Abstract

Junglerice has become a major weed in the mid-south and other areas of the United States. Glyphosate resistance has been documented in junglerice populations and is part of the reason for the increase in its prevalence. However, reduced junglerice control with glyphosate + dicamba and clethodim + dicamba mixtures has been observed in many production fields where glyphosate resistance has not yet evolved. Therefore, research was conducted to assess reduced junglerice control with glyphosate and clethodim when applied with dicamba. Adding dicamba to the spray tank with glyphosate reduced junglerice control by 27%. Adding dicamba to the spray tank with clethodim reduced junglerice control by 11%. The use of Turbo Teejet Induction (TTI) nozzles reduced junglerice control an additional 8% compared to applications with an air induction extended range (AIXR) nozzle. When a drift reduction agent (DRA) was added to dicamba mixtures with glyphosate or clethodim, junglerice control was reduced 36%. Junglerice control was similar with the glyphosate + dicamba treatment compared to the glyphosate + 2,4-D mixture. There was no interaction between nozzles and herbicide treatment. Regardless of herbicide treatment junglerice control was always lower when applied with the ultracourse TTI nozzle. Many applicators in Tennessee prefer to make one application of glyphosate + dicamba in a mixture to save time (authors' personal experience). These results show that the addition of dicamba to glyphosate or clethodim applied with labeled nozzles and a DRA results in reduced junglerice control and should be avoided.

Introduction

Junglerice has become one of the top two prevalent weeds in soybean [Glycine max (L.) Merr.] and cotton (Gossypium hirsutum L.) in much of Tennessee and across the mid-south (Perkins et al. 2021; Tahir 2007). Junglerice and barnyardgrass [Echinochloa crus-galli (L.) P. Beauv.] are the two most common Echinochloa species found in Tennessee (Perkins et al. 2021) and share many characteristics such as vast seed production, rapid C4 growth, and extended emergence periods. Several populations of junglerice have been tested for glyphosate and clethodim resistance (Perkins et al. 2021). Those studies have indicated that 15% of the populations have a 2-fold to 8-fold resistant to glyphosate, which is consistent with a report by Nandula et al. (2018) who studied selected Mississippi and Tennessee populations. Many growers have elected to grow soybean and cotton that is resistant to glyphosate + dicamba (Wechsler et al. 2019). Applying mixtures of glyphosate with dicamba provides broad-spectrum weed control with the expectation that the dicamba will control glyphosate-resistant broadleaf weeds and glyphosate will control grass weeds. However, many field reports from cotton and soybean growers in Tennessee suggest that grass weed control, particularly junglerice, from glyphosate + dicamba or clethodim + dicamba applications has been unacceptable (Perkins et al. 2021).

A decrease in herbicidal activity on grasses such as junglerice has been documented from mixtures of dicamba with glyphosate compared with only glyphosate applied alone (Flint and Barrett 1989; O’Sullivan and O’Donovan 1980). Colby (1967) has described antagonism as a result of applying two herbicides in combination, which will result in less control than what is expected based on how the individual herbicides perform alone. Herbicides such as glyphosate, dicamba, and 2,4-D and their behavior in various combinations is not fully understood for their weed-control efficacy. Both Flint and Barrett (1989) and O’Sullivan and O’Donovan (1980) have shown that antagonism can be both dependent on rates of herbicide usage and the species being evaluated. Antagonism plus herbicide resistance can lead to weed control failure after only a few years. Avoiding antagonism from herbicide mixtures can also aid in resistance management.
Antagonism has been observed with graminicides as well when applied with the auxin herbicides (Blackshaw et al. 2006; Fletcher and Drexler 1980; Mueller et al. 1989; Olson and Nalewaja 1981; Todd and Stobbe 1980). The physiological antagonism is not believed to be due to graminicide retention or absorption differences but rather to reduced translocation to meristematic tissues (Barnwell and Cobb 1994; Mueller et al. 1990). The contrast between the modes of action of these herbicides has been implicated as the cause of antagonism, wherein the acetyl CoA carboxylase–inhibiting graminicides reduce proton efflux, whereas auxin herbicides stimulate proton efflux (Barnwell and Cobb 1993; Hull and Cobb 1998). It has been reported that dicamba applications can disrupt phloem loading (Lalonde et al. 2003). Therefore, this may impact glyphosate translocation throughout the plant. In addition, the synthetic auxin response is a complicated and dynamic pathway that might be causing other physiological changes that in turn can affect the ability of glyphosate to reach its target site (e.g., sequestration). Researchers have also reported that 2,4-D decreased uptake and translocation of glyphosate, thus reducing junglerice control. Moreover, Li et al. (2020) reported that the glyphosate antagonism from 2,4-D was much higher in glyphosate-resistant junglerice than in glyphosate-susceptible populations. Researchers have reported that pretreatment with 2,4-D can upregulate cytochrome P450 in ryegrass (Lolium rigidum L.) resulting in a 10-fold increase in the plant’s tolerance to glyphosate (Han et al. 2013). Dicamba applications have been known to disrupt the natural hormone signaling, with the stimulation of ethylene biosynthesis occurring within hours of application and then growth inhibition starting within the first 24 h (Grossman 2010). There has been evidence that abscisic acid, auxins, and gibberellins can be involved with the phloem loading and unloading (Lalonde et al. 2003). This will disrupt the native hormone signaling, which impacts the herbicide transport. Additionally, glyphosate can inhibit synthesis of the amino acid tryptophan, a precursor involved in the biosynthesis of indole acetic acid (Taiz and Zeiger 2006). Hormone signaling has been described as a complex signal transduction cascade and often involves more than one phytohormone. Therefore, it is possible that the inhibition of auxin biosynthesis with concurrent exposure to high concentrations of a synthetic auxin could result in the antagonism observed.

When two herbicides are applied together in a mixture, the interactions can be described by the use of Colby’s method (Colby 1967). However, in circumstances in which one herbicide has no activity on one of the species, then Colby’s method cannot be used because the model requires control greater than 0% from both of the herbicides. Though a significant decrease in herbicidal activity from the mixture (e.g., glyphosate plus dicamba) compared with the herbicides alone with activity (e.g., glyphosate) can be considered antagonism. This methodology was used by both Flint and Barrett (1989) and O’Sullivan and O’Donovan (1980).

Another factor to consider in herbicide antagonism is the formulation of an active ingredient. For example, Kudsk and Mathiassen (2004) have reported higher levels of synergism for mixtures of commercial products compared with the technical grade laboratory products. In this scenario, the adjuvants in the commercial products may be improving the uptake of one or both products in the mixture. Nalewaja and Matysiak (1992) reported that interactions between herbicides can also differ between commercial formulations of the same active ingredient. Finally, a change in herbicide formulation cannot only impact the interaction among herbicides in its chemical structure, but could also alter the droplet spectra. Mueller and Womac (1997) reported differences in the droplet size produced between different formulations of glyphosate.

One more possible source of reduced junglerice control could be due to label application directions. Due to off-target movement (OTM) concerns, label specified dicamba formulations that may be applied POST in Xtend crops are labeled to be applied using ultracourse nozzles and a drift reduction agent (DRA; Anonymous 2019a; Anonymous 2019b). These mandated application parameters may reduce OTM, but researchers have observed a reduction in weed control (Carter et al. 2017). It is known that small droplet size is more important for spray retention on upright grass weeds compared with broadleaf weeds that have a horizontal leaf structure (Etheridge et al. 2001; McKinlay et al. 1974).

DRAs are used to modify spray characteristics to reduce spray drift, usually by minimizing small droplet formation. Previous research has shown that DRA use can increase the volume median diameter of sprays and thereby reduce spray drift (Zhu et al. 1997). Another study found a reduction in total drift deposits in field evaluations for wind speeds ranging from 2.9 to 4.9 m/s by 15% to 50% with low concentrations and up to 70% to 80% with high concentrations (Bode et al. 1976). Fietsam et al. (2004) reported that the use of a DRA with glyphosate reduced spray coverage by 6%.

Junglerice prevalence in the mid-southern United States has increased recently (Perkins et al. 2021; Tahir 2007). This could be attributed to the evolution of glyphosate resistance and the potential dicamba antagonism of glyphosate. Reduced junglerice control could be due to the labeled ultracourse droplet nozzles and DRAs that are mandated to be used for dicamba applications on dicamba-resistant soybean and cotton. The majority of hectares in Tennessee and across the mid-south receive a glyphosate + dicamba application. The U.S. Department of Agriculture reports (Wechsler et al. 2019) that in 2018, 71% of the hectares were planted with dicamba-tolerant soybean, with more than 2.2 million kg of dicamba used in the United States in this crop. With 16 million soybean hectares planted with Xtend varieties in 2019, the use of dicamba increased. A recent memorandum issued by the U.S. Environmental Protection Agency on the benefits of dicamba in dicamba-tolerant soybean production suggested that 97% of dicamba applications were mixed with glyphosate in 2018 and 2019 (Orlowski and Kells 2020). This herbicide mixture and application could be causing the reduced grass control recently observed with glyphosate and clothodim. Finally, growers reporting failure to control Palmer amaranth with glyphosate + dicamba applications have resulted in some producers using higher dicamba rates (Steckel 2019). Although using higher dicamba rates may improve Palmer amaranth control, it could also decrease glyphosate effectiveness on junglerice.

Therefore, the objective of this research was to 1) assess junglerice control with mixtures containing dicamba and 2) assess whether labeled nozzles and DRAs used in dicamba applications are reducing control; and 3) examine whether increased rates of dicamba in these mixtures resulted in less junglerice control.

Materials and Methods

Field Component

This field experiment was replicated across three locations and 2 yr for a total of six experimental site-years. The research was arranged in a randomized complete block design with a two factor-factorial treatment structure with nozzle selection and herbicide treatment
as the main factors. Plot size was 1.5 m wide and 9.1 m long in Jackson at the West Tennessee Research and Education Center (WTREC). Plots at other two locations, Milan Research and Education Center (MREC) and a grower field (Burlison, TN), were 1.5 m wide and 6 m long. Depending upon location, there were three (MREC and Burlison) or four (WTREC) replications. The two nozzles tested were Turbo Teejet Induction (TTI) 11003 nozzles and the air induction extended range (AIXR) 11003 flat-fan nozzles. The TTI 11003 nozzle is the labeled nozzle type for dicamba applications in Xtend crops and was applied at 275 kpa, which produces an ultracourse droplet (Anonymous 2021a). Likewise, the nozzle size for the AIXR was 03-orifice as well. The AIXR at the operating pressure of this boom produced a course droplet (Anonymous 2021a) and is not labeled for dicamba applications on Xtend crops. The labeled orifice size for these applications is 025 and higher (Anonymous 2021b). We chose the 03-orifice size to be able to apply these applications as directed by the label. The second factor was herbicide treatment and included a nontreated (check), glyphosate (Roundup Powermax®, Bayer Crop Protection, St. Louis, MO), clethodim (Intensity®, Loveland Products, Greensville, MS), glyphosate + clethodim, glyphosate + dicamba + clethodim, glyphosate + dicamba, glyphosate + clethodim + dicamba, glyphosate + dicamba + DRA (OnTarget®, Winfield United, Arden Hills, MN), and clethodim + dicamba + DRA. Herbicide rates were consistent throughout with glyphosate at 870 g ha⁻¹, dicamba at 560 g ha⁻¹, and clethodim at 105 g ha⁻¹. DRA was used at 0.25% vol/vol. Applications were made with a CO₂ backpack sprayer calibrated to apply at 142 L ha⁻¹. Each herbicide treatment was evaluated using each nozzle previously mentioned. Herbicides were applied when junglerice plants were 8 to 10 cm in height. Control of junglerice was visually estimated on a scale of 0% to 100% where 0% = no injury and 100% = plant death at 7, 14, and 21 d after treatment. Aboveground, fresh weight biomass data was collected at 7, 14, and 21 d after treatment. Aboveground, fresh weight biomass data was collected 21 to 28 d after treatment in a 0.2-m area by clipping plants at the soil surface. Biomass was collected and weighed using fresh weights and measured in grams. Only latest evaluations are presented here for brevity.

**Greenhouse Research**

The main field experiment was replicated in a greenhouse (Vero Beach, FL) to determine what role mixing glyphosate + dicamba or clethodim + dicamba has on control of Echinochloa species. Touchdown Hi-Tech® (glyphosate, Syngenta Crop Protection, Greensboro, NC) treatments included 0.25% vol/vol nonionic surfactant and Select Max® (clethodim, Valent U.S.A LLC., Walnut Creek, CA) treatments included 1% vol/vol crop oil concentrate in this experiment. In addition, either glyphosate or clethodim was mixed with dicamba with and without a DRA. Treatments were applied in a Generation 4 Research Track Sprayer (DeVries Manufacturing, Inc., Hollandale, MN). The track sprayer speed was calibrated to deliver 142 L ha⁻¹ and the nozzle height was set to be spraying approximately 40 to 45 cm above the crop canopy. Herbicides were applied when plants reached 8 to 10 cm in height. Junglerice control was visually estimated on a scale of 0% to 100% where 0% = no injury and 100% = plant death at 28 d after treatment. Biomass was taken 28 to 35 d after treatment. Biomass was collected by clipping plants at the soil surface and fresh weight data collected.

**Data Analysis**

This greenhouse study was arranged in a randomized complete block design with a two factor-factorial treatment structure with nozzle selection and herbicide treatment being the factors. It was blocked on site due to Echinochloa spp. population density and glyphosate resistance. Fixed effects were herbicide treatment and nozzles. Environment, replications, and any interactions of fixed by random effects were considered random in the model. Each year-location combination was considered an environment sampled at random from a population as described by Carmer et al. (1989). Designating the environments random will broaden the possible inference space the experimental results are applicable to (Carmen et al. 1989). Mean separation for individual treatment differences was performed using Fisher’s protected LSD test at P < 0.05. Post-ANOVA single degree of freedom contrasts were then used (SAS v9.4; SAS Institute, Cary, NC) to compare herbicide applications with and without dicamba as well as nozzle selection comparing AIXR flat-fan to TTI nozzles, averaged across six environments to measure the response from the addition of dicamba to the spray tank and using TTI nozzles.

**Results and Discussion**

**Field Component**

There was an overall herbicide treatment effect (P < 0.001) among treatments with glyphosate. Single-degree-of-freedom contrasts were then used to compare treatments with and without dicamba and to measure the nozzle effect and herbicide antagonism in these
parameters. When analyzing treatments across both nozzles, there was a difference between treatments that contained dicamba and treatments that did not. Initially, glyphosate alone provided 75% control (Table 1). The addition of dicamba to glyphosate resulted in 21% less control (P = 0.047). The addition of a DRA to this mixture numerically reduced control an additional 20% (P = 0.059). These results suggest that applying dicamba mixed with glyphosate as directed by the registrant’s labels (Anonymous 2019a; Anonymous 2019b) would reduce junglerice control by 40% compared with glyphosate (Anonymous 2019c) alone as directed by its label.

An application of clethodim alone provided the highest control of junglerice at 88%. A glyphosate + clethodim mixture provided similar results at 87%. Dicamba mixed with clethodim numerically reduced junglerice control by 11% from visual observations and increased junglerice biomass 45% compared to clethodim alone. Similar to glyphosate, when clethodim + dicamba was applied as directed by the dicamba registrants’ labels using the TTI nozzles and DRA, junglerice control was reduced by 22% with biomass increases of 80% compared to applying clethodim as directed by the label (Anonymous 2019d). However, it is notable that treatments containing glyphosate had higher biomass measurements. The authors suggest this could be due to more regrowth occurring in these treatments compared to clethodim.

When analyzing the two nozzles (AIXR flat-fan and TTI), there was a 7% junglerice reduction in control using the TTI nozzles (Table 2). These results are similar to those reported by Carter et al. (2017) who observed a 5% to 6% reduction in grass control. This 7% control reduction was additive to the 16% (antagonism) loss when using dicamba (Table 1), giving a total loss of 23%. These TTI nozzles are labeled for in-crop dicamba applications. This would suggest that growers should switch from TTI to AIXR nozzles if junglerice is present when using glyphosate and/or clethodim alone. In addition, these findings suggest that to achieve better junglerice control, do not mix dicamba with glyphosate and/or clethodim. These field locations are all considered to be glyphosate susceptible populations due to still achieving relatively good control, although only 75%.

### Evaluation of Increased Dicamba Rate on Glyphosate Efficacy

The 1× dicamba rate (560 g ha⁻¹) mixed with glyphosate provided 56% junglerice control (Table 3). The 1.5× use rate (840 g ha⁻¹) of dicamba mixed with glyphosate reduced grass control by an additional 14% (P < 0.001). A 2× use rate (1,120 g ha⁻¹) reduced control by an additional 9%. Biomass measurements mirrored the herbicide efficacy ratings and significantly increased as the dicamba rate increased. These results show that increased rates of dicamba resulted in decreased grass control. The addition of a DRA resulted in the greatest grass control loss compared to glyphosate + dicamba.

### Comparing Antagonism Between Dicamba and 2,4-D

There was no interaction between nozzles and herbicide treatment. Regardless of herbicide treatment junglerice control was always
lower when applied with the ultracourse TTI nozzle. No statistical differences were found between these mixtures. Junglerice control was similar with the glyphosate + dicamba treatment compared to the glyphosate + 2,4-D mixture (Table 4). Numerically, there was less antagonism from glyphosate + 2,4-D mixtures compared to the dicamba mixtures. However, there was numerically more antagonism observed from the clethodim + 2,4-D mixtures compared to dicamba.

Greenhouse Experiments

Reductions in junglerice control due to dicamba being added to glyphosate mixtures were observed in the greenhouse. However, they were not as pronounced compared with those in the field (Table 5). Glyphosate alone provided 96% control of junglerice compared with 84% control with glyphosate + dicamba (P < 0.001). However, no differences were observed (P = 0.090) when comparing clethodim (96%) to a clethodim + dicamba (97%) application. A glyphosate + dicamba application provided 84% control on junglerice, however, a glyphosate + dicamba + DRA application increased control (91%), which was similar to glyphosate alone (96%; P = 0.012). The addition of a DRA to clethodim + dicamba did not influence control (P = 0.173). There were no differences in the control with clethodim alone or in mixture (P = 0.726). The biomass data supported these results with no differences detected.

The overall better control observed particularly with the DRA in the greenhouse compared to the field would be consistent with results reported by Combellack (1982) that due to the environment and application variability in the field, less control in the field was observed compared with greenhouse applications. These results are also consistent with those reported by Perkins et al. (2021) who achieved better junglerice control with glyphosate and clethodim in the greenhouse compared to the same populations in field research.

There were observed control differences between nozzles (AIXR flat-fan vs. TTI) of 6% (P = 0.015; Table 6). These results are similar to what we observed in the field component of this research.

In conclusion, the addition of dicamba decreased junglerice control of clethodim and glyphosate in field studies. These field

### Table 4. Single degree of freedom contrasts comparing herbicide applications on junglerice control across three locations in 2019 and 2020 in Tennessee.a,b

| Herbicide treatment | Percent control | F-value | P-value | Biomass | F-value | P-value |
|---------------------|-----------------|---------|---------|---------|---------|---------|
| Glyphosate          | 96              | 18.78   | < 0.001 | 0.17    | 0.11    | 0.743   |
| Glyphosate + Dicamba| 84              | 7.01    | 0.012   | 0.67    | 0.20    | 0.663   |
| Glyphosate + Dicamba + DRA | 91 | 2.84    | 0.101   | 0.17    | 0.60    | 0.448   |
| Glyphosate          | 96              | 3.05    | < 0.01  | 5.92    | 0.024   |
| Glyphosate + Dicamba| 97              | 1.94    | 0.173   | 0.08    | 0.00    | 1.000   |
| Glyphosate + Dicamba + DRA | 93 | 0.12    | 0.726   | < 0.01  | 5.92    | 0.024   |

### Table 5. Single degree of freedom contrasts on herbicides applied with TTI nozzles comparing herbicides averaged across six populations in the greenhouse.a

| Herbicide treatment | Percent control | F-value | P-value | Biomass | F-value | P-value |
|---------------------|-----------------|---------|---------|---------|---------|---------|
| Glyphosate          | 96              | 18.78   | < 0.001 | 0.17    | 0.11    | 0.743   |
| Glyphosate + Dicamba| 84              | 7.01    | 0.012   | 0.67    | 0.20    | 0.663   |
| Glyphosate + Dicamba| 96              | 2.84    | 0.101   | 0.17    | 0.60    | 0.448   |
| Glyphosate + Dicamba| 97              | 3.05    | < 0.01  | 5.92    | 0.024   |
| Glyphosate + Dicamba + DRA | 93 | 1.94    | 0.173   | 0.08    | 0.00    | 1.000   |
| Glyphosate + Dicamba + DRA | 93 | 0.12    | 0.726   | < 0.01  | 5.92    | 0.024   |

### Table 6. Single degree of freedom contrast statement comparing nozzles for junglerice control averaged across six populations in the greenhouse.a

| Nozzle            | Percent control | F-value | P-value |
|-------------------|-----------------|---------|---------|
| AIXR flat-fan     | 96              | 6.09    | 0.015   |
| TTI               | 90              |         |         |

aAbbreviations: DRA, DRA, drift reduction agent; TTI, Turbo Teejet Induction.

bAll applications were made with TTI nozzles.
data suggest that mixtures with dicamba result in 15% less junglerice control. An additional 7% control loss was observed when the TTI nozzles were used, and an additional 16% loss occurred when a DRA was added to the spray tank. Moving forward, these data suggest separating glyphosate and/or clethodim applications with dicamba. Recommendations presented in Extension publications vary from 1 to 7 d on the length of time needed to mitigate antagonism between application of herbicides primarily used for broadleaf control compared to the herbicide targeting grasses (Barber et al. 2020; Loux et al. 2020). Future research designed to evaluate application timing of glyphosate or clethodim compared to dicamba and 2,4-D could help applicators plan the best strategy for achieving consistent grass control. A recent survey showed that on average, 40% of the fields in Tennessee have both Palmer amaranth plus *Echinochloa* spp. (Perkins et al. 2021). Growers want to control all weeds with one application of glyphosate + dicamba. However, these data show that the addition of dicamba with glyphosate or clethodim applied with labeled nozzles and DRA is resulting in reduced junglerice control and should be avoided.

Acknowledgments. We thank Syngenta for providing us the access to conduct research at their facilities in Vero Beach, Florida. In addition, we especially thank Ethan Parker, Marshall Hay, Grace Hendrix, and all employees at the Vero Beach research station for their assistance and guidance throughout this process. We also thank the support staff and technicians at the University of Tennessee West Tennessee Research and Education Center for their assistance. This research was funded in part by the Tennessee Soybean Promotion Board and CottoN Incorporated. No other conflicts of interest are noted.

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