

# Distribution of Herbicide-Resistant Johnsongrass (Sorghum halepense) in Arkansas

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In 2008, a population of johnsongrass collected from a soybean field near West Memphis, AR, in Crittenden County was confirmed resistant to glyphosate. This was the first documented case of glyphosate-resistant johnsongrass in Arkansas. The purpose of this study was to determine the geographical distribution of glyphosate-resistant johnsongrass in Arkansas crops and screen for resistance to additional herbicides. A total of 141 johnsongrass accessions were collected from 14 counties in Arkansas in the fall of 2008, 2009, and 2010 and screened for resistance to four of the most commonly used POST herbicides for johnsongrass control-imazethapyr, glyphosate, clethodim, and fluazifop. One accession potentially resistant to glyphosate (J12) and another with apparent resistance to imazethapyr (J14) were further evaluated in a dose-response experiment. The lethal dose required to kill 50% of the plants from the putative glyphosate-resistant and imazethapyrresistant accessions was higher than that of a biotype known to be susceptible to these herbicides. The J12 accession had an  $LD_{50}$  of 1,741 g at ha<sup>-11</sup> glyphosate, which was 8.5-fold greater than the susceptible biotype. The J14 accession had an  $LD_{50}$  of 73 g at ha<sup>-1</sup> imazethapyr, which was 3.7-fold greater than the LD<sub>50</sub> of the susceptible biotype. All other accessions were effectively controlled by the four evaluated herbicides. Widespread herbicide-resistant johnsongrass was not found in Arkansas, although accession J12 was resistant to glyphosate and J14 resistant to imazethapyr. Nomenclature: Clethodim; fluazifop; glyphosate; imazethapyr; johnsongrass, Sorghum halepense (L.) Pers.; soybean, Glycine max (L.) Merr.

Key words: Glyphosate resistance, mortality, weed control.

En 2008, se confirmó que una población de *Sorghum halepense* proveniente de un campo de soya cerca de Memphis Oeste, AR, en el condado Crittenden, era resistente a glyphosate. Este fue el primer caso documentado de *S. halepense* resistente a glyphosate en Arkansas. El objetivo de este estudio fue determinar la distribución geográfica de *S. halepense* resistente a glyphosate en cultivos en Arkansas y evaluar la resistencia a otros herbicidas. Un total de 141 accesiones de *S. halepense* fueron colectadas en 14 condados en Arkansas en el otoño de 2008, 2009, y 2010 y fueron evaluadas por resistencia a cuatro de los herbicidas POST más comunes usados para el control de *S. halepense*: imazethapyr, glyphosate, clethodim, y fluazifop. Una accesión potencialmente resistente a glyphosate (J12) y otra con resistencia aparente a imazethapyr (J14) fueron evaluadas en más detalle en un experimento de respuesta a dosis. La dosis letal para matar 50% de las plantas (LD<sub>50</sub>) de las accesiones con resistencia putativa a glyphosate e imazethapyr fue mayor que la de un biotipo con susceptibilidad conocida a estos herbicidas. La accesión J12 tuvo una LD<sub>50</sub> de 73 g ai ha<sup>-1</sup> de imazethapyr, la cual fue 8.5 veces mayor que la del biotipo susceptible. Todas las otras accesiones fueron controladas efectivamente por los cuatro herbicidas evaluados. No se encontró que *S. halepense* resistente a herbicidas esté ampliamente distribuido en Arkansas, aunque la accesión J12 fue resistente a glyphosate y la J14 fue resistente a imazethapyr.

Repeated use of acetyl coenzyme A carboxlyase (ACCase) inhibitors (group 1), acetolactate synthase (ALS) inhibitors (group 2), and dinotroanaline (group 3) herbicides has resulted in evolved resistances to each of these mechanisms of action

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Table 1. Johnsongrass accessions listed by county, crop, and global positioning system coordinates from which they were collected.

Га	bl	e 1	. (	Con	tinu	.ed

collected.	tioning system	coordinates	IIOIII WIIIC	ii tiley were				Latitude	Longitude
			Latitude	Longitude	Accession	County	Crop	°N	°W
Accession	County	Crop	°N	°W	J54	Desha	Soybean	34.103	91.014
T -	. 1	0 1	22 (22	01.01(	J55	Jackson	Soybean	35.571	91.250
JI	Arkansas	Soybean	33.408	91.316	J56	Jackson	Soybean	35.575	91.248
J2	Chicot	Soybean	33.347	91.294	J57	Lee	Soybean	34.895	90.791
J3	Chicot	Soybean	33.304	91.287	J58	Lee	Cotton	34.835	90.797
J4	Chicot	Soybean	33.313	91.291	159	Lee	Soybean	34.730	90.769
J5	Chicot	Soybean	33.340	91.291	J60	Lee	Cotton	34.657	90.770
J6	Chicot	Corn	33.394	91.311	J61	Lee	Soybean	34.657	90.743
J7	Chicot	Soybean	33.444	91.331	J62	Lee	Sovbean	34.657	90.833
J8	Chicot	Soybean	33.35	91.376	163	Lee	Sovbean	34.658	90.882
J9	Crittenden	Soybean	35.229	90.398	164	Lee	Sovbean	34,774	90.872
J10	Crittenden	Soybean	35.195	90.397	165	Lee	Sovbean	34.685	90.736
J11	Crittenden	Soybean	35.206	90.353	166	Lee	Soybean	34 811	90.673
J12	Crittenden	Soybean	35.198	90.121	J67	Lee	Cotton	34 833	90.645
J13	Crittenden	Soybean	35.204	90.116	168	Lonoke	Sovbean	34 600	90.706
J14	Crittenden	Soybean	35.218	90.113	169	Lonoke	Soybean	34 762	91.853
J15	Crittenden	Soybean	35.240	90.116	170	Mississippi	Soybean	35 466	90.170
J16	Crittenden	Soybean	35.243	90.127	J70 I71	Mississippi	Soybean	35 740	90.1/0
J17	Crittenden	Soybean	35.245	90.136	172	Mississippi	Soybean	35 512	90.089
J18	Crittenden	Soybean	35.247	90.176	173	Mississippi	Soybean	35 516	90.089
J19	Crittenden	Soybean	35.286	90.226	J73 174	Mississippi	Soybean	25 522	90.089
J20	Crittenden	Soybean	35.295	90.218	J/4 175	Mississippi	Soybean	35 500	90.089
J21	Crittenden	Soybean	35.295	90.212	J/J 176	Mississippi	Soybean	25 572	90.071
J22	Crittenden	Soybean	35.293	90.229	J/0 177	Mississippi	Soybean	25 (21	90.033
J23	Crittenden	Sovbean	35.310	90.169	J//	Mississippi	Soybean	35.031 25.701	89.949
124	Crittenden	Sovbean	35.314	90.182	J/8 170	Mississippi	Soybean	25 700	90.073
125	Crittenden	Sovbean	35.306	90.254	J/9	Mississippi	Soybean	))./88	90.075
126	Crittenden	Sovbean	35.307	90.267	J80	Mississippi	Soybean	35.//0 25.771	90.074
I27	Crittenden	Sovbean	35.316	90.377	J81	Mississippi	Grain sorghum	35.//1	90.073
128	Crittenden	Sovbean	35.379	90.249	J82	Mississippi	Soybean	35.//1	90.079
129	Crittenden	Soybean	35 380	90.243	J83	Mississippi	Soybean	35.5/3	90.213
130	Crittenden	Soybean	35 349	90.181	J84	Mississippi	Soybean	35.568	90.216
I31	Crittenden	Soybean	35 328	90.182	J85	Mississippi	Soybean	35.554	90.249
132	Crittenden	Soybean	35 265	90.201	J86	Mississippi	Soybean	35.484	90.251
133	Crittenden	Soybean	35 263	90.264	J87	Mississippi	Soybean	35.483	90.251
134	Crittenden	Soybean	35 263	90.271	J88	Mississippi	Soybean	35.481	90.223
125	Crittenden	Soybean	35 264	90.319	J89	Mississippi	Soybean	35.466	90.202
136	Crittenden	Soybean	35 102	90.195	J90	Mississippi	Soybean	35.464	90.199
137	Crittenden	Soybean	35.001	90.203	J91	Mississippi	Soybean	35.465	90.199
138	Crittenden	Soybean	35.000	90.203	J92	Mississippi	Soybean	35.465	90.128
120	Crittondon	Soybean	35.090	90.203	J93	Mississippi	Soybean	35.465	90.137
140	Crittondon	Soybean	35.092	90.212	J94	Mississippi	Soybean	35.462	90.129
J40 I41	Crittenden	Soybean	25 1 47	90.393	J95	Mississippi	Soybean	35.461	90.129
J41 I42	Crittenden	Soybean	3).14/ 25.140	90.597	J96	Mississippi	Soybean	35.487	90.077
J42	Crittenden	Soybean	35.149	90.556	J97	Mississippi	Soybean	35.488	90.079
J43	Crittenden	Soybean	35.109	90.229	J98	Mississippi	Soybean	35.560	91.039
J44	Crittenden	Soybean	35.081	90.231	J99	Mississippi	Soybean	35.563	91.180
J45	Cross	Soybean	35.251	90.955	J100	Monroe	Soybean	34.765	91.197
J46	Cross	Soybean	35.251	90.936	J101	Monroe	Soybean	34.766	91.226
J47	Cross	Soybean	35.275	90.791	J102	Monroe	Soybean	34.447	91.049
J48	Desha	Soybean	33.569	91.383	J103	Phillips	Soybean	34.553	90.641
J49	Desha	Soybean	33.672	91.422	J104	Phillips	Soybean	34.627	90.675
J50	Desha	Soybean	33.842	91.475	J105	Phillips	Soybean	34.583	90.692
J51	Desha	Soybean	33.868	91.480	J106	Phillips	Sovbean	34.564	90.658
J52	Desha	Soybean	33.882	91.457	J107	Phillips	Sovbean	34,570	90.820
153	Desha	Soybean	33.896	91.420	J10/	1 11111/20	Joybean	51.770	20.020

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Table 1. Continued.

			Latitude	Longitude
Accession	County	Crop	°N	°W
J108	Phillips	Soybean	34.452	90.776
J109	Phillips	Soybean	34.549	91.017
J110	Phillips	Soybean	34.601	91.032
J111	Phillips	Soybean	34.584	90.712
J112	Phillips	Soybean	34.492	90.695
J113	Phillips	Soybean	34.434	90.632
J114	Phillips	Soybean	34.416	90.666
J115	Phillips	Corn	34.493	90.648
J116	Phillips	Soybean	34.592	90.812
J117	Phillips	Soybean	34.550	90.803
J118	Phillips	Soybean	34.515	90.649
J119	Phillips	Soybean	34.435	90.726
J120	Phillips	Soybean	34.415	90.765
J121	Phillips	Soybean	34.438	90.785
J122	Phillips	Cotton	34.555	90.778
J123	Phillips	Soybean	34.273	90.973
J124	Phillips	Soybean	34.341	90.882
J125	Phillips	Soybean	34.386	90.842
J126	Phillips	Soybean	34.128	91.009
J127	Poinsett	Soybean	35.449	90.742
J128	Poinsett	Soybean	35.543	90.721
J129	Prairie	Soybean	34.845	91.423
J130	Prairie	Soybean	34.813	91.417
J131	St. Francis	Soybean	35.128	90.507
J132	St. Francis	Soybean	35.135	90.467
J133	St. Francis	Soybean	35.130	90.468
J134	St. Francis	Soybean	35.114	90.557
J135	St. Francis	Soybean	35.108	90.575
J136	St. Francis	Soybean	34.935	90.501
J137	St. Francis	Soybean	34.945	90.488
J138	St. Francis	Soybean	34.984	90.429
J139	St. Francis	Soybean	35.046	90.401
J140	St. Francis	Soybean	35.000	90.818
J141	St. Francis	Soybean	34.944	90.790

specifically in the United States and Argentina, where GR cultivars currently represent greater than 90% of the planted soybean acreage (Duke and Powles 2009). The weed management difficulties that growers had grown accustomed to before commercialization of GR crops were resolved by the in-crop use of glyphosate in soybean (Johnson et al. 2013). Extensive use of glyphosate-based weed control programs and a lack of diversity in weed management programs eventually led to the evolution of glyphosate resistance. Since the commercialization of GR crops, weed species have evolved resistance to glyphosate at an alarming rate. Currently, 24 weed species with evolved GR exist in 19 countries, including 14 species in the United States (Heap 2013).

Johnsongrass has long been considered one of the most competitive and troublesome weeds in crop production (Buchanan 1974; Elmore 1983; Webster and Coble 1997); albeit, in a recent survey, johnsongrass was listed as only the 18th most troublesome weed of soybean in the southern United States (Webster and Nichols 2012). This drastic reduction in importance can be attributed to the widespread adoption of GR crops and the efficacy of glyphosate on this weed (Webster and Nichols 2012). Most recently, the concern over this once troublesome and difficult-to-control weed has been brought to the forefront because of evolved GR (Norsworthy et al. 2008; Vila-Aiub et al. 2007). In 2005, the first incidence of GR johnsongrass occurred in the Salta Province of Argentina, when erratic control of johnsongrass with glyphosate was observed in soybean fields and fallowed ground. Further evaluation revealed that the glyphosate dose required to kill 50% of the suspected GR population was 3.5- to 10.5-fold that of a known susceptible biotype (Vila-Aiub et al. 2007). In the fall of 2007, glyphosate failed to control a population of johnsongrass effectively in a soybean field near West Memphis, AR, where glyphosate had been solely relied upon for weed control for several years (Norsworthy et al. 2008; Riar et al. 2011). This was the first confirmed case of GR johnsongrass in the United States, but confirmations in neighboring states (Mississippi 2008 and Louisiana 2010) soon followed (Heap 2013).

With resistance confirmed in johnsongrass to glyphosate and ACCase- and ALS-inhibiting herbicides in Arkansas and surrounding states (Burke et al. 2006; Heap 2013; Smeda et al. 1997), it was suspected that additional accessions of herbicideresistant johnsongrass might exist in Arkansas. However, the extent to which johnsongrass resistant to glyphosate and ACCase- and ALS-inhibiting herbicides occur in Arkansas is unknown. The objective of this research was to determine the geographical distribution of herbicide-resistant johnsongrass in Arkansas crops.

## Materials and Methods

**Collection and Plant Materials.** Johnsongrass panicles were collected from 141 agricultural fields in 14 Arkansas counties in the fall of 2008, 2009, and 2010 (Table 1; Figure 1). The number of

panicles collected was dependent on the number of plants present in a field. For instance, some fields had as few as a single clump of johnsongrass from which approximately five panicles were collected. For fields having a dense population of johnsongrass, as many as 30 to 40 panicles were collected.

A handheld global positioning system was used to record the coordinates for each sampling site. Accessions (samples) were designated as J (johnsongrass) and given a number value (1-141) (Bond et al. 2006; Norsworthy et al. 2008; Wise et al. 2009). Sites were chosen based solely on johnsongrass presence at the time of sampling in the early fall. It was not known at the time of collection what herbicides the plants had been exposed to, whether plants were present when herbicide applications occurred, or whether emergence occurred after the last herbicide application. After a 7-d drying period in a greenhouse (32/22 C day/night temperatures), seed were threshed from the panicles and combined into a single composite sample for each accession. Seed samples for accessions were stored at room temperature for approximately 4 mo to overcome seed dormancy (Harrington 1916; Taylorson and Brown 1977). Seed from each accession along with a known susceptible biotype (Azlin Seed Company, 112 Lilac Drive, Leland, MS 38756) were sown in 10-cm-diam pots containing a commercial potting media (professional growing mix, LC1 Mix, Sun Gro Horticultural Distribution Inc., Bellevue, WA 98008). After seeding, pots were placed in a greenhouse under conditions of  $32/22 \pm 3$  C day/night temperatures with a 16-h photoperiod consisting of natural light supplemented by a metal halide lighting system. All pots were irrigated as needed on a daily basis and fertilized weekly to ensure good plant growth.

The experiment was conducted in a randomized complete block design with four replications per treatment and five plants per replication for a total of 20 plants per treatment. Herbicide treatments were applied to two- to three-leaf seedling johnsongrass plants that were approximately 8- to 12-cm tall. Herbicide treatments consisted of four of the most commonly used herbicides for johnsongrass control, which represents three herbicide MOAs. A nontreated control from each accession was also included. Johnsongrass accessions were screened for resistance to glyphosate at 420 g ae ha<sup>-1</sup> (Weed Science Society of America [WSSA] group 9),

imazethapyr at 70 g ai ha $^{-1}$  (WSSA group 2), and clethodim and fluazifop at 68 and 210 g ai ha $^{-1}$ (WSSA group 1). Imazethapyr was chosen over other ALS-inhibiting herbicides because of the overwhelming number of samples recovered from soybean fields and the widespread use of imazethapyr in soybean weed control programs before commercialization of GR soybean. Applications of clethodim and fluazifop included a crop oil concentrate (Agri-Dex, Helena Chemical Co., West Helena, AR 72390) at 1% (v/v), and imazethapyr included a nonionic surfactant (NIS) (Induce, Helena Chemical Co., West Helena, AR 72390) at 0.25% (v/v), as suggested by their respective product labels. Treatments were applied inside of a stationary spray chamber with a boom containing two flat-fan 800067 nozzles (Teejet Technologies, Springfield, IL 62703) calibrated to deliver 187 L  $ha^{-1}$  at 276 kPA. After treatments were applied, pots were returned to the greenhouse.

Visual estimates of johnsongrass control were taken 14 and 21 d after treatment (DAT). Johnsongrass control was evaluated by comparing treated plants to the nontreated control for each accession. Control was assessed on a scale of 0 (no control) to 100% (complete plant mortality). Biomass of living plants was harvested immediately after the 21 DAT control evaluation. Plant shoots were clipped at the soil surface and oven-dried at 66 C for 7 d, and dry weights were determined for surviving plants. For each johnsongrass accession, dry weights of treated plants were converted to a percent dry weight reduction relative to the nontreated control for each accession.

Visual estimates of johnsongrass control and percent dry weight reduction data were subjected to ANOVA using PROC GLM in SAS (SAS, version 9.3, SAS Institute Inc., P.O. Box 8000, Cary, NC 25712). Means were separated using Tukey's honest significant difference (HSD) at a 5% level of significance. The principal objective of the experiment was to compare the response of accessions to each herbicide treatment. Therefore, accession was considered as a fixed effect, and replication was analyzed as a random effect. Accessions having 100% control or biomass reduction across all replications were excluded from the analysis.

**Dose Response.** Results of the initial screening experiment revealed that the J12 accession exhibited



Figure 1. A total of 141 Johnsongrass accessions were collected from 14 Arkansas counties (counties shaded in gray) in the Mississippi River Delta.

potential resistance to glyphosate, and the J14 accession also appeared to have reduced sensitivity to imazethapyr compared with the susceptible standard. A rate titration experiment was established to further evaluate the response of the J12 accession to glyphosate and the J14 accession to imazethapyr. Seeds from each accession and a susceptible biotype were sown in separate 45- by 60-cm trays. At the cotyledon to one-leaf stage, johnsongrass seedlings from each biotype were transplanted into 10-cmdiam pots containing potting mix. After transplanting, plants were returned to a greenhouse under the same environmental conditions as described in the previous experiment.

Seedlings of the susceptible biotype were treated with nine rates of MON 78623 (potassium salt of glyphosate), ranging from 7 to 1,680 g ae ha<sup>-1</sup>. The rate range corresponds to 1/128 to 2 times the recommended glyphosate rate of 840 g ha<sup>-1</sup>. For the J12 accession, three additional rates of 3,360, 6,720, and 13,440 g ha<sup>-1</sup> were also added. In the imazethapyr dose–response study, the susceptible biotype was exposed to rates ranging from 0.5 to 140 g ha<sup>-1</sup>, which corresponds to 1/128 to 2 times the recommended rate of 70 g ha<sup>-1</sup>. Three additional rates of 280, 560, and 1,120 g ha<sup>-1</sup> were included for the J14 accession. Applications of MON 78623 and imazethapyr contained NIS at 0.25% (v/v). Treatments were applied once seedlings had reached the two- to three-leaf stage (8 to 12 cm tall). Spray applications were made at 187 L ha<sup>-1</sup> in a stationary spray chamber with a boom containing two flat fan 800067 spray nozzles. Upon completion of all treatments, plants were returned to the greenhouse. The experiment was arranged in a completely random design with 20 replications biotype<sup>-1</sup> herbicide<sup>-1</sup> dose, and the experiment was repeated. Plant survival (live or dead) counts were taken 21 DAT. A plant was considered to be alive if at least one green leaf was present. Plant mortality data were subjected to probit analysis using JMP (Version 10, SAS Institute Inc.) to determine the  $LD_{50}$  from each biotype along with 95% confidence intervals. Means for the  $LD_{50}$  were separated using a chi-square probability test (P = 0.05).

## **Results and Discussion**

**Johnsongrass Screening.** Overall, the herbicides evaluated in this experiment were generally effective for controlling the accessions tested. At 21 DAT, average johnsongrass control across accessions was 97% for glyphosate, 95% for imazethapyr, and 99% for both fluazifop and clethodim. These herbicides yielded 100% control of most accessions evaluated. However, control across accessions varied for each herbicide; therefore, only data for accessions that were not completely controlled 21 DAT are presented. Additionally, these were the only treatments included in the analysis.

*Glyphosate*. Glyphosate at 420 g ha<sup>-1</sup>, one-half of the registered field use rate of 840 g ha<sup>-1</sup>, completely controlled 95 of the 141 accessions tested (Table 2). The 46 accessions that were not completely controlled varied in response to glyphosate. Visual estimates of control of all accessions ranged from 85 to 99%, and all but six accessions were controlled 90% through 21 DAT (Table 2). Shaw and Arnold (2002) reported greater than 93% control of seedling johnsongrass with glyphosate at 280 to 1,120 g ha<sup>-1</sup>. However, glyphosate did not effectively control the J12 accession. At 21 DAT, glyphosate resulted in only 20% control of the J12

Table 2. Response and percent dry weight reductions of 46 johnsongrass accessions 21 d after a POST application of glyphosate at 420 g ae  $ha^{-1.a}$ .

Accession <sup>b</sup>	Control	Dry weight reduction
		– % of nontreated —
I1	95	96
I4	96	97
19	97	97
J10	98	98
I11	94	95
J12	20	47
I13	94	96
J14	93	92
J17	99	98
120	97	96
I21	96	95
I23	99	99
I24	97	98
I28	97	96
136	96	95
140	99	98
I41	96	95
I43	98	96
149	98	98
150	96	95
153	91	90
J62	98	98
I68	86	90
170	95	94
I72	95	96
J74	98	98
J76	92	97
J78	88	88
J79	89	89
180	91	91
J82	99	99
J83	96	94
J84	99	99
J85	92	93
J86	98	97
J90	90	93
J93	86	88
J95	95	95
J98	99	98
J106	85	86
J109	92	90
J114	98	98
J117	96	94
J120	93	92
J127	97	98
J141	91	93
HSD <sup>c</sup>	12	11

<sup>a</sup> Herbicide applied to two- to three-leaf seedling johnsongrass.

<sup>b</sup> A total of 141 accessions were evaluated (J1–J141). Accessions that were completely controlled (100% mortality) are not presented and were excluded from the analysis.

 $^{\rm c}$  Tukey–Kramer honest significant difference (HSD) means separation within column (P < 0.05).

Table 3. Response and percent dry weight reductions of 67 johnsongrass accessions 21 d after a POST application of imazethapyr at 70 g ai  $ha^{-1.a}$ .

Accession <sup>b</sup>	Control	Dry weight reduction
		— % of nontreated ———
J1	90	92
I3	97	96
I5	90	92
I9	94	95
I10	92	92
I11	87	92
I12	87	89
I13	94	93
I14	64	14
I17	83	89
I19	97	97
120	89	89
J20 I21	94	94
122	94	93
123	93	94
123	91	93
128	90	91
129	92	95
J2) I31	93	91
132	92	94
133	90	90
136	88	88
138	97	97
J90 I40	96	92
J 10 I 4 1	89	92
J43	90	88
J 15 146	98	97
J 10 I 47	91	91
J=7 I50	86	90
154	98	96
156	97	97
J50 I61	91	92
162	94	94
J62 I65	95	94
166	90	92
168	81	87
J00 I70	89	91
J70 I72	84	90
J72 I76	87	89
J70 I78	87	89
J70 I70	87	80
J7 J 183	94	92
185	88	92 80
187	85	80
107	86	88
180	81	82
100	01 Q2	02 99
105	03	00
177 108	07	71 02
J70 I102	74 02	73 07
J105 J106	00 / 0	00 00
J100 I107	04	00
J10/ I100	۵۶ ۰ /	20
1109	ð4	89

Table	3.	Continued.
1 abic	5.	Continued.

Accession <sup>b</sup>	Control	Dry weight reduction
J111	88	89
J115	94	94
J117	90	91
J120	84	87
J121	93	93
J123	83	85
J124	88	87
J125	95	93
J126	96	96
J127	96	94
J131	91	91
J136	92	93
J137	98	98
J138	97	96
HSD <sup>c</sup>	12	11

<sup>a</sup> Herbicide applied to two- to three-leaf seedling johnsongrass. <sup>b</sup> A total of 141 accessions were evaluated (J1–J141). Accessions that were completely controlled (100% mortality) are not presented and were excluded from the analysis.

 $^{\rm c}$  Tukey–Kramer honest significant difference (HSD) means separation within column (P < 0.05).

accession, the lowest level of control of all accessions evaluated.

Dry weight reduction across accessions was similar to visual estimates of weed control observed for each accession. Much like control estimates, the lowest level of biomass reduction was observed with the J12 accession (Table 2). Applications of glyphosate at 420 g ha<sup>-1</sup> reduced dry matter production of the J12 accession 47% when compared with the nontreated 21 DAT. Minimal differences in dry weight reduction existed among other accessions, with biomass reduced at least 86%.

*Imazethapyr.* Of the four herbicides evaluated, imazethapyr was the least effective and most inconsistent. Imazethapyr at the highest application rate (70 g ha<sup>-1</sup>) provided complete control of 52% of the accessions evaluated. Response was variable among accessions for which imazethapyr failed to provide complete control 21 DAT (Table 3). Accession J14 exhibited the least sensitivity to imazethapyr compared with all other accessions, resulting in 64% control 21 DAT. Excluding J14, greater than 90% control was achieved for 39 accessions, whereas the remaining 28 accessions were controlled 81 to 89%.

In Mississippi, Riley and Shaw (1989) reported no greater than 50% control of 8- to 12-leaf

Table 4. Response and percent dry weight reduction of 10 johnsongrass accessions 21 d after a POST application of clethodim at 68 g ai  $ha^{-1}a^{a}$ .

Accession <sup>b</sup>	Control	Dry weight reduction
		%
J17	90	91
J24	94	92
J49	91	96
J54	95	91
J61	91	92
J65	93	93
J102	90	91
J103	95	95
J126	91	92
J140	89	88
HSD <sup>c</sup>	NS	6

<sup>a</sup> Herbicide applied to two- to three-leaf seedling johnsongrass.

<sup>b</sup> A total of 141 accessions were evaluated (J1–J141). Accessions that were completely controlled (100% mortality) are not presented and were excluded from the analysis.

 $^{\rm c}$  Tukey–Kramer honest significant difference (HSD) means separation within column (P < 0.05).

rhizome johnsongrass with imazethpyr applied at 70 and 105 g ha<sup>-1</sup>. In a separate field study, Shaw et al. (1990) reported less than 75% control of 15- to 60cm rhizome johnsongrass with imazethapyr applied at 70 g ha<sup>-1</sup>. In this experiment, imazethapyr was evaluated for control of seedling johnsongrass, hence the increased control. Furthermore, the johnsongrass in the above-mentioned research was larger at application than the plants treated in this research, hence the lower control observed. Efficacy of foliar herbicides has previously been reported to vary between seedling and rhizomatous johnsongrass plants, with control of seedlings often greater (Rosales-Robles et al. 1999). Inadequate translocation of herbicides allows the rhizomes to survive and regrow after applications of most POST-applied herbicides (Kells and Rieck 1979; Sprankle et al. 1975).

Dry weight reduction among accessions deviated only slightly from the levels of control observed for each accession. Twenty-one d after application, imazethapyr rendered only 14% dry matter reduction of accession J14 (Table 3). Dry weight reduction among other accessions ranged from 82 to 97%.

*Clethodim.* The johnsongrass accessions evaluated in this experiment were highly responsive to clethodim at 68 g ha<sup>-1</sup>. Across accessions, clethodim was the

most consistent and efficacious herbicide, completely controlling 131 of the 141 accessions. No differences in control were observed among accessions, with at least 89% control of all accessions achieved (Table 4). The results of this experiment are similar to those of previous research, in which it was concluded that clethodim, applied across a range of rates and johnsongrass sizes, is an effective johnsongrass control option (Bridges 1989; Jordan et al. 1996; Rosales-Robles et al. 1999).

The efficacy of clethodim on all accessions in this experiment also resulted in effective dry weight reduction compared with that of the nontreated control. Percentage dry weight reduction among accessions ranged from 88 to 96%. With the exception of accession J140, no differences could be detected for dry weight reduction among the other accessions evaluated.

Fluazifop. Fluazifop was second to clethodim in providing the most consistent and effective control of johnsongrass across accessions. Fluazifop at 210 g ha<sup>-1</sup>, the registered field use rate, provided complete (100%) control of 121 of the 141 accessions. Control of the accessions in which complete control was not obtained ranged from 83 to 97% (Table 5). Of the accessions not completely controlled, fluazifop achieved at least 90% control of all but five accessions. Control of J92 was significantly lower 21 DAT than accessions that were controlled greater than 93%, which was exactly half of the accessions (Table 5). Accession J92 was less responsive to fluazifop than most accessions evaluated, including the susceptible standard. Therefore, it is hypothesized that this accession may have a low level of resistance to fluazifop. However, because of a shortage of seed, this accession was unable to be subjected to any further evaluation to test our hypothesis.

Bridges and Chandler (1987) observed johnsongrass control of 95 and 83% over a 2-year period with fluazifop, and in two separate studies conducted by Banks and Bundschuh (1989) and Brewster and Spinney (1989), greater than 90% johnsongrass control was reported with fluazifop. The results of this experiment were also similar to that observed by Shaw et al. (1990), who reported johnsongrass control ranged from 83 to 90% when fluazifop was applied to 15- to 60-cm-tall plants.

Dry weight reduction for all accessions after an application of fluazifop was at least 87% 21 DAT

Table 5. Response and percent dry weight reduction for 20 johnsongrass accessions 21 d after a POST application of fluazifop at 210 g ai  $ha^{-1}a$ .

Accession <sup>b</sup>	Control	Dry weight reduction
		0/
J13	94	94
J14	96	95
J16	86	91
J19	97	95
J24	95	94
]36	91	91
J41	90	91
J46	97	93
J49	93	93
J54	92	88
J61	93	93
J68	95	96
J89	85	87
J92	83	87
J93	87	91
J109	90	93
J117	91	91
J123	96	94
J126	88	89
J130	94	91
HSD <sup>c</sup>	9	6

<sup>a</sup> Herbicide applied to two- to three-leaf seedling johnsongrass.

 $^{\rm b}$  A total of 141 accessions were evaluated (J1–J141). Accessions that were completely controlled (100% mortality) are not presented and were excluded from the analysis.

 $^{\rm c}$  Tukey–Kramer honest significant difference (HSD) means separation within column (P < 0.05).

(Table 5). However, with the exception of accessions J54, J89, J92, and J126, dry weight reduction of all accessions was at least 91%.

**J12 Dose Response.** The probability of death for increasing doses of glyphosate for the J12 accession and susceptible biotype are shown in Figure 2. A glyphosate dose of 205 g ha<sup>-1</sup> was needed to kill 50% of the susceptible plants. The LD<sub>50</sub> value for the susceptible plants is less than one-quarter times the registered field use rate for glyphosate. The LD<sub>95</sub> values indicate that 95% mortality of the susceptible biotype is achieved at 363 g ha<sup>-1</sup>, which is less than one-half times the recommended field rate for weed control in cotton, corn, and soybean (Anonymous 2013a). Mortality of all susceptible plants was achieved with less than the registered field use rate of 840 g ha<sup>-1</sup>.

The  $LD_{50}$  value of glyphosate for the putative resistant J12 accession was 1,741 g ha<sup>-1</sup>, an 8.5-fold increase in glyphosate dose compared with the



Figure 2. Probit analysis (y = a + bX + e) with 95% confidence intervals (dotted lines) to predict the lethal dose (dashed line = susceptible, solid line = J12 accession) of glyphosate needed to kill the susceptible biotype and the J12 accession.

susceptible biotype (Figure 2; Table 6). To achieve 95% mortality of the J12 accession, glyphosate would have to be applied at 4,069 g ha<sup>-1</sup>, which is an 11-fold increase over the susceptible biotype (Table 6), and five times the labeled field use rate of 840 g ha<sup>-1</sup>. Complete control of all treated plants of the J12 accession was accomplished at a glyphosate rate six times the labeled use rate; hence, it is concluded that the J12 accession exhibits resistance to glyphosate. Interestingly, the J12 accession was collected in Crittenden County approximately 12 km north of where the first glyphosate-resistant

Table 6. Dose needed to kill 50% of accessions J12 (glyphosate) and J14 (imazethapyr) compared with a susceptible standard.

Accession	LD <sub>50</sub>	LD <sub>95</sub>
Glyphosate <sup>a,b</sup>	g ae 1	ha <sup>-1</sup>
J12	1,741 a	4,069 a
Susceptible	205 b	363 b
R/S ratio <sup>c</sup>	8.5	11.2
Imazethapyr <sup>a,b</sup>	——— g ai l	ha <sup>-1</sup>
J14	73 a	282 a
Susceptible	20 b	36 b
R/S ratio <sup>c</sup>	3.7	7.8

 $^a$  LD\_{50} and LD\_{95} doses within a column followed by different lowercase letters are statistically different at P  $\leq$  0.05.

 $^{\rm b}$  LD\_{50} and LD\_{95} values were determined by probit analysis in JMP.

<sup>c</sup> R/S ratio was calculated by dividing the  $LD_{50}$  or  $LD_{95}$  of the resistant biotype by the  $LD_{50}$  or  $LD_{95}$  of the susceptible biotype.



Figure 3. Probit analysis (y = a + bX + e) with 95% confidence intervals (dotted lines) to predict the lethal dose (dashed line = susceptible, solid line = J14 accession) of imazethapyr needed to kill the susceptible biotype and the J14 accession.

johnsongrass population was confirmed in Arkansas (Norsworthy et al. 2008).

J14 Dose Response. The probability of death for progressing doses of imazethapyr is displayed in Figure 3. The LD<sub>50</sub> value for imazethapyr for the susceptible biotype was 20 g ha<sup>-1</sup>, which is approximately one-quarter times the recommended field use rate of 70 g ha<sup>-1</sup> (Anonymous 2013b). For the susceptible biotype, greater than 95% mortality was achieved at 36 g ha<sup>-1</sup>, which is approximately one-half of the recommended field-use rate. Based on the dose–response curve for the susceptible biotype, 99% mortality was predicted at 43 g ha<sup>-1</sup>.

For the J14 accession, the LD<sub>50</sub> value was 73 g ha<sup>-1</sup>, equivalent to a 3.7-fold reduction in sensitivity to imazethapyr compared with the susceptible biotype (Table 6). The LD<sub>95</sub> value of 282 g ha<sup>-1</sup> of imazethapyr was a 7.8-fold increase over the susceptible biotype. Based on these differences between biotypes and the inability to achieve effective control of J14 at the recommended field use rate (Figure 2), it is concluded that this accession exhibits resistance to imazethapyr.

With the previous documentation of ALSresistant johnsongrass in recent decades and the overwhelming dependence on glyphosate for weed control in virtually all U.S. cropping systems, it comes as no surprise that johnsongrass continues to exhibit resistance to the most frequently used herbicides (Riar et al. 2011). With the extensive use of ALS-inhibiting herbicides, not only in soybean but other economically important row crops, the existence of only 1 of 141 accessions exhibiting resistance to this mechanism of action was somewhat surprising. Furthermore, the massive acreage of glyphosate-resistant crops that occurred in response to the commercialization of glyphosateresistant crop technology in 1996 and exclusive reliance on glyphosate has led to resistance to glyphosate in johnsongrass and numerous other species worldwide (Heap 2013). Herbicide-resistant johnsongrass is not currently widespread in Arkansas, and in general, control can be achieved with glyphosate and ALS- and ACCase-inhibiting herbicides. However, with resistance to glyphosate and the ALS-inhibiting herbicides existing within the state and resistance to the ACCase-inhibiting herbicides existing in neighboring states, appropriate management tactics should be employed to ensure long-term sustainability of these herbicides.

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