

## Research Article

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# Survey of glyphosate-resistant junglerice (*Echinochloa colona*) accessions in dicamba-resistant crops in Tennessee

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**Abstract**

Junglerice has become a major weed in Tennessee cotton and soybean fields. Glyphosate has been relied on to control these accessions over the past two decades, but in recent years cotton and soybean producers have reported junglerice escapes after glyphosate + dicamba and/or clethodim applications. In the growing seasons of 2018 and 2019, a survey was conducted of weed escapes in dicamba-resistant (DR) crops. Junglerice was the most prevalent weed escape in these DR (Roundup Ready Xtend<sup>®</sup>) cotton and soybean fields in both years of the study. In 2018 and 2019, junglerice was found 76% and 64% of the time in DR cotton and soybean fields, respectively. Progeny from junglerice seeds collected during this survey was screened for glyphosate and clethodim resistance. Seventy percent of the junglerice accessions tested had an effective relative resistance factor to glyphosate of 3.1 to 8.5. In all, 13% of the junglerice accessions could no longer be effectively controlled with glyphosate. This research also showed that all sampled accessions could still be controlled with clethodim in a greenhouse environment, but less control was observed in the field. These data also suggest that another cause for the poor junglerice control is dicamba antagonism of glyphosate and clethodim activity.

**Introduction**

In Tennessee and other states in the midsouthern United States, junglerice and Palmer amaranth (*Amaranthus palmeri* S. Watson) are the two most troublesome weeds in cropping systems (Van Wychen 2020). Junglerice is a hexaploid, annual species (Gould et al. 1972; Yabuno 1966); that is an important weed in rice [*Oryza sativa* (L.)] production along with other agronomic cropping systems across the world (Bakkali et al. 2007; Holm et al. 1991; Valverde et al. 2000). Other *Echinochloa* spp. also can be found in Tennessee and include barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.], rice barnyardgrass [*E. phyllopogon* (Stapf) Koso-Pol.], and rough barnyardgrass [*E. muricata* (P. Beauv.) Fernald] (USDA 2020a; V. Maddox, Mississippi State University, personal communication).

Junglerice has a long-documented history of developing resistance to herbicides, including to fenoxaprop-P-ethyl (an acetyl coenzyme A carboxylase; WSSA Group 1), imazamox (an acetolactate synthase inhibitor; WSSA Group 2), quinclorac (an auxin mimic; WSSA Group 4), and propanil (a photosystem II inhibitor; WSSA Groups 5, 6, and 7) (Wright et al. 2018). The acetolactate synthase- and acetyl coenzyme A carboxylase-inhibitor resistances in this biotype have been confirmed as being nontarget site mechanisms of resistance (Chen et al. 2018; Heap 2020; Riar et al. 2013; Wright et al. 2016).

Glyphosate is the most widely used herbicide globally (Duke and Powles 2008) because of its high efficacy, broad-spectrum control and systemic mode of action (Duke et al. 2018). However, resistance to glyphosate has evolved in numerous species, including *Echinochloa*, found in glyphosate-resistant cropping systems, no-till chemical fallow areas, fence lines, and perennial crop situations (Gaines et al. 2012). The primary mechanism of action for glyphosate is the inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a key enzyme in the shikimate pathway (Steinrücken and Amrhein 1980). Glyphosate blocks the shikimate pathway, resulting in accumulation of high levels of shikimate, a decline in carbon fixation intermediates, and reduction in photosynthesis, which results in plant death (Duke et al. 2003; Duke and Powles 2008). Since the first reports of glyphosate resistance (Powles 2008; Pratley et al. 1999), 42 weed species have evolved glyphosate resistance globally (Heap 2020).

Argentina and Australia had the first reported cases of glyphosate-resistant junglerice (Gaines et al. 2012; Heap 2020). Nandula et al. (2018) confirmed glyphosate-resistant junglerice in Mississippi and Tennessee. Accessions from Mississippi had a mutation at the 106th locus of the EPSPS protein, resulting in replacement of proline for serine (Nandula et al. 2018). The

junglerice population in Tennessee had a reduced translocation mechanism of resistance to glyphosate. The hypothesis for this reduced glyphosate translocation model, proposed by Shaner (2009), is that there exists a barrier at the cellular level that prevents glyphosate from loading into the phloem. Alternatively, glyphosate could possibly be loaded into the vacuoles via a system similar to the sequestration mechanism described in Canadian horseweed [*Conyza canadensis* (L.) Cronquist] (Ge et al. 2010) and *Lolium* spp. (Ge et al. 2012).

The aforementioned glyphosate accessions from Mississippi and Tennessee are 4- and 7-fold resistant to glyphosate, respectively (Nandula et al. 2018). Gaines et al. (2012) reported a resistant population in Australia that was 8.6-fold resistant compared with a susceptible population. Another population in California was reported to be 6.6-fold resistant to glyphosate compared with a susceptible population (Alarcón-Reverte et al. 2013). A different Mississippi population has been reported to be resistant to imazamox, fenoxaprop-*p*-ethyl, quinclorac, and propanil, but not glyphosate (Wright et al. 2016, 2018). In addition, there is an increasing occurrence of multiple resistance in Arkansas, predominantly in junglerice (Rouse et al. 2018). Very high resistance levels of junglerice to quinclorac and propanil, and low-level resistance to cyhalofop have also been reported in Arkansas, due to nontarget-site resistance mechanisms (Rouse et al. 2019). The documented resistance in junglerice suggests an increasing management problem that requires attention to herbicide stewardship and design of effective management strategies.

Herbicides such as glyphosate, clethodim, sethoxydim, and quizalofop provide junglerice and barnyardgrass control in soybean and cotton (Jordan 1995; Sikkema et al. 2005; Vidrine et al. 2010). It is important to manage these herbicides and herbicide classes properly to minimize the risk of evolving further herbicide resistance. Any herbicide recommendation resulting in antagonism between two herbicide products is not an effective resistance management strategy (Norsworthy et al. 2012). Tennessee producers often use tank mixtures of glyphosate and dicamba. However, many are reporting more weed escapes from this tank mix in recent years (L.E. Steckel, personal communication).

Dicamba antagonism of glyphosate for grass control has been previously documented (Flint and Barrett 1989; Harre et al. 2020; O'Sullivan and O'Donovan 1980) and could be the reason for junglerice escapes in Tennessee cotton and soybean crops. In addition, researchers have also reported dicamba antagonism of clethodim for control of grass in soybean (Harre et al. 2020). This, coupled with the new use pattern in dicamba-resistant (DR) soybean and cotton where dicamba + glyphosate is used POST in-crop, could be a factor in the poor junglerice control. There are reports that this new use pattern for dicamba is being extensively adopted in the United States (USDA 2020b). Wechsler et al. (2019) reported that in 2018, 71% of soybean acres were planted to DR soybean, with more than 21.7 million kg of dicamba used in the United States in this crop. The U.S. Department of Agriculture reported that, in 2019, more than 95% of the cotton planted in Tennessee was to DR varieties (USDA 2020b).

We conducted a survey in 2018 and 2019 to (1) assess the frequency of junglerice accessions across Tennessee, (2) evaluate if dicamba antagonism of glyphosate is a reason for junglerice escapes, (3) determine if these junglerice escapes were evolving

**Table 1.** Accessions screened for glyphosate and clethodim resistance in Tennessee.

Population no.	Year	Site name	GPS coordinates	County	State
1	2019	Bradshaw	35.94°N, 89.26°W	Dyer	TN
2	2019	Sweeny Ridge	36.03°N, 89.53°W	Dyer	TN
3	2019	Tigertail C field	35.95°N, 89.57°W	Dyer	TN
4	2019	Ireland	33.47°N, 91.04°W	Washington	MS
5	2019	5JF	33.54°N, 90.09°W	Leflore	MS
6	2019	Smithtown 1	35.78°N, 85.92°W	Warren	TN
7	2019	Smithtown 3	35.79°N, 85.92°W	Warren	TN
8	2019	Sorrell	35.97°N, 89.34°W	Dyer	TN
9	2008	Susceptible check	From Azlin Seed Service	Washington	MS
10	2017	Susceptible check	From Azlin Seed Service	Washington	MS
11	2018	Nichols	36.17°N, 89.41°W	Dyer	TN
9	2008	Susceptible check	From Azlin Seed Service	Washington	MS
10	2017	Susceptible check	From Azlin Seed Service	Washington	MS
14	2018	Kelly Cotton	35.57°N, 89.54°W	Tipton	TN
15	2018	Knobcreek	35.52°N, 89.33°W	Haywood	TN
16	2018	Sneed 385	35.31°N, 89.80°W	Shelby	TN
17	2018	Allen	35.60°N, 89.58°W	Tipton	TN
18	2018	Sneed Rock Pile	35.28°N, 89.85°W	Shelby	TN
19	2018	Lannom	36.15°N, 88.81°W	Weakley	TN
20	2018	Milan	35.93°N, 88.72°W	Gibson	TN

<sup>a</sup>Abbreviation: GPS, global positioning system.

resistance to clethodim, and (4) to document other weed escapes in DR crops.

## Materials and Methods

### Survey

Junglerice in 108 grower-managed soybean and cotton fields was surveyed across west and middle Tennessee in 2018 and 2019. The survey was conducted as previously described by Copeland et al. (2018). Briefly, the locations for seed collection were identified by visually observing junglerice presence in the field where known dicamba + glyphosate herbicide applications were made and control failures were evident. Each population was numbered and given a corresponding site name, and information was recorded regarding global positioning system coordinates, county, and state from where the population was collected (Table 1). Because of the limited germination rate of the junglerice and number of seeds needed, only eight accessions were chosen for each year represented in the screening process.

Because greater than 95% of the cotton acreage and 70% of the soybean planted in Tennessee in these years had the DR trait (Roundup Ready Xtend<sup>®</sup>; Bayer Crop Sciences, St. Louis, MO) (USDA 2020b; Wechsler et al. 2019), these were the fields on which this survey was focused. The majority of the fields were selected because of weed control failures or after grower/consultant consultation. Approximately 200 mature junglerice seed heads were collected from each field, placed in plastic bags, and stored in a freezer at -20 C until ready for screening. Other weed species observed in these fields were included in the survey, but seeds of those plants were not collected.

**Table 2.** Weed survey in Tennessee dicamba-resistant cotton and soybean fields from 2018 and 2019.

Year	Weed species surveyed							Total fields
	Palmer amaranth <sup>a</sup>	Junglerice	Barnyardgrass	Johnsongrass	Goosegrass	Fall panicum	Waterhemp	
				%				No.
2018	NR	76	33	NR	3	12	NR	33
2019	50	64	49	25	3	11	11	75

<sup>a</sup>Abbreviation: NR, data not recorded for this species.

### Population Screening

Seeds from junglerice accessions collected were sent to the Syngenta Crop Protection laboratory (Vero Beach, FL). Approximately 50 to 75 plants (sufficient to screen for both glyphosate and clethodim resistance) were acquired from eight non-repeated accessions each year. Similar surveys have been conducted to characterize protoporphyrinogen oxidase-resistant Palmer amaranth accessions in Arkansas and Tennessee (Copeland et al. 2018; Varanasi et al. 2018). A ninth population collected in 2008 and a tenth population collected in 2017, both from Washington County, MS, by Azlin Seed Service (Leland, MS), served as the susceptible check accessions because they were known to be controlled with glyphosate at a rate of 160 g ha<sup>-1</sup>.

Plants were grown in the greenhouse from these seeds and tested for glyphosate and clethodim resistance. Greenhouse air temperature was set at 24 to 27 C; relative humidity was 60%. The study consisted of two runs and we used a randomized complete block design with three replications of each population per treatment. Seeds were first planted in flats and then transplanted to 10-cm pots with 2 plants pot<sup>-1</sup>, using a 50:50 silt loam and potting soil premix. Glyphosate Roundup Custom (glyphosate; Bayer Crop Protection, St. Louis, MO.) (Monsanto Co. 2018) was applied at 30, 90, 300, 870, and 2,600 g ai ha<sup>-1</sup> (1/30×, 1/10×, 1/3×, 1×, and 3× the labeled rate, respectively). This formulation was chosen to remove the confounding effect of surfactant present in other formulations. Clethodim (Select Max; Valent U.S.A. LLC, Walnut Creek, CA) was applied at 3.5, 10.5, 35, 105, and 315 g ai ha<sup>-1</sup> (1/30×, 1/10×, 1/3×, 1×, and 3× the labeled rate, respectively) (Valent U.S.A. 2010). All rates were determined on the basis of the 1× use rate of a labeled application (Valent U.S.A. 2010; Monsanto Co. 2018). Applications were made at 142 L ha<sup>-1</sup> with an AIXR 11015 nozzle (Teejet Technologies, Louisville, KY). Treatments were applied in a Generation 4 Research Track Sprayer (DeVries Manufacturing, Inc., Hollandale, MN). The spray deck height was set to spray approximately 40 to 45 cm above the plants. All glyphosate treatments included N-Pak ammonium sulphate at 2.5% vol/vol plus 0.25% vol/vol nonionic surfactant (WinField United, Memphis, TN), and clethodim treatments included 1% vol/vol crop oil concentrate. Applications were made when junglerice was 7.5–10 cm tall.

In 2019 and 2020, to determine if dicamba was antagonizing glyphosate and clethodim junglerice control, a field study was initiated at a location (population 20) where the preliminary data suggested glyphosate would control the weeds at 870 g ha<sup>-1</sup>, but the population showed moderate tolerance (half-maximal effective concentration [EC<sub>50</sub>] of glyphosate = 600). The treatments evaluated were glyphosate at 870 g ha<sup>-1</sup> compared with glyphosate at the same rate plus dicamba at 560 g ha<sup>-1</sup>, and clethodim at 105 g ha<sup>-1</sup> compared with clethodim at the same rate plus dicamba at 560 g ha<sup>-1</sup>. Applications were made with a CO<sub>2</sub> backpack sprayer calibrated to apply 142 L ha<sup>-1</sup> with TTI 110015 nozzles.

### Data Analysis

Junglerice control was visually assessed on a scale of 0% to 100%, where 0% indicated no injury and 100% indicated plant death at 28 d after treatment. Biomass was measured 28 to 35 d after treatment. Each plant in individual pots was clipped at the soil level to record fresh weight. All data were subjected to ANOVA with appropriate mean separation techniques.

Nonlinear regression was used to describe the response of each junglerice population to an increasing rate of glyphosate and clethodim. A sigmoidal model, as suggested by Thornley and Johnson (1990), was used (Equation 1). In this model, parameter *a* describes the asymptote or upper limit of control; parameter *c* describes the EC<sub>50</sub>, the rate needed to achieve 50% control; and the parameter *b* estimates the slope:

$$Y = a/1 + \exp[-(\text{rate} - c)/b] \quad [1]$$

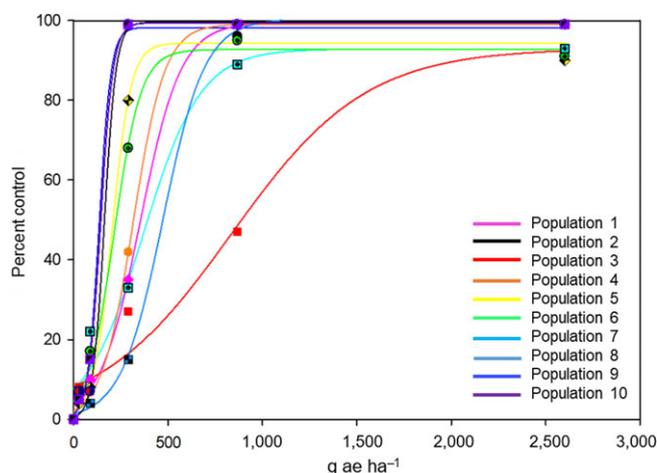
The estimate for each parameter was subjected to ANOVA using the PROC GLIMMIX procedure in SAS, version 9.4 (SAS Institute; Cary, NC). Each replication was considered a random effect in the model, because each EC<sub>50</sub> was designated as a fixed effect. Type III statistics were used to test the fixed effects and least square means were separated using the Fisher protected LSD at  $\alpha = 0.05$ . The relative resistance factor (RRF) was calculated by dividing the herbicide rate estimate that provided the EC<sub>50</sub> for the survey population by the EC<sub>50</sub> for the known susceptible population.

## Results and Discussion

### Survey

Junglerice was the most frequently found weed escape in these surveyed DR cotton and soybean fields in both years of the study (Table 2). In 2018 and 2019, junglerice was found 76% and 64% of the time, respectively. The second most commonly found weeds were barnyardgrass in 2018 and Palmer amaranth and barnyardgrass in 2019. Junglerice and barnyardgrass accessions were both present in 25% and 28% of the fields surveyed in 2018 and 2019, respectively (Table 2).

There were other notable weed escapes in 2019 in these DR cotton and soybean fields. Palmer amaranth was found in 50% of the fields, barnyardgrass in 49% of the fields, johnsongrass [*Sorghum halepense* (L.) Pers.] was found in 25% of the fields, fall panicum [*Panicum dichotomiflorum* Michx.] in 11%, tall waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] in 11%, and goosegrass [*Eleusine indica* (L.) Gaertn.] in 9% of the fields. Palmer amaranth and junglerice were the two most common weed species found. These results support the findings from a recent survey conducted by the Weed Science Society of America (Van Wychen 2020). Mixed accessions of broadleaf and grass weeds that are prone to



**Figure 1.** Glyphosate dose response by 10 accessions tested in 2019 from Tennessee. The responses of junglerice to increasing rates of glyphosate as described by Equation 1:  $Y = a/[1 + \exp[-(\text{rate} - c)/b]]$ . In this model,  $a$  describes the asymptote or upper limit of control,  $c$  describes the half-maximal effective concentration, and  $b$  estimates the slope. Dark blue (population 9) and purple (population 10) accessions were susceptible checks.

resistance development further reduce tools and tactics for weed management.

### Glyphosate-Resistance Screening Survey

The results of the 2019 survey showed that population 3 required 2,000 g ha<sup>-1</sup> glyphosate, or more than 2-fold greater than the standard label use rate, for 90% control (Figure 1). Accessions 5, 6, and 7 needed 870 g ha<sup>-1</sup> to obtain 90% control. Those accessions, along with accessions 2, 3, and 8, required five times more glyphosate to achieve 100% control than did the susceptible checks (accessions 9 and 10) (Table 3).

The results of the 2018 survey showed that nine of the 10 junglerice accessions surveyed could be controlled with the rates used in this study (Figure 2). However, population 18 was controlled 80% at 2,800 g ha<sup>-1</sup>, which was more than 3-fold the labeled rate. Accessions 17, 19, and 20 required 870 g ha<sup>-1</sup> to achieve better than 90% control, or approximately the standard labeled full rate (Monsanto Co. 2018). Even though those accessions would be controlled with the labeled 1× rate, it is notable that almost six times more glyphosate was needed to achieve 100% control than in the susceptible check accessions (Table 3).

### Half-Maximal Effective Concentration

In 2018, the EC<sub>50</sub> for the three most susceptible accessions (i.e., 9, 10, and 13) ranged from 110 to 160 g ae ha<sup>-1</sup> glyphosate (Table 3). Population 18 had the highest level of resistance (EC<sub>50</sub>, 1,230 g ae ha<sup>-1</sup>). This equates to an RRF of 8.5-fold, compared with the most susceptible accessions. Accessions 14, 15, 16, 17, and 19 were all similar, with EC<sub>50</sub> values ranging from 400 to 580 g ae ha<sup>-1</sup> glyphosate. These would equate to a 4- to 5-fold more resistance to glyphosate than the most susceptible accessions.

In 2019, population 3 showed the highest level of glyphosate resistance (EC<sub>50</sub> = 1,080 g ae ha<sup>-1</sup>), and had an RRF of 8 when

**Table 3.** Response of junglerice accessions to increasing rates of glyphosate in 2018 and 2019 in Tennessee.

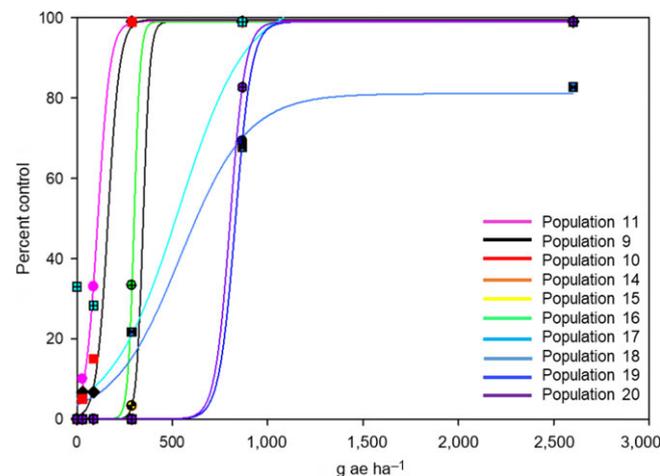
Year <sup>a</sup>	Population	EC <sub>50</sub> parameter		
		a <sup>b</sup>	estimate <sup>c</sup>	b ± SEM <sup>b,c</sup>
		%	g ha <sup>-1</sup>	g ha <sup>-1</sup>
2018	18	NA	1,230 a	184 ± 25 a
	19	99	680 b	17 ± 10 c
	20	99	680 b	15 ± 1 c
	17	99	580 bc	11 ± 3 c
	16	99	400 c	9 ± 1 c
	15	99	490 bc	11 ± 2 c
	14	99	490 bc	11 ± 2 c
	13	99	110 d	31 ± 2 b
	9 <sup>d</sup>	99	160 d	31 ± 3 b
	10 <sup>d</sup>	99	140 d	31 ± 1 b
F value		1.00	9.22	3.21
Df		9, 20	9, 18	9, 18
P value		0.474	<0.001	0.019
2019	3	102	1,080 a	440 ± 13 a
	8	100	470 b	110 ± 3 bc
	7	94	410 b	170 ± 10 b
	2	100	380 bc	110 ± 9 bc
	4	99	350 c	90 ± 9 cd
	6	93	230 d	80 ± 11 cd
	5	94	200 de	50 ± 5 cd
	9 <sup>d</sup>	99	160 ef	30 ± 3 d
	10 <sup>d</sup>	99	140 ef	30 ± 1 d
	1	98	130 f	30 ± 4 d
F value		0.53	5.96	5.07
Df		9, 20	9, 18	9, 18
P value		0.838	<0.001	0.002

<sup>a</sup>Abbreviations: Df, degrees of freedom; EC<sub>50</sub>, half-maximal effective concentration; NA, not applicable.

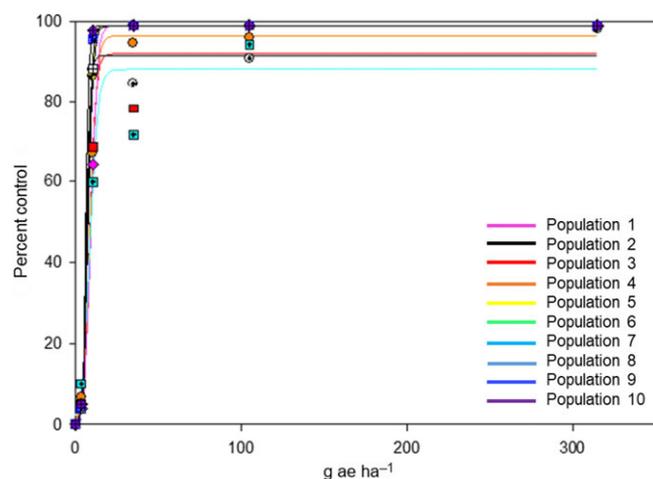
<sup>b</sup>In this model, the  $a$  parameter describes the asymptote or upper limit of control, the  $c$  parameter describes the EC<sub>50</sub>, and the  $b$  parameter estimates the slope.

<sup>c</sup>Means not followed by a common letter are significantly different ( $P < 0.05$ ).

<sup>d</sup>Accessions used as susceptible checks.



**Figure 2.** Glyphosate dose response of 10 junglerice accessions tested in 2018 in Tennessee. The responses of 10 accessions to increasing rates of glyphosate as described by Equation 1:  $Y = a/[1 + \exp[-(\text{rate} - c)/b]]$ . In this model,  $a$  describes the asymptote or upper limit of control,  $c$  describes the half-maximal effective concentration, and the  $b$  estimates the slope. Populations 9 (black line) and 10 (red line) accessions were the susceptible checks. Accessions 9 and 10 and 14 and 15 were similar and overlapped, resulting in the thicker black line at the top of the graph.



**Figure 3.** Clethodim dose response of 10 junglerice accessions tested in Tennessee in 2019. The responses of 10 accessions to increasing rates of clethodim as described by Equation 1:  $Y = a/[1 + \exp[-(\text{rate} - c)/b]]$ . In this model,  $a$  describes the asymptote or upper limit of control,  $c$  describes the half-maximal effective concentration, and  $b$  estimates the slope.

compared with susceptible accessions (i.e., 1, 9, and 10). Accessions 2, 7, and 8 had  $EC_{50}$  values of 380, 410, and 470, respectively, and an RRF ranging from 2.5 to 3.6. The RRF of 3.6–8.0 found in this survey would be similar to the 4- to 7-fold RRF reported by Nandula et al. (2018). Those authors reported 13% less glyphosate being transported out of the leaf in Tennessee accessions showing 4- to 7-fold more resistance. Accessions 5, 6, 4, and 2 had  $EC_{50}$  values of 200, 230, 350, and 380 g ae ha<sup>-1</sup>, respectively, or an RRF of 2. That lower level of resistance would be similar to what Nandula et al. (2018) reported for a glyphosate-resistant population in Mississippi, in which the mechanism of resistance was the well-documented, single-nucleotide substitution of T for C at the codon 106 position, resulting in a proline-to-serine substitution (Powles and Preston 2006; Yu et al. 2015).

The parameter  $b$  estimates the slope on the model. Most notably, the two most resistant accessions (population 18 in 2018 and population 3 in 2019) had an RRF >8. The standard error (Table 3) for the slope indicates that the most resistant accessions were 13 to 25 times in order of magnitude different compared with the 18 other accessions.

Accessions did not differ in screening for clethodim at different use rates (Figure 3). The  $EC_{50}$  for these junglerice accessions ranged from 5 to 18 g ae ha<sup>-1</sup> clethodim (Table 4). No difference ( $P = 0.483$ ) was observed from these accessions in terms of the  $EC_{50}$  parameter estimate. From these data, we suggest clethodim can still be an effective management option for controlling these grasses.

### Dicamba Antagonism of Glyphosate and Clethodim

Field studies (Figure 4) of junglerice population number 20 showed that the 870 g ha<sup>-1</sup> rate of glyphosate and the 105 g ha<sup>-1</sup> rate of clethodim provided 80% control compared with 100% control with the same treatments in the greenhouse. This is consistent with the findings of Combella (1982), who reported that, due to

**Table 4.** Tennessee junglerice accession responses to increasing rates of clethodim parameter estimates in 2019.<sup>a</sup>

Population <sup>b</sup>	$a$	$EC_{50}$ parameter estimate	$b$
	%	g ha <sup>-1</sup>	g ha <sup>-1</sup>
3	92	8	1.5
8	99	8	1.3
7	96	18	8.7
2	99	10	2.0
4	99	8	1.4
6	92	6	1.0
5	99	8	1.4
9	99	7	1.1
10	99	5	0.5
1	97	11	2.9
F value	1.45	0.98	1.00
Df	9, 20	9, 20	9, 20
P value	0.235	0.483	0.473

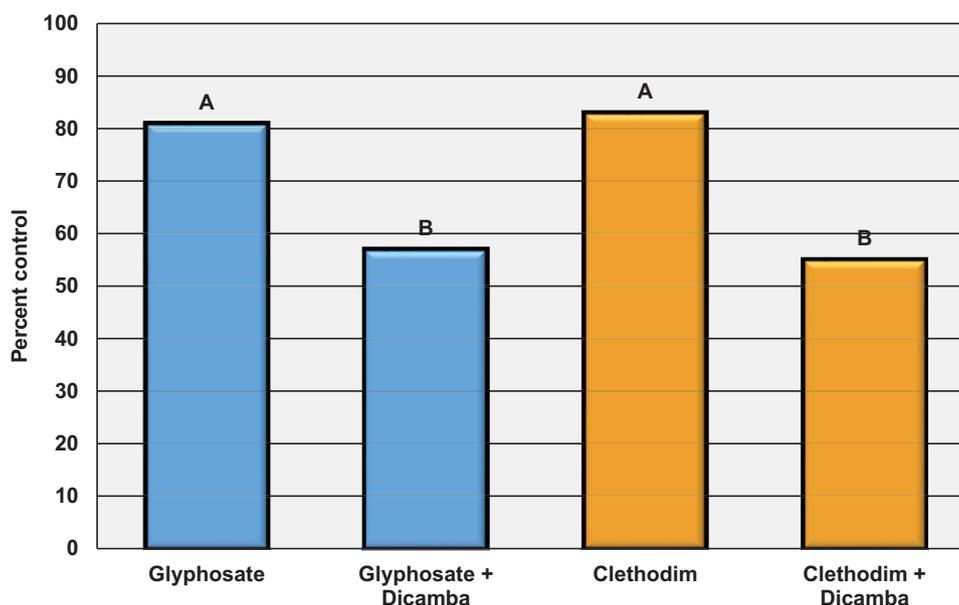
<sup>a</sup>Estimates for  $a$  (rate that provided maximum control);  $c$ , the  $EC_{50}$ ; and  $b$ , the point on the model where an exponential increase in rate was required to observe a subsequent increase in control (see Equation 1 in the text).

<sup>b</sup>Abbreviations: Df, degrees of freedom;  $EC_{50}$ , half-maximal effective concentration.

environment and application variability, field applications can result in less control compared with greenhouse applications. The addition of dicamba to glyphosate reduced junglerice control 25% compared with glyphosate alone. Similarly, clethodim + dicamba provided 6.5% less junglerice control than clethodim alone. These data suggest that part of the junglerice escapes in DR crops could be due to dicamba antagonizing the glyphosate and clethodim. This would be consistent with other studies in which grass control by glyphosate and clethodim was reduced when these herbicides were tank mixed with dicamba (Flint and Barrett 1989; O'Sullivan and O'Donovan 1980).

Our survey showed that 70% of the junglerice accessions tested had an effective glyphosate RRF of 2.5 to 8.5, suggesting glyphosate-resistance evolution has occurred in Tennessee. Several junglerice accessions have evolved resistance to glyphosate applied at 870 g ha<sup>-1</sup>. The resistant accessions exhibited 8.5-fold resistance to glyphosate compared with their most susceptible accessions. These data indicate that junglerice escapes in DR cotton and soybean fields are due, in part, to an evolution of glyphosate resistance in approximately 13% of junglerice accessions surveyed in Tennessee. We also showed that all accessions screened could still be controlled with clethodim in a greenhouse environment but less control was seen in the field. These findings also imply that a significant cause of the poor junglerice control is dicamba antagonizing the glyphosate and clethodim activity. These results suggest that the poor junglerice control in 64% to 76% of the DR fields in the survey was due to a combination of glyphosate resistance and dicamba antagonism of glyphosate and clethodim.

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**Figure 4.** Field comparison results from 2019 and 2020 in Tennessee using single degree-of-freedom contrast statements comparing junglerice control 21 d after application with glyphosate at 870 g ha<sup>-1</sup> to glyphosate at 870 g ha<sup>-1</sup> + dicamba at 560 g ha<sup>-1</sup> and clethodim at 105 g ha<sup>-1</sup> compared with clethodim at 105 g ha<sup>-1</sup> + dicamba 560 g ha<sup>-1</sup>.

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