Characterization and Management of Glyphosate-Resistant Junglerice

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DEDICATION

I would like to dedicate this research to my family. First and foremost, my parents Amy and Paul Perkins. Your love, guidance, and support along the way have meant the world to me. I could not have gotten to where I am today without your help. To my brother Clint, you have always been there for me. Even though we find something to argue about every once in a while, we always bounce back and have each other's back the next minute. I would also like to dedicate this to my grandparents who have always had an interest in my education and what new research I participated in each day. In addition, I would like to dedicate this research to Mr. Eddie Anderson. You took me under your wing before I was in high school and always gave your time when I needed something or had a question. Thank you for instilling a love for both agriculture and the Dyer County Fair in me.

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ABSTRACT

Junglerice has become a major weed in Tennessee and across the mid-south. Glyphosate resistance and dicamba antagonism has resulted in the reported control failures and rise in prevalence. Junglerice was the most prevalent weed escape in cotton and soybean fields across Tennessee from 2018 to 2020. In all, 13% of the junglerice accessions could no longer be effectively controlled with glyphosate. Due to poor in-crop control, it has been recommended to start clean when trying to control junglerice and other grasses. Therefore, research was conducted to determine the best burndown methods utilizing dicamba, glufosinate, or paraquat. A sequential application of glyphosate two weeks after the initial application was applied and provided the greatest control of junglerice regardless of burndown option. A dicamba + glyphosate, glufosinate + clethodim, or paraquat + clethodim application all provided the greatest control of junglerice (98, 90, and 90% respectively) amongst the different burndown options. On average, adding dicamba to the tank with glyphosate or clethodim reduced junglerice control by 19%. Labeled (TTI) nozzles reduced junglerice control an additional 8% and a drift reduction agent (DRA) reduced junglerice control an additional 16%. No antagonism differences were observed between dicamba and 2,4-D applications with glyphosate or clethodim. 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. Sequential treatments of glyphosate and/or clethodim preceding or following dicamba were examined. Overall, a glyphosate + clethodim application provided the most complete junglerice control regardless of

timing. These data confirm that leaving dicamba out of the tank will mitigate antagonizing junglerice control. Glyphosate + clethodim applications in the future will aid in resistance management as well as the rise in glyphosate-resistant junglerice populations.

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INTRODUCTION

A major challenge to global food and fiber production is posed by weed species that infest crops that reduce quality and yield. For the past half century, the challenge of weeds has been managed with herbicides to remove these pests. The evolution of herbicide resistant weeds poses a threat to herbicide efficacy and crop yields (Powles and Yu 2010). Currently, there are 262 known weed species with populations that are resistant to one or more herbicides (Heap 2020). Mechanisms of herbicide resistance can be grouped into two categories, target site resistance and non-target site resistance (Powles and Yu 2010). Junglerice (*Echinochloa colona* L.) has become a growing problem over the past few years to growers in Tennessee and across the Mid-south. Glyphosate is a highly effective tool for managing these *Echinochloa* species in cotton (*Gossypium hirsutum* L.) and soybean [*Glycine max* (L.) Merr.] (Payne and Oliver 2000; Scott et al. 2017).

Junglerice

Junglerice (*Echinochloa colona* (L.) is a hexaploid (2n = 6x = 54) annual species (Gould et al. 1972; Yabuno 1966). Junglerice is an important weed in rice [*Oryza sativa* (L.)] production systems and other agronomic crops across the world (Bakkali et al. 2007; Holm et al. 1991; Valverde et al. 2000). Species in the *Echinochloa* genus include some of the worst weeds in US rice (Muenscher 1955). The *Echinochloa* complex has become a common weed in soybean and cotton across the Mid-south (Webster 2012; 2013). Other *Echinochloa* spp. that are closely related to junglerice are barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] and rice barnyardgrass [*Echinochloa phyllopogon* (Stapf) Koso-Pol.].

Junglerice and barnyardgrass share many weedy characteristics with Palmer amaranth (*Amaranthus palmeri* Wats.). These characteristics include extensive seed production, rapid C_4 growth, and extended emergence periods. However, it is not as competitive with crops as are the *Amaranthus* species (Cowan et al. 1998). *Echinochloa* emergence takes place from mid-April to late September and is highly dependent upon location (Bagavathiannan et al. 2011b). Bagavathiannan et al. (2011a) estimated that barnyardgrass produces up to 31,500 seed per plant when emerging in the row-middle of soybean. Vail and Oliver (1993) reported that a yield reduction in soybean was estimated to be 0.25% per plant per meter row.

One biotype of junglerice in Sunflower County, MS has been confirmed to having resistance to four herbicides, imazamox (an acetolactate synthase (ALS) inhibitor "Group 2"), fenoxaprop-P-ethyl (an acetyl coenzyme A carboxylase (ACCase) inhibitor "1"), quinclorac (an auxin mimic "4"), and propanil (a photosystem II inhibitor "5, 6, 7"), each with different mechanisms of action (Wright et al. 2018). The ALS- and ACCase-inhibitor resistances in this biotype have been confirmed as being non-target site mechanisms of resistance (Riar et al. 2013; Wright et al. 2016).

Additionally, junglerice has evolved resistance to seven different chemical classes. These include the synthetic auxins "4" (i.e. quinclorac), (ALS) inhibitors "2", (ACCase) inhibitors "1", photosystem II inhibitors "5, 6, 7", microtubule inhibitors "3", long-chain fatty-acid inhibitors and lipid synthesis inhibitors "8, 16" (Heap, 2018; Chen et al. 2018). Herbicide resistant simulation models suggest that applying multiple effective sites of action (SOA) can be a highly effective method for preventing evolution of herbicide resistance (Bagavathiannan et al. 2013; Bagavathiannan et al. 2014; Busi et

al. 2019; Diggle et al. 2003). Herbicides such as glyphosate, clethodim, sethoxydim, and quizalofop are available in soybean and cotton which have been reported to provide good control on junglerice and barnyardgrass (Jordan 1995; Scott et al. 2015; Sikkema et al. 2005; Vidrane et al. 1995). However, these herbicides must be managed properly to minimize the risk of evolving further resistance. An herbicide recommendation resulting in antagonism between two herbicide products is not an effective resistance management strategy (Norsworthy et al. 2012).

Antagonistic Factors in Controlling *Echinochloa*

A decrease in herbicidal activity on grasses has been documented from tank mixtures of dicamba with glyphosate compared to glyphosate alone (Flint and Barrett 1989; O'Sullivan and O'Donovan 1980). Colby (1967) described antagonism as a result of applying two herbicides in combination which results in less control than what was expected based on how the individual herbicide application performs alone.

The behavior of glyphosate, glufosinate, dicamba, and 2,4-D in various combinations is not fully understood with respect to weed efficacy. In addition, the extent of nozzle selection and spray volume and its effect on herbicide antagonism is not well understood. Small droplet size is more important for the retention on upright, grass weeds compared with broadleaf weeds with a horizontal leaf structure (McKinlay et al. 1974; Etheridge et al. 2001). Both Flint and Barrett (1989) and O'Sullivan and O'Donovan (1980) showed that the documentation of antagonism can be both dependent on rates of the herbicides and the species being evaluated. A sound herbicide program is needed to minimize the evolution of resistance, especially when

controlling herbicide-resistant weed populations. A situation where mixing two herbicides results in antagonism should be avoided (Norsworthy et al. 2012).

When two herbicides are applied together in a mixture, the interactions can be described by the use of Colby's method (Colby 1967). In circumstances where an herbicide provides no POST activity on a given species (e.g. dicamba on junglerice), then Colby's method cannot be used because the model requires control greater than 0% from both of the herbicides. However, a significant decrease in herbicidal activity from the mixture (e.g. glyphosate plus dicamba), compared with the straight herbicide with activity alone (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 for antagonism is the formulation of an active ingredient. For example, Kudsk and Mathiassen (2004) reported higher levels of synergism for mixtures of commercial products compared to the technical grade laboratory products. This would indicate that the adjuvants in the commercially available products may be improving the uptake of one or both products in the mixture. In addition, the identification of interactions between herbicides can also differ between commercial formulations of the same active ingredient (Nalewaja and Matysiak 1992). Finally, a change in formulations may not only impact the interaction among herbicides in a physiochemical fashion, but it could also alter the droplet spectra. Mueller and Womac (1997) reported differences in the droplet size produced between different formulations of glyphosate.

It is not clear as to why dicamba and 2,4-D antagonize the activity of glyphosate on grass species. It is known that dicamba applications can disrupt phloem loading, and

thus may be impacting glyphosate translocation throughout the plant. Additionally, the synthetic auxin response is a complicated and dynamic pathway that might be causing other physiological changes which in turn could affect the ability of glyphosate to reach its target site (e.g. sequestration). Dicamba applications are known to disrupt natural hormone signaling, with the stimulation of ethylene biosynthesis occurring within hours of application and growth inhibition setting in within the first 24 hours (Grossman 2010). There is evidence suggesting that abscisic acid, auxins and gibberellins are involved with the phloem loading and unloading (Lalonde et al. 2003). This disrupts native hormone signaling which impacts the herbicide transport. Additionally, glyphosate inhibits synthesis of the amino acid tryptophan, a precursor involved in the plant biosynthesis of indole acetic acid (Taiz and Zeiger 2006). Hormone signaling is described as a complex of a 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. Flint and Barrett (1989) have reported that the reduced uptake and translocation of glyphosate in the presence of 2,4-D could account for the reduced control in glyphosate activity in these grass weed species. The different 2,4-D formulations such as the sodium salt and butoxyethyl ester formulation was found to be more antagonistic than the diethanolamine formulation (Nalewaja and Matysiak 1992). It was proposed that the antagonism of glyphosate activity by 2,4-D was due to the reactions affecting absorption.

Not much research has been done on the antagonism observed when tank mixing dicamba with clethodim or other grass products. Mueller et al. (1990) has

reported observing reductions in both the adsorption and translocation of halyoxyfop-methyl when tank-mixed with 2,4-D and applied to johnsongrass. Other studies have shown when mixing Group 1 herbicides with synthetic auxins that the antagonism is caused by either a reduction in adsorption or translocation (Culpepper et al. 1999; Olsen and Nalewaja 1981).

Glyphosate Resistance

The most widely used and successful herbicide globally is glyphosate (Duke and Powles 2008). Glyphosate has an annual use rate of 300 million pounds in the United States alone in recent years. This is in part due to its high efficacy, broad spectrum of control and systemic mode of action (Duke et al. 2018). However, resistance to glyphosate has evolved in numerous species 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 the 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, a key enzyme in the shikimate pathway (Steinrucken and Amrhein 1980). Blockage by glyphosate from this pathway causes the accumulation of high levels of shikimic acid, a decline in carbon fixation intermediates and a reduction in photosynthesis that results in plant death (Duke et al. 2003; Duke and Powles 2008). This evolution of glyphosate resistance by numerous species including *E. colona*, further demonstrates the need for improved glyphosate stewardship practices. Continued glyphosate sustainability as the world's most used herbicide is threatened. The threat comes from the overuse, reliance, and the lack of diverse weed control tactics, resulting in intense selection pressure for glyphosate-resistant weeds (Powles 2008). Since the first reports (Powles et al. 2008; Pratley et al.

1999), 42 weed species populations globally have now evolved glyphosate resistance (Heap 2019).

Herbicides have been the main tool available for control of *Echinochloa* spp. and have been in use for several decades. *E. colona* populations have confirmed resistance to one or more herbicide mechanisms of action, including acetyl-CoA carboxylase inhibitors, acetolactate synthase inhibitors, photosystem II inhibitors, synthetic auxins (cellulose inhibitors), and 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) inhibitor (glyphosate) in the following locations: Argentina, Australia, Bolivia, Colombia, Costa Rica, Egypt, El Salvador, Guatemala, Honduras, Iran, Nicaragua, Panama, the United States, and Venezuela (Heap 2018). Glyphosate-resistant junglerice was first found in Argentina and Australia (Gaines et al. 2012; Heap 2012). Nandula et al. (2018) confirmed glyphosate-resistant *E. colona* in Mississippi and Tennessee. The Mississippi population has a mechanism of resistance due to a mutation at the 106th loci of the EPSPS protein, resulting in replacement of proline for serine (Nandula et al. 2018). The Tennessee population had a reduced translocation mechanism of resistance to glyphosate. The glyphosate translocation model proposed by Shaner (2009), which hypothesized the existence of a barrier at the cell level which prevents glyphosate to load into the phloem, may have a role in the resistant Tennessee population. The glyphosate from this Tennessee population could possibly be loaded into the vacuoles via a system akin to the sequestration mechanism described in *E. canadensis* (Ge et al. 2010) and *Lolium* spp. (Ge et al. 2012). *E. colona* populations from Mississippi and Tennessee have been confirmed to be four- and seven-fold resistant to glyphosate, respectively (Nandula et al. 2018). Gaines et al. (2012) reported that a resistant

population in Australia was 8.6-fold resistant compared to a susceptible population. A glyphosate-resistant population in California reported to having 6.6-fold resistance compared to a susceptible population (Alarcon-Reverte et al. 2013). In addition, a Mississippi population has been reported possessing resistance to four mechanisms of action, but not glyphosate (Wright et al. 2016, 2018). This indicates the growing problem of resistance to a broad spectrum of herbicides in *E. colona* from the midsouthern US

Objectives

Junglerice and barnyardgrass have become major weeds across the Mid-South cotton and soybean fields. When both Palmer amaranth and *Echinochloa spp.* are present, then weed management becomes more complex. Soybean and cotton producers attempting to control these weed species with glyphosate + dicamba often results in inconsistent control of junglerice and barnyardgrass. Based on previous research, separating these two applications is required to increase grass control. In addition, understanding the prevalence of junglerice populations in Tennessee with glyphosate resistance is needed in order to better construct sound weed management for its corn (Z*ea mays* L.), soybean and cotton producers. Utilizing clethodim to control these *Echinochloa species* is one option to combat GR biotypes. Further research is needed to quantify how much each antagonism factor is contributing to overall poor junglerice and barnyardgrass control is needed. Additionally, research to determine effective methods for controlling these *Echinochloa* populations by reducing the number of herbicide applications and sustaining current weed control techniques is critical.

1) To evaluate the distribution of glyphosate-resistant populations of junglerice and barnyardgrass in Tennessee.

2) To evaluate antagonism of control when tank mixing dicamba + glyphosate or dicamba + clethodim.
3) To evaluate different sequential application techniques and timing effects on the control of *Echinochloa* populations.

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CHAPTER I
SURVEY OF GLYPHOSATE-RESISTANT JUNGLERICE (*ECHINOCHLOA COLONA*) ACCESSIONS IN DICAMBA-RESISTANT CROPS IN TENNESSEE

A version of this chapter was originally published by Clay M. Perkins, Thomas C. Mueller and Lawrence E. Steckel:

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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®) 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, 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 (Steinrucken and Amrhein 1980). Glyphosate blocks the shikimate pathway, resulting in accumulation of high levels of shikimic acid, 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 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®; 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.

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 nonrepeated 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^{-1}.

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^{-1}, 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^{-1} (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^{-1} (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^{-1} 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^{-1}, but the population showed moderate tolerance (half-maximal effective concentration [EC_{50}] of glyphosate = 600). The treatments evaluated were glyphosate at

870 g ha^{-1} compared with glyphosate at the same rate plus dicamba at 560 g ha^{-1}, and clethodim at 105 g ha^{-1} compared with clethodim at the same rate plus dicamba at 560 g ha^{-1}. Applications were made with a CO_2 backpack sprayer calibrated to apply 142 L ha^{-1} 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_{50}, the rate needed to achieve 50% control; and the parameter *b* estimates the slope:

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

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_{50} 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_{50} for the survey population by the EC_{50} 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 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^{-1} 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^{-1} 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^{-1}, which was more than 3-fold the labeled rate. Accessions 17, 19, and 20 required 870 g ha^{-1} 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_{50} for the three most susceptible accessions (i.e., 9, 10, and 13) ranged from 110 to 160 g ae ha^{-1} glyphosate (Table 3). Population 18 had the highest level of resistance (EC_{50}, 1,230 g ae ha^{-1}). 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_{50} values ranging from 400 to 580 g ae ha^{-1} 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_{50} = 1,080 g ae ha^{-1}), and had an RRF of 8 when 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^{-1}, 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^{-1} 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^{-1} rate of glyphosate and the 105 g ha^{-1} rate of clethodim provided 80% control compared with 100% control with the same treatments in the greenhouse. This is

consistent with the findings of Combellack (1982), who reported that, due to 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^{-1}. 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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Appendix

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

Population Number	Year	Site ID	GPS	County	State
1	2019	Bradshaw	35.9589 -89.5390	Dyer	TN
2	2019	Sweeny Ridge	36.0302 -89.5316	Dyer	TN
3	2019	Tigertail C Field	35.9567 -89.5789	Dyer	TN
4	2019	Ireland	33.479 -91.044	Washington	MS
5	2019	5JF	33.5456 -90.0965	Leflore	MS
6	2019	Smithtown 1	35.7862 -85.9239	Warren	TN
7	2019	Smithtown 3	35.7940 -85.9227	Warren	TN
8	2019	Sorrell	35.9749 -89.3437	Dyer	TN
9	2008	Susceptible Check	Azlin source	Washington	MS
10	2017	Susceptible Check	Azlin source	Washington	MS
11	2018	Nichols	36.1732 -89.4179	Dyer	TN
9	2008	Susceptible Check	Azlin source	Washington	MS
10	2017	Susceptible Check	Azlin source	Washington	MS
14	2018	Kelly Cotton	35.5794 -89.5458	Tipton	TN
15	2018	Knobcreek	35.5259 -89.3318	Haywood	TN
16	2018	Sneed 385	35.3137 -89.8046	Shelby	TN
17	2018	Allen	35.6002 -89.5825	Tipton	TN
18	2018	Sneed Rock Pile	35.2837 -89.8596	Shelby	TN
19	2018	Lannom	36.1573 -88.8169	Weakley	TN
20	2018	Milan	35.9390 -88.7229	Gibson	TN

[a]Abbreviation: GPS, global positioning system.

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

Weed Species Surveyed							
Palmer amaranth	Junglerice	Barnyardgrass	Johnsongrass	Goosegrass	Fall Panicum	Waterhemp	Total Fields
2018							
%							number
-[a]	76	33	-	3	12	-	33
2019							
%							number
50	64	49	25	3	11	11	75

[a]Abbreviation; NR, data not recorded for these species.

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

Population	a	2018 EC50 Parameter Estimate	b ± SEM	Population	a	2019 EC50 Parameter Estimate	b ± SEM
	%	g ha^{-1}	g ha^{-1}		%	g ha^{-1}	g ha^{-1}
18	-	1230 a	184 ± 25 a	3	102	1080 a	440 ± 13 a
19	99	680 b	17 ± 10 c	8	100	470 b	110 ± 3 bc
20	99	680 b	15 ± 1 c	7	94	410 b	170 ± 10 b
17	99	580 bc	11 ± 3 c	2	100	380 bc	110 ± 9 bc
16	99	400 c	9 ± 1 c	4	99	350 c	90 ± 9 cd
15	99	490 bc	11 ± 2 c	6	93	230 d	80 ± 11 cd
14	99	490 bc	11 ± 2 c	5	94	200 de	50 ± 5 cd
13	99	110 d	31 ± 2 b	9[a]	99	160 ef	30 ± 3 d
9[a]	99	160 d	31 ± 3 b	10[a]	99	140 ef	30 ± 1 d
10[a]	99	140 d	31 ± 1 b	1	98	130 f	30 ± 4 d
F-Value	1.00	9.22	3.21	F-Value	0.53	5.96	5.07
Df	9, 20	9, 18	9, 18	Df	9, 20	9, 18	9, 18
P-Value	0.474	< 0.001	0.019	P-Value	0.838	< 0.001	0.002

[a]Abbrevations: Df, degrees of freedom; EC_{50}, half-maximal effective concentration; NA, not applicable.

[b]In this model, the *a* parameter describes the asymptote or upper limit of control, the *c* parameter describes the EC_{50}, and the *b* parameter estimates the slope.

[c]Means not followed by a common letter are significantly different ($P < 0.05$).

[d]Accessions used as susceptible check.

Table 4. Tennessee junglerice accession responses to increasing rates of clethodim parameter estimates in 2019.[a]

Population	a	EC50 Parameter Estimate	b
	%	g ha^{-1}	g ha^{-1}
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

[a]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).

[b]Abbreviations: Df, degrees of freedom; EC_{50}, half-maximal effective concentration.

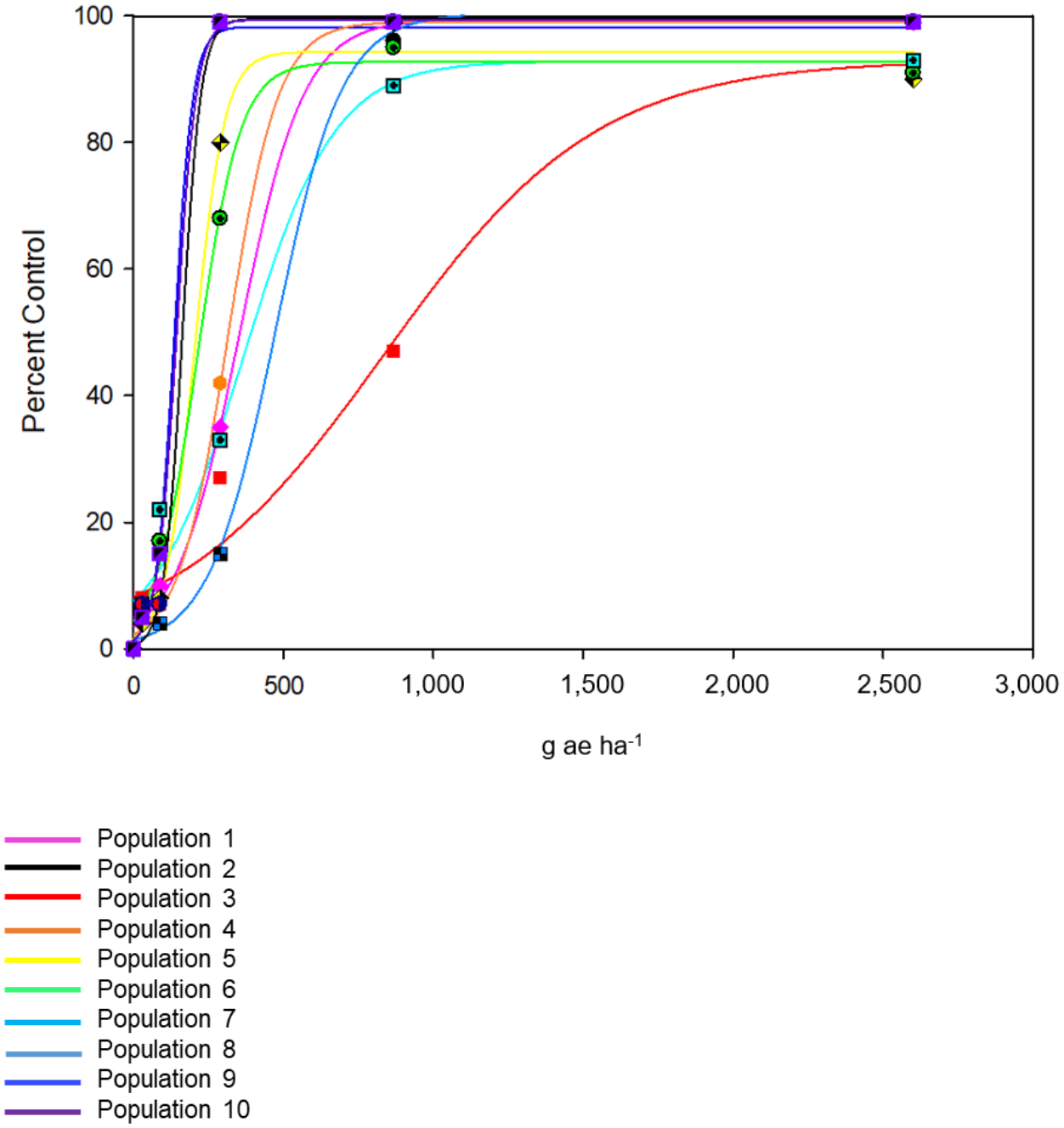

Figure 1. Glyphosate dose response by 10 accessions tested in 2019 from Tennessee. The responses of junglerice to increasing rates of glyphosate as described by Equation 1: Y = a/{1 + exp[−(rate − c) /b]}. In this model, *a* describes the asymptote or upper limit

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.

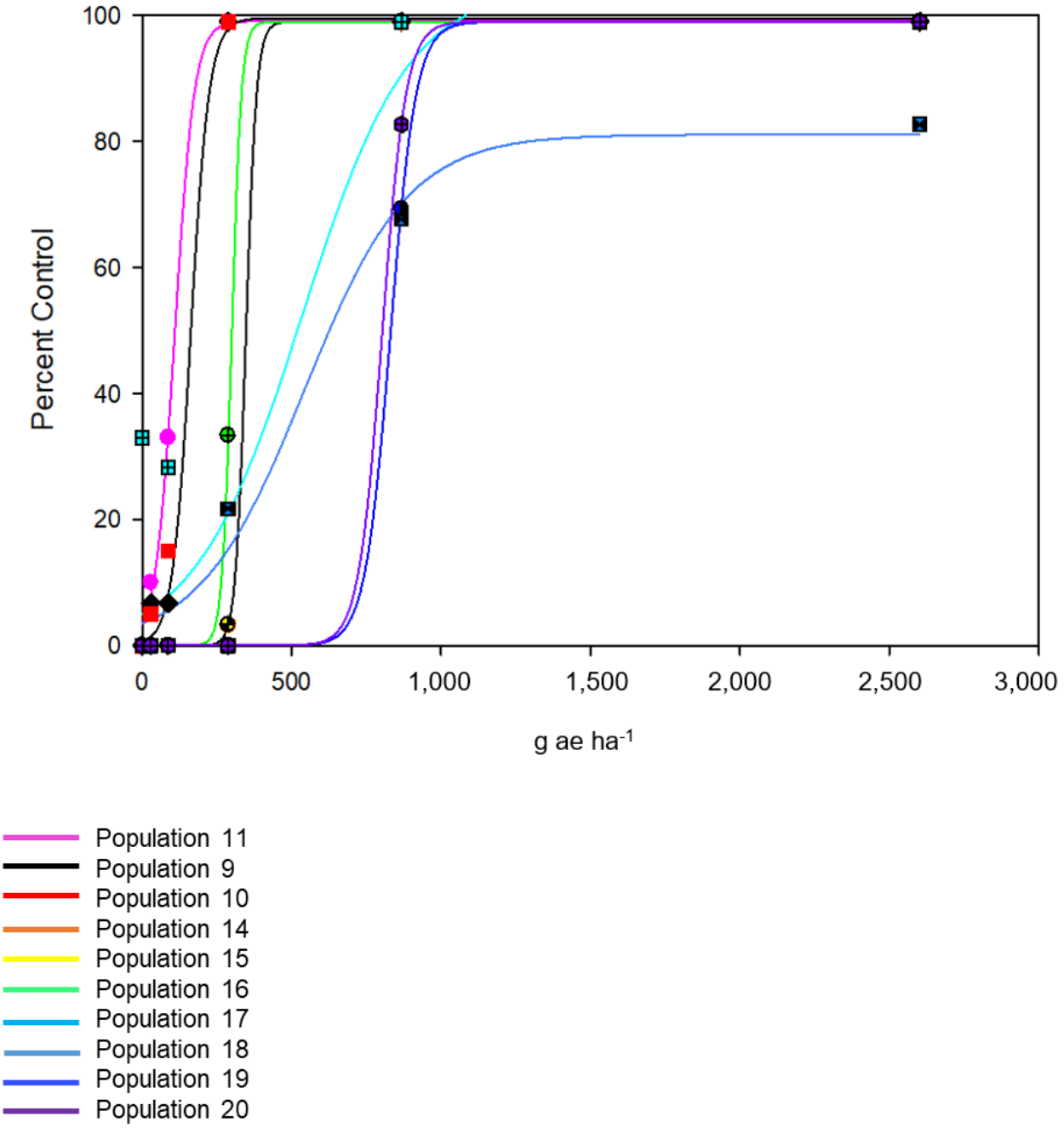

Figure 2. Glyphosate dose response of 10 junglerice accessions tested in 2018 in Tennessee. The responses of 10 accessions to increasing rates of glyphosate as described by Equation 1: Y = a/{1 + exp[−(rate − c) /b]}. In this model, *a* describes the

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.

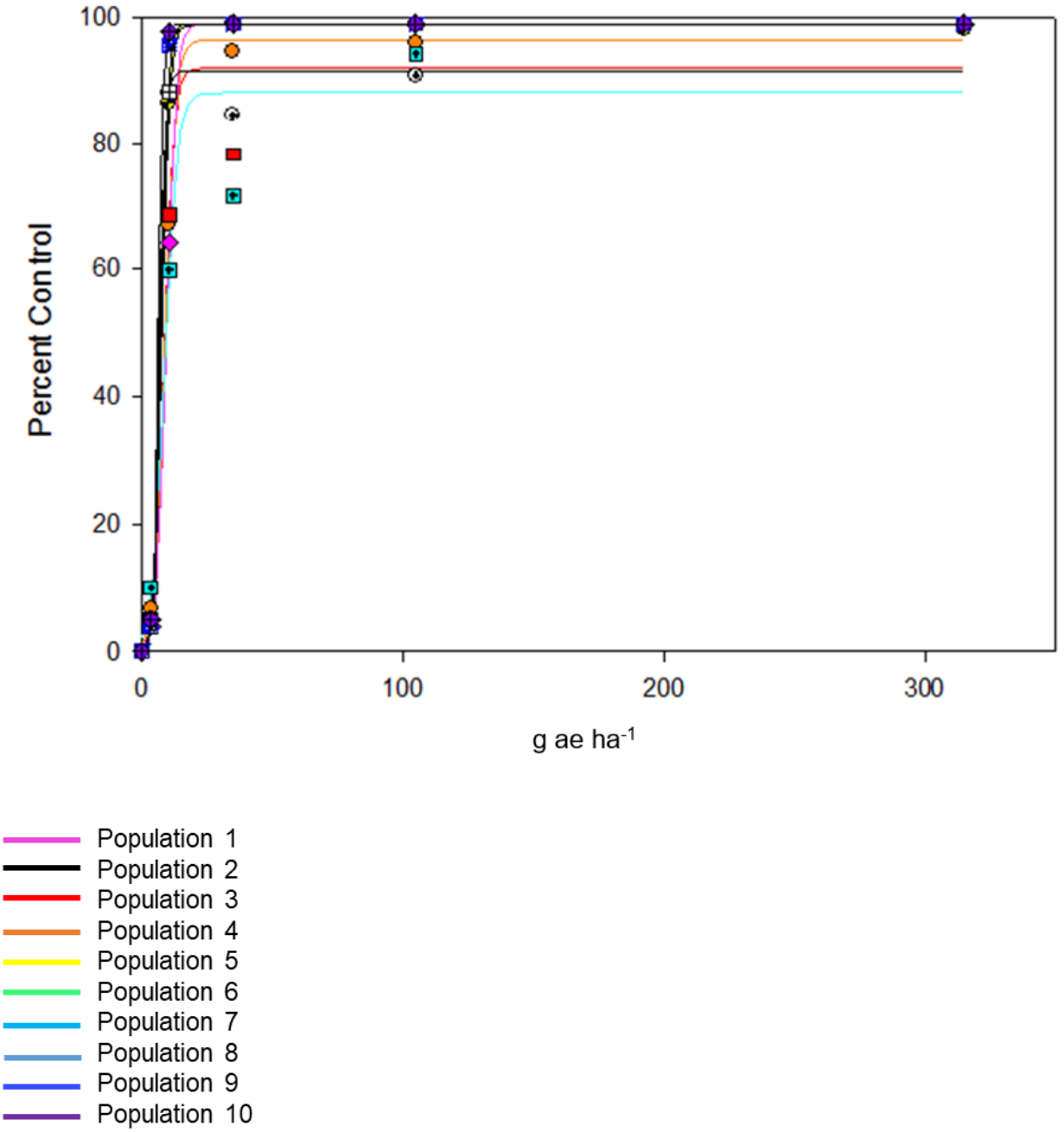

Figure 3. Clethodim dose response of 10 junglerice accessions tested in Tennessee in 2019. The responses of 10 accessions to increasing rates of clethodim as described by Equation 1: Y = a/{1 + exp[−(rate − c) /b]}. In this model, *a* describes the asymptote or

upper limit of control, *c* describes the half-maximal effective concentration, and *b* estimates the slope.

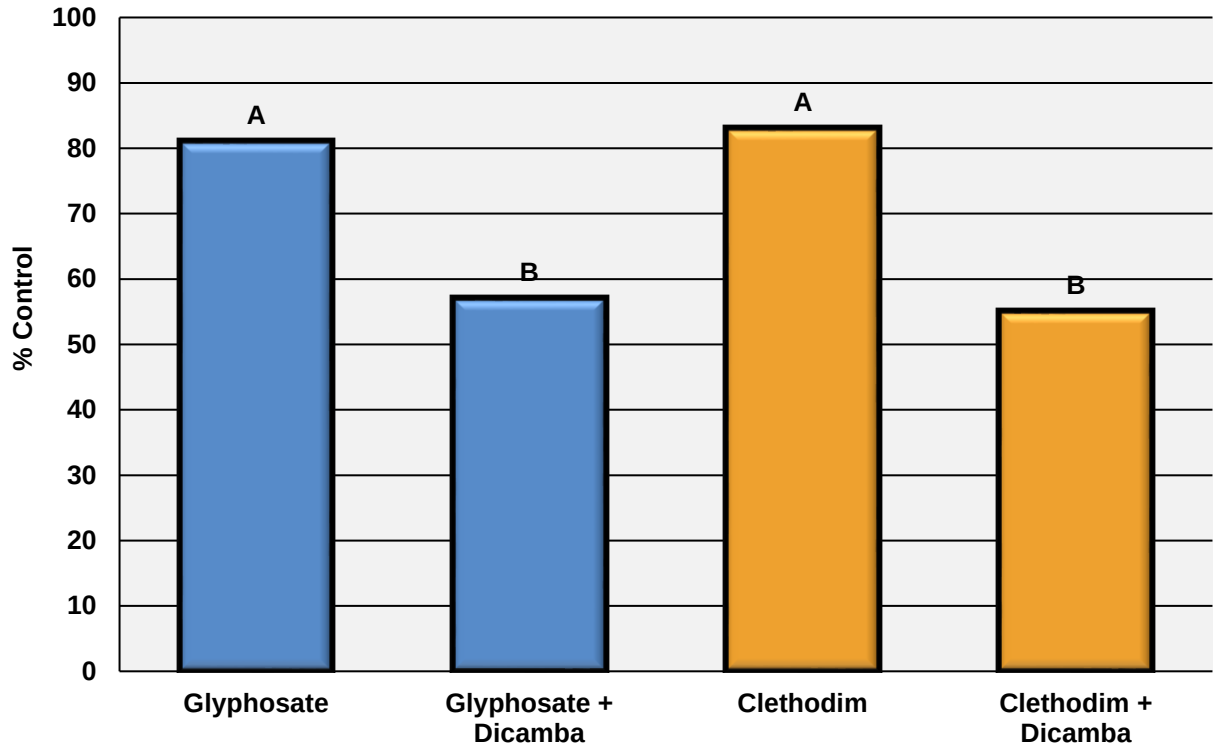

Figure 4. Field comparison results from 2019 and 2020 in Tennessee using single degree-of-freedom contrast statements comparing junglerice control 21 d after application with glyphosate at 870 g ha^{-1} to glyphosate at 870 g ha^{-1} + dicamba at 560 g ha^{-1} and clethodim at 105 g ha^{-1} compared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

CHAPTER II
JUNGLERICE CONTROL WITH GLYPHOSATE AND CLETHODIM AS INFLUENCED BY DICAMBA AND 2,4-D TANK-MIXES

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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 ultra-course 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. 2020a; Tahir 2007). Junglerice and barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] are the two most common *Echinochloa* species found in Tennessee (Perkins et al. 2020) and share many characteristics such as vast seed production, rapid C_4 growth, and extended emergence periods. Several populations of junglerice have been tested for glyphosate and clethodim resistance (Perkins et al. 2020). 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. 2020).

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 ultra-course 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. 2020a; 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 ultra-course 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 clethodim. 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 years 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 ultra-course 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, Greenville, MS), glyphosate + clethodim, glyphosate + dicamba (Engenia, BASF Corporation, Ludwigshafen, Germany), clethodim + 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 CO_2 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 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.

A second group of field experiments was conducted in 2020 at three locations. The purpose of this study was to analyze the effect of increasing the dicamba rate with mixtures of glyphosate. The same methods were used as described for the previous field experiment. Herbicide treatments include a nontreated (check), glyphosate, glyphosate + dicamba (1× rate), glyphosate + dicamba (1.5× rate), glyphosate + dicamba (2× rate), glyphosate + dicamba (1× rate) + DRA, glyphosate + dicamba (1.5× rate) + DRA, and glyphosate + dicamba (2× rate) + DRA. 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 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.

A third field experiment was conducted in 2019 at Jackson at WTREC and then repeated in 2020 again at WTREC and MREC. The purpose of this study was to analyze antagonism from glyphosate/clethodim + dicamba mixtures compared with applications of glyphosate/clethodim + 2,4-D (Enlist, Corteva Agrisciences, Wilmington, DE). The same methods were used as described for the previous field experiment. Herbicide treatments include a nontreated (check), glyphosate, clethodim, glyphosate + clethodim, glyphosate + dicamba, clethodim + dicamba, glyphosate + 2,4-D, and

clethodim + 2,4-D. 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}. 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 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 (Carmer et al. 1989). Mean separation for individual treatment differences was performed using Fisher's protected LSD test at $P \leq 0.05$. 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 5). 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 6). 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 5), 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 7). 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 ultra-course 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 8). 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 9). 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. (2020) 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 10). 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 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. 2020). 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.

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Appendix

Table 5. Single degree of freedom contrasts comparing glyphosate and/or clethodim to those herbicides mixed with dicamba or dicamba plus DRA on junglerice across six environments in Tennessee. All applications made with TTI nozzles.

Herbicide Treatment	Percent Control %	F-Value	P-Value	Biomass[a] g m^{-2}	F-Value	P-Value
Glyphosate	75	4.28	0.047	147	1.49	0.241
Glyphosate + Dicamba	54			121		
Glyphosate + Dicamba	54	3.86	0.059	121	3.37	0.086
Glyphosate + Dicamba + DRA	34			167		
Glyphosate	75	15.71	< 0.001	147	0.62	0.444
Glyphosate + Dicamba + DRA	34			167		
Clethodim	88	1.30	0.263	57	4.42	0.053
Clethodim + Dicamba	77			82		
Clethodim + Dicamba	77	1.30	0.262	82	0.67	0.426
Clethodim + Dicamba + DRA	66			102		
Clethodim	88	5.03	0.032	57	6.88	0.019
Clethodim + Dicamba + DRA	66			102		
Glyphosate + Clethodim	87	1.66	0.208	83	0.58	0.457
Glyphosate + Clethodim + Dicamba	75			94		

[a]Biomass is recorded in grams m^2

Table 6. Single degree of freedom contrasts comparing AIXR Flat Fan nozzles to TTI nozzles to those herbicides mixed with dicamba or dicamba plus DRA on junglerice across six environments in Tennessee.

Nozzle	Percent Control %	F-Value	P-Value
AIXR Flat Fan	83	5.16	0.025
TTI	76		

Table 7. Observed antagonism with increasing rates of dicamba mixtures with glyphosate (870 g ha^{-1}) with/without a drift reduction agent (DRA) on junglerice control across three locations in 2020 in Tennessee. All applications made with TTI nozzles.

Herbicide Treatment	Dicamba Dose[a]	Percent Control %	Biomass[c] g m^{-2}
Glyphosate at 870 g ha^{-1}			
1	0	75 a*	94 bc
2	560	56 b	89 c
3	840	42 c	88 c
4	1,120	33 d	131 ab
Glyphosate at 870 g ha^{-1} + DRA			
5	560	31 de	124 abc
6	840	25 ef	106 abc
7	1,120	19 f	138 a

[a]560 g ha^{-1} represents a 1x labeled use rate of dicamba
[b]DRA = drift reduction agent

[c]Biomass is recorded in grams m^2

*Means followed by the same letter are not significantly different according to Fisher's protected LSD at $P<0.05$

Table 8. Single degree of freedom contrast comparing glyphosate/clethodim + dicamba applications to glyphosate/clethodim + 2,4-D applications on junglerice control across three locations in 2019 and 2020 in Tennessee. All applications made with TTI nozzles.

Herbicide Treatment	Percent Control %	Biomass[a] g m^{-2}	F-Value	P-Value
Glyphosate + Dicamba	58	65	0.11	0.748
Glyphosate + 2,4-D	74	73		
Clethodim + Dicamba	67	-	0.89	0.366
Clethodim + 2,4-D	63	74		

[a]Biomass is recorded in grams m^2

Table 9. Single degree of freedom contrasts on herbicide applied with TTI nozzles comparing of glyphosate vs glyphosate + dicamba, clethodim vs clethodim + dicamba, glyphosate + dicamba vs glyphosate + dicamba + DRA, and clethodim + dicamba vs clethodim + dicamba + DRA, average across six populations in the greenhouse.

Herbicide Treatment	Percent Control %	F-Value	P-Value	Biomass[a] g m^{-2}	F-Value	P-Value
Glyphosate	96	18.78	< 0.001	0.17	0.11	0.743
Glyphosate + Dicamba	84			0.67		
Glyphosate + Dicamba	84	7.01	0.012	0.67	0.20	0.663
Glyphosate + Dicamba + DRA	91			0.33		
Glyphosate	96	2.84	0.101	0.17	0.60	0.448
Glyphosate + Dicamba + DRA	91			0.33		
Clethodim	96	3.05	0.090	< 0.01	5.92	0.024
Clethodim + Dicamba	97			0.08		
Clethodim + Dicamba	97	1.94	0.173	0.08	0.00	1.000
Clethodim + Dicamba + DRA	93			0.08		
Clethodim	96	0.12	0.726	< 0.01	5.92	0.024
Clethodim + Dicamba + DRA	93			0.08		

[a]Biomass is recorded in grams

Table 10. Single degree of freedom contrast statement comparing nozzles (AIXR Flat Fan vs TTI) on junglerice control, averaged across six populations in the greenhouse.

Nozzle	Percent Control %	F-Value	P-Value
AIXR Flat Fan	96	6.09	0.015
TTI	90		

CHAPTER III
JUNGLERICE (*ECHINOCHLOA COLONA*) CONTROL WITH SEQUENTIAL APPLICATIONS OF GLYPHOSATE AND CLETHODIM TO DICAMBA

A version of this chapter was originally published by Clay M. Perkins, Thomas C. Mueller and Lawrence E. Steckel:
Perkins CM, Mueller TC, Steckel LE (2021) Junglerice (*Echinochloa colona*) control with sequential applications of glyphosate and clethodim to dicamba. Weed Technol *In Press.*

Abstract

Junglerice is becoming more prevalent in Tennessee, Arkansas and Mississippi row crop fields. The evolution of glyphosate-resistant junglerice populations is one reason for the increase. Another possible explanation is that glyphosate and clethodim grass activity is being antagonized by dicamba. This question has led to research to examine if sequential applications alleviate antagonism observed with dicamba plus glyphosate and/or clethodim mixtures and determine if 24 h, 72 h or 168 h sequential treatments of those herbicides can improve junglerice control. Glyphosate + clethodim applications provided >90% junglerice control. The observed levels of antagonism varied by whether the location of the test was in the greenhouse or the field and the timing of applications. In the greenhouse, clethodim + dicamba provided excellent control while in the field the same treatment showed over a 30% reduction in junglerice control compared with clethodim alone. However, control was restored by using a mixture of glyphosate + clethodim without dicamba. The environment at the time of application and relative glyphosate-resistance (GR) level of the junglerice influenced the overall control of these sequential applications. Clethodim applied first followed by dicamba at 72 or 168 h, better control was observed compared with applying dicamba followed by clethodim. Overall, tank mixing glyphosate + clethodim provided the most complete junglerice control regardless of timing. These data confirm that leaving

dicamba out of the tank will mitigate herbicide antagonism on junglerice control. These data would also indicate that avoiding dicamba and glyphosate tank mixtures will also improve the consistency of control with glyphosate-susceptible junglerice.

Introduction

Junglerice is one of the predominant weeds in soybean (*Glycine max* (L.) Merr.) and cotton (*Gossypium hirsutum* L.) across the Mid-south and in particular Tennessee (Perkins et al. 2020; Tahir 2007). Junglerice and barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] are the two most common *Echinochloa* species found in Tennessee (Perkins et al. 2020). Junglerice is a warm-season annual grass that grows rapidly, has prolific seed production, and an extended emergence period. Testing for herbicide resistance of junglerice in Tennessee indicated that 15% of junglerice populations have a 2 to 8-fold resistance level to glyphosate which is consistent to what Nandula et al. (2018) found on selected Mississippi and Tennessee populations (Perkins et al. 2020).

The increase in junglerice prevalence across the Mid-south, is believed to be due to the evolution of glyphosate resistance as well as dicamba antagonism of glyphosate (Perkins et al. 2021). Poor junglerice control could be compounded by using the ultra-course nozzles and drift reduction agents (DRA) that are mandated for dicamba applications (Perkins et al. 2020). The majority of the hectares across the Mid-south, including Tennessee, are receiving at least one glyphosate + dicamba application. USDA reports that in 2018, 71% of the hectares were planted in dicamba-tolerant soybean and over 2.2 million kilograms of dicamba were applied in-crop in the United States (Wechsler et al. 2019). Dicamba use increased in 2019 with 16 million soybean

hectares planted to Xtend® varieties (Bayer Crop Protection, St. Louis, MO). A recent EPA memorandum on benefits of dicamba in dicamba-tolerant soybean production suggested that 97% of the dicamba applications were applied with glyphosate in 2018 and 2019 (Orlowski and Kells 2020). The frequent co-application of dicamba with glyphosate and/or clethodim could result in reduced grass control recently observed in the mid-south. In addition, growers have reported Palmer amaranth (*Amaranthus palmeri* S. Wats.) control failures with glyphosate + dicamba applications which resulted in some producers using higher dicamba rates (Steckel 2019). While using higher dicamba rates may improve Palmer amaranth control, it has been reported to decrease glyphosate effectiveness on junglerice (Perkins et al. 2021).

Colby (1967) describes herbicide 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 herbicide performs alone. In circumstances where an herbicide provides no POST activity on a given species (e.g. dicamba on junglerice), then Colby's method cannot be used because the model requires control greater than 0% from both of the herbicides. A decrease in glyphosate activity on junglerice plus other grass species has been documented from mixtures of glyphosate + dicamba compared with glyphosate applied alone (Perkins et al. 2021; O'Sullivan and O'Donovan 1980). In addition, antagonism of graminicides have been observed in other studies when they are applied with auxin herbicides (Blackshaw et al. 2006; Fletcher and Drexler 1980; Mueller et al. 1989; Olson and Nalewaja 1981; Todd and Stobbe 1980). Minton et al. (1989b) reports that antagonism and synergism responses may vary with the herbicides

used in tank mixtures or sequential applications, and responses may also differ depending on the grass species to be controlled. Minton et al. (1989a) reports decreased control of barnyardgrass where sethoxydim or quizalofop was tank-mixed with the broadleaf herbicides imazaquin, chlorimuron, or lactofen. They also reported antagonism when either grass product was applied 24 h after imazaquin or lactofen but not with chlorimuron. Tank mix combinations of broadleaf and grass herbicides have been reported to provide less grass control than expected (Byrd and York 1987; Croon and Merkle 1988; Grickar and Boswell 1987; Minton et al. 1989a; Minton et al. 1989b; Vidrine 1989). Myers and Coble (1992) reports that sequential applications of broadleaf and grass herbicides have been used to overcome antagonism.

Antagonism plus herbicide resistance can lead to weed control failure after only a few years. Avoiding antagonism from herbicide applications will assist in resistance management as well. Therefore, the objective of this research was to (1) examine if sequential applications alleviate antagonism observed with dicamba plus glyphosate and/or clethodim mixtures on junglerice control and (2) determine if 24 h, 72 h or 168 h sequential treatments of those herbicides can improve the consistency of junglerice control.

Materials and Methods

Greenhouse Research

A greenhouse experiment was replicated to determine how applications of glyphosate or clethodim made either 24 h or 72 h before or after an application of dicamba would impact weed control. This study was conducted on six non-repeating

collected populations from Tennessee similar to Perkins et al. (2020). The study design is 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^{-1}, using a 50:50 silt loam and potting soil premix. The treatment list contains a non-treated (check), glyphosate (Touchdown Hi-Tech®, Syngenta Crop Protection, Greensboro, NC), clethodim (Select Max®, Valent U.S.A. LLC., Walnut Creek, CA), glyphosate + dicamba (Engenia, BASF Corporation, Ludwigshafen, Germany), and clethodim + dicamba. Touchdown Hi-Tech® applications included 0.25% v/v NIS (non-ionic surfactant) and Select Max® applications included 1% v/v COC (crop oil concentrate) for this experiment. Treatments were applied in a Generation 4 Research Track Sprayer (DeVries Manufacturing, Inc., Hollandale, MN) using a TTI 11003 nozzle. The track sprayer speed was calibrated to deliver 140 L ha^{-1} and the nozzle height was set to spray approximately 45 cm above the crop canopy. Applications were made when plants reached 8-10 cm in height. Greenhouse air temperature was set at 24 to 27 C and 60% relative humidity. Junglerice control was visually estimated on a scale of 0 to 100% where 0 = no injury and 100 = plant death at 28 days after treatment. Plant biomass was taken 28 to 35 days after treatment. Biomass was collected by clipping plants at the soil surface and collecting fresh weights.

Field Research

Because the greenhouse data indicated that a 72 h sequential application mitigated antagonism, field studies having a 72 h and 168 h sequential timing were conducted. The research was arranged in a randomized complete block design.

Individual plot size was 1.5 m wide and 9.1 m long at the West Tennessee Research and Education Center (WTREC) in Jackson, TN. Plots at two other 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 herbicide treatments included a non-treated (check), glyphosate (Roundup Powermax®, Bayer Crop Protection, St. Louis, MO), clethodim (Intensity®, Loveland Products, Greenville, MS), glyphosate + clethodim, glyphosate + dicamba (Engenia, BASF Corporation, Ludwigshafen, Germany), clethodim + dicamba, and glyphosate + clethodim + dicamba. In addition, glyphosate and clethodim applications were made at 72 h and 168 h, preceding or following dicamba. 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}.

Applications were made with a CO_2 pressurized backpack sprayer calibrated to apply at 140 L ha^{-1} using a TTI 11003 nozzles. Applications were made when junglerice plants were 8-10 cm in height. Control of junglerice was visually estimated at 7, 14, and 21 days after final treatment on a scale of 0 to 100% where 0 = no injury and 100 = plant death. Above-ground, fresh weight biomass data was collected 21 to 28 days after treatment in a 0.2 m^2 area by clipping plants at the soil surface and weighing to the nearest gram. Only the latest evaluations are presented.

Data Analysis

The authors did not run Colby's method to determine relative antagonism levels. The reason for this is that in circumstances where a herbicide provides no POST activity

on a given species (e.g. dicamba on junglerice), then Colby's method cannot be used because the model requires control greater than 0% control from both of the herbicides.

At all the given locations, there was both representation from glyphosate-resistant and -susceptible populations of junglerice. This was documented by Perkins et al. 2020 where 13% of the populations from these fields was found to be glyphosate-resistant. However, much more than 13% of the junglerice population was surviving dicamba + glyphosate applications which suggested dicamba antagonism of glyphosate activity on junglerice (Perkins et al. 2020).

In order to discriminate lack of junglerice control between glyphosate-resistance and dicamba antagonism of glyphosate, populations were blocked on site due to differences in junglerice population density and glyphosate resistance. Block four typically had the highest proportion of glyphosate-resistant junglerice population. The authors suggest that blocking in this fashion did help sequester the error (CV <10) caused by the glyphosate-resistant populations. Fixed effects were herbicide treatments. Environment, replication, 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 (Carmer et al. 1989). Mean separation for individual treatment differences was performed using Fisher's Protected LSD test at $p \leq 0.05$. 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.

Results and Discussion

Greenhouse Study

There was an overall herbicide effect ($P < 0.001$) among treatments with glyphosate and clethodim. Glyphosate alone provided 83% junglerice control (Table 11). Glyphosate + dicamba tank-mix provided numerically less (68%) junglerice control, but this difference was not quite significant at an alpha of 0.06. This is 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. The treatment where dicamba was applied 24 h after the glyphosate application resulted in the lowest level of junglerice control (59%). Conversely, when glyphosate was applied 24 h after dicamba, control was similar to glyphosate applied alone. When the glyphosate application was separated from the dicamba application for 72 h, regardless of order, junglerice control was not different than glyphosate applied alone. Biomass results in all cases but one supported the control data with no difference detected amongst the treatments (Table 11).

The clethodim treatment provided 92% control of junglerice (Table 12), and clethodim + dicamba tank-mix provided similar control (86%). When dicamba was applied 24 h after clethodim, control was consistent with glyphosate alone or in a tank-mix of clethodim + dicamba. These results are consistent with Minton et al. (1989a) who reported no antagonism with clethodim when tank mixed with 2,4-DB on barnyardgrass. However, control was lower (65%) when clethodim was applied 24 hours after the dicamba application. When dicamba was applied 72 h after clethodim, junglerice control was reduced by 20%, whereas, when clethodim was applied 72 hours after dicamba,

junglerice control was similar to glyphosate alone (93%). With the exception of the treatment where clethodim was applied 24 h after dicamba, the biomass data reflected the control data. These results are consistent with Minton et al. (1989a,b) who reported that fluazifop-P was antagonized by tank mixing with 2,4-DB.

Field Studies

An effect of herbicide treatments on junglerice control was observed in the field studies ($P < 0.001$). Glyphosate alone provided 59% control (Table 13). The poor junglerice control by glyphosate would suggest that at least one of the locations contained both a segregating glyphosate-resistant and -susceptible population. The glyphosate + dicamba application provided similar junglerice control (48%). These results are similar to Perkins et al. (2020) where 57% junglerice control with glyphosate + dicamba tank-mix was reported, but glyphosate alone provided 82% control. This was also similar to Flint and Barrett (1989) who observed antagonism with dicamba tank mixes on johnsongrass. The glyphosate + clethodim treatment provided the greatest junglerice control at 91%. These results are consistent with Perkins et al. (2020) who reported that 15% of Tennessee junglerice populations could no longer be controlled with glyphosate, but clethodim was still effective. Similarly, a glyphosate + clethodim + dicamba application provided 81% control of junglerice. Glyphosate control was reduced when dicamba was sprayed 72 h before or after glyphosate, (55% and 53%, respectively). Similar control was found with a 168 h sequential. From these data, however, a glyphosate + clethodim application preceding or following a dicamba application at both 72 and 168 h provided the best control on junglerice. Biomass

results supported visual control data with no differences detected amongst the glyphosate or clethodim treatments.

Antagonism was observed from a clethodim + dicamba application which provided only 63% control where clethodim alone provided 86% ($P < 0.001$; Table 14). This differs from Minton et al. (1989a) who reported that 2,4-DB did not reduce control of barnyardgrass by clethodim. We observed that adding glyphosate to the clethodim + dicamba tank-mix improved control (81%), but mixing glyphosate with clethodim did not improve junglerice control (91%) over clethodim alone. When dicamba followed clethodim at 72 or 168 h later junglerice control was similar to clethodim applied alone. Similarly, dicamba applied first followed 72 h later with clethodim did not reduce junglerice control over clethodim applied alone. However, if clethodim was applied 168 h after dicamba then junglerice control was greatly reduced (61%). Biomass results were similar and supported these data with no differences detected.

The addition of dicamba decreased junglerice control by glyphosate and clethodim in some but not all of our studies. As would be anticipated, where GR junglerice existed in the population, control with glyphosate was poor regardless of whether or not dicamba was added. In some of these environments, dicamba hindered clethodim control of junglerice. The level of antagonism documented observed varied by timing of sequential applications. In the greenhouse, dicamba + clethodim provided excellent control while in the field the same treatment showed over a 30% reduction in junglerice control compared with clethodim alone. However, resistance or antagonism was overcome by using a mixture of glyphosate + clethodim. Ultimately, the question of

whether junglerice control could be improved by applying glyphosate and waiting 24, 72 or 168 h to apply dicamba or vice versa was not clearly answered. The authors suggest that the relative glyphosate-resistance level of the junglerice influenced the overall control of these sequential applications. However, clethodim applied first followed at either 72 or 168 h by dicamba provided consistently better control than applying dicamba followed by clethodim.

Tank mixing glyphosate + clethodim provided the most consistent junglerice control regardless of different application intervals. These data confirm that leaving dicamba out of the tank will avoid the possibility of it antagonizing control of junglerice with clethodim. These data along with Perkins et al. (2020) would also indicate that avoiding dicamba and glyphosate tank mixtures will also improve the consistency of control with glyphosate-susceptible junglerice. A survey by Perkins et al. (2020) reported that on average, 40% of the fields in Tennessee have both Palmer amaranth plus *Echinochloa* species present at harvest. Thus, the control of both junglerice and Palmer amaranth in the same field can be improved by not co-applying dicamba with glyphosate.

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Appendix

Table 11. Junglerice control comparing 24 and 72 h sequential applications of glyphosate applications preceding and following dicamba application across 6 environments from Tennessee in the greenhouse.

Sequential Timing	Herbicide Treatment	Percent Control %	Biomass[a] g m^{-2}
Alone	Glyphosate	83 abc	0.67 b
Tank Mix	Glyphosate + Dicamba	68 cd	1.92 b
24 Hr. Sequential	Glyphosate fb Dicamba	59 d	1.08 b
	Dicamba fb Glyphosate	68 cd	0.5 b
72 Hr. Sequential	Glyphosate fb Dicamba	76 bcd	0.25 b
	Dicamba fb Glyphosate	87 ab	0.42 b
	F-Value	4.01	2.81
	Df	11, 191	11, 132
	P-Value	< 0.001	0.003

Means not followed by a common letter are significantly different (P < 0.05).

[a]Biomass is recorded in grams m^{-2}

Table 12. Junglerice control comparing 24 and 72 h sequential applications of clethodim applications preceding and following dicamba application across 6 environments from Tennessee in the greenhouse.

Sequential Timing	Herbicide Treatment	Percent Control %	Biomass[a] g m^{-2}
Alone	Clethodim	92 ab	2.00 b
Tank Mix	Clethodim + Dicamba	86 ab	1.75 b
24 Hr. Sequential	Clethodim fb Dicamba	87 ab	2.33 b
	Dicamba fb Clethodim	65 d	1.25 b
72 Hr. Sequential	Clethodim fb Dicamba	66 cd	5.25 a
	Dicamba fb Clethodim	93 a	0.01 b
	F-Value	4.01	2.81
	Df	11, 191	11, 132
	P-Value	< 0.001	0.003

Means not followed by a common letter are significantly different (P < 0.05).

[a]Biomass is recorded in grams m^{-2}

Table 13. Junglerice control comparing 72 h and 168 h sequential applications of glyphosate and glyphosate + clethodim applications preceding and following dicamba application across 3 environments in Tennessee in 2020.

Sequential Timing	Herbicide Treatment	Percent Control %	Biomass[a] g m^{-2}
Alone	Glyphosate	59 de	120.0 abc
	Glyphosate + Clethodim	91 a	53.0 c
Tank Mix	Glyphosate + Dicamba	48 e	133.8 ab
	Glyphosate + Clethodim + Dicamba	81 ab	93.9 abc
3 Day Sequential	Glyphosate fb Dicamba	55 de	133.6 ab
	Dicamba fb Glyphosate	53 de	131.8 ab
	Glyphosate + Clethodim fb Dicamba	81 ab	95.1 abc
	Dicamba fb Glyphosate + Clethodim	87 a	73.5 abc
7 Day Sequential	Glyphosate fb Dicamba	68 bcd	92.0 abc
	Dicamba fb Glyphosate	60 de	100.0 abc
	Glyphosate + Clethodim fb Dicamba	87 a	135.0 a
	Dicamba fb Glyphosate + Clethodim	86 a	79.3 abc
	F-Value	5.27	1.16
	Df	17, 34	17, 17
	P-Value	< 0.001	0.381

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

[a]Biomass is recorded in grams m^{-2}

Table 14. Junglerice control comparing 72 h and 168 h sequential applications of clethodim and glyphosate + clethodim applications preceding and following dicamba application across 3 environments in Tennessee in 2020.

Sequential Timing	Herbicide Treatment	Percent Control %	Biomass[a] g m^{-2}
Alone	Clethodim	86 ab	59.8 abc
	Glyphosate + Clethodim	91 a	53.0 c
Tank Mix	Clethodim + Dicamba	63 cde	72.3 abc
	Glyphosate + Clethodim + Dicamba	81 ab	93.9 abc
3 Day Sequential	Clethodim fb Dicamba	78 abc	70.6 ab
	Dicamba fb Clethodim	82 ab	72.9 abc
	Glyphosate + Clethodim fb Dicamba	81 ab	95.1 abc
	Dicamba fb Glyphosate + Clethodim	87 a	73.5 abc
7 Day Sequential	Clethodim fb Dicamba	84 ab	84.8 abc
	Dicamba fb Clethodim	61 cde	57.3 bc
	Glyphosate + Clethodim fb Dicamba	87 a	135.0 a
	Dicamba fb Glyphosate + Clethodim	86 a	79.3 abc
	F-Value	5.27	1.16
	Df	17, 34	17, 17
	P-Value	< 0.001	0.381

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

[a]Biomass is recorded in grams m^{-2}

CHAPTER IV
EFFICACY OF BURNDOWN WITH SEQUENTIAL APPLICATIONS FOR JUNGLERICE (*ECHINOCHLOA COLONA*) CONTROL

Abstract

Areas reporting problems controlling junglerice has continued to expand in Tennessee. Both glyphosate resistance and herbicide antagonism have been documented in causing poor control of junglerice. In Tennessee, glyphosate resistance has been estimated in 15% of junglerice populations. In addition, dicamba tank mixtures with glyphosate and/or clethodim have been reported to reduce junglerice control. Due to poor in-crop control, starting clean has taken on added importance when trying to control junglerice. Therefore, research was conducted to determine the best burndown methods utilizing clethodim, dicamba, glufosinate, glyphosate, or paraquat. A dicamba + glyphosate, glufosinate + clethodim, or paraquat + clethodim application provided the greatest control of junglerice (98, 90, and 90% respectively). Regardless of which herbicides were initially applied, making a follow-up application of glyphosate two weeks later provided optimal control of junglerice. In Tennessee, based on these data, a glyphosate + clethodim application at 14 DBP is recommended to control junglerice, other grasses and some broadleaves, and then to apply paraquat at planting to control remaining weed species present. Subsequent applications of glyphosate or glyphosate + clethodim will provide optimal results in controlling junglerice and other grasses.

Introduction

The Mid-south has seen a significant increase in the population of junglerice (*Echinochloa colona* (L.) Link). It has become one of the most predominant weed species found in soybean (*Glycine max* (L.) Merr.) and cotton (*Gossypium hirsutum* L.) fields (Perkins et al. 2020; Perkins et al. 2021a; Tahir 2007). Junglerice and

barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] have become the most common *Echinochloa* species found in the midsouth USA (Perkins et al. 2020). These two species share many characteristics, such as vast seed production, rapid C_4 growth, and extended emergence periods. Glyphosate resistance has been documented across the midsouth. A recent survey estimated that 15% of the populations are 2 to 8-fold more tolerant to glyphosate than the most susceptible populations which is consistent to what Nandula et al. (2018) found on selected Mississippi and Tennessee populations.

The increase of junglerice prevalence across the Mid-south is believed to be not only due to the evolution of glyphosate resistance, but also because of auxin antagonism of glyphosate on junglerice (Perkins et al. 2021b). This antagonism could be enhanced by using the ultra-coarse nozzles and drift reduction agents (DRA) that are mandated for dicamba applications. Another survey estimated a majority of the soybean and cotton hectares across the Mid-south and Tennessee are receiving at least one glyphosate + dicamba application. USDA has reported that in 2018, 71% of the hectares were planted in dicamba-tolerant soybean with 2.2 million kilograms of dicamba being applied in-crop in the United States (Wechsler et al. 2019). Dicamba use increased in 2019 with 16 million soybean hectares planted to Xtend® (Bayer Crop Protection, St. Louis, MO) varieties. A recent EPA memorandum on benefits of dicamba in dicamba-tolerant soybean production suggested that 97% of the dicamba applications were being mixed with glyphosate in 2018 and 2019 (Orlowski and Kells 2020). The antagonism from this mixture is likely contributing to poor grass control. In addition, growers have reported Palmer amaranth (*Amaranthus palmeri* S. Wats.) control failures

with glyphosate + dicamba applications which resulted in some producers using higher dicamba rates (Steckel 2019). While using higher dicamba rates may improve Palmer control, it has been reported to decrease glyphosate effectiveness on junglerice (Perkins et al. 2021b).

Paraquat is a non-selective contact herbicide (photosystem I electron diverter (Group 22); Sagar 1987). One benefit as an effective burndown herbicide is that due to chemical reaction of tightly binding to soil particles, paraquat deactivates on contact with soil. As such, no biologically active residues remain in the soil allowing planting to be carried out immediately. Paraquat's many unique properties have resulted in making it the herbicide of choice for 25 million farmers worldwide (Brown et al. 2004). Paraquat's rapid action gives farmers confidence that weeds have been controlled and the need for follow-up applications are reduced. Most extension publications today show paraquat to be less effective on grasses than broadleaves (Barber et al. 2021; Steckel et al. 2021). Buker et al. (2002) evaluated grass control with tank mixtures of paraquat and graminicides. They reported an increase in control of goosegrass (*Eleusine indica*) when mixing paraquat with sethoxydim or clethodim compared with paraquat alone.

Glufosinate is also known as a non-selective herbicide and can also be used in burdowns (Blair-Kerth et al. 2001; Gardner et al. 2006a). However, glufosinate control of annual grasses can be marginal, especially in less than ideal growing conditions (Beyers et al. 2002; Coetzer et al. 2002; Corbett et al. 2004; Culpepper et al. 2000; Hill et al. 1997; Steckel et al. 1997; York and Culpepper 2004). Grass regrowth may occur on plants not completely killed by glufosinate, and new plants may start to emerge after

a single application (Coetzer et al. 2002). Randell et al. (2020) reported that glufosinate + glyphosate applications can improve the effectiveness of grass control compared with glufosinate alone. Antagonism has been reported from glufosinate + graminicides, such as clethodim, on annual grasses and barnyardgrass (Burke et al. 2005; Gardner et al. 2006b; Irby et al. 2007; Eytcheson and Reynolds 2019).

Givens et al. (2009) reported that between 20 and 76% of growers are utilizing a preplant burndown application. They noted that the most frequently used herbicides for spring preplant burndown applications were glyphosate and 2,4-D. Glyphosate use was reported at a higher percentage in cotton and soybean fields in a burndown application. Current best management practices of known troublesome and herbicide resistant weeds include planting intro weed-free fields, keeping fields as weed-free as possible, and applying herbicides at the recommended weed size (Norsworthy et al. 2012). Starting weed-free is an important first step to help maintain adequate weed control. Vollmer et al. (2019) found that two sequential spring applications were needed to provide a weed-free seedbed. Starting weed-free is an important first step to help maintain adequate weed control. Adequate control of Palmer amaranth was achieved with timely applications of an effective PRE herbicide followed by effective POST residual herbicides when the crop was planted weed-free (Bell et al. 2015; Whitaker et al. 2010).

Therefore, the objective of this research was to (1) evaluate junglerice control with dicamba, glufosinate, and paraquat burndown options and (2) determine the efficacy of tank mixtures of these herbicides with glyphosate and clethodim.

Materials and Methods

The research was arranged in a randomized complete block design with herbicide treatment as the main factor. Plot size was 1.5 m wide and 9 m long in Jackson at the West Tennessee Research and Education Center (WTREC). Plots at two other locations, Milan Research and Education Center (MREC) and a grower field (Golddust, TN) were 1.5 m wide and 6 m long. Depending upon location, there were three (MREC and Golddust) or four (WTREC) replications. The herbicide treatments can be found in Table 15 and were a non-treated (check), glyphosate (Roundup Powermax®, Bayer Crop Protection, St. Louis, MO), clethodim (Intensity®, Loveland Products, Greenville, MS), glyphosate + clethodim, glufosinate (Liberty®, BASF Corporation, Florham Park, NJ), glufosinate + glyphosate, glufosinate + clethodim, dicamba (Engenia, BASF Corporation, Florham Park, NJ), glyphosate + dicamba, clethodim + dicamba, paraquat (Gramoxone® SL 2.0, Syngenta, Greensboro, NC), paraquat + glyphosate, and paraquat + clethodim. Herbicide treatments were replicated with and without a follow-up application of glyphosate made two weeks after the initial application. 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}. Applications were made with a CO_2 back pack sprayer calibrated to apply at 142 L ha^{-1} using a TTI 11003 nozzle. Applications were made when junglerice plants were 8-10 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 days after treatment.

Data Analysis

Populations were blocked on site due to *Echinochloa* spp. population. Fixed effects were herbicide treatments. 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 (Carmer et al. 1989). Mean separation for individual treatment differences was performed using Fisher's Protected LSD test at $p \leq 0.05$ (SAS v9.4; SAS Institute; Cary, NC).

Results and Discussion

Dicamba Burndown

Glyphosate and clethodim treatments provided 94 and 85% control respectively, at 14 DAA (Table 16). When glyphosate or clethodim was applied mixed with dicamba, junglerice control was reduced (67%). Similar results with dicamba + glyphosate and dicamba + clethodim were reported by Perkins et al. (2021a). Glyphosate alone or tank mix glyphosate with clethodim provided the best control of junglerice (94 and 95% respectively). At 35 DAA, glyphosate and clethodim applied alone provided 74 and 79% control of junglerice, respectively. The tank mix of glyphosate + clethodim gave similar control. A dicamba + glyphosate application reduced junglerice control. However, the dicamba + glyphosate tank mixed provided similar control as clethodim alone.

When glyphosate was applied 14 days after the initial application, control improved. All treatments provided similar and good control except dicamba alone followed by glyphosate. A dicamba + glyphosate application followed by glyphosate

provided 98 percent control of junglerice after 5 weeks (Table 16). Similarly, a glyphosate + clethodim application followed by glyphosate provided 96 percent control.

Glufosinate Burndown

Glufosinate alone provided 68% junglerice control at 14 DAA (Table 17). Poor control of annual grasses by glufosinate has been previously reported (Burke et al. 2005; Corbett et al. 2004; Norris et al. 2002). Glufosinate + clethodim provided similar control to glufosinate + glyphosate and better control than glufosinate alone. At 35 days after initial application, poor control was observed with all treatments (≤ 35%). There were no differences among treatments as junglerice had recovered. No antagonism was observed from a glufosinate + clethodim application, which conflicts with previous research on grasses (Burke et al. 2005; Gardner et al. 2006a; Irby et al. 2007; Eytcheson and Reynolds 2019).

A glyphosate application two weeks following initial application markedly improved junglerice control (≥ 86%; $P < 0.001$; Table 17) with all treatments providing similar control. These glufosinate options provided good control of junglerice, however, numerically not as much as the burndown options of glyphosate and clethodim alone and in tank mix.

Paraquat Burndown

After 14 days, paraquat alone provided only 52% control of junglerice (Table 18). Glyphosate tank mixed with paraquat did not improve junglerice control (59%), however, the addition of clethodim to paraquat increased control (95%). These data are similar to what Buker et al. (2002) found when tank mixing paraquat plus clethodim compared

with paraquat alone. At 35 DAA, poor control was observed from all treatments. At 35 DAA, paraquat + clethodim provided better control than the other treatments, but was still not satisfactory (50%).

A follow-up application of glyphosate two weeks after the initial application greatly improved control ($P < 0.001$; Table 18). From this data, a paraquat burndown application at planting and then a glyphosate application two weeks later will provide acceptable control of junglerice (87 – 90%).

In conclusion, a follow-up application of glyphosate two weeks after the initial application, regardless of burndown option, substantially improved control of junglerice. These data suggest that the best control of junglerice can be achieved with a glyphosate + clethodim application at burndown to control junglerice and then applying paraquat at planting to control any broadleaves present. A subsequent application of glyphosate or glyphosate + clethodim will provide excellent control of junglerice and should assist in resistance management by utilizing two effective modes of action in controlling junglerice.

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Appendix

Table 15. Herbicide treatment list containing common name, trade name, and manufacturer.

Treatment	Common Name	Trade Name	Manufacturer
1	Glyphosate	Roundup Powermax®	Bayer Crop Protection
2	Clethodim	Intensity®	Loveland Products
3	Glyphosate + Clethodim	Roundup Powermax® + Intensity®	Bayer Crop Protection + Loveland Products
4	Glufosinate	Liberty®	BASF Corporation
5	Glufosinate + Glyphosate	Liberty® + Roundup Powermax®	BASF Corporation + Bayer Crop Protection
6	Glufosinate + Clethodim	Liberty® + Intensity®	BASF Corporation + Loveland Products
7	Dicamba	Engenia	BASF Corporation
8	Dicamba + Glyphosate	Engenia + Roundup Powermax®	BASF Corporation + Bayer Crop Protection
9	Dicamba + Clethodim	Engenia + Intensity®	BASF Corporation + Loveland Products
10	Paraquat	Gramoxone®	Syngenta
11	Paraquat + Glyphosate	Gramoxone® + Roundup Powermax®	Syngenta + Bayer Crop Protection
12	Paraquat + Clethodim	Gramoxone® + Intensity®	Syngenta + Loveland Products

Table 16. Junglerice control at burndown with dicamba options and following up with a glyphosate application two weeks after initial application in Tennessee across three environments.

	Percent Control %[a]		
Herbicide Treatment	**14 DAA***	**35 DAA**	**21 DAB* fb Glyphosate**
Glyphosate	94 a	74 bc	94 a
Clethodim	85 b	79 b	96 a
Glyphosate + Clethodim	95 a	78 b	96 a
Dicamba	0 d	0 d	79 b
Dicamba + Glyphosate	67 c	62 c	98 a
Dicamba + Clethodim	67 c	74 bc	97 a
F-value	283.3	44.0	
Df	5, 33	11, 51	
P-value	< 0.001	< 0.001	

[a]Means followed by the same letter are not significantly different according to Fisher's protected LSD at P < 0.05.
*DAA – Days After "A" Application; DAB – Days After "B" Application

Table 17. Junglerice control at burndown with glufosinate options and following up with a glyphosate application two weeks after initial application in Tennessee across three environments.

	Percent Control %[a]		
Herbicide Treatment	**14 DAA***	**35 DAA**	**21 DAB* fb Glyphosate**
Glufosinate	68 b	22 b	86 a
Glufosinate + Glyphosate	80 ab	31 b	87 a
Glufosinate + Clethodim	88 a	35 b	90 a
F-value	3.9	40.8	
Df	2, 20	5, 33	
P-value	0.037	< 0.001	

[a]Means followed by the same letter are not significantly different according to Fisher's protected LSD at P < 0.05.
*DAA – Days After "A" Application; DAB – Days After "B" Application

Table 18. Junglerice control at burndown with paraquat options and following up with a glyphosate application two weeks after initial application in Tennessee across three environments.

	Percent Control %[a]		
Herbicide Treatment	**14 DAA***	**35 DAA**	**21 DAB* fb Glyphosate**
Paraquat	52 b	21 c	88 a
Paraquat + Glyphosate	59 b	14 c	87 a
Paraquat + Clethodim	95 a	50 b	90 a
F-value	23.3	39.1	
Df	2, 8	5, 24	
P-value	< 0.001	< 0.001	

[a]Means followed by the same letter are not significantly different according to Fisher's protected LSD at P < 0.05.
*DAA – Days After "A" Application; DAB – Days After "B" Application

CONCLUSION

The overall goal of this research was to characterize junglerice and learn of better management options in controlling one of the top weed species found in Tennessee. The first objective of the research was to survey the spread of junglerice and quantify its presence across the state. Junglerice was found in 65% of the fields (N = 108) surveyed in Tennessee. Barnyardgrass followed at a 50% infestation rate, with Palmer amaranth present in 56% of fields, and 28% of the fields were infested with both junglerice and barnyardgrass. In addition, 41% of the fields were infested with both Palmer amaranth and *Echinochloa* spp. From this survey, we collected several populations to measure the level of resistance. The survey showed that 70% of the junglerice accessions had an effective glyphosate RRF of 2.5 to 8.5, suggesting glyphosate-resistance has evolved in Tennessee. These data indicate that junglerice population escapes in dicamba-resistant (DR) cotton and soybean field are due, in part, to glyphosate resistance in approximately 13% of junglerice accessions surveyed from Tennessee. From these accessions, it was observed that all were still controlled by clethodim in a greenhouse environment but less control was seen under field conditions. These data also imply that a significant cause of the poor junglerice control is dicamba antagonizing the glyphosate and/or clethodim activity. It is suggested that the poor junglerice control in the majority DR fields in the survey was due to a combination of glyphosate resistance and dicamba antagonism of glyphosate and clethodim.

These results lead to my second objective of measuring the level of antagonism from glyphosate/clethodim + dicamba applications. This was assessed by making

dicamba tank mix applications, and also determining whether labeled nozzles and drift reduction agents (DRAs) used in these applications are reducing control, and also investigating whether increased dicamba rates are resulting in less junglerice control. Field results show that on average, 15% less junglerice control was observed when mixing glyphosate with dicamba. An additional 7% loss of control was observed when the TTI nozzles were used, and an additional 16% loss occurred when a DRA was added. Greenhouse results show that antagonism from tank mixing dicamba was still evident, however, not as pronounced as the field results. It was observed as increased rates of dicamba resulted in decreased grass control. The data suggest that separating glyphosate and/or clethodim applications with dicamba will provide better junglerice control. There were no statistical differences between dicamba and 2,4-D applications mixed with glyphosate or clethodim. However, there was a numerical reduction in antagonism from glyphosate + dicamba applications to glyphosate + 2,4-D applications. There was more antagonism observed from clethodim + 2,4-D mixtures compared to dicamba. Mixing dicamba with glyphosate or clethodim using labeled nozzles and a DRA is causing reduced junglerice control and should be avoided. Sequential applications of these products are recommended for greater junglerice control.

The third objective of my research was to determine if sequential applications could alleviate antagonism observed with dicamba plus glyphosate and/or clethodim mixtures and if 24 h, 72 h, or 168 h sequential application intervals have a similar impact on junglerice control. From these data, it was clear that a lone glyphosate + clethodim application provided better junglerice control than when a sequential

application of dicamba was made, regardless of the application intervals. Utilizing a 72 or 168 h interval preceding or following a dicamba application with glyphosate + clethodim provided the best sequential method on control of junglerice.

The fourth and final objective of this research was to evaluate junglerice control with dicamba, glufosinate, and paraquat burndown options as well determining if tank mixtures of those with glyphosate and clethodim are effective. These data showed a follow-up application of glyphosate two weeks after initial application, regardless of burndown options, provided the greatest control of junglerice. A dicamba + glyphosate, glufosinate + clethodim, or paraquat + clethodim application all provided the greatest control of junglerice (98, 90, and 90% respectively) amongst the different burndown options. In Tennessee, from these data, using a glyphosate + clethodim application at burndown to control junglerice, other grasses, and some broadleaves, and then applying paraquat at planting to control the remainder broadleaves present appears to be the best recommendation for overall weed control. A follow-up application of glyphosate or glyphosate + clethodim application will provide excellent control of junglerice and others grasses. A glyphosate + clethodim application is also expected to assist in managing potential resistance of junglerice to these herbicides. It is also recommended to start clean in controlling junglerice, and this data supports that recommendation.

Overall, leaving dicamba out of the tank when applying glyphosate, clethodim, or glyphosate + clethodim applications will improve control of junglerice. Tank mixing glyphosate with clethodim in the future will aid in resistance management and the better

control of glyphosate-resistant junglerice. Starting clean in grass management is key and glyphosate + clethodim helps achieve this goal. Rotating applications of dicamba with glyphosate + clethodim are then recommended in POST applications of controlling Palmer amaranth and junglerice.

VITA

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