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Short title: Southcentral US Johnsongrass

Sensitivity of Southcentral U.S. Johnsongrass Accessions to Selected Herbicides

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Abstract

New technologies in grain sorghum allow for the use of multiple acetyl CoA carboxylase- (ACCase) or acetolactate synthase- (ALS) inhibiting herbicides 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 ones with potential to become available for use in grain sorghum, johnsongrass seeds were collected from 2017 to 2021 in Arkansas, Kansas, Texas, and Oklahoma and were screened for sensitivity to fluazifop, quizalofop, nicosulfuron, and imazamox. Additionally, glyphosate sensitivity was evaluated because of its use before planting or postharvest. Quizalofop resulted in 100% mortality of all johnsongrass accessions. Of the johnsongrass accessions evaluated, 89% were completely controlled with glyphosate. The ALS inhibitors nicosulfuron and imazamox resulted in 100% mortality of all Oklahoma accessions, but failures occurred on samples from other states. One accession from Kansas, 12 from Texas, and eight from Arkansas were found to have reduced sensitivity to nicosulfuron and imazamox. If producers plan to plant grain sorghum in areas with johnsongrass populations, an ACCaseinhibitor herbicide will most likely 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.

Nomenclature: 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 % yield loss in crops such as cotton (*Gossypium hirsutum* L.), soybean [*Glycine max* (L.) Merr*.*], corn (*Zea mays* L.), and grain sorghum (*Sorghum bicolor* (L.) Moench) (Klein and Smith 2020). While the introduction of glyphosate in the 1970s and glyphosate-resistant crops in the 1990s significantly decreased johnsongrass infestations in cotton, corn, and soybean. The genetic similarity between grain sorghum and johnsongrass, which are members of the genus *Sorghum*, 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 and was brought to the southern United States (US) in the 1800s as a forage crop (Mitch 1987). Johnsongrass has the ability to grow greater than two meters tall and create large quantities of biomass, which was optimal for forage producers but made it detrimental as a weed. Johnsongrass quickly escaped managed cultivation, spreading via seeds and rhizomes. Rhizomes are horizontally growing underground stems from which new plants can develop, and one single johnsongrass plant can produce up to 5,000 rhizomes in one growing season (McWhorter 1971). Rhizomes are often responsible for escapes or herbicide control failures. Herbicides that control aboveground growth must also be able to translocate and control rhizomes below ground, the lack of which can lead to regrowth through the production of new rhizomatous shoots. Therefore, producers must successfully control johnsongrass before rhizome development at the 5-leaf stage (Horowitz 1972).

New herbicide resistance technologies are being researched to help grain sorghum producers better control johnsongrass, allowing producers to utilize either ACCase or ALS inhibitors for grass control in grain sorghum (Pinkerton 2020). Currently, an ACCase inhibitorresistant technology [developed by S&W seed company (Longmont, CO)] is commercially available in grain sorghum, known as Double Team[™], with resistance to quizalofop herbicide (FirstAct™, ADAMA Ltd., Raleigh, NC). The University of Arkansas System Division of Agriculture and Texas A&M AgriLife Research jointly developed TamArk™ grain sorghum from a known johnsongrass population with resistance to quizalofop and fluazifop, with a mutation different from that in Double Team™. The two ALS inhibitor technologies in grain sorghum include a genetic line developed by Corteva (Indianapolis, IN) known as Inzen[™] with resistance to nicosulfuron and a line developed by Alta seeds (Amarillo, TX) and UPL (King of Prussia, PA) known as igrowth[™] with resistance to imazamox (Pinkerton 2020). While lines resistant to glyphosate are unavailable, the herbicide is important for johnsongrass control across the US in fallow areas, before 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 control in broadleaf crops such as cotton and soybean. 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 % (Byrd and York 1987; Clegg 1987). Quizalofop also effectively controlled similar grasses in broadleaf crops (Brewster and Spinney 1989; Sanders et al. 2021). Recently, quizalofop has been utilized for grass control in wheat (*Triticum aestivum* L.) through CoAXium[™] wheat production system developed by Colorado Wheat Research Foundation and Albaugh (St. Joseph, MO), in rice (*Oryza sativa* L.) through the Provisia[™] technology developed by BASF (Beaumont, TX), and Max-Ace[™] technology from Rice Tec (Alvin, TX) (Kumar et al. 2020; Lancaster et al. 2018; Sanders et al. 2021; Tarundeep et al. 2019).

Nicosulfuron and imazamox can control grasses in both broadleaf and grass crops (Dobbels and Kapusta 1993; Geier et al. 2004; Gubbiga et al. 1995; Nelson et al. 1998). Nicosulfuron was used primarily for grass and broadleaf control in corn before the introduction of glyphosate-resistant crops in the mid to late 1990s. Nicosulfuron can control johnsongrass by greater than 90 % in production situations and is also desirable to producers because of the low herbicide use rate (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 and postemergence (POST) applications primarily in wheat, corn, and rice but also other broadleaf and grass crops (Bond and Walker 2011; Jimenez et al. 2015; Larson et al. 2000). Although imazamox was not previously used specifically for johnsongrass control, it has successfully controlled 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 (Kumar et al. 2023; 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 eight resistant accessions were found out of 59 johnsongrass accessions evaluated, three being resistant to nicosulfuron and five 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 before 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

The herbicide resistance evaluations presented in this study were conducted in two locations: the University of Arkansas, Fayetteville, AR (*hereafter* AR), and Texas A&M University, College Station, TX (*hereafter* TX). The resistance screenings were conducted under greenhouse conditions using standard protocols in both locations.

Resistance screenings at AR

The greenhouse study was conducted twice at the Arkansas Agricultural Research and Extension Center in Fayetteville, AR. This completely randomized design evaluated five herbicides (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; the coordinates were not recorded for samples collected in Kansas. 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 some johnsongrass accessions' low seed germination percentage, not all herbicides were evaluated on all accessions collected. The initial number of plants per tray was recorded before herbicide application. Then, at 28 days after application (DAA), the final number of living plants was recorded per tray, and the survival percentage was calculated.

Resistance screenings at TX

The greenhouse experiment was conducted twice at the Texas A&M University Norman Borlaug greenhouse complex at College Station, TX, during February-May 2022 and January-March 2024. The experiment was arranged in a completely randomized design. A total of 34 johnsongrass accessions collected from across Texas from 2017 to 2022 were evaluated for four herbicides (quizalofop, nicosulfuron, imazamox, and glyphosate) (Table 1; Figure 1). For each accession, a minimum of 15 mature johnsongrass panicles were randomly collected, and the geocoordinates of the sampling sites were recorded using a handheld GPS. The samples were dried, threshed, and placed in cold storage (4 C). Before being used in this study, johnsongrass samples were transferred to room temperature for a week and were sandpaper scarified to break dormancy. Johnsongrass seeds from each accession were planted in individual plastic trays (50 cm x 25 cm) filled with a potting soil mix (Pro-line C/20, Jolly Gardener). These trays were placed in a greenhouse maintained at 28 C/22 C (day/night) temperature regime with a 14-hour photoperiod and watered as needed. The seedlings were transplanted into 6-six cell trays (one plant/cell) filled with the potting soil mix at the single-leaf stage. Herbicide applications were made to 2- to 3-leaf stage johnsongrass, using a track-sprayer (DeVries, Hollandale, MN) fitted with a flat fan nozzle (TeeJet XR110015) that was calibrated to deliver a spray volume of 140 L ha⁻¹ at 276 kPa pressure, and an operating speed of 4.8 kph (Table 2). Like the AR screening, not all herbicides were evaluated on all accessions due to limited seed availability and germination issues. The number of seedlings treated per accession for each herbicide ranged from 6 to 63. The experiment was repeated twice. At 28 DAA, plant survival (0 or 1) and % injury (0 to 100%) were recorded. Priority was given to resistance screening for imazamox and quizalofop, given the current market availability of the igrowth[®] and Double Team[®] sorghum cultivars, with resistance to imazamox and quizalofop, respectively.

Results and Discussion

Fluazifop. Out of the 117 johnsongrass accessions collected and screened, 113 were evaluated for sensitivity to fluazifop. The mean mortality of the 113 johnsongrass accessions screened to fluazifop was 2% (Table 3). Only four accessions evaluated had less than complete mortality (all from AR), with two, AR5 and AR7, showing 4% survival and the other two accessions, AR8 and AR9, at 80 and 94% survival, respectively (Table 4). This indicates fluazifop resistance in AR8 and AR9, especially considering the surviving plants showed no more than 5% injury from fluazifop. AR8 and AR9 are putative-resistant accessions but require dose-response evaluations to determine the resistance level. While johnsongrass resistant to fluazifop has been found previously in Arkansas, likely due to its use in broadleaf crops such as cotton and soybean, it has not been widespread (Johnson et al. 2014; Norsworthy et al. 2007; Schwartz-Lazaro 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 expansion of resistance in the future.

Quizalofop. A total of 104 johnsongrass accessions were evaluated for sensitivity to quizalofop (Table 3); other accessions were not tested due to limited seed supply or lack of germination. The quizalofop application resulted in 100% mortality of all accessions evaluated in AR and TX (Table 3). Quizalofop-resistant johnsongrass has never been reported (Heap 2023). Interestingly, 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 resistant to fluazifop but not to other ACCase-inhibiting herbicides. Hence, quizalofop would be a highly effective option for johnsongrass control in grain sorghum technologies such as $\text{TamArk}^{\text{TM}}$ or Double Team^{TM} , which will allow POST 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 resistance has not been widespread (Bagavathiannan and Norsworthy 2014; Werle et

al. 2016; Heap 2023). Nicosulfuron at 47 g ha⁻¹ resulted in the complete mortality of 80% of the johnsongrass accessions evaluated in AR, whereas complete mortality was only achieved for 15% of the samples screened in TX at a nicosulfuron rate of 36 g ha⁻¹. Of the samples evaluated in AR with survivors, none of the surviving plants exhibited more than 40% injury from the herbicide (Table 4). Johnsongrass accessions having survivors were found in Arkansas, Texas, and Kansas, all states with previously documented nicosulfuron-resistant johnsongrass (Table 4). There were 16 accessions screened in TX, with plant survival to nicosulfuron ranging from 0 to 100%, and injury to the surviving plants ranging from 20 to 80%. These accessions also exhibited reduced sensitivity to imazamox like those in the AR screening (Table 4). Johnsongrass accessions surviving nicosulfuron, especially with minimal injury, are worrisome with the new Inzen™ sorghum technology being released that allows producers to use nicosulfuron for POST control of the weed 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 incorporating different effective herbicide sites of action in the following crop to control any potential johnsongrass escapes.

Imazamox. Of the 69 johnsongrass accessions evaluated in AR for sensitivity to imazamox, 58 had no survival following treatment. The other 11 accessions had survival ranging from 2 to 40% (Table 4). Accessions with <80% mortality were found in Arkansas, Texas, and Kansas (Table 4). Of the 34 johnsongrass accessions screened at TX, 14 were completely controlled with imazamox, and survival of the remaining accessions ranged from 9 to 96%, with injury on the survivors ranging from 5 to 78% (Table 4). One notable observation was that the accession TX56 had only 1% survival following imazamox, yet the injury was negligible in the survivors (Table 4), indicating a high level of resistance at a low frequency within the population. Another notable observation was that six accessions from AR 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 (Heap 2023; Merotto et al. 2009; Riar et al. 2015). 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 the mortality of all johnsongrass plants in 57 of the 64 accessions evaluated at AR. Of the seven accessions with plants surviving glyphosate, survival percentages ranged from 6 to 86% (Table 4). Injury to plants surviving glyphosate was 40 - 60% in accessions AR3, AR5, and AR40, which would indicate that there is a high likelihood that these plants are resistant to the herbicide. All 15 johnsongrass accessions screened in TX were susceptible to glyphosate (Table 3). The number of glyphosate-resistant johnsongrass accessions has been increasing since the mid-2000s due to the frequent use of the herbicide in crops like corn, cotton, and soybean, where weeds such as johnsongrass were prevalent (Heap 2023). Although glyphosate is not available for POST in-crop use in grain sorghum, many producers use it for fall and spring burndown of johnsongrass and as an effective POST option in the following crop (Smith and Scott 2010). While glyphosate is still 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 prominent each growing season as the reliance on the same herbicides continues due to the lack of options for successful Johnsongrass control. Based on this screening, quizalofop is the best option for producers to use for POST johnsongrass control in Double Team™ grain sorghum or the soonto-be-registered TamArk™ grain sorghum. However, other effective control options should be utilized with quizalofop to ensure maximum control and reduce the risk of 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 sites of action; albeit johnsongrass is not listed as a controlled weed on the FirstAct label. Lower levels of control would be expected under dryland conditions compared to the greenhouse since growing conditions are optimal in the greenhouse. Overall, producers must know which herbicides are effective in specific fields and develop programs incorporating integrated weed management strategies to mitigate further resistance.

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

^a Crop present or last crop grown before seed collection.

NA indicates that data is not available.

	Common			
Trade name	name	Rate	Manufacturer	Location
		ai g		
		ha^{-1}		
Screening at AR				
Fusilade DX	Fluazifop	105	Syngenta Crop Protection, Greensboro, NC	
			LLC	
Assure II	Quizalofop	46	Amvac Chemical Corp.	Newport Beach, CA
ImiFlex	Imazamox	53	UPL	King of Prussia, PA
Zest	Nicosulfuron	47	Corteva Agriscience	Indianapolis, NC
Roundup	Glyphosate	962^{a}	Bayer CropScience	Research Triangle
PowerMax				Park, NC
Screening at TX				
Assure II	Quizalofop	45	Amvac Chemical Corp.	Beach, CA, Newport
				IN
Beyond	Imazamox	53	BASF	Florham Park, NJ
Accent Q	Nicosulfuron	36	Corteva Agriscience	Indianapolis, NC
Roundup	Glyphosate	785	Bayer CropScience	Research Triangle
PowerMax				Park, NC

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

 a g ae ha $^{-1}$

Table 3. Survival of johnsongrass accessions from Arkansas, Texas, Oklahoma, and Kansas to different herbicides^a.

^aDescriptive statistics were generated from mortality data.

 b g ae ha⁻¹</sup>

Table 4. Johnsongrass accessions surviving herbicide treatment and injury to surviving plants. Accessions are sorted by survival within a herbicide and screening location.

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