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Distribution of Herbicide-Resistant Shattercane and Johnsongrass Populations in Sorghum Production Areas of Nebraska and Northern Kansas

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ABSTRACT

Overreliance on acetolactate synthase (ALS)-inhibiting herbicides for weed control during the 1990s resulted in selection of ALS-resistant shattercane [*Sorghum bicolor* (L.) Moench ssp. *drummondii* (Nees ex Steud.) de Wet ex Davidse] biotypes in Nebraska. The objective of this study was to assess the baseline presence of ALS-resistance in 190 shattercane and 59 johnsongrass [*Sorghum halepense* (L.) Pers.] populations collected across northern Kansas, northwestern Missouri, and southern Nebraska in 2013. In 2014, a preliminary field experiment was conducted to evaluate the presence of herbicide resistance in the aforementioned populations. Treatments consisted of four herbicides (clethodim {2-[1-[[(E)-3-chloroprop-2-enoxy]amino] propylidene]-5-(2-ethylsulfanylpropyl)cyclohexane-1,3-dione}, glyphosate [N-(phosphonomethyl) glycine], imazethapyr [5-ethyl-2-(4-methyl-5-oxo-4-propan-2-yl-1H-imidazol-2-yl) pyridine-3-carboxylic acid], and nicosulfuron {2-[(4,6-dimethoxypyrimidin-2-yl)carbamoylsulfamoyl]-N,N-dimethylpyridine-3-carboxamide}) applied at labeled rates. Clethodim and glyphosate controlled all shattercane and johnsongrass populations evaluated. Putative imazethapyr and nicosulfuron (ALS-inhibiting herbicides) resistant populations were further exposed to a dose–response study under greenhouse conditions. Five shattercane and five johnsongrass populations were confirmed resistant to imazethapyr. Four shattercane and three johnsongrass populations were confirmed resistant to nicosulfuron. All ALS-resistant shattercane and johnsongrass populations were collected in Nebraska except for one johnsongrass population, resistant to nicosulfuron, that was collected in Kansas. Acetolactate synthase-resistance persists, even though ALS-inhibitors have not been widely used to control shattercane and johnsongrass for more than 15 yr, indicating the lack of a strong fitness cost associated with ALS-resistance. Therefore, shattercane and johnsongrass should be properly managed before and during the commercialization of ALS-tolerant grain sorghum [*Sorghum bicolor* (L.) Moench ssp. *bicolor*] (expected in 2017), especially in regions where ALS-resistance has been confirmed.

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Grain sorghum is ranked as the fifth most important cereal crop in the world after wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), corn (*Zea mays* L.), and barley (*Hordeum vulgare* L.), and is the third most common cereal planted in the United States, trailing corn and wheat (Defelice, 2006; USDA-NASS, 2015). Sorghum is a warm season C4 grass species that is highly efficient in the conversion of solar energy and use of water. Sorghums are cultivated throughout the world for food, fodder, syrup, and biofuel production. In the United States, the crop is primarily used for livestock feed and is ranked second after corn for ethanol production (Paterson, 2008). In spite of the agronomic potential and food value of grain sorghum, the land area of sorghum production has declined in many parts of the United States (USDA-NASS, 2015), in part because the number of options for weed management in sorghum is limited. Most post-emergence (POST) herbicides labeled for grain sorghum are effective on broadleaf weed species but have only limited activity on annual grasses. Consequently, pre-emergence (PRE) herbicides are the primary option for annual grass control in grain sorghum (Hennigh et al., 2010). However, grain sorghum is often grown in dry environments and the absence of adequate soil moisture often reduces the activation and efficacy of PRE herbicide treatments (Hennigh et al., 2010).

Acetolactate synthase-inhibiting herbicides, also known as acetohydroxyacid synthase (AHAS)-inhibitors, are commonly used to control grass weeds in certain broadleaf and grass crops (Hennigh et al., 2010). However, conventional grain sorghum is susceptible to ALS-herbicides that have grass activity. The ALS-inhibiting herbicides are effective in small quantities (grams of active ingredient per hectare), allow for wide application windows, have high safety margins on labeled crops, are effective on a broad spectrum of weed species, typically possess soil residual activity, and have low mammalian toxicities (Tranel and Wright, 2002). However, there are at least eight known mutation sites in the ALS gene that confer resistance

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Abbreviations: ALS, acetolactate synthase; AMS, ammonium sulfate; COC, crop oil concentrate; DAP, days after planting ; DAT, days after treatment; GR, growth reduction; POST, post-emergence; PRE, pre-emergence; VE, visual evaluation; WSSA, Weed Science Society of America.

to ALS-inhibiting herbicides (Tranel et al., 2015). Owing to the versatility of these herbicides, the selective pressure on weed populations imposed by herbicide use, and the number of genetic mutations that can confer resistance, numerous weed species have evolved resistance to one or multiple ALS chemistries. There have been 153 reported ALS-resistant weed species worldwide, and 47 are in the United States (Heap, 2015).

In 2004, a shattercane population exhibiting resistance to ALS-inhibiting herbicides was identified in Kansas. Using conventional breeding, a project was then initiated by scientists at Kansas State University with the objective to introgress the ALS-resistant gene from the shattercane population into grain sorghum germplasm and ultimately commercialize grain sorghum varieties with tolerance to ALS-inhibiting herbicides (Tuinstra and Al-Khatib, 2008). DuPont (Wilmington, DE) has acquired the license of the ALS herbicide tolerance trait from Kansas State University and has branded the technology as "Inzen". Nicosulfuron (herbicide in the ALS-sulfonylurea family), an effective active ingredient for the control of weedy annual grasses, is the herbicide intended to be labeled for the technology. The ALS-tolerant grain sorghum varieties are expected to be on the market in 2017 (D.W. Saunders and K. L. Carlson, personal communication, 2015). This technology has the potential to improve weed control options in grain sorghum production by allowing for POST control of grass weeds (Hennigh et al., 2010). Moreover, the technology has strong potential to increase the use of grain sorghum in crop rotations and expand its production in environments where grain sorghum is better adapted than corn, but where corn is typically cultivated because of the availability of more herbicide options.

Despite the potential of the Inzen technology, the coexistence of sympatric weedy relatives poses some threats to its adoption and potential lifespan. The main concerns are (i) crop-to-weed gene flow that would increase the frequency of the ALS-resistance allele in shattercane and johnsongrass populations, (ii) the difficulty of controlling weeds that are already ALS-resistant and (iii) selection for additional resistant biotypes due to overreliance on the technology. Shattercane and johnsongrass are troublesome weedy sorghums in agronomic crops worldwide, especially in grain sorghum production (Hans and Johnson, 2002; Kegode and Pearce, 1998; Stahlman and Wicks, 2000). Shattercane is a de-domesticated sorghum with many similarities to grain sorghum. Shattercane and grain sorghum are both diploid ($2x = 20$), sexually compatible, and may be cross-pollinated by wind, which can result in hybridization where floral synchrony occurs (Defelice, 2006; Sahoo et al., 2010; Schmidt et al., 2013). Johnsongrass is typically a tetraploid ($2x = 40$), rhizomatous, perennial, self-pollinated weed species that can propagate vegetatively and reproduce sexually. Despite the difference in ploidy levels, johnsongrass and grain sorghum have been reported to outcross and produce hybrids (Arriola and Ellstrand, 1996; Arriola and Ellstrand, 1997). Sahoo et al. (2010) and Arriola and Ellstrand (1997) reported that shattercane × sorghum hybrids and johnsongrass × sorghum hybrids, respectively, had similar ecological fitness to the wild-type parents with respect to several metrics (i.e., biomass and seed production). Thus, there is apparently no barrier to prevent the transfer of any beneficial or neutral traits from the crop to weedy relatives (Arriola and Ellstrand, 1997; Sahoo et al., 2010). This indicates that any neutral or beneficial trait to shattercane or johnsongrass would likely persist in the weedy relatives infesting agricultural fields, even in the absence of selection.

The ALS-resistant shattercane populations were first detected in Nebraska during the early 1990s, when ALS-inhibiting herbicides were the main POST option to control shattercane in corn (Lee et al., 1999). After 1996, glyphosate-tolerant corn and soybean [*Glycine max* (L.) Merr.] were widely adopted. The ALS-resistant shattercane populations were no longer a concern in corn–soybean rotations because glyphosate is a very effective herbicide on shattercane. The ALS-resistant shattercane and ALS-resistant johnsongrass populations have been reported in eight and four U.S. states, respectively (Heap, 2015). Moreover, johnsongrass populations resistant to glyphosate and ACCase-inhibitors have been reported (Heap, 2015), making management of this perennial species even more challenging. To our knowledge, there have been no reports of herbicide-resistant johnsongrass in Kansas, Missouri, and Nebraska.

Since ALS-resistance traits reportedly have little to no impact on the ecological fitness of weed populations in the absence of selective pressure by ALS-inhibiting herbicides (Davis et al., 2009; Park et al., 2004; Sibony and Rubin, 2003; Tranel and Wright, 2002), we hypothesized that the ALS-resistance trait in shattercane would still be detected in Nebraska where resistance was reported by Lee et al. (1999), even though management practices have switched to glyphosate-based systems for at least 15 yr. The objective of this study was to evaluate the current frequency and distribution of ALS-resistant shattercane and johnsongrass populations in Kansas, Missouri, and Nebraska. Knowledge of the distribution of herbicide-resistant weedy sorghums will be important to indicate regions where shattercane and johnsongrass populations should be firmly managed before and after the introduction of the Inzen technology.

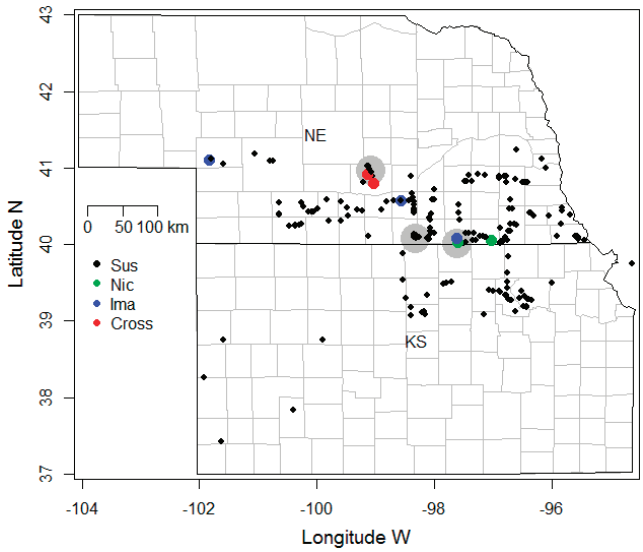

Fig. 1. Sampling distribution of shattercane populations from Kansas (KS) and Nebraska (NE). "Sus" = population susceptible to all herbicides tested in this study, "Nic" = resistant to nicosulfuron, "Ima" = resistant to imazethapyr, and "Cross" = cross-resistant to the acetolactate synthase (ALS) herbicides tested (nicosulfuron and imazethapyr). The three gray circles represent the regions in Nebraska (Buffalo, Webster, and Thayer Counties) where ALS-resistant shattercane populations were detected by Lee et al. (1999).

MATERIALS AND METHODS

Seed Collection

Seeds from shattercane and johnsongrass populations were collected between August and November of 2013 from agricultural fields and non-cropped areas from Kansas, Missouri, and Nebraska with the objective to evaluate the frequency and distribution of herbicide resistance.

Shattercane Seed Collection and Preparation. Shattercane panicles were collected from 190 random locations across northern Kansas and southern Nebraska where shattercane was detected in or surrounding agricultural fields (Fig. 1; Supplemental Table S1). Extra effort was taken to collect as many populations as possible in Buffalo, Webster, and Thayer Counties, regions where ALS-resistant shattercane populations were first reported in Nebraska (Lee et al., 1999). The panicles of at least 20 mature plants were collected at each location. Shattercane density and distribution varied across locations, but panicles within a population were collected from plants located as far apart as possible (at least 1 m apart for small populations). The majority of the shattercane populations were collected from the center and/or edges of corn, soybean, and sorghum fields. The GPS coordinates for each population were recorded using a handheld global positioning system. Populations were designated as shattercane (S-) and given a number (1–190) based on county and state where they were collected (Supplemental Table S1). After collection, seeds were allowed to dry for at least 10 d under greenhouse conditions (24/19 °C day/night temperature). When dry, seeds were manually threshed from the panicles and combined into a single composite sample for each population. Seed samples were stored at 4°C for approximately 3 mo to overcome seed dormancy. Fifty seeds were then counted by a seed counter and placed in properly labeled paper envelopes.

Johnsongrass Seed Collection and Preparation. Johnsongrass panicles were collected from 59 random locations across northern Kansas, northwestern Missouri, and southern Nebraska where johnsongrass was detected in or surrounding agricultural fields (Fig. 2; Supplemental Table S2). Seeds from at least 50 mature panicles were collected for most populations. Johnsongrass density and distribution varied across locations, but seeds within a population were collected from panicles located as far apart as possible (at least 1 m apart for small populations). The majority of the johnsongrass populations were collected from roadsides, railroads, rights of way, and edges of corn, soybean or sorghum fields. Populations were designated as johnsongrass (J-) and given a number (1–59) based on county and state where they were collected (Supplemental Table S2). After collection, seeds were processed and stored similarly to shattercane seeds. Sixty seeds with dark caryopses were manually selected, counted, and placed in properly labeled paper envelopes.

Field Screening Experiment

A field experiment was conducted in 2014 at the University of Nebraska Agricultural Research and Development Center (ARDC, 41.161 N 96.424 W) as the preliminary screening to detect and select putative herbicide-resistant shattercane and johnsongrass populations. The shattercane and johnsongrass studies were conducted separately, but in adjacent areas of the same field. Four grain sorghum rows were planted as borders in between each of four herbicide treatments within each species to minimize drift.

Site Description. The selected site had been in a corn–soybean rotation for the past 10 yr and had no history of shattercane or johnsongrass infestations. The soil was a Yutan silty clay loam (33% clay, 54% silt, 13% sand, 3% organic matter, and 5.6 pH).

Shattercane Field Screening. The field was tilled twice (16 and 27 May 2014) using a tandem disk to eliminate emerged weeds and prepare a seedbed before planting. On 29 May 2014, 50 seeds from each shattercane population were sown at 1.5- to 2-cm depth in 3 m rows using a cone planter (each 3 m row was considered a plot). Herbicide treatments were applied at 26 d after planting (DAP) when most shattercane and the control sorghum plants were at the five- to six-leaf stage (20–28 cm tall).

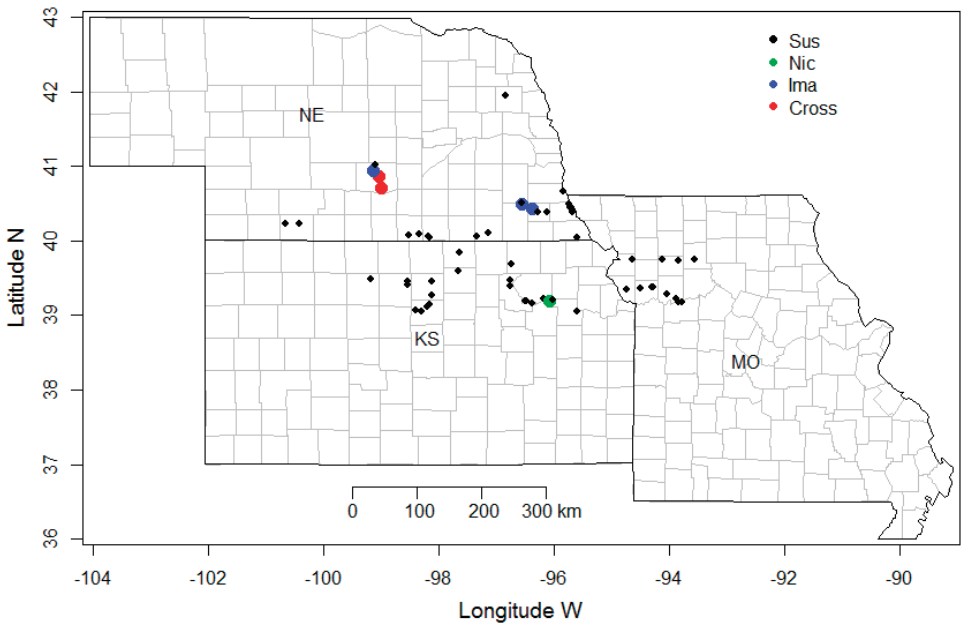

Fig. 2. Sampling distribution of johnsongrass populations from Kansas (KS), Missouri (MO), and Nebraska (NE). "Sus" = population susceptible to all herbicides tested in this study, "Nic" = resistant to nicosulfuron, "Ima" = resistant to imazethapyr, and "Cross" = cross-resistant to the acetolactate synthase (ALS) herbicides tested (nicosulfuron and imazethapyr).

Johnsongrass Field Screening. The field was prepared for planting as mentioned above. On 28 May 2014, 60 seeds from each johnsongrass population were sown at 1.5- to 2-cm depth in 3-m rows using a cone planter. Herbicide treatments were applied at 34 DAP when most johnsongrass plants were at the five- to six-leaf stage (24–40 cm tall) and the control sorghum plants were at the six- to seven-leaf stage (45–53 cm tall).

Herbicide Treatments. Four herbicides were applied at labeled rates: clethodim at 76.5 g a.i. ha^{-1} (Weed Science Society of America [WSSA] group 1), glyphosate at 867 g a.e. ha^{-1} (WSSA group 9), imazethapyr at 70 g a.i. ha^{-1} (WSSA group 2; imidazolinone family), and nicosulfuron at 35 g a.i. ha^{-1} (WSSA group 2; sulfonylurea family). Application of clethodim included a crop oil concentrate (COC) at 1% v/v + 2800 g ha^{-1} ammonium sulfate (AMS); for glyphosate, 1428 g ha^{-1} AMS; for imazethapyr, COC at 1.25% v/v + 2017 g ha^{-1} AMS; and for nicosulfuron, COC at 1% v/v + 2240 g ha^{-1} AMS. Herbicides were sprayed with a tractor-mounted sprayer calibrated to deliver 140 L ha^{-1} using AIXR11002 nozzle tips (TeeJet Technologies, Spraying Systems Co., Wheaton, IL) at a pressure of 241 kPa. These four herbicides, which represent three modes of action, consist of commonly used chemicals for shattercane and johnsongrass control in row crops. Moreover, nicosulfuron will be recommended for the Inzen technology.

Study Design. The shattercane study had 200 rows per block per herbicide treatment, one planted to each of the 190 shattercane populations, one to conventional grain sorghum (susceptible control, Pioneer 87P06), one to Inzen (ALS-tolerant control, Pioneer YSA3527), and eight were left unplanted and used as blank plots to assure proper seed placement by the cone planter, no off-site seed movement, and absence of natural weedy sorghum emerging from the seed bank. The johnsongrass study had 64 rows per block per herbicide treatment, one planted to each of the 59 johnsongrass populations, one to sorghum, one to Inzen, and three were left unplanted and used as blank plots. The experiment for each herbicide was conducted in a randomized complete block design with four replicate blocks.

Data Collection. The number of established plants in each plot was counted 7 d before herbicide application. Visual evaluations of plant growth (VE; on a scale of 1 to 10 as suggested by Anderson et al. (1998), with 1 being dead and 10 being completely healthy) and plant mortality (%; calculated based on the number of plants alive before and after herbicide application) data were taken on a population basis at 21 d after treatment (DAT). Populations with VE ranging from 1 to 3, 4 to 7, and 8 to 10 were considered susceptible (dead plants), intermediate (stunted plants, with the main culm injured or dead but new tillers growing back) and resistant (light interveinal chlorosis and/or plant stunting to no detectable injury) when exposed to the labeled herbicide rate, respectively (Anderson et al., 1998). The mortality data were used to support the visual evaluations. Means and standard errors for each response variable were estimated and used to decide the putative herbicide-resistant populations (populations showing VE > 3 and mortality < 100%) that should be further subjected to a dose–response study under greenhouse conditions (Tables 1 and 2).

Table 1. Response of the putative resistant shattercane populations detected in the field screening conducted in the summer of 2014.† Mortality and visual evaluation (VE)‡ data were collected at 21 d after treatment (DAT).

	Imazethapyr		
Population	Plants $plot^{-1}$	Mortality	VE
		%	
S-31	39 (2)§	82 (4)	5 (1)
S-46	16 (4)	15 (6)	6 (1)
S-58	21 (1)	29 (11)	6 (1)
S-63	16 (4)	42 (10)	6 (1)
S-105	37 (2)	89 (3)	4 (0)
S-117	32 (1)	39 (8)	5 (0)
S-136	28 (4)	96 (2)	3 (1)
S-177	44 (2)	59 (9)	4 (0)
S-178	39 (2)	84 (7)	4 (0)
S-179	39 (2)	81 (3)	8 (0)
Inzen	43 (1)	0 (0)	10 (0)
	Nicosulfuron		
Population	Plants $plot^{-1}$	Mortality	VE
		%	
S-58	25 (3)	0 (0)	9 (0)
S-63	14 (2)	15 (9)	9 (0)
S-105	35 (3)	66 (18)	5 (1)
S-113	21 (4)	91 (7)	4 (0)
S-134	11 (1)	88 (4)	4 (0)
S-177	42 (2)	98 (2)	4 (1)
S-178	41 (1)	35 (15)	6 (1)
Inzen	40 (2)	0 (0)	10 (0)

† The study was conducted at the University of Nebraska Agricultural Research and Development Center (ARDC) near Mead, NE. Plants were treated at 26 d after planting (DAP) with the labeled rate of imazethapyr and nicosulfuron (70 and 35 g a.i. ha^{-1}, respectively).

‡ Populations with VE ranging from 4 to 7 and 8 to10 were considered intermediate (stunted plants, with the main culm dead or injured dead but new tillers growing back) and resistant (light interveinal chlorosis and/or plant stunting to no detectable injury), respectively, when exposed to the labeled herbicide rate (adapted from Anderson et al., 1998).

§ Mean with ± 1 SE in parentheses.

Dose-Response Study

Putative herbicide-resistant populations were exposed to dose-response studies in the greenhouse for confirmation of herbicide resistance using the seeds collected in the fall of 2013 (Tables 1 and 2). Treatments consisted of eight herbicide doses: 0, 0.5, 1, 2, 4, 8, 16, and 32 times the labeled field rate of the herbicide being evaluated. The respective adjuvants for each herbicide were included, as for the field study.

Glumes of shattercane and johnsongrass seeds were mechanically removed using a rub board, seeds were pre-germinated on wet germination paper, and two seedlings with exposed radicles were transplanted to a 3.8-cm wide by 21-cm high cone-tainer filled with potting mix (Berger BM1 All-Purpose Mix, Berger Peat Moss Ltd., Saint-Modeste, QC, Canada). Placement of seeds on wet germination paper was considered the starting point of the study (0 DAP). At 7 DAP, plants were thinned to one plant per cone-tainer. At 11 and 18 DAP, 30 mL of a nutrient solution containing 15 g of 20–20–20 water soluble fertilizer (Peters General Purpose Fertilizer, Scotts, Marysville, OH) diluted in 3.785 L of water was applied to each cone-tainer. Herbicide treatments were applied at 21 DAP when sorghum, shattercane, and johnsongrass plants were at three- to four-leaf stage (22–30 cm tall). Herbicide treatments were

Table 2. Response of the putative resistant johnsongrass populations detected in the field screening conducted in the summer of 2014.† Mortality and visual evaluation (VE)‡ data were collected at 21 d after treatment (DAT).

Imazethapyr			
Population	Plants plot^{-1}	Mortality	VE
		%	
J-10	11 (1)§	72 (7)	4 (1)
J-12	19 (1)	76 (10)	4 (1)
J-18	11 (0)	44 (11)	4 (0)
J-35	41 (1)	43 (4)	6 (0)
J-36	41 (2)	3 (1)	9 (0)
J-37	28 (4)	18 (4)	8 (0)
J-38	25 (3)	73 (5)	4 (0)
J-40	1 (0)	75 (25)	4 (3)
J-41	1 (0)	67 (33)	3 (2)
J-44	4 (1)	27 (10)	6 (0)
J-55	33 (3)	68 (2)	4 (0)
Inzen	46 (2)	0 (0)	10 (0)
Nicosulfuron			
Population	Plants plot^{-1}	Mortality	VE
		%	
J-15	2 (0)	63 (24)	5 (2)
J-35	37 (6)	49 (10)	8 (0)
J-36	31 (2)	1 (1)	9 (0)
Inzen	43 (2)	0 (0)	10 (0)

† The study was conducted at the University of Nebraska Agricultural Research and Development Center (ARDC) near Mead, NE. Plants were treated at 34 d after planting (DAP) with the labeled rate of imazethapyr and nicosulfuron (70 and 35 g a.i. ha^{-1}, respectively).

‡ Populations with VE ranging from 4 to 7 and 8 to 10 were considered intermediate (stunted plants, with the main culm dead or injured dead but new tillers growing back) and resistant (light interveinal chlorosis and/or plant stunting to no detectable injury), respectively, when exposed to the labeled herbicide rate (adapted from Anderson et al., 1998).

§ Mean with ± 1 SE in parentheses.

delivered using 140 L ha^{-1} carrier volume and TP8001E flat-fan nozzle tip (TeeJet Technologies, Spraying Systems Co., Wheaton, IL) at a pressure of 241 kPa within a spray chamber (Research Track Sprayer; DeVries, Hollandale, MN).

Visual evaluation data were collected on an individual plant basis at 21 DAT as described previously. Plants were then harvested, dried to constant weight at 60°C, dry weight of individual plants was recorded, and dry weight reduction, or growth reduction (GR), at 21 DAT was calculated (compared to untreated plants within the same population). Greenhouse conditions during the study were set at 24/19°C day/night cycle with a 16-h photoperiod provided by metal halide lamps to supplement natural daylight. Plants were watered daily.

Two susceptible shattercane (S-13 and S-125), two susceptible johnsongrass (J-14 and J-52) and Inzen sorghum were included in the dose–response experiments as our control populations. The control shattercane and johnsongrass populations were selected from the field screening because of high susceptibility to all herbicides tested and good germination rates (data not shown). The study for each herbicide was conducted separately in a completely randomized design using three replicates (cone-tainers) per population per dose. The experiment was replicated in time (first and second run started on 11 Aug. and 29 Sept. 2014, respectively).

Dose-Response Curves. The four parameter log-logistic function was fit to the GR data of each population regressed on herbicide dose as the explanatory variable (x; g a.i. ha^{-1}):

$$y = c + \{d - c/1 + \exp[b(\log x - \log e)]\} \quad [1]$$

where y is the response variable (GR), c is the lower limit (theoretical minimum for y normalized to 0%), d is the upper limit (normalized to 100%), b is the relative slope around e, and e is the GR_{50} (inflection point, mid-point or estimated herbicide dose when y = 50%). The model parameters for each population-herbicide combination were estimated using the DRC package in R statistical software (R Foundation for Statistical Computing, Wien, Austria) (Knezevic et al., 2007). GR_{50} was used in this research as our response variable to compare the resistance level across populations as suggested by Heap (2015). Additionally, populations with low GR_{50} but with consistent number of plants having VE ≥ 4 at rates equal or greater than the labeled herbicide rate were also considered resistant [a similar approach has been taken by Kruger et al. (2009)]. The relative level of resistance of each population was expressed by calculating the ratios between the GR_{50} value of the population-herbicide combination of interest and the GR_{50} value of the susceptible population in the same species treated with the same herbicide.

RESULTS AND DISCUSSION

Field Screening

The preliminary field study was found to be an efficient way of screening multiple populations with multiple herbicides to identify putative resistance. Temperature and soil moisture were appropriate for grain sorghum, shattercane, and johnsongrass establishment and growth during the study (21.8, 35.4, and 8.1°C, and 226 mm for average, maximum, and minimum temperature and cumulative precipitation during the study, respectively). Average (range) number of plants per plot in the shattercane and johnsongrass studies were 29 (2–44) and 10 (1–41), respectively. More than 40 plants per plot were observed in the two control sorghum plots (Tables 1 and 2). The cone planter properly delivered the seeds of each population to the appropriate depth and the research area was confirmed to be free of weedy-sorghum species (no shattercane or johnsongrass detected in the blank plots). Natural infestations of velvetleaf (*Abutilon theophrasti* Medik.), common waterhemp (*Amaranthus rudis* Sauer), and volunteer soybean was detected and were hand-hoed from the research plots as they emerged.

Shattercane field screening showed that 10 populations treated with imazethapyr had live plants at 21 DAT, with one classified as resistant (VE ≥ 8; surviving plants with vigorous growth) and nine as intermediate (4 ≤ VE ≥ 7; surviving plants with the main culm dead or highly stunted and regrowth of small tillers) (Table 1). Seven shattercane populations treated with nicosulfuron had live plants at 21 DAT, with two populations being classified as resistant and five populations classified as intermediate (Table 1). All shattercane populations evaluated were susceptible to glyphosate and clethodim (VE < 3 and mortality = 100%). Johnsongrass field screening showed that 11 populations treated with imazethapyr had surviving plants at 21 DAT (two resistant and nine intermediate) and three populations treated with nicosulfuron had surviving plants at 21 DAT (two resistant and one

intermediate) (Table 2). All johnsongrass populations evaluated were susceptible to glyphosate and clethodim. Therefore, glyphosate and clethodim are still very effective herbicide options to manage weedy sorghum in rotational years in northern Kansas, northwestern Missouri, and southern Nebraska. Continuous use of these two herbicides have selected for resistant johnsongrass biotypes in other parts of the United States (Heap 2015); indicating that resistance is likely to occur if these herbicides are solely and continuously used. The number of shattercane and johnsongrass plants surviving the imazethapyr and nicosulfuron treatments varied across populations, indicating that the resistance alleles are not present in all plants within populations (Tables 1 and 2). As expected, conventional sorghum was susceptible to imazethapyr and nicosulfuron, whereas Inzen sorghum was classified as resistant to both herbicides, indicating that the tolerance trait in Inzen confers cross-resistance to ALS herbicides. The control sorghum varieties used in this study were susceptible to clethodim and glyphosate.

Shattercane and johnsongrass populations with low emergence in the field screening (average of three or less established plants plot^{-1}) were grown in square plastic pots (13 cm wide by 15 cm high) in the greenhouse and treated with the labeled rate of imazethapyr and nicosulfuron at 21 DAP (20 plants per treatment). All populations with low field emergence were confirmed to be susceptible to ALS-inhibiting herbicides at 21 DAT (data not shown).

Dose Response

According to the results of field screening, all shattercane and johnsongrass populations were controlled by both clethodim and glyphosate but not by imazethapyr and nicosulfuron (Tables 1 and 2). Thus, dose-response studies were conducted only with imazethapyr and nicosulfuron on putative-resistant shattercane and johnsongrass populations.

The dose-response studies showed that five and four shattercane populations were confirmed to be resistant to imazethapyr (S-46, S-58, S-63, S-117, and S-179) and nicosulfuron (S-58, S-63, S-105, and S-178), respectively (Table 3). Five and three johnsongrass populations were confirmed to be resistant to imazethapyr (J-35, J-36, J-37, J-40, and J-44) and nicosulfuron (J-15, J-35, and J-36), respectively (Table 4). The level of resistance varied across populations.

Populations S-46 and S-58 were highly resistant to imazethapyr and populations S-58 and S-63 highly resistant to nicosulfuron (at least a 100-fold difference in the GR_{50} when compared to the most susceptible population) (Table 3). The remaining shattercane populations confirmed resistant to imazethapyr and nicosulfuron presented a lower level of resistance (20-fold difference in GR_{50} when compared to the most susceptible population). Population J-36 was highly resistant to imazethapyr and nicosulfuron (>1000-fold difference in the GR_{50} when compared to the most susceptible population; Table 4). The remaining johnsongrass populations confirmed resistant to imazethapyr and nicosulfuron displayed a 2- to 270-fold difference in the GR_{50} when compared to the most susceptible population. Inzen sorghum was highly resistant to imazethapyr and nicosulfuron (>1000-fold difference in the GR_{50} when compared to the most susceptible population).

Since resistance alleles were not fixed for most populations (frequency <1), these dose-response results reflect the current response of each population and not the resistance level of resistant individuals within a population. For instance, S-63 and S-179 presented a low frequency of resistant plants in the population when treated with imazethapyr, and were associated with a relatively low GR_{50}, but surviving plants exposed to 2 X the labeled rate (VE ≥ 5) were observed (data not shown). Population J-15 had a very low frequency of plants resistant to nicosulfuron and was associated with a GR_{50} that did not differ from that of the susceptible populations, but surviving plants exposed to 8 X the labeled rate were observed (data not shown).

Resistance to ALS-inhibiting herbicides is typically conferred by a single, nuclear-encoded gene that is either dominant or semidominant (has incomplete dominance) resulting in a dominant inheritance pattern (Preston and Mallory-Smith, 2001). Therefore, dominant homozygous and heterozygous plants are likely to survive ALS-inhibiting herbicide treatment (with heterozygous plants often being more injured). Although this was not the intent of the study, the current frequency of resistant individuals (homozygous resistant plus heterozygous plants) within the confirmed resistant populations can be roughly estimated using the mortality data collected in the field study (Tables 1 and 2), whereas mortality represents the frequency of susceptible plants (%) and (100 – mortality) represents the frequency of resistant plants (%) within a population. In the

Table 3. Effective dose of imazethapyr and nicosulfuron (a.i. ha^{-1}) to cause 50% growth reduction (GR_{50})† on confirmed acetolactate synthase (ALS)-resistant shattercane populations.‡

Imazethapyr			Nicosulfuron		
Population	GR_{50}	Fold§	Population	GR_{50}	Fold
S-13	0.12 (0.01)¶	1.0	S-13	0.11 (0.01)	1.0
S-125	0.12 (0.01)	1.0	S-125	0.12 (0.01)	1.1
S-179	2.38 (2.12)	19.7	S-178	2.16 (1.33)	20.0
S-63	2.85 (1.27)	23.7	S-105	2.22 (2.19)	20.5
S-117	2.86 (1.38)	23.8	S-63	23.07 (21.7)	213.1
S-58	14.49 (6.62)	120.3	S-58	400.26 (133.55)	>1000
S-46	161.30 (56.27)	>1000			
Inzen	>2400	>1000	Inzen	196.37 (20.48)	>1000

† The imazethapyr or nicosulfuron dose needed to cause 50% GR was estimated using a nonlinear logistic model.

‡ S-13 and S-125 were included in the dose–response study as our known susceptible populations. Inzen represent the ALS-resistant control in this study.

§ Fold was calculated by dividing the GR_{50} of the population by the GR_{50} of the most susceptible population within the same species.

¶ GR_{50} ± 1 SE in parentheses.

absence of selection by ALS-inhibiting herbicides, the presence of the resistance alleles is assumed not to influence plant fitness. According to Hardy–Weinberg theory, in populations wherein the resistance allele was close to fixation before adoption of glyphosate-based cropping systems, the resistant allele would be expected to be present in extant populations in the homozygous form. However, in populations wherein the resistant allele was relatively recent before adoption of glyphosate-based systems, the resistance allele would be expected to be present in extant populations in the heterozygous form (Roughgarden, 1998). Due to the lack of reduced ecological fitness in the absence of herbicide selection, the frequency of the resistant allele likely did not change since the introduction of glyphosate systems at locations where ALS-resistant populations were detected (assuming no random genetic drift). For instance, resistance was almost fixed in population J-36 (mortality ≤3% when exposed to imazethapyr or nicosulfuron), indicating that ALS-inhibiting herbicides were probably no longer effective at managing this johnsongrass population. Conversely, some populations had a low frequency of resistant individuals (i.e., J-15 when treated with nicosulfuron and S-179 when treated with imazethapyr), indicating that resistance alleles were likely at initial stages of introgression when growers stopped using ALS-inhibiting herbicides for management of these weedy-sorghum populations.

To obtain and compare the "true" resistance level of resistant individuals across populations, these should be exposed to multiple generations of selection to favor individuals carrying the resistance allele and then expose them to dose–response studies. Since our objective was to detect the baseline frequency of resistant populations, dose response was conducted only with the generation of seeds collected from the field in the fall of 2013. According to the results of individual herbicide screenings, two shattercane (S-58 and S-63) and two johnsongrass populations (J-35 and J-36) appeared to be cross-resistant to nicosulfuron and imazethapyr. According to Kruger et al. (2009), for confirmation of cross-resistance, plants within a population should also be exposed to both herbicides simultaneously because sometimes a population carries biotypes with different resistance types, yet individuals are not resistant to both (further work will be conducted to confirm individuals' cross-resistance to ALS herbicides in these populations).

Distribution of Resistance

All confirmed shattercane populations resistant to imazethapyr and/or nicosulfuron were collected in Nebraska (Fig. 1). All confirmed johnsongrass populations resistant to imazethapyr and/or nicosulfuron came from Nebraska, but one population resistant to nicosufuron that was found in Kansas (J-15, Pottawatomie County; Fig. 2). All of these populations came from areas where corn has been traditionally cultivated and where ALS-inhibiting herbicides were widely used in the past. To our knowledge, this is the first report of ALS-resistant johnsongrass populations in Kansas and Nebraska. Interestingly, the two cross-resistant shattercane populations (S-63 and S-58) as well as the two cross-resistant johnsongrass populations (J-35 and J-36) and the imazethapyr-resistant johnsongrass population J-37 came from Buffalo County, Nebraska. One nicosulfuron- (S-178) and one imazethapyr- (S-179) resistant population came from Thayer County, Nebraska. Lee et al. (1999) detected ALS-resistant shattercane populations in Buffalo, Webster, and Thayer Counties; resistance was still detected by the current study in two of these counties. This is a strong indicator that the ALS-resistance trait has little to no fitness cost to weedy populations in the absence of selection by ALS-inhibiting herbicides, corroborating observations of Davis et al. (2009), Park et al. (2004), and Sibony and Rubin (2003).

The level of resistance to either or both imazethapyr and nicosulfuron varied across and within regions (Tables 3 and 4). For instance, populations S-178 and S-179 were 9 km apart and showed different types of resistance (to nicosulfuron and imazethapyr, respectively). Moreover, S-58 and S-63 were 15 km apart and were cross-resistant to imazethapyr and nicosulfuron; however, S-58 plants had a higher level of resistance to both herbicides when compared to S-63 (GR_{50} was higher for S-58 than S-63; Table 3). Populations J-35, J-36, and J-37 were within a radius of 17 km; J-36 plants had a higher level of resistance to both herbicides when compared to J-35 plants (GR_{50} was higher for J-36 than J-35; Table 4), and J-37 population was resistant only to imazethapyr. Given the different response observed to multiple ALS-inhibiting herbicides, Lee et al. (1999) suggested that the populations evaluated in their study developed resistance independently. According to Tranel et al. (2015) there are eight confirmed sites of ALS gene mutation that confer resistance to ALS-inhibiting herbicides (Ala_{122}, Pro_{197}, Ala_{205}, Asp_{376}, Arg_{377}, Trp_{574}, Ser_{653}, and

Table 4. Effective dose of imazethapyr and nicosulfuron (a.i. ha^{-1}) to cause 50% growth reduction (GR_{50})† on confirmed acetolactate synthase (ALS)-resistant johnsongrass populations‡.

Imazethapyr			Nicosulfuron		
Population	GR_{50}	Fold§	Population	GR_{50}	Fold
J-14	0.31 (0.14)¶	1.0	J-52	0.12 (0.01)	1.0
J-52	0.42 (0.20)	1.3	J-15	0.12 (0.01)	1.0
J-35	0.74 (0.31)	2.3	J-14	0.15 (0.06)	1.2
J-44	27.32 (14.10)	87.2	J-35	0.61 (0.44)	4.9
J-37	38.62 (19.30)	123.2	J-36	446.44 (79.85)	>1000
J-40	84.47 (16.91)	269.5			
J-36	>2400	>1000			
Inzen	>2400	>1000	Inzen	196.37 (20.48)	>1000

† The imazethapyr or nicosulfuron dose needed to cause 50% GR was estimated using a nonlinear logistic model.

‡ J-14 and J-52 were included in the dose-response study as our known susceptible populations. Inzen represent the ALS-resistant control in this study.

§ Fold was calculated by dividing the GR_{50} of the population by the GR_{50} of the most susceptible population within the same species.

¶ GR_{50} ± 1 SE in parentheses.

Gly_{654}). At each site, multiple amino acid substitutions are possible. Common amino acid substitutions reported in the ALS gene of resistant weeds are Ala_{122} to Thr, Val, and Tyr; Pro_{197} to Thr, His, Arg, Leu, Gln, Ser, Ala, Ile, and Tyr; Ala_{205} to Val; Asp_{376} to Glu; Arg_{377} to His; Trp_{574} to Leu, Gly, and Met; Ser_{653} to Thr, Asn, Ile; and Gly_{654} to Glu and Asp (Tranel et al., 2015). Substitutions at Pro_{197} and Trp_{574} have been the most common across several weed species. The specific amino acid substitution at each site may confer different types and levels of resistance to different ALS herbicide families (Tranel et al., 2015). For instance, substitution of Ala_{122} to Tyr results in resistance to both imidazolinones and sulfonylureas, whereas substitution of Ala_{122} to Thr results in resistance to imidazolinones only. All nine confirmed substitutions on Pro_{197} confer resistance to the sulfonylureas but not necessarily to the imidazolinones and the other ALS herbicide families (Tranel et al., 2015). Given our field and dose–response results, we hypothesize that for most resistant populations detected in this research, (i) populations evolved ALS-resistance independently rather than the same event being dispersed via seeds or pollen and (ii) different mutations were selected on (mutation site and/or amino acid substitution within the ALS-gene). DNA sequencing work will be conducted in all resistant populations to test these hypotheses.

If growers are willing to adopt the Inzen sorghum technology, shattercane and johnsongrass should be properly managed before and during its adoption. The results of this research indicate that if growers have observed ALS-resistant shattercane and/or johnsongrass in the past and these species are still present, they are likely to still carry the resistance alleles. Use of ALS-inhibiting herbicides in Inzen will favor selection of ALS-resistant plants. If the technology is used continuously, within a few generations of selection most individuals will carry the resistance allele and the technology will quickly lose its value. Moreover, if surviving plants (either resistant or escapes) are present at the end of the season, crop-to-weed gene flow may have occurred. If so, the frequency of the resistance allele in that population in subsequent generations would be expected to increase rapidly with further ALS-inhibiting herbicide applications. Modeling work has indicated that crop rotation and proper management with effective herbicides (i.e., glyphosate and clethodim) during non-sorghum years will be key strategies to postpone fixation of the resistance allele in weedy sorghums and maintain their population densities at low levels following deployment of Inzen technology (Werle et al., 2015).

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