.

When evaluating percent seed reduction, no significance was observed with rate or application timing. Seed production per was reduced 99% or greater when fluazifop was applied, regardless the application timing or rate (Tables 7 and 8).

TamArk™ grain sorghum injury. Low levels of injury, no more than 6%, were observed with applications of fluazifop-butyl to TamArk™ grain sorghum (Tables 9). TamArk™ grain sorghum injury was higher when fluazifop-butyl was applied to 5- to 6-leaf compared to 2- to 3-leaf grain sorghum, resulting in 6% and 4% injury, respectively (Table 9). No differences in TamArk™ grain sorghum injury were observed when analyzed by rate and application timing (Table 6).

TamArk™ grain sorghum consistently reached the heading stage earlier when treated with fluazifop-butyl compared to nontreated plots. However, the relative heading date was not significantly affected by stage at application or application rate. It is unclear why the grain sorghum headed earlier following fluazifop-butyl applications.

Practical Implications. Fluazifop-butyl applications to johnsongrass greater than 6-leaf did not result in control greater than 90% regardless of the rate or number of applications. The highest level of johnsongrass control with fluazifop-butyl was achieved when johnsongrass ranged from the 2- and 6-leaf stage with either a single or sequential applications. If a single application is utilized, the fluazifop-butyl rate must be 210 g ai ha^{-1}. An application of 105 g ai ha^{-1} will result in sufficient johnsongrass control if followed by another application of 105 g ai ha^{-1} approximately 3 weeks later. Regardless of fluazifop-butyl rate or timing, johnsongrass seed production was nearly eliminated. No data were collected on rhizome production. While the number of seeds entering the soil seed bank will be reduced, johnsongrass plants still have the potential to reproduce if rhizome production is not controlled.

No more than 6% injury to TamArk™ grain sorghum was observed at both application timings. Fluazifop-butyl applications before the 6-leaf stage resulted in acceptable injury, making the size of johnsongrass the most critical aspect for application timing. It is important to note that herbicide resistance to ACCase inhibitors is present in some grain sorghum-producing states and could become more problematic if grain TamArk™ sorghum is not correctly managed. Therefore, fluazifop-butyl should not be relied upon solely for johnsongrass control in grain sorghum but instead used in a program approach with residual herbicides such as chloroacetamides or atrazine as well as other biological, cultural, and mechanical control options to develop an integrated weed management strategy. Utilization of multiple strategies and not solely relying on one will help mitigate future johnsongrass resistance to fluazifop-butyl.

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Tables

Table 1. Analysis of variance for johnsongrass response in Marianna and Keiser, AR in 2021.

Independent variables	P-value			
	Control			Mortality
	14 DAFA[a]	21 DAFA	28 DAFA	
Fluazifop-butyl	<0.0001	<0.0001	0.0002	0.0011
Application timing	0.0308	0.0215	<0.0001	<0.0001
Application number	0.0027	0.0016	<0.0001	<0.0001
Fluazifop-butyl X Application timing	0.3958	0.1607	0.7526	0.0796
Fluazifop-butyl X Application number	0.4286	0.2323	0.8121	0.8674
Application timing X Application number	0.3469	0.4003	0.4540	0.0679
Fluazifop-butyl X Application timing X Application number	0.4084	0.3452	0.0840	0.0295

Table 2. Visible johnsongrass control by fluazifop-butyl rate at 14, 21, and 28 days after final application (DAFA), averaged over application stage, application number, and location.[a]

	Control		
Fluazifop-butyl	14 DAFA	21 DAFA	28 DAFA
g ai ha^{-1}	---------------------------------------%---------------------------------------		
105	87 B	90 B	92 B
210	94 A	96 A	97 A

[a] Means within a column followed by the same letter are not significantly different based on Fisher's protected LSD (0.05)

Table 3. Visible estimates of johnsongrass control by johnsongrass stage at application at 14, 21, and 28 days after final application (DAFA), averaged over application rate, type, and location.[a]

Stage at application	Control		
	14 DAFA	21 DAFA	28 DAFA
	--	%	--
2- to 3-Leaf	90 B	92 B	95 A
5- to 6-Leaf	93 A	96 A	97 A
8- to 9-Leaf	89 B	90 B	88 B

[a] Means within a column followed by the same letter are not significantly different based on Fisher's protected LSD (0.05)

Table 4. Visible estimates of johnsongrass control by application number at 14, 21, and 28 days after final application (DAFA), averaged over application rate, stage, and location.[a]

Application number	Control		
	14 DAFA	21 DAFA	28 DAFA
	--	%	--
Single	88 B	91 B	88 B
Sequential [b]	93 A	95 A	98 A

[a] Means within a column followed by the same letter are not significantly different based on Fisher's protected LSD (0.05)

[b] Sequential applications were made 21 days after the initial application

Table 5. Percent mortality of johnsongrass by application rate, type, and timing of fluazifop averaged over location 28 days after the final application.[a]

Fluazifop-butyl	Application number	Stage at application	Mortality	
g ai ha^{-1}			%	
105	Single	2- to 3-Leaf	87	B
		5- to 6-Leaf	70	C
		8- to 9-Leaf	58	D
	Sequential[b]	2- to 3-Leaf	91	AB
		5- to 6-Leaf	99	A
		8- to 9-Leaf	83	BC
210	Single	2- to 3-Leaf	90	AB
		5- to 6-Leaf	95	AB
		8- to 9-Leaf	66	D
	Sequential	2- to 3-Leaf	99	A
		5- to 6-Leaf	99	A
		8- to 9-Leaf	87	B

[a] Means within a column followed by the same letter are not significantly different based on Tukey's HSD (0.05)

[b] Sequential applications were made 21 days after the initial application

Table 6. Analysis of variance for TamArk™ grain sorghum injury and johnsongrass control, mortality, and seed reduction in Fayetteville and Marianna, AR in 2021.

	P-value							
	Crop injury			Control				
Independent variables	14 DAFA	21 DAFA	28 DAFA	14 DAFA	21 DAFA	28 DAFA	Mortality	Seed reduction
Fluazifop-butyl	0.3490	0.7070	0.2639	0.0125	0.0071	0.0093	0.0087	0.9452
Application stage	0.0467	0.9705	0.2180	0.0342	0.0169	0.0592	0.1922	0.9776
Fluazifop-butyl X Application stage	0.9005	0.9237	0.7315	0.0957	0.1094	0.2679	0.0862	0.9857

Table 7. Visible estimates of johnsongrass control from fluazifop-butyl initially applied to 2- to 3-leaf and 5- to 6-leaf TamArk™ grain sorghum and rated 14, 21, and 28 days after final application (DAFA) and johnsongrass mortality and seed production, averaged over application rate and location.[a]

	Control				
Application stage	14 DAFA	21 DAFA	28DAFA	Mortality	Seed reduction[b]
	----------------	----------------	%	----------------	----------------
2- to 3-leaf	97 A	98 A	98 A	94	99
5- to 6-leaf	90 B	92 B	93 B	90	99

[a] Means within a column followed by the same letter are not significantly different based on Fisher's protected LSD (0.05)

[b] Seed reduction is calculated relative to the nontreated

Table 8. Visible estimates of johnsongrass control by fluazifop rate at 14, 21, and 28 days after final application (DAFA) and johnsongrass mortality averaged over application timing and location.[a]

	Control				
Fluazifop-butyl	14 DAFA	21 DAFA	28DAFA	Mortality	Seed reduction[c]
(g ai ha^{-1})	%				
140	84 B	91 C	92 B	84 B	99
210	92 A	95 B	95 A	92 A	99
140 fb 140[b]	96 A	98 A	98 A	96 A	99

[a] Means within in a column followed by the same uppercase letter are not significantly different based on Fisher's protected LSD (0.05)

[b] Followed by (fb) initial application followed by a second application 21 days later

[c] Seed reduction is calculated relative to the nontreated

Table 9. Injury to TamArk[TM] grain sorghum based on stage at initial application at 14, 21, and 28 days after final application (DAFA) and relative heading, averaged over application rate and location.[a]

Stage at application	Injury 14 DAFA	Injury 21 DAFA	Injury 28DAFA	Relative heading[b]
	---------------------------%	------------------------		d
2- to 3-Leaf	4 B	4	4	-2
5- to 6-Leaf	6 A	4	4	-2

[a] Means within a column followed by the same letter are not significantly different based on Fisher's protected LSD (0.05)

[b] Negative numbers represent days before the nontreated, and positive numbers represent days after the nontreated.

General Conclusions

Control of grass weeds, specifically johnsongrass, is an ongoing challenge for grain sorghum producers across the US. The continued evolution of resistance to commonly used herbicides such as glyphosate applied prior to planting makes control difficult. Furthermore, current resistance to acetolactate synthase-inhibiting herbicides in johnsongrass populations may result in failure of this herbicide site of action in fields as new grain sorghum traits are adopted. New acetyl CoA carboxylase (ACCase)-resistant grain sorghum traits may give producers more effective options for grass control based on few documented johnsongrass populations being found resistant to this class of herbicides.

TamArk ™ grain sorghum, with resistance to some ACCase-inhibiting herbicides, could provide producers the option to utilize one specific ACCase-inhibiting herbicide, but could allow producers to choose from multiple herbicides from the aryloxyphenoxypropionate or phenylpyrzolin families, if labeled. TamArk™ grain sorghum showed low sensitivity to these later two families of herbicides, often with <10% injury. While high levels of sensitivity were observed with herbicides from the cyclohexanedione family (>80% injury), these herbicides provide producers an option for control of volunteer plants or johnsongrass that could become resistant.

When ACCase inhibitors safe on TamArk™ grain sorghum were evaluated for efficacy on common grass weeds, many resulted in high levels of control of johnsongrass, broadleaf signalgrass, Texas panicum, and barnyardgrass. Fluazifop-p-butyl, quizalofop-p-ethyl, and fenoxaprop-p-ethyl, all safe for application on TamArk™, resulted in >90% control of these grasses.

Johnsongrass control can be achieved using fluazifop-p-butyl in multiple combinations including single applications of 210 g ai ha^{-1} or sequential applications of 105 g ai ha^{-1} 21 days apart. Either of these treatments resulted in >90% control of naturally occurring johnsongrass accessions while resulting in no more than 6% injury to TamArk^{TM} grain sorghum. However, applications must be timely. The highest level of johnsongrass control occurred when applications of fluazifop-p-butyl were made to johnsongrass at the 5- to 6-leaf stage which resulted in >92% control.