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Evaluation of Fenclorim Safener for Use in Rice with Group 15 Herbicides

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by

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Abstract

The development of herbicide resistance and the lack of effective herbicides to control problematic weeds has caused Arkansas rice (*Oryza sativa* L.) production to pursue alternative sites of action. Currently, very long-chain fatty acid elongase inhibitors are not labeled for U.S. rice production but have been widely used for Asian rice production systems. Previous research has demonstrated the utility of acetochlor and pyroxasulfone to provide in-season weed control for Arkansas rice production, but variable crop tolerance has been observed. Additionally, acetochlor at 1,260 g ai ha^{-1} elicited less rice injury when seeds were treated with a herbicide safener seed treatment of fenclorim at 2.5 g ai kg^{-1} of seed relative to without the fenclorim seed treatment. Therefore, trials were conducted in 2020 and 2021 to evaluate rice tolerance and weed control with pyroxasulfone, microencapsulated acetochlor, and a fenclorim seed treatment.. In-season applications of acetochlor provided better control of weedy rice and barnyardgrass with earlier application timings and increasing rates. The fenclorim seed treatment enhanced crop tolerance to acetochlor applied delayed-preemergence (DPRE) averaged over acetochlor rate. Also, rice demonstrated good tolerance to acetochlor applied DPRE at 1,260 g ai ha^{-1} with a fenclorim seed treatment at 2.5 g ai kg^{-1} of seed, which led to $\leq$ 19% rice injury, $\geq$ 88% barnyardgrass control, and $\geq$ 45% weedy rice control 28 days after treatment. Other studies evaluated the fenclorim seed treatment dose for acetochlor applied DPRE at 1,260 g ai ha^{-1}. The fenclorim seed treatment rate of 2.5 g ai kg^{-1} of seed reduced rice injury from acetochlor relative to no fenclorim and provided comparable heights and number of shoots to the nontreated check at each evaluation. Increasing from 2.5 to 5 g kg^{-1} of seed provided no additional improvements for tolerance, and rice tolerance to acetochlor from fenclorim at < 2.5 g kg^{-1} of seed was inconsistent and not commercially viable due to variable tolerance. Across 16 common Arkansas rice cultivars, DPRE acetochlor at 1,260 g ai ha^{-1} caused ≤ 24% injury, and rice planted under adverse growing conditions exhibited < 20% injury with acetochlor and fenclorim. Regardless of the fenclorim seed treatment, rice demonstrated good tolerance to fall applications of acetochlor but not pyroxasulfone. Pyroxasulfone at the low and high rate, respectively, caused 39 and 47% injury 28 days after emergence and averaged over the fenclorim seed treatment. Additionally, weedy rice control ranged from 48 to 0% with acetochlor, and the fenclorim seed treatment did not influence weed control. Based on the results of these experiments, the fenclorim seed treatment will provide adequate crop tolerance to microencapsulated acetochlor but not to pyroxasulfone. Additionally, in both studies evaluating weed control, the fenclorim seed treatment did not influence weed control, indicating that the safening response for cultivated rice is not reciprocated to adjacent weeds. Should microencapsulated acetochlor be registered for use in U.S. rice production with the addition of the fenclorim seed treatment, rice producers would have a new, effective site of action to control problematic weeds without compromising crop tolerance.

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Chapter 1

General Introduction and Review of Literature

Rice Overview

Rice (*Oryza sativa* L.) serves as the most consumed grain in several countries across the globe. Consumption has increased steadily over the past several years, and USDA estimates 163,526,000 hectares of rice were produced and 488,269,000 metric tons consumed globally in the 2018/19 growing season (Foreign Agriculture Service 2019). Rice producers face several challenges to meet the demand of the growing world population; furthermore, the shift of young labor from rural to urban society reduces the workforce required for rice production (Prasad et al. 2017). Most rice production in the central United States (US) occurs along the gulf coast of Southeast Texas, Eastern Arkansas, West Mississippi, Louisiana, and Southeast Missouri. Along the Mississippi River and gulf coast, almost all production is devoted to long-grain rice while California produces mainly short-medium grain varieties.

Arkansas Rice Production

Arkansas rice generates about 48% of the US crop, and the most commonly planted conventional, long-grain inbred rice cultivar is 'Diamond', which encompasses 11% of Arkansas rice hectares (Hardke 2021). In Arkansas, fall and spring tillage are utilized to prepare seedbeds for planting, and most planting occurs in silt loam soils. Rice usually serves as an annual rotational crop with soybean [*Glycine max* (L.) Merr.], and planting takes place from the last week in March until early June (Hardke 2020). Most (86%) of Arkansas rice hectares are drill-seeded with 10% dry broadcast and 4% water broadcast seeded.

Lack of different herbicide sites-of-action (SOA) for rice producers has led to resistant weeds across the state of Arkansas (Carey et al. 1995). Particularly, improper stewardship of Clearfield technology led to weedy rice (*Oryza sativa* L.) resistance to acetolactate synthase (ALS)-inhibiting herbicides (Burgos et al. 2008). The need for more SOAs for Arkansas rice growers to combat resistance is an increasing issue.

Rice Weed Control

Weeds can hinder growers in several ways, including contamination of seed during harvest and milling, reduction of harvesting feasibility and efficiency, competition for water, nutrients, and sunlight, and increased input costs to mechanically or chemically control weeds. Weeds also serve as hosts for disease and insect pests (Smith 1988). The top three problematic weeds in Arkansas flooded rice rated by respondents during 2020 were barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.], Cyperus spp., and weedy rice (Butts et al. 2022). Additionally, the average annual cost to control weeds in rice was reported at \$266.40 ha^{-1}. In 2007, the extra cost of treating herbicide-resistant weeds was approximately \$65 ha^{-1}. (Norsworthy et al. 2007). Additionally, consultants requested more research to improve broadleaf-weed control. In the past, growers produced rice without rotation and relied on the same SOA herbicides repetitively. Now weeds have developed resistance to many of the technology options available to growers (Barber et al. 2020; Heap 2022)

Barnyardgrass

Barnyardgrass in Arkansas is resistant to six SOAs making this weed one of the most difficult to control in rice (Heap 2022). Barnyardgrass can be identified by its long, narrow leaves, being taller than rice, green to purple panicle inflorescence, and lack of a ligule at the leaf collar. In Arkansas, barnyardgrass is resistant to acetyl-coenzyme A carboxylase, propanil, quinclorac, clomazone, and ALS-inhibiting herbicides (Barber et al. 2020). Barnyardgrass is resilient due to its genetic diversity, and in a study published in 1988, barnyardgrass alone caused up to 70% yield loss when left uncontrolled in a rice production system (Smith 1988). The environments in which producers grow rice, whether drill- or water-seeded, is an ideal environment for barnyardgrass growth, and the plant is very competitive when allowed to persist in a rice crop (Norsworthy et al. 2007). Barnyardgrass needs to be removed from rice as early as possible due to its ability to reduce yield by lodging and competing with the cultivated crop for important nutrients in the soil (Talbert and Burgos 2007).

Weedy rice

Weedy rice has the highest potential for reducing yield in rice at 82% if left to interfere all season (Smith 1988). Weedy rice, being the weedy relative of the cultivated crop, is more difficult to control than other weeds in rice production systems (Burgos et al. 2014). Furthermore, traited rice cultivars have the potential to cross with weedy rice populations, which allowed Clearfield-resistant traits to transfer to the weedy rice population leading to herbicide resistance (Burgos et al. 2008). Weedy rice looks like cultivated rice; however, its growth habit is different from that of the cultivated crop as it is taller and produces more tillers. Grain from weedy rice can be black-hull or straw-hull, either of which reduce grain quality of the rice crop when harvested (Talbert and Burgos 2007). Weedy rice and cultivated rice vary due to weedy rice having a lighter color, profuse tillering, low grain weight, and easily shattered seeds, which ultimately makes weedy rice less desirable as a crop than the cultivated relative.

Herbicide Options

Clearfield® and FullPage® Rice

This technology was discovered by accident through a breeding program to develop varieties with higher yield, and Louisiana State University (LSU) released the first two commercial varieties as CL121 and CL141 (Croughan 2004). In 2003, LSU released CL161 to replace the subsequent varieties due to higher yield and increased tolerance to the imidazolinone family of herbicides belonging to the Weed Science Society of America's Group 2 SOA. FullPage rice was released by RiceTec in 2019 for use with Preface and Postscript herbicides (Barber et al. 2020; Boyd 2019). Newpath™ (imazethapyr) was the primary herbicide used for Clearfield rice. Clearpath, a combination of both imazethapyr and quinclorac, was also formulated for use in Clearfield systems. Newpath offers growers the ability to use a herbicide with both foliar and residual control of weeds. Generally, imazethapyr is applied preemergence and followed by a postemergence application. Currently, six problematic weeds in rice have resistance to ALS herbicides: Palmer amaranth (*Amaranthus palmeri* S. Watson), Pennsylvania smartweed [*Persicaria pensylvanica* (L.) M. Gomez], rice flatsedge (*Cyperus iria* L.), yellow nutsedge (*Cyperus esculentus* L.) barnyardgrass, and weedy rice (Barber et al. 2020; Heap 2022). The largest risk with the Clearfield technology is the potential cross-pollination between weedy rice and the imidazolinone-resistant cultivars (Sudianto et al. 2013).

Provisia™ and Max-Ace® Rice

Quizalofop is an acetyl-coenzyme A carboxylase- (ACCase)-inhibitor sold under the trade name Provisia™ for Provisia rice systems and HighCard™ for Max-Ace rice systems that allows for grass weed control in the respective cultivars (Lancaster 2017; Robb 2019). The Provisia cultivar was developed by BASF through a breeding program to provide growers with the ability to control weeds such as weedy rice and barnyardgrass; the first cultivar (PVL01) was released in 2018 (Hardke 2020). Quizalofop has been used in broadleaf crop systems such as cotton and soybean to control grasses (Abit 2010). However, quizalofop tends to be antagonized in tank-mixtures. Specifically, in a recent study conducted at LSU, antagonism was noted when quizalofop was mixed with clomazone and/or pendimethalin (Osterholt et al. 2019). Lastly, because of the outcrossing potential between Provisia rice cultivars and weedy rice species, the risk for outcross is likely similar to that of Clearfield rice cultivars, that have been shown to transfer the resistance trait to weedy rice biotypes (Gealy et al. 2015).

Propanil & Quinclorac

Propanil has been around for over forty years, but its use has now become limited for barnyardgrass control in rice (Barber et al. 2020). Propanil belongs to the WSSA Group 5 photosystem II inhibitors and is a contact herbicide. In 1990, Poinsett County, Arkansas, reported resistance of barnyardgrass to propanil because of years of repetitive use in continual rice cropping practices (Carey 1994). Furthermore, propanil has never been able to control weedy rice (Barber el al. 2020). Quinclorac became available for rice growers in Arkansas shortly after the first reports of barnyardgrass resistance to propanil (Talbert and Burgos 2007). Quinclorac is a synthetic auxin, WSSA Group 4 herbicide most commonly known under the trade name of Facet L®. This herbicide became the new staple for use until barnyardgrass was reported resistant to quinclorac in 1999 (Lovelace et al. 2007).

Cyhalofop and Fenoxaprop

Cyhalofop (Clincher™) and fenoxaprop (Ricestar HT™) belong to the ACCase-inhibitor WSSA Group 1 SOA herbicides. Both herbicides are selective and only offer control of grasses in a rice crop (Barber et al. 2020). These herbicides offer an SOA that can be applied early –

postemergence and after flooding, and cyhalofop provides substantial control of barnyardgrass up to ten days post-flood and is also effective for some other grass weeds such as Amazon sprangletop [*Diplachne panicoides* (J. Presl) A.S. Hitchc] and broadleaf signalgrass [*Urochloa platyphylla* (Munro ex C. Wright) R.D. Webster]. As with quizalofop, cyhalofop and fenoxaprop may be antagonized in a tank-mixture with some broadleaf herbicides making control of a diverse weed spectrum more difficult (Osterholt et al. 2019).

Clomazone

Clomazone is the active ingredient of Command 3ME® sold by FMC and belongs to the Group 13 SOA (diterpene synthesis inhibitor). Clomazone can be applied up to 14 days before planting and up to 3-leaf rice growth stage. This herbicide offers residual control of grasses in rice such as barnyardgrass, Amazon sprangletop, and broadleaf signalgrass (Barber et al. 2020). A secondary application of a postemergence herbicide will be required to remove broadleaves and sedges before flooding. Clomazone controls weeds by inhibiting an enzyme responsible for the synthesis of carotenoid pigments (Zimdahl 2018). This results in bleaching of the plant, and after 75% of the plant’s foliage has bleached, the plant senesces. Clomazone has the potential to volatilize, and improvements of the formulation have reduced some clomazone volatility; however, injury can readily be observed by bleaching symptomology. Clomazone can sometimes elicit symptomology in rice, although research has shown that symptoms are benign and pose little risk of permanent injury and yield reduction (Zhang et al. 2005).

Proposed Herbicides and Seed Treatment

Very Long-Chain Fatty Acid Inhibitors

Very long-chain fatty acid inhibitors (VLCFA) belong to the Weed Science Society of America's Group 15 SOA. VLCFA consist of three chemical families, with the largest being chloroacetamides. This family contains the active ingredients of acetochlor and metolachlor. The pyrazole family contains the active ingredient of pyroxasulfone. Group 15 herbicides cause cessation of all cell division in the shoots of emerging plants. Elongated tissues such as shoots and roots then distort, which results in plant death (Babczinski et al. 2012). VLCFA do not provide control of emerged weeds and most require at least 1.25 cm of rain or shallow tillage to be activated (Anonymous 2018). Shallow tillage places the herbicide within the top few centimeters of soil where soil moisture can then activate the herbicide. Currently, the only documented resistant species to VLCFA in Arkansas is Palmer amaranth, which is important to consider when growers select herbicides for a weed management program (Heap 2022).

Warrant® is a microencapsulated formulation of acetochlor for weed control in soybean, cotton, corn, peanut, sorghum, and sugar beets (Anonymous 2018). Warrant contains 359 g of ai L^{-1} and is applied at 1.26 kg of ai ha^{-1} when sprayed at a medium rate for soybean. This herbicide is currently sold by Bayer and offers preemergence control of small-seeded broadleaves and annual grasses. Dual II Magnum contains the active ingredient *S*-metolachlor and a herbicide safener known as benoxacor. Benoxacor provides enhanced tolerance to corn (*Zea mays* L.) by increasing the metabolism of metolachlor (Rowe et al. 1991). In one such study, benoxacor significantly reduced the injury to corn from applications of metolachlor by 34% (Bernards et al. 2006). *S*-metolachlor is produced by Syngenta® for preemergence control of grasses, sedges, and small-seeded broadleaves in soybean, corn, cotton, and several horticulture crops (Anonymous 2021). Rates of application of *S*-metolachlor range from 1070 to 2140 g ai ha^{-1}.

Herbicide Formulation

Microencapsulation (ME) contains one or more active ingredients surrounded by an organic/inorganic polymer (Becher 2010). Encapsulation allows for slower degradation of the herbicide active ingredient(s) and makes the herbicide release slowly through diffusion into the soil. Microencapsulation makes the herbicide much safer to handle and provides extended residual; however, ME formulations tend to settle in solution and require agitation. Emulsified concentrate (EC) formulations typically consist of the active ingredient, a solvent, and an emulsifier that allows for suspension within water (Fishel 2010). Emulsified concentrate formulations are more desirable than ME; because this type of formulation requires mild agitation to prevent settling out while in suspension, and EC formulations are the least abrasive second only to solution formulations (Martin et al. 2011).

The caveat to an EC versus a ME is the increased chance of phytotoxicity in the crop due to the herbicide active ingredient being readily available for uptake by the plant (Becher 2010; Fishel 2010). When an EC herbicide is applied, the amount of active ingredient available for uptake peaks shortly after activation and declines rapidly thereafter (Scher 1990; Dowler et al. 1999). This peak could then exceed the level at which the crop can tolerate the herbicide if the crop is at a susceptible growth stage; however, herbicide concentrations for ME formulations remain lower in soil due to the diffusion process of the active ingredient moving from inside the polymer coating into soil solution. This controlled release of an ME herbicide also provides consistent concentration over a longer duration providing enhanced crop tolerance and greater potential duration of weed control when compared to that of an EC application at the same g ai ha^{-1}. EC formulations also have a potential dermal hazard (Becher 2010; Fishel 2010; Martin et al. 2011). The emulsifying agent of an EC herbicide has the capability to penetrate human skin and foliar wax layers allowing the active ingredient to be rapidly absorbed; whereas, ME provides a barrier prohibiting the active ingredient from being rapidly absorbed by skin or vegetation (Tsuiji 2001).

Very long-chain fatty acid elongase inhibitors in rice

Currently, VLCFA herbicides are not labeled for use in rice in the United States; however, chloroacetamides are widely used in Asian rice production for the control of weedy rice and other annual grasses that may hinder rice production (Chauhan et al. 2017). Recent research conducted at the University of Arkansas showed that one-leaf applications or later are the safest for rice; however, earlier applications enhanced weed control significantly (Fogleman 2018). Fogleman found that microencapsulation was the main factor to safely apply acetochlor in rice without causing significant injury. The EC formulation caused greater injury because the active ingredient was immediately available and taken up by the rice shoot during elongation of the germinated seed (Fishel 2010; Fogleman 2018). Furthermore, during preliminary studies conducted at the University of Arkansas, field studies showed that fenclorim applied as a seed treatment was unable to effectively safen rice to applications of EC acetochlor (Norsworthy and Brabham, unpublished data). Microencapsulation plays an important role in safening rice to applications of acetochlor and will still be required to adequately safen rice with fenclorim to applications of chloroacetamides.

Fenclorim and Pretilachlor

Fenclorim (4, 6-dichloro-2-phenyl-pryrimidine) is a safener developed by Ciba Geigy in the 1980s for use in water-seeded and transplanted rice to safen applications of pretilachlor (Quadranti and Ebner 1983). Pretilachlor is a VLCFA belonging to the chloroacetamide family labeled in Asian rice production systems. Sofit® is a mixture of fenclorim and pretilachlor for the control of barnyardgrass and sedges (*Cyperus* spp). Water-seeded rice was injured by pretilachlor applied alone on the day of planting and up to four days after planting (Quadranti and Ebner 1983). Injury was not observed if applications were made six days or more after planting or at any time after transplanting rice; however, weed control dropped significantly six days after planting for barnyardgrass and rushes (*Scirpus* spp). The premix of fenclorim and pretilachlor compared to pretilachlor alone reduced injury to rice and varied insignificantly in weed control. In another experiment, applications of the herbicide safener fenclorim and the herbicide pretilachlor were utilized to analyze effects on rice germination and yield. For this study, applications of fenclorim increased yields by greater than 50% when used in combination with preemergence pretilachlor (Chen et al. 2013). The yield increase observed indicates a safening effect by fenclorim to the rice when in the presence of the herbicide pretilachlor.

Fenclorim can safen rice to pretilachlor in two ways. The first is by inducing the plant to produce glutathione S-transferase (GST), which allows the plant to effectively metabolize the herbicide (Usui et al. 2001; Han and Hatzios 1990). The second being through reduced uptake of pretilachlor when seedlings were treated with fenclorim before pretilachlor. Han and Hatzios studied radiolabeled pretilachlor and some of the metabolites associated with detoxification of pretilachlor. During this study, reduction in uptake was determined to be a minor mechanism in how fenclorim safens rice to applications of pretilachlor. Fenclorim significantly increased the amount of glutathione-pretilachlor conjugates recovered from the plant indicating that fenclorim increased pretilachlor metabolism through GST upregulation. GST facilitates the reaction of reduced glutathione to conjugate with the electrophilic position on the chloroacetamide molecule (Shahzad et al. 2017;Usui et al. 2001; Wu et al. 1999). This glutathione conjugate is then water soluble and less toxic; the conjugated chloroacetamide can then be transported to the vacuole to be further detoxified. While in the vacuoles the conjugated chloroacetamide loses its chlorine group in order to further breakdown the herbicide.

Researchers believe that the same GSTs that are produced when rice seedlings are treated with pretilachlor are also produced when seedlings were treated with fenclorim (Wu et al. 1999). The two GSTs identified in this study were also upregulated to the highest amount 48 hours after treatment of the safener which coincides with other publications (Han and Hatzios 1990; Scarponi et al. 2005; Usui 2001). This ultimately indicates that applications should be made no sooner than two to three days after planting rice if fenclorim is applied as a seed treatment.

Applications of fenclorim and pretilachlor in mixture interfere with some of the metabolic processes involving protein and starch production within the shoots of rice plants (Scarponi et al. 2005). This was determined to be a product of the reduction in pretilachlor persistence by fenclorim-induced GST; however, the reductions in metabolites were non-lethal and did not hinder dry weight of the shoots, which indicated no stress to the rice plant. This study also considered the detoxification period of treatments with fenclorim and pretilachlor alone and in mixture concluding that applications of fenclorim reduced the persistence of pretilachlor in the shoot by 48 hours. The rice plant also accumulates twice as much fenclorim when applied in combination with pretilachlor versus the amount accumulated when only fenclorim is applied. This indicates that the metabolic process responsible for detoxifying pretilachlor and fenclorim in mixture favors pretilachlor, and the shortened persistence of pretilachlor indicates the safening effect caused by fenclorim (Scarponi et al. 2005; Wu et al. 1999)

One of the disadvantages of applying a chloroacetamide and fenclorim in combination as a broadcast application is the potential induction of GSTs in both weedy rice and other problematic weed species. In one such study that looked at the upregulation of GSTs in both rice and early watergrass (*Echinochloa oryzicola* Vasing), pretilachlor-induced GSTs were present in the shoots of both rice and early watergrass, but higher levels were detected in rice (Usui et al. 2001). When looking at fenclorim-induced GSTs, early watergrass showed little to no GST activity while rice showed high levels of GST activity when treated with fenclorim. This study ultimately concludes that for early watergrass an overspray application of fenclorim would not influence GST regulation; however, this does not prove that other weeds could not be affected by fenclorim applications. Furthermore, research has indicated that fenclorim induction of GSTs is greatest when fenclorim is applied directly to the roots of rice (Deng and Hatzios 2002). During this study, shoots and roots were treated with pretilachlor or fenclorim resulting in a two-fold increase in RNA transcripts responsible for GST production when the safener was applied to the roots. Since fenclorim provides rice with enhanced tolerance to pretilachlor through the conjugation of the pretilachlor active group with glutathione, other chloroacetamides such as acetochlor or metolachlor should be safened by applications of fenclorim. However, molecular shape and structure could potentially influence the level of safety that fenclorim provides for certain chloroacetamides (Shazhad et al. 2017). Lastly, rice already produces GSTs when exposed to pretilachlor; the safener causes rice to overproduce GSTs before the uptake of pretilachlor, speeding up the detoxification.

Significance of Problem

Growers and consultants are looking for new means of weed control and more SOAs for rice production. Introducing VLCFA herbicides to rice production in the US, specifically Arkansas, would help improve control of noxious weeds that might otherwise hinder growers. Bringing in this group of chemistries would also provide another SOA to combat against resistance for rice producers. Furthermore, because the use of a seed treatment, rather than genetically modifying a crop to promote tolerance, the potential for outcrossing to the weedy rice population is nullified. VLCFA herbicides have the capability of controlling weedy rice, sedges, and barnyardgrass, three of the most problematic weeds in rice, but without the use of the herbicide safener fenclorim, rice injury can be unacceptable. At this time, fenclorim is used as a tank-mixture partner with pretilachlor for broadcast applications in Asian rice production systems. The previously-mentioned studies have evaluated fenclorim using agar growing media, seeds soaked in a solution of fenclorim and water to be water seeded, and foliar and root applications. Currently there is no peer-reviewed published data on the safening effects of fenclorim when applied as a seed treatment for drill-seeded rice; however, preliminary studies have shown the utility of fenclorim for safening applications of microencapsulated acetochlor. Introduction of these herbicides with a fenclorim seed treatment and combining them with other postemergence and preemergence applications will provide growers the ability to control a broad-spectrum group of weeds with much greater feasibility.

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Chapter 2

Rice Tolerance to Acetochlor with a Fenclorim Seed Treatment

Abstract

Rice producers in the U.S. need a new, effective residual herbicide to control weedy rice and herbicide-resistant barnyardgrass. Acetochlor is a very-long chained fatty acid elongase inhibitor (VLCFA) that is currently not labeled for rice production. Previous research has demonstrated the efficacy of acetochlor to provide in-season weed control in rice; however, undesirable injury is common. Thus, trials were initiated in 2020 and 2021 to evaluate 1) rice cultivar tolerance to microencapsulated (ME) acetochlor with the use of a fenclorim seed treatment at 2.5 g ai kg^{-1} of seed, 2) a dose-response of a fenclorim seed treatment with ME acetochlor, and 3) rice tolerance to fenclorim and ME acetochlor under cool, wet conditions. For all trials, acetochlor was applied delayed-preemergence (4 to 7 days after planting). In the dose-response trials and in the presence of acetochlor, the fenclorim seed treatment rate of 2.5 g ai kg^{-1} reduced rice injury and increased rice plant heights and shoot numbers relative to acetochlor without fenclorim and was comparable to the nontreated control in all evaluations. Additionally, in the cultivar screening, 14 of 16 cultivars exhibited < 20% injury with acetochlor at 1,260 g ai ha^{-1} and fenclorim at 2.5 g ai kg^{-1} 2 weeks after emergence (WAE) at the Pine Tree Research Station (PTRS). At the Rice Research and Extension Center (RREC) 2 and 4 WAE and PTRS 4 WAE, all cultivars exhibited < 20% injury with acetochlor and fenclorim. The fenclorim seed treatment in the presence of acetochlor provided comparable rice plant height, shoot numbers, groundcover, and rough rice yield to the nontreated control. Under cool, wet conditions, rice injury without fenclorim ranged from 15 to 60% with acetochlor at 1,050 g ai ha^{-1}, while injury from acetochlor with the fenclorim seed treatment ranged from 0 to 20%. Based on the results of

these experiments, the fenclorim seed treatment appears to safen an assortment of rice cultivars from injury caused by ME acetochlor .

Nomenclature: Acetochlor; fenclorim; rice, *Oryza sativa* L.

Introduction

With approximately 1,229,000 hectares of rice planted across the U.S., rice production grosed over $3-billion in total production in 2020 (NASS 2021). However, weed control remains one of the primary factors limiting rice production, especially when problematic weeds have developed resistance to typical rice herbicides (Barber et al. 2020; Butts et al. 2022; Heap 2022). Three of the five most problematic weeds of Arkansas rice include barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv], Amazon sprangletop [*Diplachne panicoides* (J. Presl) A.S. Hitchc], and weedy rice (*Oryza sativa* L.). These grasses can cause potential yield losses up to 82% if left uncontrolled throughout the season (Smith 1988).

Within the mid-southern U.S., barnyardgrass has developed resistance to propanil (WSSA Group 5 photosystem II inhibitor), quinclorac (WSSA Group 4 synthetic auxin), clomazone (WSSA Group 13 1-deoxy-D-xyulose-5-phosphate synthase inhibitor), fenoxaprop/cyhalofop (WSSA Group 1 acetyl-coenzyme A carboxylase inhibitors), and WSSA Group 1 acetolactate synthase (ALS) inhibitors (Heap 2022). Additionally, barnyardgrass resistance to thiobencarb (WSSA Group 8 lipid synthesis inhibitors) has been reported in California rice production. In comparison to barnyardgrass, Amazon sprangletop and weedy rice have been reported resistant to only a few herbicides. Amazon sprangletop has developed resistance to cyhalofop and fenoxaprop, while weedy rice has developed resistance to imidazolinone herbicides (ALS inhibitors) through backcrossing with Clearfield® rice (Dauer et al. 2018; Gealy et al. 2015). A survey conducted in 2012 with Mississippi and Arkansas rice consultants rated the "control of herbicide-resistant weeds" as the number one concern, leading to the need for new effective sites of action for rice producers (Norsworthy et al. 2013).

Current rice recommendations suggest overlapping residual herbicides to limit the dependence on postemergence herbicides to control weeds prior to flooding (Barber et al. 2020). However, the lack of preemergence residual herbicide options to control weedy rice pressures producers to plant non-transgenic, herbicide-resistant cultivars to allow for control of emerged weedy rice. Even with optimum applications, escapes are inevitable and threaten the durability of current options (Bagavathiannan and Norsworthy 2012). Therefore, mid-southern rice producers need a non-traited residual control option for weedy rice and an alternative site of action for residual barnyardgrass control.

Very long-chained fatty acid elongase (VLCFA)-inhibiting herbicides are currently unavailable for use in U.S. rice production but are labeled in Asian rice production systems. Previous research has evaluated incorporating VLCFA herbicides into current rice herbicide programs for the past several years (Avent et al. 2020; Bertucci et al. 2019; Fogleman 2018; Godwin 2017; Norsworthy et al. 2019). An experiment evaluated acetochlor application timings in rice and determined that undesirable injury occurred at delayed preemergence (DPRE) and spiking timings. However, good rice tolerance was observed when applications occurred at 1- to 2-leaf stage or later (Fogleman et al. 2019; Godwin et al. 2018). Additionally, microencapsulated (ME) formulations provided better rice tolerance than emulsifiable concentrate formulations of acetochlor (Fogleman et al. 2019).

Acetochlor absorption occurs through root and shoot uptake of germinated seedlings emerging through the soil and provides little to no control of emerged weeds (Babczinski et al. 2012). Consequently, control of emerged weeds with early postemergence applications of acetochlor alone is not possible (Anonymous 2018; Babczinski et al. 2012). A DPRE application timing is ideal for weed control since most rice producers plant to a seedbed with no weeds present. However, Fogleman (2017) and Godwin (2018) have demonstrated the variability in rice tolerance to ME acetochlor at this application timing. Therefore, a herbicide safener seed treatment has been pursued to provide adequate rice tolerance to acetochlor.

Fenclorim was developed by Ciba-Geigy in the 1980s and released as a mixture with pretilachlor, another chloroacetamide herbicide similar to acetochlor (Quadranti and Ebner 1983). Pretilachlor has been widely used to control weeds in Asian rice production systems when fenclorim is used in conjunction with the herbicide to mitigate injury (Chauhan et al. 2014; Chen et al. 2013). Fenclorim mitigates rice injury from pretilachlor by reduced total uptake and improved degradation of the herbicide (Scarponi et al. 2003). Additionally, fenclorim causes an upregulation of several metabolic responses in rice (Chen et al. 2013; Deng and Hatzios 2002; Hu et al. 2020; Scarponi et al. 2005; Shahzad et al. 2017; Usui et al. 2001; Wu et al. 1999).

Upregulation of glutathione-*S*-transferase (GST) genes is considered the primary metabolic pathway in which pretilachlor detoxification is improved by fenclorim. Moreover, a recent study has illustrated the wide variety of metabolic processes upregulated by fenclorim and identified that fenclorim reduces lipid peroxidation and reactive oxygen species production induced by pretilachlor (Hu et al. 2020). Pretilachlor detoxification is well linked to GST activity, and GST enzymes have been well described to react with foreign compounds with similar molecular shape and structure (Deng and Hatzios 2002; Shahzad et al. 2017; Wu et al. 1999). Therefore, the metabolic processes induced by fenclorim for pretilachlor will likely be reciprocated to acetochlor. Furthermore, both herbicides belong to the same chemical family, and acetochlor only lacks two hydrogen-saturated carbons (C) compared to pretilachlor.

In the summer of 2019 in Fayetteville, AR, preliminary studies were conducted with 'Diamond' rice and a technical grade fenclorim seed treatment at 0.25 and 2.5 g ai kg^{-1} of seed. The fenclorim seed treatment improved rice tolerance to DPRE applications of emulsifiable concentrate (EC) and microencapsulated (ME) acetochlor. However, commercial tolerance was not observed with the EC formulation (Avent et al. 2020). Additionally, the fenclorim seed treatment at 2.5 g ai kg^{-1} provided adequate tolerance to the lower rates of ME acetochlor, but not at 1,260 g ai ha^{-1}. The fenclorim seed treatment at 2.5 g ai kg^{-1} of seed safened rice from acetochlor and <10% injury was observed 21 days after treatment with acetochlor at 1,260 g ai ha^{-1}. However, these trials were micro-plots initiated in the middle of the summer; therefore, yield data could not be collected, and injury was likely not representative of what would occur in typical rice-growing conditions. Thus, trials were initiated to 1) determine an optimum fenclorim seed treatment rate to provide adequate rice tolerance to acetochlor, 2) evaluate common Arkansas rice cultivars tolerance to acetochlor and fenclorim, and 3) determine if fenclorim can provide adequate rice tolerance to acetochlor under cool, wet growing conditions.

Materials and Methods

Fenclorim dose response. Two experiments were initiated at the Rice Research and Extension Center (RREC) near Stuttgart, AR, on May 3, 2020, and April 23, 2021 to evaluate the safening effects of varying fenclorim rates on rice. These trials were established on a Dewitt silt loam soil composed of 27% sand, 54% silt, and 19% clay with a soil pH of 6.2 and 1.7% organic matter. Each trial was managed culturally and for pest management based on University of Arkansas Cooperative Extension Services recommendations for direct-seeded, delayed-flooded rice production. Both trials were amended preplant based on University of Arkansas System Division of Agriculture Marianna Soil Test Lab fertility recommendations with no preplant nitrogen. The site was cultivated at trial establishment to remove any weeds present, and the entire site was over-sprayed with clomazone at 336 g ai ha^{-1} at the time of planting.

'RT 7521 FP' and 'RT 7321 FP' (Table 1) were planted at 36 seeds m^{-1} of row at a 1-cm depth with a 10-row Almaco (Nevada, Iowa) cone drill on 19-cm-wide rows. All rice seed was base treated with clothianidin, carboxin, thiram, metalaxyl, fludioxonil, and gibberellins at 0.75, 0.38, 0.33, 0.16, 0.03, and 0.04 g ai kg^{-1} of seed, respectively. Plots were 1.5 m by 5.2 m with 1.5 m between plots within a block and a 0.9-m alley between blocks. Lastly, all herbicide applications, including treatments and over-sprays, were made using a CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} at 276 kPA with AIXR 110015 (TeeJet Technologies, Glendale Heights, IL) nozzles at 4.8 km hr^{-1}. Urea (46-0-0) was applied at 316 kg ha^{-1} less than six hours before flooding.

The experiments were conducted as a three-factor factorial within a randomized complete block design with four replications. Factor A was the two different cultivars (Table 1). Factor B (herbicide) consisted of no acetochlor or a DPRE application of ME acetochlor at 1,260 g ai ha^{-1}. Lastly, factor C was fenclorim rates of 0, 0.625, 1.25, 2.5, and 5 g ai kg^{-1} of seed, allowing for a total of 20 treatments. Since these experiments were focused on tolerance, all plots were kept weed-free using conventional rice herbicides and hand-removal. Evaluations included an average of five rice plant heights, an average of two 0.5-m^{-1} of row shoot counts, and visual rice injury 2 and 4 weeks after emergence (WAE). Rice injury was evaluated on a 0 to 100 scale, with 0 being no injury and 100 representing crop death (Frans and Talbert 1977). Rough rice grain yield was collected following crop maturity using an Almaco small-plot combine harvesting the entire plot, and grain was adjusted to 12% moisture.

Initially, regression analysis with a nonlinear three-parameter model was considered; however, due to poor R^2 (< 0.50), an analysis of variance (ANOVA) was deemed more appropriate. All data distributions were analyzed using the distribution platform of JMP Pro 16.1 (SAS Institute Inc, Cary, NC), and heights, shoots, and yield were normally distributed while injury was gamma-distributed. Data distribution selections were based on best fit using least log-likelihood and Akaike information criterion. All data were subjected to ANOVA, and means were separated using Fisher's protected LSD at an alpha level of 0.05. Dunnett's procedure ($\alpha = 0.05$) was conducted to evaluate if the relative shoots, heights, and yields were comparable to the nontreated (no fenclorim, no acetochlor). Normally distributed data were analyzed within JMP Pro using the fit-model platform, while injury was analyzed using the generalized linear mixed model add-in with a gamma distribution (Gbur et al. 2012).

All quantitative data are reported relative to the nontreated control for each cultivar and each year. Since the objective of this experiment was to determine the optimum fenclorim seed treatment rate to use with ME acetochlor, all data were analyzed separately by year and herbicide effect considering there were differences in rainfall activation both years.

Cultivar screening. Two experiments were initiated in the spring of 2021 to evaluate differential cultivar response to applications of acetochlor with and without fenclorim seed treatment. On April 19, 2021, the first trial was initiated at the Pine Tree Research Station (PTRS) near Colt, AR, on a Calloway silt loam (11% sand, 70% silt, 19% clay, 7.8 pH, and 1.69% organic matter). The second trial was established on April 20, 2021, at the RREC on a Dewitt silt loam soil composed of 27% sand, 54% silt, and 19% clay with a soil pH of 5.5 and 1.8% organic matter. Each trial was managed culturally and for pest management based on University of Arkansas Cooperative Extension Services recommendations for direct-seeded, delayed-flooded rice production. Both trials were amended preplant based on Marianna Soil Test Lab fertility recommendations with no preplant nitrogen. Both RREC and PTRS were cultivated before trial establishment to remove any weeds present, and both sites were over-sprayed with clomazone at 336 g ai ha^{-1} at the time of planting. Sixteen different cultivars were planted at a 1-cm depth with a 10-row Almaco cone drill on 19-cm wide rows at 36, 52, and 72 seeds m^{-1} of row for hybrid, Max Ace®, and inbred cultivars, respectively (Table 1). All rice seed was base treated and all herbicide treatments and overspray applications were applied the same as the dose response experiment. Urea (46-0-0) was applied at 316 kg ha^{-1} less than six hours before flooding.

The objective of this experiment was not to determine the differences of each cultivar but rather to determine if fenclorim provided a safening effect for each cultivar. The experiments were conducted over 16 different cultivars as a two-factor factorial within a randomized complete block design with four replications. The factors included 1) no acetochlor or DPRE application of ME acetochlor at 1,260 g ai ha^{-1} and 2) a fenclorim seed treatment of 0 or 2.5 g ai kg^{-1} of seed, allowing for a total of 64 treatments. Since these experiments focused on tolerance, all plots were kept weed-free using conventional rice herbicides and hand-removal. Evaluations included an average of five rice plant heights, an average of two 0.5-m^{-1} of row shoot counts, and visual rice injury 2 and 4 WAE. An unmanned aerial system [DJI Mavic 2 (DJI Technology Co., LTD., Nanshan, Shenzhen, China)] captured groundcover images 5 WAE. Overhead images were then analyzed using Field Analyzer (Green Research Services, LLC., Fayetteville, AR). Green pixel counts were measured to determine percentage groundcover. Rough rice grain yield was collected at harvest with a small-plot combine, and grain was adjusted to 12% moisture for yield estimates. The center four rows of each plot at PTRS were harvested and seven rows were harvested at RREC.

All data distributions were analyzed using the distribution platform of JMP Pro 16.1 (SAS Institute Inc, Cary, NC). Data distribution selections were based on best fit using least log-likelihood and Akaike information criterion. Heights, shoots, groundcover, and yield were normally distributed, and injury was gamma-distributed. All data were subjected to ANOVA, and means were separated using Fisher's protected LSD at an alpha level of 0.05. Normally distributed data were analyzed using the fit model platform in JMP Pro, while injury was analyzed using the GLIMMIX procedure in SAS version 9.4 (SAS Institute Inc, Cary, NC) (Gbur et al. 2012). Site was analyzed separately due to differences in rainfall activation which caused varying injury between the two locations.

Growth chamber experiment. Two growth chamber experiments were initiated at the Milo J. Shult Research and Extension Center, Fayetteville, AR, in 2021 to evaluate rice injury potential from applications of acetochlor with and without fenclorim under cool, wet conditions. Both growth chambers were set to provide a 12-hr photoperiod with day and nighttime temperatures of 23.8 C and 12.8 C, respectively. Light intensity was set to 1000 µmol m^{-2} s^{-1}. Before trial initiation, a silt loam soil was collected and sieved. Soil analysis was conducted at the University of Arkansas Diagnostic Lab in Fayetteville, Arkansas using loss on ignition for organic matter and the hydrometer method for texture resulting in a 6.4 pH and 2.3, 20, 66, and 14% organic matter, sand, silt, and clay, respectively. The soil was then dried at 33 C for 2 weeks, and 8 kg was added to 11.4 L pots. Soil bulk density and volumetric field capacity were calculated using Soil Plant Air Water software (USDA ARS, Washington DC) with inputs of soil texture and organic matter to determine how much water was required to maintain 100% field capacity of the soil (Equation 1):

volumetric field capacity ÷ bulk density × 100% × mass of soil = water needed (31.5% ÷ 1.42 × %100 × 8000g = 1775g of water)

Temperatures and soil moisture were set to provide a “worst-case” scenario for rice tolerance to a chloroacetamide herbicide. Under these conditions, rice should accumulate only 15 growing degree units each day, prolonging elongating root and shoot exposure to acetochlor. At 100% field capacity, maximum acetochlor efficacy should be expected (Dhareesank et al. 2006). The experiment was designed as a three-factor factorial completely randomized design with four replications. Factors consisted of with and without a fenclorim seed treatment at 2.5 g ai kg^{-1} of seed; with and without ME acetochlor at 1,050 g ai ha^{-1}; and planting depths of 0.6 and 2.5 cm. 'Diamond' rice was planted at 40 seeds pot^{-1} (30.5 cm diameter) at 80% field capacity. Acetochlor was applied five days after planting (DPRE) using a spray chamber calibrated at 187 L ha^{-1} with two flat-fan 1100067 nozzles (TeeJet, Glendale Heights, IL).

After application, pots were watered to 100% field capacity and maintained every three days. Evaluations included visual injury estimates (0 to 100, with 0 being no injury and 100 being rice death), an average of five rice plant heights per pot, and shoot counts in the pot at 1 and 4 WAE. Rice aboveground biomass was collected at the final evaluation timing and weighed after drying at 60 C for 5 days until constant mass. Soil in pots was flooded at approximately 3 WAE (4-leaf to tillering growth stage), and urea at 316 kg ha^{-1} was applied immediately after flooding to simulate field conditions. The experiment was analyzed within JMP Pro 16.1 (SAS Institute, Cary, NC), and all data were subjected to ANOVA. Distributions were checked using the distribution platform, and all distributions were normal except injury, which was gamma-distributed. Data distribution selections were based on best fit using least log-likelihood and Akaike information criterion. All data were pooled over the two different experimental runs which was considered random, and means were separated using Fisher's protected LSD ($\alpha = 0.05$).

Results and Discussion

Fenclorim dose response. Injury to rice from acetochlor at 1,260 g ai ha^{-1} was 30% in 2020 and 66% in 2021 at 2 WAE in the absence of fenclorim (Table 2). The variation in injury from one year to the next is likely caused by an activating rainfall occurring 7 days after acetochlor application in 2020 when the rice was spiking as compared to 3 days after application in 2021 when the rice had yet to emerge. The increase in injury for 2021 is expected since chloroacetamide herbicides are highly dependent upon water activation and the emerging rice was at a more susceptible growth stage than the emerged rice in 2020 (Babczinski et al. 2012). In similar studies evaluating DPRE applications of acetochlor with rates ranging from 630 to 1,570 g ai ha^{-1}, injury varied from 18 to 89% (Fogleman et al 2019; Godwin et al. 2018; Norsworthy et al. 2019).

Rice injury from the fenclorim seed treatment in the absence of acetochlor was ≤10% for all rates of the safener at both evaluations (Table 2). Slight rice injury from seed treatments in has been observed, where carbendazim reduced root length of the crop relative to no seed treatment (Sandhya et al. 2018). The injury associated with the fenclorim treatments 2 WAE was due to a delay in emergence of the crop. Across several field studies, fenclorim generally delayed emergence by 1 to 2 days, which caused the rice to appear stunted. By 4 WAE, rice in plots with fenclorim-treated seed generally recovered or even surpassed growth of rice not treated with fenclorim. In recent greenhouse trials, fenclorim-applied to rice at 2.5 g ai kg^{-1} of seed caused an increase in root and shoot biomass compared to non-treated plants by 4 WAE (Norsworthy, non-published data).

The fenclorim seed treatment increased rice tolerance to acetochlor, and lowered the year-to-year variability in injury (Table 2). In both years and evaluation timings, injury trended downward as the fenclorim seed treatment rate increased. By 4 WAE, the fenclorim seed treatment rate of 2.5 g ai kg^{-1} of seed reduced injury compared to fenclorim rates ranging from 0 to 1.25 g ai kg^{-1} of seed. Increasing the fenclorim rate beyond 2.5 g ai kg^{-1} of seed did not further reduce injury to rice. Since fenclorim at 5 g ai kg^{-1} of seed provided no extra benefit compared to 2.5 g ai kg^{-1}, the recommended seed treatment rate should remain at 2.5 g ai kg^{-1} of seed to reduce the potential cost for producers.

A significant effect of the fenclorim seed treatment rate on rice shoot counts in the absence of acetochlor occurred 2 WAE in 2020 (Table 3); however, at all other timings and years, this effect was not significant and appears to be due to field variability. When comparing fenclorim seed treatment rates for plots treated with acetochlor, rice shoots were greatest for the two highest rates of fenclorim at 4 WAE in both years. Additionally, according to a Dunnett's test, the number of shoots in the presence of acetochlor and fenclorim at 0 or 0.625 g ai kg^{-1} were less than the nontreated control (no fenclorim and no acetochlor) at 2 WAE (31 to 66% reduction) and 4 WAE (33 to 46% reduction). Conversely, fenclorim rates of 2.5 and 5 g ai kg^{-1} of seed were comparable to the nontreated control in both evaluation timings and years. Godwin et al. (2018) observed a 44% reduction in shoots following a DPRE application of acetochlor at 1,050 g ai ha^{-1}.

Acetochlor reduced the average height of rice numerically (27%) and significantly (35%) at 2 WAE in 2020 and 2021, respectively, when seed did not receive the fenclorim treatment (Table 4). Meanwhile, rice heights with acetochlor and fenclorim at 2.5 g ai kg^{-1} were greater than rice treated with acetochlor and no fenclorim at each evaluation. Previous research reported an 18% reduction in rice height approximately 4 weeks after treatment with ME acetochlor applied DPRE averaged over acetochlor at 1,050 and 2,100 g ai ha^{-1} (Fogleman et al. 2019). In 2020 and 2021, higher injury levels were observed without fenclorim, which likely caused the height reductions. Similar to shoots and injury, rice height was less impacted by acetochlor if fenclorim of 2.5 or 5.0 g ai kg^{-1} of seed was employed. Additionally, fenclorim rates of 0.625 and 1.25 g ai kg^{-1} of seed resulted in shorter rice than the nontreated control in 2021 at 2 WAE.

The rates of fenclorim did not influence rough rice grain yield in the presence or absence of acetochlor (Table 5). The lack of a response is likely due to the ability of hybrid rice to tiller and compensate for stand loss. Across other field studies by previous researchers, acetochlor affected yield in some trials but not others. Fogleman and others (2019) reported 14 to 22% reductions in yield from acetochlor applied DPRE at 1,050 g ai ha^{-1}. In another experiment with the same acetochlor application timings, yield was reduced numerically by 1% in 2015 and significantly by 42% in the following year, indicating the variable influence acetochlor has on rice yield (Godwin et al. 2018).

The fenclorim seed treatment rate of 2.5 g ai kg^{-1} of seed was derived from the maximum use rate of pretilachlor and fenclorim of 450 and 225 g ai ha^{-1}, respectively (Chauhan et al. 2013; Quadranti and Ebner 1983). With an inbred rice cultivar planting rate of 90 kg of seed ha^{-1}, the amount of fenclorim ha^{-1} is equivalent to the foliar use rate. Conversely, when planting a hybrid cultivar at 30 kg ha^{-1}, as opposed to 90 kg ha^{-1}, the amount of fenclorim ha^{-1} is reduced by a third. However, the amount of fenclorim per seed is equivalent across cultivars regardless of planting rate; because the application rate is based on weight of seed, not area treated. Therefore, based on the results from this experiment, the optimum seed treatment rate appears to be 2.5 g ai kg^{-1} of seed. Injury, average height, and number of shoots obtained with acetochlor plus fenclorim seed treatment at 2.5 g ai kg^{-1} of seed were comparable to the nontreated control. Additionally, rice treated with acetochlor in the presence of fenclorim at 2.5 g ai kg^{-1} of seed showed greater tolerance to the herbicide compared to acetochlor-treated rice without fenclorim.

Cultivar screening. Rice injury at PTRS was >40% without fenclorim when acetochlor was applied across all cultivars (Table 6). In comparison, rice injury following acetochlor was <20% when seed were treated with fenclorim across all cultivars, and a reduction in injury relative to non-fenclorim treatments within a cultivar was observed for all evaluations at this location. Similarly, at the RREC location, reductions in injury with the addition of fenclorim were observed for all cultivars and evaluations except 'Titan' at 4 WAE; however, injury overall for this cultivar was ≤13%. Among three previous studies evaluating rice tolerance to DPRE-applied acetochlor, injury ranged from 18 to 89% with four separate cultivars: ‘CL151,’ ‘CL111,’ CL172,’ and ‘PVL01’ (Fogleman et al. 2019; Godwin et al. 2018; Norsworthy et al. 2019).

All cultivars exhibited <20% injury with acetochlor and fenclorim, except for 'DG263L' and 'XP753’ 2 WAE at PTRS where 23 and 24% injury were observed (Table 6). However, a reduction in yield was not observed for DG263L or XP753 with acetochlor and fenclorim. Previous research has reported up to 35% bleaching of rice with clomazone applied at 1,120 g ai ha^{-1}, but rice yield was not negatively impacted (Zhang et al. 2005). At PTRS, 7 out of 16 cultivars suffered reductions in rough rice grain yield when acetochlor was applied without fenclorim. However, grain yield was never reduced at either site for any cultivar when acetochlor was used in conjunction with the fenclorim seed treatment.

Frans and Talbert (1977) historically classified crop injury at 20 to 30% as a slight effect that is unlikely to persist; however, rice producers may not accept >20% injury. Therefore, if ME acetochlor were to become labeled for use in rice with a fenclorim seed treatment, the rate of acetochlor should likely be reduced from 1,260 g ai ha^{-1}. Future studies should consider reducing the acetochlor rate and continue to screen XP753 and DG263L, which appear to be more susceptible to acetochlor injury. Regardless, the safening potential of a fenclorim seed treatment to provide <20% injury to acetochlor at 1,260 g ai ha^{-1} across 14 of 16 cultivars is quite promising.

A safening response is defined in this study as a significant improvement relative to rice treated with acetochlor without fenclorim. For rice height, shoots, and groundcover, there were 160 total observations (Table 7). Out of these observations, fenclorim improved rice tolerance to acetochlor for 70 assessments, and there were no rice evaluations where fenclorim increased rice sensitivity to the herbicide. Of those 70 observations, rice treated with fenclorim and acetochlor was comparable to the nontreated control 60 times.

The lack of a safening effect for the other 90 of 160 observations is due to heights being a poor predictor for rice tolerance to acetochlor (Table 7). Of the 64 observations for heights, 46 were insignificant and 25 provided no trend numerically, indicating that acetochlor does not always affect rice heights. Furthermore, only 16 of 64 observations for height resulted in a significant interaction, where fenclorim provided a safening effect to acetochlor. For shoots and groundcover data, 35 of 64 and 21 of 32 observations had significant differences, respectively. For all significant P-values of shoots and groundcover, fenclorim elicited a safening response to acetochlor, and the acetochlor plus fenclorim treatment achieved numerically greater shoots or groundcover than acetochlor alone for the remaining, insignificant observations. Significant differences were likely not detected due to overall variation in quantitative data from field variability or the lack of reduction in height or groundcover caused by acetochlor; hence, a significant improvement was not possible. To better detect differences, future studies should include more replications and height may be of little value when evaluating rice tolerance to acetochlor with fenclorim.

A previous study evaluating pretilachlor and fenclorim with three different genetic lines of rice reported similar safening responses, with varying tolerance for each rice cultivar to pretilachlor alone (Deng and Hatzios 2002). Pretilachlor reduced root lengths by 67, 54, and 34% for rice lines 'Teqing,' 'Koshihikari,' and 'Lemont,' respectively. Conversely, the addition of fenclorim to pretilachlor caused rice root growth to be similar or greater than the nontreated control of each cultivar. Similarly, the fenclorim seed treatment improved rice tolerance to acetochlor by reducing injury or improving height, shoots, or groundcover across 16 different rice cultivars, illustrating the feasibility of a ME chloroacetamide herbicide option with a fenclorim seed treatment across most cultivars currently grown in the mid-southern U.S. rice region.

Growth chamber experiment. Rice in the growth chamber experiment accumulated 15 growing degree units each day at the daily maximum and minimum temperatures of 23.8 and 12.7 °C. Under this temperature regime and at 100% field capacity, conditions represent a worst-case scenario for rice injury after the DPRE application of acetochlor. At 1 WAE, rice height averaged over planting depth was reduced from 6.6 cm without acetochlor and fenclorim to 2.9 cm when acetochlor was applied in the absence of fenclorim (Table 8). Additionally, the delay in emergence, which causes the appearance of shorter rice, can be observed when comparing with and without fenclorim in the absence of acetochlor at 1 WAE. By 4 WAE, rice height and shoot counts were similar among treatments, except when treated with acetochlor at 1,050 g ai ha^{-1} in the absence of fenclorim.

Rice aboveground biomass was also improved by the fenclorim seed treatment, averaged over planting depth (Table 8). In the absence of the herbicide, fenclorim increased aboveground biomass by 3 g, and in the presence of acetochlor, fenclorim provided a safening effect improving aboveground biomass from 16.4 g without fenclorim to 23.7 g with fenclorim. Furthermore, with acetochlor and the fenclorim seed treatment, rice aboveground biomass was comparable to the nontreated control. The increase in aboveground biomass in the absence of acetochlor is likely due to improved root growth caused by fenclorim. Recent greenhouse research evaluating rice with and without fenclorim seed treatments demonstrated an increase in root biomass from the addition of the fenclorim seed treatment (Avent and Norsworthy, nonpublished data). The increased root growth could result in improved nutrient uptake which would allow greater accumulation in aboveground biomass.

Planting depth as the main effect or within any interaction did not significantly influence heights, shoots, or biomass (P-value > 0.05). However, planting depth influenced injury, and deeper planting depth reduced visual injury in treatments with acetochlor and no fenclorim. Injury from shallow to deeper planting depth decreased from 41 to 29% at 1 WAE and from 35 to 23% at 4 WAE, respectively (Table 9). In treatments with acetochlor plus fenclorim, little difference was observed in the injury rates across the planting depths (from 7 to 11%), indicating that planting depth would not provide a secondary improvement with the addition of the fenclorim seed treatment.

The injury reduction obtained by the deeper planting depth without fenclorim is likely due to placing the seed and roots below the activated herbicide zone, allowing rice to emerge in a lower concentration of herbicide and then push through the more concentrated herbicide layer. Previous research has demonstrated a reduction in grain sorghum [*Sorghum bicolor* (L.)

Moench] phytotoxicity with *S*-metolachlor due to planting in deeper soil depths that were treated with the herbicide (Procópio et al. 2001). The recommended seeding depth of rice is 1.3 cm, and based on the results of this experiment, a deeper planting depth would not provide sufficient tolerance to rice with acetochlor at 1,050 g ai ha^{-1} without the addition of the fenclorim seed treatment.

It is important to note that rice injury from acetochlor without the fenclorim seed treatment averaged over planting depth and evaluation timing ranged from 15 to 60%. In comparison, acetochlor injury with the addition of fenclorim ranged from 0 to 20% (data not shown). Rice injury from acetochlor alone is still highly variable despite controlled conditions provided by a growth chamber. However, with the addition of fenclorim, rice injury was <20%, and the variability in tolerance was reduced from a difference of 45 percentage points to only 20 percentage points. Based on the results of this experiment, the cultivar Diamond under these less than ideal conditions (cool and wet) appears tolerant to acetochlor at 1,050 g ai ha^{-1} if treated with fenclorim at 2.5 g ai kg^{-1} of seed.

Practical implications. Fenclorim has been well described as a safener when used in mixture with pretilachlor in Asian transplanted rice (Chen et al. 2013; Deng and Hatzios 2002; Hu et al. 2020; Quadranti and Ebner 1983; Scarponi et al. 2003 and 2005; Usui et al. 2001; Wu et al. 1999). To date, only one publication has described the safening potential of a fenclorim seed treatment to acetochlor (Avent et al. 2020). The experiments conducted in 2020 and 2021 demonstrate the safening capability of a fenclorim seed treatment under typical drill-seeded rice production systems, which encompasses ~85% of Arkansas rice production (Hardke 2021). With the fenclorim seed treatment of 2.5 g ai kg^{-1} of seed and ME acetochlor applied DPRE at 1,260 g ai ha^{-1}, rice exhibited $\leq$ 24% injury across all trials and cultivars.

Based on the results of the fenclorim dose-response experiment, the optimum rate of the fenclorim seed treatment appears to be 2.5 g ai kg^{-1} of seed. Comparable tolerance levels were observed with 5 g ai kg^{-1} of seed; however, the 2.5 g ai kg^{-1} rate was sufficient and would be a more affordable solution for producers than 5 g ai kg^{-1} of seed. Fenclorim seed treatment rates lower than 2.5 g ai kg^{-1} of seed provided less consistent safening. Future studies should also consider a rate response of acetochlor on a heavy clay soil texture since acetochlor activity is negatively correlated with increasing clay content (Reinhardt and Nel 1990), and these studies were conducted on a silt loam soil.

If fenclorim and acetochlor become labeled in U.S. rice production, some initial delay in emergence from the fenclorim seed treatment might be observed. However, without comparing with and without fenclorim, the effects of the fenclorim seed treatment may not be apparent. Across all trials, no adverse effects in the form of stand or yield were observed from the fenclorim seed treatment. Additionally, acetochlor would provide an alternative site of action to control herbicide-resistant barnyardgrass populations that are common throughout mid-southern U.S. rice. Acetochlor would also provide a non-traited option for controlling weedy rice if the tolerance from fenclorim provided to the cultivated rice was not reciprocated to weedy rice.

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Tables

Table 1. List of cultivars, designation, producer, and seeding rate.

Cultivar	Designation	Producer	Experiment	Seeding rate
				seeds m^{-1} of row
RT 7521 FP	Long-grain hybrid	RiceTec, Inc., Alvin, TX	FDR CS[a]	36
RT 7321 FP	Long-grain hybrid	RiceTec, Inc., Alvin, TX	FDR CS	36
XP 753	Long-grain hybrid	RiceTec, Inc., Alvin, TX	CS	36
RTV 7231 MA	Long-grain pureline	RiceTec, Inc., Alvin, TX	CS	52
PVL02	Long-grain pureline	Horizon Ag, LLC., Memphis, TN	CS	72
PVL03	Long-grain pureline	Horizon Ag, LLC., Memphis, TN	CS	72
CLL15	Long-grain pureline	Horizon Ag, LLC., Memphis, TN	CS	72
CLL16	Long-grain pureline	Horizon Ag, LLC., Memphis, TN	CS	72
CLL17	Long-grain pureline	Horizon Ag, LLC., Memphis, TN	CS	72
CLJ01	Long-grain pureline	Horizon Ag, LLC., Memphis, TN	CS	72
Diamond	Long-grain pureline	UADA, Stuttgart, AR	CS GC	72
Jewel	Long-grain pureline	UADA, Stuttgart, AR	CS	72
Jupiter	Medium-grain pureline	UADA, Stuttgart, AR	CS	72
Lynx	Medium-grain pureline	UADA, Stuttgart, AR	CS	72
Titan	Medium-grain pureline	UADA, Stuttgart, AR	CS	72
DG263L	Long-grain pureline	Nutrien Ag Solutions, Inc. Saskatoon, Saskatchewan, Canada	CS	72

[a] Abbreviations: FDR, fenclorim dose response; CS, cultivar screening; GC growth chamber; UADA, University of Arkansas System Division of Agriculture

Table 2. Effect of fenclorim seed treatment doses on rice injury with and without acetochlor and averaged over RT 7321 FP and RT 7521 FP.

	Injury							
	Acetochlor at 0 g ai ha^{-1}				Acetochlor at 1,260 g ai ha^{-1}			
	2 WAE[a]		4 WAE		2 WAE		4 WAE	
Fenclorim rate	2020	2021	2020	2021	2020	2021	2020	2021
g ai kg^{-1} of seed	%							
0	0	0	0	0	30 A[b]	66 A	22 A	70 A
0.625	9	3	1	3	27 A	41 B	17 AB	36 B
1.25	9	4	1	4	23 AB	34 B	13 BC	24 C
2.5	10	2	1	5	18 B	15 C	5 D	13 D
5	10	3	3	2	19 B	19 C	8 CD	14 D
P-value[c]	NA[d]	NA	NA	NA	0.0104	< 0.0001	0.0004	< 0.0001

[a] Abbreviation: WAE, weeks after emergence

[b] Means within a column not containing the same letter are significantly different according to Fisher's protected LSD (α=0.05)

[c] P-values were generated using the generalized linear mixed model add in with a gamma distribution within JMP Pro 16.1

[d] P-values for rice injury without acetochlor were not displayed since injury was relative to the nontreated check which is always 0%. The presence of the fenclorim seed treatment did cause an effect but injury was ≤ 10% and no differences ($P < 0.05$) were observed for the fenclorim doses ≥ 0.625 g ai kg^{-1} of seed.

Table 3. Effect of fenclorim seed treatment doses on rice shoots with and without acetochlor and averaged over cultivar.

	Relative shoots[a]							
	Acetochlor at 0 g ai ha^{-1}				Acetochlor at 1,260 g ai ha^{-1}			
	2 WAE[b]		4 WAE		2 WAE		4 WAE	
Fenclorim rate	2020	2021	2020	2021	2020	2021	2020	2021
g ai kg^{-1} of seed	%							
0	100 AB[c]	100	100	100	57 C*[d]	44 C*	67 C*	54 B*
0.625	80 B	113	95	110	69 C*	61 BC*	66 C*	60 B*
1.25	109 A	118	79	108	97 A	78 AB	81 BC	70 B*
2.5	90 AB	102	93	105	81 B	93 AB	99 A	97 A
5	81 B	111	86	108	83 B	81 AB	96 AB	105 A
P-value[e]	0.0343	0.2740	0.2780	0.2744	< 0.0001	0.0003	0.0025	< 0.0001

[a] Average shoots in nontreated at 2 WAE were 24 and 20 m^{-1} of row and at 4 WAE were 42 and 30 m^{-1} of row for 2020 and 2021, respectively

[b] Abbreviations: WAE, weeks after emergence

[c] Means within a column not containing the same letter are significantly different according to Fisher's protected LSD (α=0.05)

[d] An asterisk indicates a mean significantly different from the nontreated (no fenclorim and no acetochlor) according to Dunnett's Test (α=0.05)

[e] P-values were generated using the fit model platform with JMP Pro 16.1

Table 4. Effect of fenclorim seed treatment doses on rice heights with and without acetochlor and averaged over cultivar.

	Relative height[a]							
	Acetochlor at 0 g ai ha^{-1}				Acetochlor at 1,260 g ai ha^{-1}			
	2 WAE[b]		4 WAE		2 WAE		4 WAE	
Fenclorim rate	2020	2021	2020	2021	2020	2021	2020	2021
g ai kg^{-1} of seed	%							
0	100	100	100	100	73 B[c]	65 B*[d]	79 B*	90 BC
0.625	97	104	99	103	86 AB	73 B*	95 A	101 AB
1.25	105	104	103	91	87 A	74 B*	100 A	88 C
2.5	101	109	101	104	92 A	100 A	101 A	108 A
5	100	113	98	109	83 AB	88 A	100 A	110 A
P-value[e]	0.8169	0.7260	0.9060	0.1039	0.0363	0.0004	0.0001	0.0039

[a] Average heights in nontreated at 2 WAE were 17 and 11 cm and at 4 WAE were 35 and 15 cm for 2020 and 2021, respectively

[b] Abbreviations: WAE, weeks after emergence

[c] Means within a column not containing the same letter are significantly different according to Fisher's protected LSD ($\alpha=0.05$)

[d] An asterisk indicates a mean significantly different from the nontreated (no fenclorim and no acetochlor) according to Dunnett's Test ($\alpha=0.05$)

[e] P-values were generated using the fit model platform with JMP Pro 16.1

Table 5. Effect of fenclorim seed treatment doses on rough rice yields with and without acetochlor and averaged over cultivar.

	Relative yield[a]			
	Acetochlor at 0 g ai ha^{-1}		Acetochlor at 1,260 g ai ha^{-1}	
Fenclorim rate	2020	2021	2020	2021
g ai kg^{-1} of seed	--------------------- % ---------------------------			
0	100	100	123	84
0.625	112	97	118	83
1.25	100	94	115	97
2.5	103	102	108	97
5	104	103	115	97
P-value[b]	0.6602	0.4303	0.2147	0.1039

[a] Average rough rice yield in nontreated were 10,200; 7,600; 10,500; and 8,400 kg ha^{-1} for cultivars 7521 and 7321 and 2020 and 2021, respectively

[b] P-values were generated using the fit model platform with JMP Pro 16.1

Table 6. Rice cultivar injury and yield as influenced by acetochlor and fenclorim.

			Injury				Yield	
			2 WAE[a]		4 WAE			
Cultivar	Fenc[b]	Acet[c]	PTRS	RREC	PTRS	RREC	PTRS	RREC
	g ai kg^{-1}		------------------------- % ------------------------				------------ kg ha^{-1} -----------	
RT7321FP	no	None	-	-	-	-	11200	10700
		Treated	76 A[d]	75 A	74 A	65 A	9700	10200
	yes	None	4 C	4 C	0 C	5 B	11000	10700
		Treated	18 B	17 B	20 B	13 B	11100	10900
P-value[e]			< 0.0001	< 0.0001	< 0.0001	0.0052	0.1534	0.6180
RT7521FP	no	None	-	-	-	-	10500	11000
		Treated	55 A	42 A	63 A	55 A	10600	10200
	yes	None	5 C	5 B	0 C	4 B	11400	10600
		Treated	14 B	9 B	15 B	20 B	10600	10700
P-value			0.0003	0.0006	< 0.0001	0.0138	0.3050	0.3101
CLJ01	no	None	-	-	-	-	6400	9000
		Treated	81 A	50 A	70 A	53 A	5800	8300
	yes	None	1 C	2 C	0 C	3 B	6600	8200
		Treated	19 B	10 B	19 B	11 B	6400	8600
P-value			< 0.0001	< 0.0001	< 0.0001	0.0157	0.7771	0.1389
CLL 15	no	None	-	-	-	-	7400	8300
		Treated	39 A	24 A	55 A	28 A	6100	8300
	yes	None	3 C	6 B	0 C	3 B	6700	8100
		Treated	17 B	9 B	8 B	9 B	7100	8500
P-value			0.0006	0.0043	< 0.0001	0.0015	0.1241	0.6812
CLL 16	no	None	-	-	-	-	8300	8400
		Treated	40 A	28 A	53 A	28 A	7900	8300
	yes	None	4 C	1 B	0 C	1 C	8300	7500
		Treated	10 B	10 B	6 B	14 B	8200	8200
P-value			< 0.0001	0.0037	< 0.0001	0.0016	0.7011	0.2328
CLL 17	no	None	-	-	-	-	8400	8000
		Treated	51 A	35 A	68 A	34 A	7800	7700
	yes	None	5 C	3 C	0 C	5 B	8600	8100
		Treated	14 B	7 B	11 B	18 B	8100	8600
P-value			< 0.0001	< 0.0001	< 0.0001	0.0370	0.8104	0.4641
DG263L	no	None	-	-	-	-	10800	10400
		Treated	70 A	41 A	68 A	45 A	9300	9600
	yes	None	4 C	3 C	0 C	1 C	11500	10000
		Treated	23 B	12 B	16 B	13 B	10200	10600
P-value			<0.0001	0.0016	< 0.0001	0.0011	0.7631	0.1226
Diamond	no	None	-	-	-	-	8200	9200
		Treated	56 A	49 A	58 A	43 A	6500	8900
	yes	None	5 C	5 B	0 C	3 B	8800	9200
		Treated	14 B	7 B	9 B	10 B	7900	9800
P-value			< 0.0001	0.0003	< 0.0001	0.0313	0.3624	0.4431
Jewel	no	None	-	-	-	-	8200 A	10000 A
		Treated	65 A	31 A	66 A	38 A	7400 B	9000 B
	yes	None	4 C	1 C	0 C	3 B	8300 A	9100 B
		Treated	15 B	7 B	9 B	13 B	8600 A	9700 AB
P-value			< 0.0001	< 0.0001	< 0.0001	0.0104	0.0221	0.0065
Jupiter	no	None	-	-	-	-	9000 A	8700
		Treated	40 A	16 A	51 A	17 A	6400 B	7900
	yes	None	4 C	3 B	0 C	0 B	8400 A	7800
		Treated	12 B	7 B	13 B	0 B	7700 A	8700
P-value			< 0.0001	0.0130	< 0.0001	< 0.0001	0.0472	0.2468

Table 6. (Cont.)

			Injury				Yield	
			2 WAE		4 WAE			
Cultivar	Fenc	Acet	PTRS	RREC	PTRS	RREC	PTRS	RREC
			%				kg ha^{-1}	
Lynx	no	None	-	-	-	-	9100 A	8300
		Treated	34 A	28 A	54 A	18 A	6300 B	8200
	yes	None	2 C	4 B	0 C	1 B	9100 A	8200
		Treated	10 B	7 B	6 B	5 B	8500 A	8600
P-value			<0.0001	0.0004	<0.0001	0.0276	0.0358	0.6163
RT7231MA	no	None	-	-	-	-	7500	8300
		Treated	76 A	23 A	68 A	15 A	7200	7400
	yes	None	5 C	4 C	0 C	0 C	7800	7900
		Treated	16 B	12 B	10 B	5 B	7000	8200
P-value			0.0002	<0.0010	<0.0001	0.0004	0.4600	0.2923
PVL02	no	None	-	-	-	-	8400 A	7300
		Treated	44 A	35 A	65 A	34 A	6300 B	6700
	yes	None	2 C	1 C	0 C	1 B	7900 A	7300
		Treated	11 B	10 B	19 B	11 B	7800 A	7800
P-value			0.0002	<0.0001	<0.0001	0.0068	0.0035	0.4946
PVL03	no	None	-	-	-	-	7900 A	8700
		Treated	75 A	36 A	69 A	39 A	6700 B	8600
	yes	None	0 C	2 C	0 C	1 B	7600 A	8500
		Treated	15 B	8 B	11 B	10 B	7700 A	9100
P-value			<0.0001	<0.0001	<0.0001	0.0047	0.0477	0.4884
Titan	no	None	-	-	-	-	9700 A	8200
		Treated	38 A	16 A	44 A	13	6300 B	8100
	yes	None	3 C	1 B	0 C	8	8900 A	7500
		Treated	13 B	5 AB	6 B	8	8800 A	8700
P-value			0.0001	0.0117	<0.0001	0.1777	0.0354	0.2219
XP753	no	None	-	-	-	-	10800 AB	10400
		Treated	83 A	64 A	73 A	58 A	7100 C	9700
	yes	None	1 C	2 C	0 C	8 B	11300 A	9800
		Treated	24 B	20 B	19 B	13 B	9900 B	10000
P-value			<0.0001	0.0003	<0.0001	0.004	0.0216	0.4816

[a] Abbreviations: WAE, weeks after emergence; Fenc, fenclorim; Acet, acetochlor; PTRS, Pinetree Research Station; RREC, Rice Research and Extension Center

[b] Fenclorim seed treatment rate of 0 and 2.5 g ai kg^{-1} of seed for no and yes, respectively

[c] Acetochlor rate of 0 and 1,260 g ai ha^{-1}

[d] Means within a column for each cultivar not containing the same letter are significantly different according to Fisher's protected LSD (α=0.05)

[e] P-values were determined using SAS version 9.4 and the GLIMMIX procedure with a gamma distribution for injury and JMP Pro 16.1 using the fit model platform for yield data

Table 7. Cultivar height, shoots, and coverage in response to acetochlor and fenclorim.

			Average height				Average shoots				Coverage	
			2 WAE[a]		4 WAE		2 WAE		4 WAE		5 WAE	
Cultivar	Fenc[b]	Acet[c]	PTRS	RREC	PTRS	RREC	PTRS	RREC	PTRS	RREC	PTRS	RREC
			cm				count m^{-1}				%	
RT7321FP	no	none	8 A[d]	11 A	8 AB	22	24 A	36 A	24	38	30 A	48
		treated	4 B	6 B	8 B	21	8 B	16 B	16	16	8 B	16
	yes	none	8 A	10 A	8 AB	19	24 A	36 A	24	40	26 A	56
		treated	7 A	10 A	9 A	20	24 A	34 A	20	30	24 A	38
P-value[e]			0.0164	0.0008	0.0214	0.5787	0.0237	0.0087	0.4903	0.1594	0.0050	0.0821
RT7521FP	no	none	7 A	11 A	9	19	32 A	34 A	26	36	24	50
		treated	5 B	7 C	8	19	16 B	18 B	18	20	20	30
	yes	none	8 A	10 B	9	19	32 A	40 A	26	36	26	56
		treated	7 A	9 B	8	19	26 A	54 A	22	30	26	40
P-value			0.0151	0.003	0.7969	0.1522	0.0138	0.012	0.1147	0.0734	0.2361	0.7314
CLJ01	no	none	5 A	13 A	9	19	46 AB	54 A	44	50 A	28	46
		treated	3 B	7 B	8	19	10 C	28 B	24	16 B	8	26
	yes	none	6 A	12 A	9	18	56 A	52 A	44	44 A	36	48
		treated	5 A	11 A	9	19	40 B	52 A	38	44 A	26	40
P-value			0.0461	0.002	0.2435	0.5001	0.0123	0.0104	0.1519	0.0032	0.0832	0.0832
CLL 15	no	none	8	13	10	19	56 A	56 A	42	52	34 A	58
		treated	6	10	9	18	30 B	38 B	22	46	18 B	40
	yes	none	7	11	10	18	52 A	56 A	42	54	36 A	62
		treated	6	10	9	18	46 A	58 A	34	48	38 A	52
P-value			0.1325	0.0983	0.8074	0.4319	0.0096	0.0138	0.1678	0.7228	0.0151	0.0641
CLL 16	no	none	8	13	9	20	54 A	58	38	50 A	34 A	48
		treated	7	10	9	20	30 B	40	34	32 B	6 B	34
	yes	none	7	13	9	21	54 A	62	40	48 A	36 A	56
		treated	7	11	9	19	50 A	56	42	52 A	30 A	40
P-value			0.0528	0.0626	0.6709	0.2711	0.0042	0.1114	0.4009	0.0126	0.0001	0.7100
CLL 17	no	none	8 A	11	9 A	20	50 A	48	38	40	26 A	50 B
		treated	5 C	9	7 B	20	20 B	36	22	28	10 B	28 C
	yes	none	7 AB	12	8 AB	19	50 A	52	38	44	30 A	56 A
		treated	7 B	10	8 AB	17	44 A	54	34	44	34 A	48 B
P-value			0.0003	0.622	0.0354	0.1983	0.0011	0.1016	0.0769	0.0747	0.0006	0.019
DG263L	no	none	8	11	9	18	60 A	62 A	46 A	50 A	28 A	58 A
		treated	4	7	7	18	20 C	32 C	22 C	26 B	18 B	30 B
	yes	none	7	10	9	17	54 A	60 A	38 AB	54 A	36 A	58 A
		treated	6	8	8	15	44 B	52 B	32 B	48 A	34 A	52 A
P-value			0.1313	0.1878	0.2146	0.0944	0.0001	0.0501	0.0307	0.0384	0.0261	0.0041

Table 7 (Cont.)

			Average height				Average shoots				Coverage	
			2 WAE		4 WAE		2 WAE		4 WAE		5 WAE	
Cultivar	Fenc	Acet	PTRS	RREC	PTRS	RREC	PTRS	RREC	PTRS	RREC	PTRS	RREC
			------------------------ cm ------------------------				------------------ count m^{-1} ----------------				---------- % ----------	
Diamond	no	none	9 A	13 A	10	18 A	50 A	56 A	50 A	48	28	44 A
		treated	6 C	9 B	7	16 B	24 B	34 B	24 C	34	14	26 B
	yes	none	8 AB	11 AB	9	16 AB	54 A	54 A	34 B	44	42	48 A
		treated	7 B	11 AB	8	18 A	52 A	54 A	34 B	42	34	48 A
P-value			0.0115	0.0430	0.0766	0.0112	0.0410	0.0261	0.0012	0.3007	0.2734	0.0394
Jewel	no	none	8 A	11 A	8	18	64 A	60 A	44	48	34 A	44 A
		treated	5 C	9 C	7	15	20 B	40 B	18	32	4 C	30 B
	yes	none	7 A	11 A	9	17	60 A	58 A	50	50	30 AB	48 A
		treated	6 B	9 B	8	17	52 A	54 A	36	48	20 B	46 A
P-value			0.0246	0.0371	0.5662	0.2258	0.0013	0.0200	0.0880	0.0976	0.0042	0.0463
Jupiter	no	none	6	11	9	17	60 A	64 A	50	60 A	28 A	44 A
		treated	4	9	8	17	28 B	46 B	28	42 B	6 B	30 B
	yes	none	6	11	9	18	58 A	58 A	50	54 A	30 A	50 A
		treated	5	10	8	18	54 A	58 A	34	54 A	26 A	52 A
P-value			0.0787	0.4831	0.4567	0.2316	0.0063	0.024	0.3799	0.0163	0.0023	0.0500
Lynx	no	none	7	11	9	19	56 A	56 A	46 A	52	28	52
		treated	5	9	8	19	36 B	38 B	26 B	40	22	36
	yes	none	7	11	8	18	54 A	58 A	44 A	46	32	54
		treated	6	10	9	18	48 A	64 A	44 A	46	32	54
P-value			0.1630	0.4278	0.2249	0.8195	0.0133	0.0229	0.0312	0.0648	0.4071	0.1241
RT7231MA	no	none	5 A	10	8	23	36 A	40	34 A	44	32 A	42 A
		treated	3 B	10	8	20	10 B	28	22 B	34	14 B	32 B
	yes	none	5 A	10	8	21	36 A	44	32 A	42	34 A	50 A
		treated	5 A	8	8	22	30 A	38	34 A	40	30 A	48 A
P-value			0.0035	0.5959	0.1370	0.1033	0.0262	0.4957	0.0096	0.1652	0.0041	0.0142
PVL02	no	none	7	11	9	20	54 A	56 A	50	50	28 A	52 A
		treated	5	8	8	19	34 B	34 B	28	40	16 B	34 B
	yes	none	7	12	8	19	54 A	58 A	52	52	28 A	56 A
		treated	6	10	8	20	54 A	52 A	40	54	26 A	56 A
P-value			0.1535	0.1558	0.8904	0.5608	0.0146	0.0472	0.3154	0.2647	0.0020	0.0370

Table 7 (Cont.)

			Average height				Average shoots				Coverage	
			2 WAE		4 WAE		2 WAE		4 WAE		5 WAE	
Cultivar	Fenc	Acet	PTRS	RREC	PTRS	RREC	PTRS	RREC	PTRS	RREC	PTRS	RREC
			------------------------ cm			------------------------	------------------ count m^{-1}			----------------	---------- %	--------
PVL03	no	none	6	13 A	8	19	48 AB	54 A	32	48 A	28 A	58 A
		treated	5	10 B	8	20	22 C	34 B	26	24 B	8 B	32 B
	yes	none	6	12 A	8	23	56 A	58 A	40	50 A	28 A	66 A
		treated	6	12 A	8	22	42 B	54 A	38	44 A	26 A	56 A
P-value			0.2888	0.0135	0.5089	0.7508	0.0172	0.0366	0.6504	0.0273	0.0002	0.0361
Titan	no	none	7	11	9 A	18	54 A	56	46	54	40 A	58 A
		treated	5	10	8 B	20	28 B	48	30	46	8 B	36 C
	yes	none	6	11	9 A	19	56 A	54	48	58	38 A	54 AB
		treated	6	10	9 A	18	56 A	58	38	50	36 A	46 B
P-value			0.2061	0.0172	0.0002	0.1640	0.0071	0.2386	0.6148	0.9430	<0.0001	0.0382
XP753	no	none	8 A	9	8	18	26	34	18	32	28 B	42
		treated	4 C	7	7	20	8	22	14	14	4 C	30
	yes	none	7 AB	10	8	19	28	34	22	32	40 A	48
		treated	6 B	8	8	19	20	28	16	24	40 A	44
P-value			0.0136	0.5415	0.6523	0.3037	0.1801	0.6524	0.7860	0.2177	0.0008	0.493

[a] Abbreviations: WAE, weeks after emergence; Fenc, fenclorim; Acet, acetochlor; PTRS, Pinetree Research Station; RREC, Rice Research and Extension Center

[b] Fenclorim seed treatment rate of 0 and 2.5 g ai kg^{-1} of seed

[c] Acetochlor rate of 0 and 1,260 g ai ha^{-1}

[d] Means within a column for each cultivar not containing the same letter are significantly different according to Fisher's protected LSD (α=0.05)

[e] P-values were determined using JMP Pro 16.1 using the fit model platform for all data

Table 8. Effect of acetochlor and the fenclorim seed treatment on rice heights, shoots, and aboveground biomass averaged over planting depth.

		Heights				Shoots				Aboveground biomass	
Acetochlor	Fenclorim[a]	1 WAE[b]		4 WAE		1 WAE		4 WAE		4 WAE	
g ai ha^{-1}		---------- cm ----------				---- count pot^{-1} ----				g	
0	No	6.6	A[c]	31.7	A	31	A	43	A	23.1	B
	Yes	5	B	32.4	A	31	A	46	A	26.1	A
1,050	No	2.9	C	26.4	B	21	B	32	B	16.4	C
	Yes	4.7	B	29.2	A	30	A	44	A	23.7	AB
P-value[d]		< 0.0001		0.0178		0.0002		0.0001		0.0374	

[a] Fenclorim seed treatment rate of 0 and 2.5 g ai kg^{-1} of seed

[b] Abbreviations: WAE, weeks after emergence

[c] Different letters within each column indicate a significant difference between treatments; means separated using Fisher's protected LSD (α = 0.05)

[d] P-values determined using JMP Pro 16.1 with the fit model platform

Table 9. Effect of planting depth, acetochlor, and the fenclorim seed treatment on rice injury.

			Injury	
Planting depth	Acetochlor	Fenclorim[a]	1 WAE[b]	4 WAE
cm	g ai ha^{-1}		--------------- (%) -----------------	
0.6	0	No	-	-
		Yes	5 C[c]	2 D
	1,050	No	41 A	35 A
		Yes	7 C	6 CD
2.5	0	No	-	-
		Yes	3 C	1 D
	1,050	No	29 B	23 B
		Yes	8 C	11 C
P-value[d]			0.0052	0.0027

[a] Fenclorim seed treatment rate of 0 and 2.5 g ai kg^{-1} of seed

[b] Abbreviations: WAE, weeks after emergence

[c] Different letters within each column indicate a significant difference between treatments; means separated using Fisher’s protected LSD ($\alpha = 0.05$)

[d] P-values determined using JMP Pro 16.1 with the fit model platform

Chapter 3

Evaluation of Rice Tolerance and Weed Control with Acetochlor and Fenclorim

Abstract

Many problematic weeds have evolved resistance to herbicides in mid-southern U.S. rice fields. With the lack of new effective herbicides, rice producers seek alternatives that are currently not labeled for rice production. Very-long chain fatty acid elongase (VLCFA) inhibitors are currently not labeled for U.S. rice but are labeled for use in other U.S. row cropping systems and Asian rice production. Previous research has demonstrated the utility of VLCFA inhibitors for weed control in rice; however, these herbicides induce variable amounts of injury to the crop when applied early in the growing season. Therefore, experiments were initiated in 2020 and 2021 at the Rice Research and Extension Center near Stuttgart, AR, to evaluate rice tolerance and weed control with acetochlor and a fenclorim (herbicide safener) seed treatment. Three rates of a microencapsulated formulation of acetochlor (630, 1,260, and 1,890 g ai ha^{-1}), four application timings [preemergence (PRE), delayed-preemergence (DPRE), spiking, and 1-leaf], and without or with the fenclorim seed treatment (2.5 g kg^{-1} of seed) were used to evaluate rice tolerance, weedy rice control, and barnyardgrass control. Acetochlor applied DPRE at 1,260 g ai ha^{-1} provided better weedy rice and barnyardgrass control than 1-leaf applications at the same rate. Acetochlor rates of 1,260 and 1,890 g ai ha^{-1} reduced barnyardgrass and weedy rice densities greater than 630 g ai ha^{-1}. The fenclorim seed treatment did not influence weedy rice or barnyardgrass control but did reduce injury for DPRE applications. Based on these results, acetochlor can be safely applied to rice DPRE ($\leq$ 19% injury) at 1,260 g ai ha^{-1} when the seed is treated with fenclorim, leading to $\geq$ 88% barnyardgrass and $\geq$ 45% weedy rice control 28 days after treatment.

Nomenclature: Acetochlor; fenclorim; barnyardgrass, *Echinochloa crus-galli* (L.) P. Beauv; weedy rice, *Oryza sativa* L.; rice, *Oryza sativa* L.

Introduction

Rice is one of the most consumed grains globally, and within the United States (U.S.), Arkansas is the leading rice producer (Foreign Agriculture Service 2021). However, arguably on of the most limiting factor for rice production in Arkansas is weed control. With rice weed control, the availability of only a few sites of action (SOA) limits producers and has led to some problematic weeds developing herbicide resistance to many of the commonly used modes of action (Barber et al. 2020; Heap 2022).

Two of the most problematic weeds for rice producers to control in Arkansas are barnyardgrass and weedy rice (Butts et al. 2022). Barnyardgrass has developed resistance to five different SOAs across the Midsouth. Within Arkansas, barnyardgrass has resistance to propanil [Weed Science of America (WSSA) Group 5 photosystem II inhibitors]; quinclorac and florypyrauxifen-benzyl (WSSA Group 4 synthetic auxins); clomazone (WSSA Group 13 1-deoxy-D-xylulose 5-phosphate synthase inhibitors); and imazethapyr, penoxsulam, and bispyribac-sodium [WSSA Group 2 acetolactate synthase (ALS) inhibitors] (Barber et al 2020; Heap 2022). Additionally, Mississippi has confirmed resistance to the active ingredient fenoxaprop, a WSSA Group 1 acetyl CoA carboxylase inhibitor (Heap 2022; Lovelace et al. 2007; Talbert and Burgos 2007). Without the previously mentioned herbicides, rice producers have only a select few herbicides to control barnyardgrass, indicating the need for an alternative SOA for producers (Barber et al. 2020).

The third most problematic weed of rice, weedy rice, is resistant to only one known SOA in the Midsouth, ALS inhibitors (Heap 2022). Furthermore, since weedy rice and cultivated rice are the same species, weedy rice is tolerant to the same herbicides as cultivated rice (Barber et al. 2020). Therefore, to control resistant populations of weedy rice, growers must use either water-seeded practices with thiobencarb (WSSA Group 8 lipid synthesis inhibitors) or quizalofop-P-resistant (Provisia or Max-Ace) rice, which utilizes the active ingredient quizalofop-P (Barber et al. 2020; Lancaster 2017). However, most Arkansas rice producers plant drill-seeded rice, and with quizalofop-P-resistant rice, the potential for outcrossing to weedy rice has already been demonstrated with imidazolinone-resistant rice technology (Burgos et al. 2008; Gealy et al. 2015; Hardke 2020; Shivrain et al. 2007). Thus, mid-southern rice producers need an alternative method for controlling weedy rice within a nontransgenic, drill-seeded production system.

Currently, very long-chained fatty acid elongase- (VLCFA) inhibiting herbicides are not labeled for U.S. rice production; however, Asian rice production systems use a VLCFA uncommon to the U.S., pretilachlor (Chen et al. 2013; Quadranti and Ebner 1983). Herbicides that inhibit VLCFA disrupt the biosynthesis of saturated and unsaturated fatty acids longer than 18 carbons (C) in length. These fatty acids are important for various lipids, particularly the lipids that facilitate cell division, which are needed in root and shoot growth of emerging seedlings (Babczinski et al. 2012). Additionally, VLCFA herbicides provide residual control of grasses and small-seeded broadleaf weeds but offer little to no control of emerged weeds (Anonymous 2018; Barber et al. 2020).

The use of acetochlor, another chloroacetamide herbicide more efficacious than pretilachlor can provide substantial weed control in rice production systems (Fogleman 2018; Godwin 2017; Norsworthy et al. 2019). Furthermore, Godwin (2017) demonstrated that acetochlor applied at early application timings provided significantly better weed control than later applications. However, VLCFA inhibitors such as acetochlor are water-activated residual herbicides, and earlier applications also posed an increased risk to injure rice (Babczinski et al. 2012; Fogleman et al. 2019).

When evaluating an emulsifiable concentrate formulation (EC) versus a microencapsulated formulation (ME), the ME formulation of acetochlor elicited significantly less rice phytotoxicity than the EC (Fogleman et al. 2019). The decrease in rice injury with the ME formulation was due to the controlled release of the active ingredient which distributes the soil concentration of the herbicide over time rather than being immediately available for uptake (Bernards et al. 2006; Dowler et al. 1999). However, high variability in rice tolerance with microencapsulated acetochlor has also resulted in unacceptable crop injury (Fogleman et al. 2019; Godwin et al. 2018). Therefore, the need for a secondary enhancement for rice tolerance to chloroacetamides drove the consideration of including a herbicide safener as a seed treatment.

Fenclorim, the seed safener, works in several ways to reduce the phytotoxicity of chloroacetamides in rice. Fenclorim reduced total uptake and persistence of pretilachlor and increased glutathione-*S*-transferase (GST) enzyme activity, the primary pathway by which rice metabolizes pretilachlor (Chen et al. 2013; Scarponi et al. 2003, 2005; Usui et al. 2001). While fenclorim provides enhanced rice tolerance to pretilachlor, previous research has demonstrated the ability of a fenclorim seed treatment to reduce acetochlor injury in rice (Avent et al. 2020). Although the fenclorim seed treatment did not provide adequate crop tolerance to EC acetochlor, fenclorim at 2.5 g kg^{-1} seed provided acceptable crop tolerance to microencapsulated acetochlor at 1,260 g ai ha^{-1}.

Because current research has not demonstrated rice tolerance and weed control with acetochlor and a commercial fenclorim seed treatment, experiments were conducted to determine the influence of a fenclorim seed treatment with various application timings and acetochlor rates.

The objectives evaluated barnyardgrass and weedy rice control as well as rice tolerance. In consideration of previous research, the hypotheses for this experiment were that earlier application timings and increasing rates of acetochlor would increase weed control, and the fenclorim seed treatment would not influence weed control but would reduce rice injury from acetochlor.

Materials and Methods

Experimental design. The experiment was designed as a randomized complete block with a three-factor factorial treatment structure with four replications. The three factors were fenclorim seed treatments of 0 and 2.5 g of fenclorim kg^{-1} of seed; herbicide application timings of preemergence (PRE), delayed-preemergence (DPRE), spiking, and 1-leaf rice; and three rates of ME acetochlor at 630, 1,260, and 1,890 g ai ha^{-1}. Additionally, rice with both fenclorim rates were planted without no herbicides applied to allow for comparisons for a total of 26 possible treatments. The experiment was initiated in the spring of 2020 and 2021 at the Rice Research and Extension Center (RREC) near Stuttgart, AR, on a Dewitt silt loam composed of 27.1% sand, 54.4% silt, and 18.5% clay, a soil pH of 5.6, and 1.8% organic matter. Each study was managed culturally and for pest management based on University of Arkansas Cooperative Extension Services recommendations for direct-seeded, delayed-flooded rice production. Soil fertility was amended preplant and based on UADA Marianna Soil Test and Research Laboratory recommendations with no preplant nitrogen. The field was cultivated before trial initiation to remove any emerged weeds and produce a fine seedbed. Urea (46-0-0) was applied at 316 kg ha^{-1} before flooding the entire field.

The rice cultivar 'Diamond' was planted at 72 seeds m^{-1} of row on May 11, 2020, and April 28, 2021, with a base seed treatment of clothianidin, carboxin, thiram, metalaxyl, fludioxonil, and gibberillins at 0.75, 0.38, 0.33, 0.16, 0.03, and 0.04 g ai kg^{-1} of seed, respectively. Plots were 1.5 m wide and 5.2 m long, with 1.5 m between plots in each block and 0.9 m between each block. Nine rows of rice were planted on 19-cm row spacings to a 1.5-cm depth. Preemergence applications were applied the day of planting, and DPRE applications were made after the rice seed had germinated but before emergence (four and seven days after planting for 2020 and 2021, respectively). Spiking and 1-leaf applications went out at the appropriate rice stages (Table 1). All herbicide applications were applied with a CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} at 276 kPA and 4.8 kph with four AIXR 110015 nozzles (TeeJet, Glendale Heights, IL) spaced 51 cm apart.

Data Collection and Analysis. Rice phytotoxicity was visually evaluated relative to the nontreated control 14, 21, and 28 days after treatment (DAT) +/- 3 days on a 0 to 100 scale, with 0 representing no injury and 100 being plant death (Frans and Talbert 1977). Additionally, barnyardgrass and weedy rice control were visually evaluated relative to the nontreated control 14, 21, and 28 DAT from 0 to 100, with 0 being no control and 100 being no weeds present (Frans and Talbert 1977). Quantitative assessments included densities of weedy rice and barnyardgrass from two randomly established 0.25 m^2 quadrats per plot, counted 28 days after rice emergence.

All data distributions were checked using JMP pro version 16.1 (SAS Institute Inc, Cary, NC) and found to be gamma-distributed. Data distribution selections were based on best fit using least log-likelihood and Akaike information criterion. Data were analyzed by year due to herbicide activation from rainfall (Figures 1 and 2). Since evaluations of injury and weed control occurred seven days apart for three weeks and evaluations showed increases or decreases, an analysis of variance (ANOVA) with repeated measures was used to determine the differences between treatments and evaluation timings. An unstructured covariance structure was selected for repeated measures analysis based on the model of best fit. All other data were subjected to ANOVA. All data were analyzed using SAS version 9.4 with the GLIMMIX procedure with a gamma distribution (Gbur et al. 2012). Means were separated using Tukey's honestly significant difference test with an $\alpha = 0.05$.

Results and Discussion

Rice Injury. In the absence of fenclorim averaged over acetochlor rates, rice tolerance to the herbicide generally increased in 2020 and 2021 as application timing was delayed (Table 2). The increased tolerance with delayed application timing was highly evident in 2021 when 85% injury was observed at 14 DAT following a PRE application in the absence of fenclorim and only 3 to 4% injury occurred following spiking and 1-leaf applications, respectively. At 14 DAT, averaged over acetochlor rate, and without fenclorim, rice injury in 2020 and 2021 decreased from 33 and 45% (DPRE) to 17 and 3% (spiking), and 22 and 4% (1-leaf), respectively (Table 2). In similar studies with acetochlor applied at 1,050 g ai ha^{-1}, injury decreased as application timing was delayed (Godwin et al. 2018). Godwin et al. (2018) reported that in 2016 at 14 DAT, injury was 89% following DPRE, 43% following spiking, and 10% following a 1- to 2-leaf application. The excessive injury following the DPRE was attributed to a 10 cm rainfall event that occurred 4 DAT (Godwin et al. 2018), emphasizing the variability of acetochlor activity based on activation timing (Babczinski et al. 2012). In other research with quizalofop-P-resistant rice, injury at 14 days following a DPRE application of ME acetochlor increased from 51% at a rate of 1,050 g ai ha^{-1} to 73% following 1,470 g ai ha^{-1}, respectively (Norsworthy et al. 2019). Similarly, in 2020 and 2021 at 28 DAT, averaged over herbicide application timing, and without fenclorim, injury

28 DAT increased from 33 to 53% and 66 to 79% following acetochlor at 1,260 and 1,890 g ai ha^{-1} in 2020 and 2021, respectively (Table 3).

Emerging crops and weeds uptake acetochlor through roots and shoots shortly after germination. Activation and incorporation of acetochlor typically requires at least 1.3 cm of rainfall or irrigation (Anonymous 2018; Babczinski et al. 2012). In 2020, rice was planted into adequate moisture to allow for germination; however, an activating rainfall did not occur until seven days after the PRE application (Figure 1). In 2021, the PRE application was activated by rainfall the next day (Figure 2), resulting in increased rice injury and weed control. Additionally, the fenclorim seed treatment did not statistically improve tolerance at 21 and 28 DAT for PRE applications in 2021 (Table 2). In general, fenclorim reduced injury by 6 to 23 percentage points for PRE applications averaged over herbicide rate in 2021; however, the lack of substantial safening is likely a function of the activation timing of the herbicide. Conversely, fenclorim reduced injury for DPRE applications of acetochlor averaged over herbicide rate by 29 to 43 percentage points in 2021.

When evaluating the uptake and conjugation of fenclorim and pretilachlor, a herbicide closely related to acetochlor, in rice shoots, previous research has shown that fenclorim uptake did not occur until 48 hours after treatment, and pretilachlor uptake occurred 24 hours after treatment (Scarponi et al. 2003). Additionally, GST activity for fenclorim-treated rice did not statistically separate from the nontreated control until 48 hours after treatment, and a reduction in pretilachlor persistence from fenclorim-treated shoots was not observed until 72 hours after treatment. Therefore, in theory, the time of rice uptake of acetochlor should be at least 48 hours after the rice has absorbed fenclorim.

A reduction in rice injury was observed at all evaluation timings with the addition of fenclorim for PRE applications in 2020 averaged over herbicide rate (Table 2). Rice was planted into moist soil and germinated prior to the first activating rainfall event, which likely contributed to the enhanced tolerance in 2020 versus the greater injury observed in 2021 following the PRE application. In general, the fenclorim seed treatment reduced injury for all applications of acetochlor occurring earlier than 1-leaf. The lack of a safening response for the 1-leaf applications is likely due to fenclorim no longer improving conjugation of a chloroacetamide herbicide by 5 DAT (Scarponi et al. 2003). However, Scarponi and others evaluated foliar-applied fenclorim. The persistence and uptake of fenclorim as a seed treatment has not been studied and GST activity could be prolonged, which may explain why safening can still be observed for the spiking treatments.

It is important to note that for 2020, <20% rice injury was observed with the fenclorim seed treatment when acetochlor was applied at 630 and 1,260 g ai ha^{-1} at all application timings and evaluation timings (Table 3). However, in 2021, injury ranged from 37 to 91% with the fenclorim seed treatment at all rates and evaluation timings for PRE applications (data not shown). Additionally, in 2021, <20% injury was observed with the fenclorim seed treatment for 630 and 1,260 g ai ha^{-1} at all evaluation timings for DPRE or later application timings. Therefore, PRE applications of acetochlor and rates greater than 1,260 g ai ha^{-1} should be discouraged if the use of fenclorim and acetochlor becomes registered in U.S. rice production systems.

In general, rice injury from acetochlor at 630 g ai ha^{-1} averaged over application timing and the fenclorim seed treatment did not increase as evaluation timings progressed from 14 to 28 DAT; however, applications of 1,260 g ai ha^{-1} in 2021 and 1,890 g ai ha^{-1} for both years showed an increase in injury from 14 to 28 DAT (Table 3). Because injury did not decrease as evaluation timings progressed, the evaluations may not have continued long enough to capture the recovery of rice from acetochlor injury. Future studies should consider continuing evaluations further into the growing season.

Weed Control. Acetochlor applied DPRE at 1,260 g ai ha^{-1} controlled barnyardgrass 88 to 96% 21 and 28 DAT, respectively, for both years (Table 4). Similarly, in other research, control was 62 to 88% and 63 to 90% with acetochlor applied at 1,050 and 1,470 g ai ha^{-1}, respectively (Norsworthy et al. 2019). In still other research, Fogleman (2018) reported barnyardgrass control of 77 and 94% averaged over DPRE applications of acetochlor at 1,050 and 1,470 g ai ha^{-1} 14 and 28 DAT, respectively. In 2020 and 2021, a 1-leaf application of 630 g ai ha^{-1} did not achieve comparable barnyardgrass control to PRE and DPRE applications at the same rate 21 and 28 DAT, indicating that as application timing delayed, barnyardgrass control decreased. Furthermore, no rate of acetochlor applied at the 1-leaf timing controlled barnyardgrass 80%, while DPRE applications of 1,260 g ai ha^{-1} achieved ≥ 88% barnyardgrass control 21 and 28 DAT for both 2020 and 2021. The reduction in control with delayed application timing is attributed to emergence of barnyardgrass prior to the acetochlor treatment. It is well documented that acetochlor provides only residual weed control (Babczinski et al. 2012).

In 2020 and 2021, weedy rice control trended similarly to barnyardgrass control (Table 5). As application timing was delayed and as rates decreased, weedy rice control generally decreased. PRE and DPRE applications of acetochlor at 1,260 g ai ha^{-1}, averaged over presence and absence of fenclorim, provided better weedy rice control than spiking and 1-leaf applications at the same rate 28 DAT. Additionally, the lowest rate of acetochlor did not achieve comparable weedy rice control to the highest rate of acetochlor 28 DAT for each application timing in both years. In previous research, weedy rice control was better with DPRE applications, averaged over acetochlor rates, than control with 1- to 2-leaf applications (Fogleman 2018).

Relative barnyardgrass and weedy rice densities trended similarly to visually estimated control. For both years, acetochlor at 1,260 and 1,890 g ai ha^{-1} provided a greater reduction in barnyardgrass and weedy rice densities 28 days after emergence averaged over application timing and fenclorim (Table 6). Additionally, DPRE applications reduced weedy rice and barnyardgrass densities greater than 1-leaf applications averaged over acetochlor rate and fenclorim. Therefore, the optimum timing of acetochlor applications for weedy rice and barnyardgrass control appears to be at the DPRE timing, which coincides with Fogleman 2018; Norsworthy et al. 2019.

For all barnyardgrass and weedy rice evaluations, there was never a significant main effect of fenclorim or interaction with the seed safener ($P > 0.05$). These results would indicate that fenclorim aids rice protection and does not negatively affect the level of weed control provided by acetochlor. Originally, fenclorim was used with pretilachlor in spray solution (Quadranti and Ebner 1983). Broadcast applications of fenclorim could potentially reduce herbicide efficacy by providing enhanced metabolism of chloroacetamides. Field studies conducted in 2021 demonstrated a 10 to 20% reduction in broadleaf signalgrass [*Urochloa platyphylla* (Munro ex C. Wright) R.D. Webster] control when fenclorim was added to spray solution with acetochlor at 1,260 g ai ha^{-1} (Norsworthy et al., unpublished data). However, with the application of fenclorim as a seed treatment, the herbicide safener is directly placed in-furrow, where only cultivated rice receives enhanced tolerance to acetochlor.

Practical Implications. Based on previous and current research, applications of acetochlor at 1,050 to 1,260 g ai ha^{-1} should provide adequate control of barnyardgrass and suppression of weedy rice (Fogleman 2018; Norsworthy et al. 2019). If labeled, the addition of acetochlor to current rice herbicide programs would provide residual barnyardgrass control, including control of populations known to be resistant to the PRE-applied herbicides clomazone and quinclorac. Furthermore, acetochlor would also provide some weedy rice suppression to aid postemergence applications in imidazolinone- or quizalofop-P-resistant rice. Reducing the number and size of weeds present at the time of postemergence applications would reduce selection pressure and prolong the efficacy of the current herbicide options available to rice producers.

In general, higher levels of rice injury and weed control were observed for PRE and DPRE applications in 2021 as compared to 2020 due to the adverse growing conditions with greater total rainfall, activation of the PRE applications, and cooler conditions in 2021 (Table 1). However, rice injury was < 20% for DPRE applications of acetochlor at 1,260 g ai ha^{-1} or less with the fenclorim seed treatment. Other preemergence herbicides such as clomazone can bleach rice up to 35% without causing any yield loss (Zhang et al. 2005). Based on visual estimates of weed control, weed densities, and visual estimates of crop injury, the optimum application timing and rate for acetochlor in these trials was 1,260 g ai ha^{-1} applied DPRE with fenclorim-treated rice seed at 2.5 g kg-seed^{-1}. For this treatment, acetochlor controlled weedy rice 45 to 69% at 28 DAT and barnyardgrass control of 88 to 89% (Tables 4 and 5). Acetochlor at the same rate and timing caused as much as 74% injury in the absence of the fenclorim seed treatment. In comparison, adding the fenclorim seed treatment reduced rice injury to no more than 19%. However, current research with acetochlor and fenclorim has been conducted predominately on silt loam soils, which encompass only 50% of Arkansas rice hectares (Hardke 2021). Future studies should consider a rate response of acetochlor on different textured soils since acetochlor activity is negatively correlated with clay content (Reinhardt and Nel 1990).

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Tables

Table 1. List of dates for cultural management practices, herbicide applications, and total rainfall from planting until flooding.

Year	Planting/PRE[a]	DPRE	Spiking	1-Leaf	Flooding	Harvest	Rainfall (cm)
2020	May 11	May 14	May 21	May 25	Jun. 11	Sep. 29	24.3
2021	Apr. 28	May 06	May 12	May 15	Jun. 12	Nov. 02	38.0

[a] Abbreviations: PRE, preemergence; DPRE, delayed-preemergence

Table 2. Influence of application timing, evaluation timing, and fenclorim seed treatment on rice (*Oryza sativa* L.) injury.

		Injury					
		2020			2021		
Timing	Fenclorim	14 DAT[a]	21 DAT	28 DAT	14 DAT	21 DAT	28 DAT
		%					
PRE	Without	40 A[b]	42 A	47 A	85 A	72 A	91 A
	With	17 BC	17 B	16 CD	62 B	64 AB	85 A
DPRE	Without	33 A	39 A	39 A	45 C	59 B	65 B
	With	13 CD	15 B	15 D	16 D	22 C	22 E
Spiking	Without	17 BC	15 B	27 B	3 E	20 CD	49 C
	With	5 D	5 C	15 D	2 E	18 CD	23 E
1-leaf	Without	22 B	20 B	25 BC	4 E	14 CD	35 D
	With	16 BC	13 BC	13 D	2 E	8 D	25 DE
P-value[c]		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
RM P-value[d]		0.6025			0.0892		

[a] Abbreviations: DAT, days after treatment; PRE, preemergence; DPRE, delayed-preemergence; RM, repeated measures

[b] Means within a column for the fenclorim by timing interaction not containing the same letter are different according to Tukey's HSD (α=0.05)

[c] P-values were generated using the GLIMMIX procedure without repeated measures in SAS 9.4 with a gamma distribution

[d] RM P-values were generated using the GLIMMIX procedure with repeated measures in SAS 9.4 with a gamma distribution

Table 3. Influence of acetochlor rate, evaluation timing, and fenclorim seed treatment on rice (*Oryza sativa* L.) injury.

		Injury					
		2020			2021		
Rate	Fenclorim	14 DAT[a]	21 DAT	28 DAT	14 DAT	21 DAT	28 DAT
g ai ha^{-1}		%					
630	Without	17 FGH[b]	17 FGH	18 FG	25 GHIJ	31 EFGH	35 EFG
	With	6 I	8 I	9 HI	16 J	20 IJ	24 GHIJ
1,260	Without	29 CDE	30 CD	33 BC	37 EF	42 DE	66 B
	With	12 GHI	12 GHI	13 GHI	22 HIJ	27 FGHI	38 E
1,890	Without	39 B	40 B	53 A	40 E	51 CD	79 A
	With	20 EFG	19 FG	23 DEF	24 HIJ	37 EF	54 C
RM P-value[c]		0.0159			< 0.0001		

[a] Abbreviations: DAT, days after treatment; PRE, preemergence; DPRE, delayed-preemergence; RM, repeated measures

[b] Means within a year for the fenclorim by herbicide timing interaction not containing the same letter are different according to Tukey's HSD (α=0.05)

[c] RM P-values were generated using the GLIMMIX procedure with repeated measures in SAS 9.4 with a gamma distribution

Table 4. Influence of application timing, acetochlor rate, and evaluation timing on barnyardgrass control.

		Barnyardgrass control					
		2020			2021		
Timing	Rate	14 DAT[a]	21 DAT	28 DAT	14 DAT	21 DAT	28 DAT
	g ai ha^{-1}	%					
PRE	630	87 AB[b]	82 ABC	73 ABCDEF	75 ABCD	83 ABC	91 AB
	1260	91 A	83 ABC	84 ABC	86 ABC	92 AB	95 A
	1890	94 A	86 AB	88 AB	95 AB	95 AB	98 A
DPRE	630	77 ABCD	72 ABCDEF	75 ABCDEF	33 EFG	80 ABC	69 BCD
	1260	93 A	88 AB	89 AB	47 CDEF	96 A	88 AB
	1890	92 A	90 A	87 AB	69 ABCD	98 A	95 A
Spiking	630	51 FG	50 GF	52 DEFG	15 HI	14 I	38 EF
	1260	69 ABCDEF	69 ABCDEF	59 BCDEF	53 BDEC	74 ABCD	74 ABCD
	1890	80 ABC	80 ABC	83 ABC	52 BDFEC	70 ABCD	89 AB
1-leaf	630	19 I	26 HI	36 GH	19 HIJ	24 GH	35 EF
	1260	56 CDEF	59 BCDEF	51 EFG	24 FGH	22 GH	66 BCD
	1890	77 ABCD	77 ABCD	63 ABCDEF	44 DEF	44 DEF	73 ABCD
	RM P-value[c]	0.0042			< 0.0001		

[a] Abbreviations: DAT, days after treatment; PRE, preemergence; DPRE, delayed-preemergence; RM, repeated measures

[b] Means within a year not containing the same letter are different according to Tukey's HSD (α=0.05)

[c] P-values were generated using the GLIMMIX procedure with repeated measures in SAS 9.4 with a gamma distribution

Table 5. Influence of application timing, acetochlor rate, and evaluation timing on weedy rice control.

		Weedy rice control					
		2020			2021		
Timing	Rate	14 DAT[a]	21 DAT	28 DAT	14 DAT	21 DAT	28 DAT
	g ai ha^{-1}	%					
PRE	630	22	22	18 D	49	50	50 EF
	1260	39	41	48 B	66	70	65 C
	1890	52	56	73 A	81	75	79 AB
DPRE	630	22	23	24 CD	21	38	49 EF
	1260	38	46	45 B	29	62	69 BC
	1890	37	58	76 A	49	76	83 A
Spiking	630	21	15	11 D	4	10	23 G
	1260	39	26	20 CD	26	32	55 DE
	1890	42	43	49 B	23	31	69 BC
1-leaf	630	22	22	23 CD	0	11	22 G
	1260	26	28	24 CD	12	14	37 F
	1890	41	40	38 BC	23	24	44 EF
	P-value[c]	0.2069	0.1479	< 0.0001	0.1412	0.0512	0.0025
	RM P-value[d]	0.4974			0.1781		

[a] Abbreviations: DAT, days after treatment; PRE, preemergence; DPRE, delayed-preemergence; RM, repeated measures

[b] Means within a column for the fenclorim by timing interaction not containing the same letter are different according to Tukey's HSD (α=0.05)

[c] P-values were generated using the GLIMMIX procedure without repeated measures in SAS 9.4 with a gamma distribution

[d] RM P-values were generated using the GLIMMIX procedure with repeated measures in SAS 9.4 with a gamma distribution

Table 6. Influence of herbicide application timing and acetochlor rate on barnyardgrass and weedy rice densities evaluated 28 days after emergence

	Weed densities			
	2020		2021	
Factor	Barnyardgrass[a]	Weedy rice[b]	Barnyardgrass	Weedy rice
Herbicide timing	-------------------------------- % of nontreated	--------------------------------		
PRE[a]	13 B[b]	43 C	4 D	55 B
DPRE	16 B	34 D	17 C	30 C
Spiking	17 B	63 B	25 B	72 A
1-leaf	29 A	113 A	28 A	58 B
P-value	0.0002	< 0.0001	< 0.0001	< 0.0001
Herbicide rate				
g ai ha^{-1}				
630	32 A	73 A	27 A	64 A
1,260	15 B	56 B	13 B	47 B
1,890	12 B	45 B	12 B	44 B
P-value	< 0.0001	< 0.0001	< 0.0001	0.0003

[a] Average barnyardgrass densities in the nontreated were 19 and 26 m^{-2} for 2020 and 2021, respectively

[b] Average weedy rice densities in the nontreated were 19 and 21 m^{-2} for 2020 and 2021, respectively

[c] Abbreviations: PRE, preemergence; DPRE, delayed-preemergence

[d] Means within a column for each factor level not containing the same letter are different according to Tukey's HSD (α=0.05)

[e] P-values were generated using the GLIMMIX procedure in SAS 9.4 with a gamma distribution

Figures

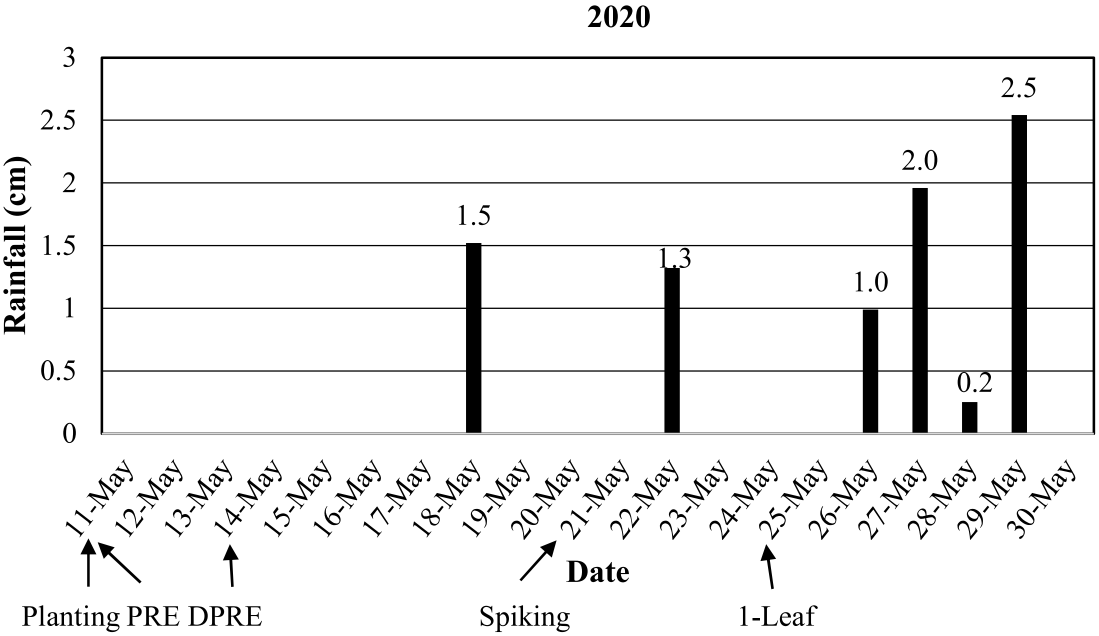

Figure 1. Rainfall amount each day for three weeks following planting at the Rice Research and Extension Center near Stuttgart, AR in 2020 totaling 8.5 cm of rain. Abbreviations: PRE, preemergence; DPRE, delayed-preemergence

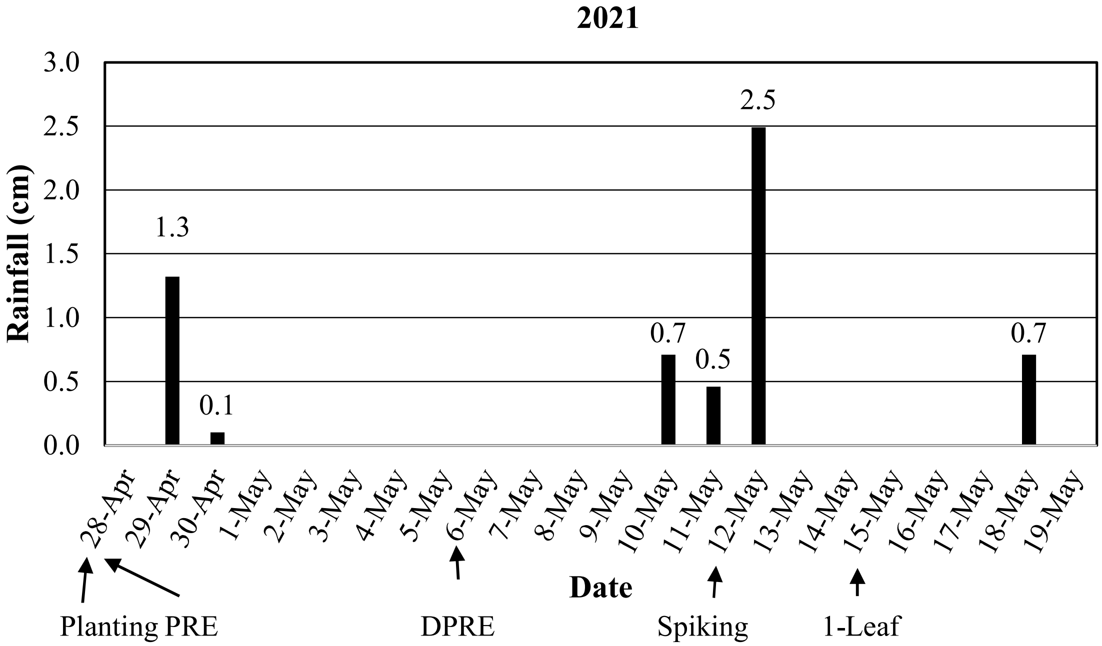

Figure 2. Rainfall amount each day for three weeks following planting at the Rice Research and Extension Center near Stuttgart, AR in 2021 totaling 5.8 cm of rain. Abbreviations: PRE, preemergence; DPRE, delayed-preemergence

Chapter 4

The Evaluation of a Fenclorim Seed Treatment to Reduce Rice Phytotoxicity from Fall-Applied Acetochlor and Pyroxasulfone

Abstract

Very long-chained fatty acid elongase (VLCFA)-inhibiting herbicides are currently not labeled within U.S. rice production but have been used in Asian rice production to suppress weedy rice and control other annual grasses and small-seeded broadleaves. Therefore, separate experiments were initiated in the fall of 2019 and 2020 to evaluate rice tolerance and weedy rice control with acetochlor and pyroxasulfone in the following spring without and with a fenclorim seed treatment (2.5 g ai kg^{-1} of seed). A three-factor experiment within a randomized complete block design was implemented with the factors being 1) herbicide choice (acetochlor or pyroxasulfone), 2) a low or high herbicide rate (acetochlor applied at 2,100 and 4,200 g ai ha^{-1}, and pyroxasulfone applied at 237 and 475 g ai ha^{-1}), and 3) with or without the fenclorim seed treatment. Throughout the experiments, rice exhibited good tolerance to acetochlor with injury being < 10% averaged over the seed treatment. The fenclorim seed treatment reduced injury averaged over herbicide form and rate from 28 to 21% 28 days after the emergence (DAE) of rice for both trial types. Fenclorim also improved rough-rice yield averaged over herbicide form and rate by 12 and 18 percentage points for the two different studies. However, at no point did rice demonstrate good tolerance (<20% injury) to pyroxasulfone with or without fenclorim. The fenclorim seed treatment or any interaction containing fenclorim did not influence weedy rice control, and weedy rice control with acetochlor ranged from 48 to 0%. Findings from this experiment demonstrate the effects of a fenclorim seed treatment for fall-applied acetochlor and pyroxasulfone and the efficacy of acetochlor and pyroxasulfone for controlling weedy rice.

Based on this research, acetochlor may provide a viable herbicide option to apply in the fall to suppress weedy rice.

Nomenclature: Acetochlor; pyroxasulfone; fenclorim; rice, *Oryza sativa* L.; weedy rice, *Oryza sativa* L.

Introduction

Weedy rice is one of the most troublesome weeds for flooded-rice producers to control, ranking third in most problematic weeds by Arkansas survey respondents (Butts et al. 2022). Weedy rice can compete with cultivated rice, reducing grain yield and quality through contamination (Ottis et al. 2005). Weedy rice can cause up to 82% yield loss if left uncontrolled season long (Smith 1988). Additionally, with weedy rice and cultivated rice being the same species and nearly indistinguishable early in the season, postemergence control options are limited to non-transgenic, herbicide-resistant cultivars (Burgos et al. 2008; Croughan 2004). Currently, the only preemergence option to control weedy rice in conventional cultivated rice is by water-seeding the crop in conjunction with applications of thiobencarb (WSSA Group 8 lipid synthesis inhibitor) (Barber et al. 2020). Additionally, producers can utilize crop rotation to control weedy rice with other herbicides.

For postemergence chemical control of weedy rice, producers must plant quizalofop-P- or imidazolinone-resistant rice and utilize the respective herbicide options for each trait. However, some weedy rice populations have developed resistance to the imidazolinone herbicide family through outcrossing between cultivated rice and weedy rice (Burgos et al. 2008; Gealy et al. 2015; Shivrain et al. 2007). Nevertheless, in non-resistant populations, high levels of control with both technologies can still be achieved, providing ≥ 95% control of weedy rice (Avila et al. 2005; Lancaster 2017). However, escapes are inevitable, even with proper application timings and methods, due to various other factors affecting the efficacy of either herbicide (Bagavathiannan and Norsworthy 2012). With weedy rice escapes, the potential for outcrossing is low (0.1099 to 0.434%) (Shivrain et al. 2007), but due to the fecundity of weedy rice, a few plants can quickly become an infestation (Burgos et al. 2014).

With the majority of Arkansas rice production utilizing drill-seeded practices (85.7%) rather than water-seeded (4.7%) (Hardke 2021), Arkansas rice producers need a preemergence herbicide option to control weedy rice in drill-seeded systems. Additionally, using a preemergence herbicide that controls weedy rice would reduce the number of plants that need to be controlled with postemergence applications, potentially reducing the number of escapes that could outcross back to the weedy rice population (Norsworthy, personal communication 2021). Based on these criteria, the use of very long-chained fatty acid elongase (VLCFA)-inhibiting herbicides has been investigated for the past several years in mid-southern United States rice production systems (Avent et al. 2020; Bertucci et al. 2019; Fogleman 2018; Godwin 2017).

In Asian rice production systems, VLCFA herbicides have been widely used (Chen et al. 2013; Han and Hatzios 1991). In three studies conducted in 2012 in southern Vietnam, varying rates of pretilachlor (chloroacetamide, WSSA Group 15 VLCFA) were evaluated for grass, sedge, and broadleaf weed control with weedy rice panicle counts and biomass assessments. All doses of pretilachlor reduced the weed density and biomass of all species groups compared to the nontreated (Chauhan et al. 2014). Furthermore, weedy rice panicles and biomass were reduced from treatments of pretilachlor (20 to 80% and 15 to 54%, respectively). Yield collected at harvest showed an increase from pretilachlor treatments, but in-season evaluations of rice tolerance were not evaluated, and yield benefits are likely a function of weed control. However, this study and most rice planted in Asia practice water seeding or transplanted rice culture, which is not the primary form of rice crop establishment within the mid-southern United States.

Based on research in the southern U.S., rice tolerance to VLCFA herbicides can vary with formulation and time of application. Godwin and others (2018) evaluated rice tolerance as a function of application timing (delayed-preemergence, spiking, 1- to 2-leaf, and 3- to 4-leaf) and rate for both acetochlor (chloroacetamide, WSSA Group 15 VLCFA) and pyroxasulfone (pyrazole, WSSA Group 15 VLCFA). In these experiments, pyroxasulfone-induced rice injury ranged from 4 to 100%, depending on application timing and activation. Likewise, acetochlor-induced phytotoxicity ranged from 0 to 89%, with higher injury occurring at earlier application timings. Therefore, at the conclusion of this study, pyroxasulfone was not recommended for consideration of in-season applications due to the risk of undesirable injury. However, acetochlor showed promising results, with good rice tolerance at the 1- to 2-leaf and 3- to 4-leaf application timings, but not earlier.

Additional studies have demonstrated the influence of acetochlor formulation, rate, and application timing on rice phytotoxicity (Fogleman et al. 2019). Applications of an emulsifiable concentrate formulation of acetochlor elicited an average of 48% injury, while microencapsulation lowered injury on average to 22% when applied delayed-preemergence. Additional evaluations showed again that the safest application timing was at the 1- to 2-leaf stage of rice and that a microencapsulated formulation should be utilized for adequate crop tolerance. Furthermore, studies have considered the use of WSSA Group 15 herbicides as fall applications for weedy rice control (Bertucci et al. 2019). With fall applications of various WSSA Group 15 herbicides, including acetochlor and pyroxasulfone, rice injury was ≤ 11% and provided 59 and 53% weedy rice control, respectively. Despite good tolerance in this study, the variation in rice tolerance across other studies to WSSA Group 15 herbicides, particularly in-season use, called to question if a secondary enhancement for crop tolerance was needed.

Fenclorim is a herbicide safener developed by Ciba-Geigy in the 1980s for use with pretilachlor to improve rice tolerance to the herbicide (Quadranti and Ebner 1983). The safening ability of fenclorim has been well described to cause an upregulation of glutathione-*S*-transferase (GST) genes, which increases GST production (Scarponi et al. 2003; Chen et al. 2013; Shahzad et al. 2017; Usui et al. 2001; Wu et al. 1999). Additionally, pretilachlor detoxification has been well documented as GST mediated. Therefore, increased metabolism of pretilachlor may be facilitated with the treatment of fenclorim, which has demonstrated improved crop tolerance to pretilachlor. With acetochlor belonging to the same family as pretilachlor, fenclorim should and has caused enhanced crop tolerance to acetochlor in the form of a fenclorim seed treatment (Avent et al. 2020, 2021).

Rice tolerance and safening by fenclorim are called to question with pyroxasulfone as it has not yet been tested and belongs to the pyrazole family of WSSA Group 15 herbicides. Furthermore, whether a fenclorim seed treatment would enhance crop tolerance to fall applications of acetochlor or influence weedy rice control has yet to be tested. Therefore, two types of experiments were conducted to evaluate the effect of a fenclorim seed treatment on rice tolerance to acetochlor and pyroxasulfone and to determine whether fenclorim influences the efficacy of these herbicides on weedy rice.

Materials and Methods

Site Description. Four field trials were initiated at the Pine Tree Research Station in the Fall of 2019 and 2020. Two trials were conducted each year, and in each year, one trial was focused on tolerance and kept weed-free throughout the season. The other trial focused on weedy rice control and was kept free of all other weeds. For clarification, the two types of trials will be referred to as a tolerance trial and a weedy rice trial. The tolerance trial was conducted on a Calloway silt loam (11.3% sand, 69.5% silt, and 19.2% clay) and a Calhoun silt loam (11.7% sand, 69.8% silt, and 18.5% clay) with a soil pH of 7.8 and 1.7% organic matter in 2020 and 2021, respectively. Both weed control trials were located approximately 3.5 km from the tolerance trials and were conducted on a Loring silt loam with 8.3% sand, 78.7% silt, and 13% clay with a pH of 7.7 and 2.3% organic matter.

Experimental Setup. All trials were conducted as a three-factor factorial within a randomized complete block design with four replications. Factor A (herbicide choice) consisted of fall-applied herbicides of none, pyroxasulfone (Zidua® SC herbicide, BASF Corporation, Research Triangle Park, NC), or acetochlor (Warrant herbicide, Bayer Crop Science, St. Louis, MO). Factor B (rate) consisted of a low or high rate of each herbicide with the low and high rate of acetochlor being 2,100 and 4,200 g ai ha^{-1} and pyroxasulfone being 237 and 475 g ai ha^{-1}, respectively. Lastly, the fenclorim seed treatment was factor C being either 0 or 2.5 g ai kg^{-1} of seed. All plot dimensions were 1.5 m by 5.2 m with 1.5 m between plots within a block and a 0.9-m alley between blocks. For all studies, fields were prepped through cultivation and leveled before trial establishment, followed by applying the herbicide treatments (Table 1.) Fall applications were made using a CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} at 276 kPA with AIXR 110015 (TeeJet Technologies, Glendale Heights, IL) nozzles at a speed of 4.8 km hr^{-1}. ‘PVL01’ (Horizon Ag LLC, Memphis, TN) was planted to a 1-cm depth at 72 seeds m^{-1} of row (Table 1) using a 9-row drill with a 19-cm row spacing. All rice seed was base treated with clothianidin, carboxin, thiram, metalaxyl, fludioxonil, and gibberillins at 0.75, 0.38, 0.33, 0.16, 0.03, and 0.04 g ai kg^{-1} of seed, respectively.

Each study was managed culturally and for pest management based on University of Arkansas Cooperative Extension Services recommendations for direct-seeded, delayed-flooded rice production. All trials were amended preplant for fertility based on the Marianna Soil Test Lab recommendations with no preplant nitrogen. All trials were over-sprayed with glyphosate at 1,260 g ae ha^{-1} two to three weeks before planting, and clomazone was applied at 336 g ai ha^{-1} at planting. For the weed control studies, applications were made at the 2- to 3-leaf stage of rice and preflood to control other weeds with halosulfuron-methyl, thifensulfuron-methyl, and fenoxaprop-p-ethyl, each with NIS (0.25% v/v) at 62.5, 7.5, and 122 g ai ha^{-1}, respectively. The tolerance studies were treated similarly with halosulfuron-methyl and thifensulfuron-methyl at the 2- to 3-leaf stage of rice and preflood, but the 2- to 3-leaf application also included propanil and thiobencarb, both at 3360 g ai ha^{-1}, and a preflood application of quizalofop-P-ethyl (Provisia™ herbicide, BASF Corporation, Research Triangle Park, NC) at 119 g ai ha^{-1}. Urea (46-0-0) was applied at 316 kg ha^{-1} 1 to 12 hours before flooding rice.

Data Collection and Analysis. Rice injury was visually evaluated at 14 and 28 days after crop emergence (DAE). Visual injury estimates were relative to the nontreated control, with 0 being no injury and 100 being complete crop death (Frans and Talbert 1977). For both studies, five rice plant heights from each plot were measured, and rice stands were counted in two 0.5 m of row at 14 and 28 DAE. Rough rice yield estimates were collected at maturity in all trials using a Kubota, small-plot combine set to harvest the four center rows of each plot. Yield data were estimated with the harvested weight and moisture adjustments to 13%. Weedy rice control was visually evaluated at 14 and 28 DAE on a 0 to 100 scale for the two weed control studies, with 0 being no control and 100 being no weedy rice present (Frans and Talbert 1977). Additionally, weedy rice densities were collected from two established 1 m^2 quadrats in each plot 14 and 28 DAE.

All data distributions were checked using the distribution platform within JMP Pro 16.1 (SAS Institute Inc, Cary, NC). Data distribution selections were based on best fit using least log-likelihood and Akaike information criterion. Injury and weedy rice densities were gamma distributed, yield, height, and shoot data were normally distributed, and weedy rice control was beta distributed. Gamma distributions were analyzed using the generalized linear mixed model add-in for JMP Pro, and normally distributed data were analyzed using the fit model platform of JMP Pro. Beta distributions were analyzed using SAS 9.4 with the GLIMMIX procedure (Gbur et al. 2012). All data were subjected to analysis of variance and considered significant at $P \leq 0.05$. Means were separated using Fisher's protected LSD at an alpha equal to 0.05. Because the weed control and tolerance trials were only 3.5 km apart each year, injury, height, and shoot density data were pooled over study type and year, with study type, year, and block being treated as random effects for data analysis (Blouin et al. 2011). Yield data were analyzed by trial type, whether tolerance or weed control due to weedy rice confounding rough rice yield. Weedy rice densities and control were analyzed by year due to an earlier planting date in 2021.

Results and Discussion

Rice Tolerance. Similar to Bertucci et al. (2019), a low level of rice injury was observed from fall applications of acetochlor (< 10%); however, pyroxasulfone caused unacceptable rice injury (> 30%) (Tables 2 and 3). At 14 DAE, acetochlor averaged over rate and use of fenclorim injured rice only 5% while pyroxasulfone increased injury to 44%. Increased rice phytotoxicity from pyroxasulfone has been observed in other research when 90 and 150 g ai ha^{-1} were applied delayed-preemergence, causing 33 and 55% injury to rice two weeks after treatment, respectively

(Godwin et al. 2018). While Godwin et al. (2018) did not statistically compare acetochlor and pyroxasulfone, acetochlor applied delayed-preemergence only caused 3 and 1% injury two weeks after treatment at rates of 630 and 1050 g ai ha^{-1}, respectively, demonstrating a difference in sensitivity of rice to pyroxasulfone and acetochlor.

To further illustrate the risk associated with fall-applied pyroxasulfone in rice relative to acetochlor, the low and high rate of acetochlor caused 3 and 8% injury to rice 28 DAE, and the low and high rate of pyroxasulfone caused 39 and 47% injury, averaged over the fenclorim seed treatment (Tables 2 and 3). Additionally, the use of a fenclorim seed treatment decreased the extent of injury to rice from 28% in its absence to 21% injury with the seed treatment 28 DAE, averaged over herbicide choice and rate. These results indicate that a fenclorim seed treatment does provide slight safening to fall applications of acetochlor and pyroxasulfone. In previous studies, a fenclorim seed treatment on rice reduced injury by 20 and 17 percentage points when the crop was treated with acetochlor at 1,260 g ai ha^{-1} when applied delayed-preemergence (Avent et al. 2020, 2021).

There was no evaluation for which fenclorim negatively affected rice plant height or shoot numbers when treated with acetochlor or pyroxasulfone, regardless of rate (data not shown). Furthermore, the main effects of fenclorim for rice plant height and shoots were insignificant (Table 2). The reduction in injury from fenclorim for fall applications of acetochlor and pyroxasulfone is likely associated with improved groundcover or biomass and reduced chlorosis, albeit these were not directly evaluated in these experiments.

On a trial-by-trial basis, the safening effect from the fenclorim seed treatment was not highly apparent for fall applications. The effect of fenclorim on injury 28 DAE was only evident when comparing the treatments across all four trials. The fenclorim seed treatment may not be as effective in safening WSSA Group 15 herbicides in rice when applied the previous fall compared to what was seen previously with delayed preemergence applications. Differences in fall applications versus delayed preemergence applications are likely due to the herbicide being immediately absorbed by germinating rice prior to the fenclorim having a chance to cause an upregulation of metabolic enzymes responsible for deactivating the herbicide when applied in the fall. Averaged over herbicide choice and rate, the fenclorim seed treatment only safened rice by 7 percentage points in this study when averaged over all four trials.

Use of the fenclorim seed treatment, averaged over herbicide choice and rate, did result in greater rough rice grain yields in both the tolerance and weed control trials (Table 3). The improved yields may be partly a result of the weedy rice suppression in the weed control trials, but in the tolerance trials, other factors would have had to cause this increase. Recent greenhouse research has revealed that a fenclorim seed treatment can increase rice root growth by more than 60% at 30 DAE (Avent and Norsworthy, nonpublished data). The increased root growth could result in improved nutrient uptake and possibly grain yield. Yield improvement through enhanced crop tolerance has been observed and reported in other studies where the presence of fenclorim as a seed treatment and in solution with pretilachlor improved rice yield (Avent et al. 2021; Chen et al. 2013). Similar to injury data, findings from the rough rice yield data show that rice exhibited excellent tolerance to acetochlor while pyroxasulfone reduced yields from 5 to 30%, meaning that pyroxasulfone would not be a viable option for fall applications before planting rice at the rates tested.

Weedy Rice Control. Weedy rice control and densities from 2020 and 2021 were analyzed separately due to the reduced efficacy of acetochlor in 2021 (Table 2 and 4). In 2021, acetochlor at averaged across rates resulted in 5% control of weedy rice 14 DAE and 0% control 28 DAE.

In 2020, weedy rice control was 30% averaged over acetochlor rates 28 DAE. The low level of control in 2021 can be attributed to reduced preemergence herbicide efficacy with high plant densities (Braverman et al. 1985). As weed density increases, the effectiveness of a preemergence herbicide often decreases. In nontreated plots, weedy rice density averaged 103 plants m^{-2} in 2020 at 28 DAE compared to 350 plants m^{-2} in 2021. The lower density in 2020 is likely a result of later planting compared to 2021 (Table 1) and the use of glyphosate to control earlier emerged weedy rice plants at the time of planting.

While Bertucci et al. (2019) reported an average weedy rice control across herbicide rates of 43 and 34% weeks after planting with acetochlor and pyroxasulfone, respectively, this study showed an increase in weedy rice control for the two different herbicides (Table 2 and 4). In 2020 at 28 DAE, acetochlor achieved the lowest control at 21 and 38% for the low and high rates, respectively. In the same year and evaluation timing, pyroxasulfone provided better control than acetochlor with the low and high rate providing 61 and 73% control, respectively. The trends in weedy rice control are similar to the injury observed on rice, where both pyroxasulfone rates caused greater injury than both rates of acetochlor.

Weedy rice density had a similar response to the evaluated treatments as did weedy rice control in both years (Table 4). In all cases except 14 DAE in 2021, herbicide choice and rate were significant main effects, with acetochlor reducing weedy rice density less than pyroxasulfone and increasing herbicide rates reducing weedy rice density. In general, for 2020, acetochlor and pyroxasulfone maintained consistent control for both evaluations; however, in 2021, weedy rice control with acetochlor and pyroxasulfone trended downward, which is likely due to the increase in weedy rice densities. While herbicide choice and rate influenced weedy rice control and densities, fenclorim or any interaction containing the factor did not influence weedy rice control or densities ($p \geq 0.1430$). Application of fenclorim to seed and use of the material as a seed treatment is distinctly different from how fenclorim has been used commercially in the past. Fenclorim has mainly been used as an additive to foliar sprays of VLCFA-inhibiting herbicides to safen the herbicide in rice (Quadranti and Ebner 1983). With weedy rice being genetically synonymous with cultivated rice, the broadcast application of VLCFA-inhibiting herbicides and fenclorim should reduce the efficacy of the herbicide. However, with the precise placement of fenclorim as a seed treatment, the safening provided to cultivated rice was not reciprocated to weedy rice.

Practical Applications. These experiments show the efficacy of fall-applied acetochlor and pyroxasulfone for weedy rice suppression in cultivated rice. Pyroxasulfone at either application rate demonstrated ≥ 41% control of weedy rice at all evaluation timings (Table 3); however, at no point did rice exhibit good tolerance (<20%) to pyroxasulfone applications even with the fenclorim seed treatment. Acetochlor provided ≥ 21% control of weedy rice in 2020 at all evaluation timings, and with or without the fenclorim seed treatment, rice injury was ≤ 10%. Even at these low levels of injury, there was a yield benefit associated with using the fenclorim seed treatment; therefore, using the fenclorim seed treatment should be encouraged. With the weedy rice suppression provided under lower weedy rice densities and acceptable tolerance to fall applications of acetochlor, these practices could be used in conjunction with quizalofop-P- and imidazolinone-resistant rice plantings to potentially reduce escapes and further protect these traits from outcrossing to the weedy rice population.

Currently, neither of these herbicides are labeled for in-crop use in rice, and both have plant back intervals that prevent rice planting following a fall application (Anonymous 2018, 2021). If the applications of acetochlor evaluated in these studies become labeled, there should be no concern with applying up to 2,100 g ai ha^{-1} in the fall followed by planting of fenclorim-treated rice the subsequent spring. However, these studies were conducted only on a silt loam textured soil, which encompasses approximately 50% of Arkansas rice hectares (Hardke 2021). Both organic matter and clay content are negatively correlated to the bioactivity of acetochlor (Reinhardt and Nel 1990); therefore, similar studies should be conducted on other soil textures with varying levels of weedy rice infestations to verify the results of these studies. Nevertheless, data presented here show the potential for fall applications of acetochlor to provide weedy rice suppression without causing excessive injury to rice. The use of acetochlor would provide an alternative site of action for rice producers to control weedy rice and other problematic weeds in rice.

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Tables

Table 1. Selected dates of cultural management practices and annual rainfall for each trial

Year x Trial	Fall application	Planting	Number of days[a]	Rainfall (cm)[b]
2020 tolerance	Oct. 10	May. 03	206	102.8
2020 weedy rice control	Oct. 10	Apr. 27	200	100.3
2021 tolerance	Oct. 7	Apr. 13	189	66.6
2021 weedy rice control	Oct. 22	Apr. 13	174	59.0

[a] days from application until rice planting

[b] cumulative rainfall from application until rice planting

Table 2. Analysis of variance table listing P-values for visual rice injury, rice heights, rice shoots, rough rice yield, weedy rice control, and weedy rice densities as influenced by the effects and interactions of herbicide choice, herbicide rate, and the fenclorim seed treatment.

	Site and year random[a]						Year random[b]	
	Injury		Heights		Shoots		Yield	
	14 DAT[c]	28 DAT	14 DAT	28 DAT	14 DAT	28 DAT	Tol	WC
Effect								
Herbicide choice	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0374
Herbicide rate	0.0023	0.0009	0.0255	0.0027	0.0008	0.3665	0.0083	0.0060
Fenclorim	0.6603	0.0420	0.8783	0.9811	0.4691	0.5043	0.0046	0.0003
Herbicide choice × herbicide rate	0.0825	0.0056	0.0153	0.0580	0.0001	0.0009	0.0004	0.3809
Herbicide choice × fenclorim	0.7098	0.6572	0.5692	0.9786	0.1308	0.7275	0.5225	0.5032
Herbicide rate × fenclorim	0.9301	0.8694	0.9208	0.1285	0.8184	0.5234	0.2767	0.2837
Herbicide choice × Herbicide rate × Fenclorim	0.8041	0.7094	0.6553	0.9243	0.3239	0.5396	0.6868	0.4710

	Year analyzed separately							
	Weedy rice control[d]				Weedy rice densities[e]			
	14 DAT		28 DAT		14 DAT		28 DAT	
	2020	2021	2020	2021	2020	2021	2020	2021
Effect								
Herbicide choice	0.0002	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Herbicide rate	0.8620	0.0016	0.6411	0.1139	0.0064	0.1062	< 0.0001	0.0352
Fenclorim	0.3200	0.7398	0.3758	0.6565	0.9770	0.7481	0.7119	0.7481
Herbicide choice × herbicide rate	0.1260	0.4398	0.0303	0.1139	0.2238	0.1893	0.3488	0.4361
Herbicide choice × fenclorim	0.2177	0.4623	0.1430	0.6565	0.7532	0.3876	0.9847	0.3876
Herbicide rate × fenclorim	0.5900	0.6606	0.4673	0.7840	0.9084	0.1181	0.8355	0.1181
Herbicide choice × Herbicide rate × Fenclorim	0.2362	0.5293	0.3758	0.7840	0.7477	0.1700	0.8304	0.1700

[a] P-values were generated using the JMP Pro 16.1 generalized linear mixed model add-in with a gamma distribution for injury and the fit model platform for heights and shoots, with site-year and block as a random effect

[b] P-values were generated using the JMP Pro fit model platform with year and block as a random effect.

[c] Abbreviations: DAT, days after treatment; Tol, tolerance trials; WC, weed control trials

[d] P-values were generated using the GLIMMIX procedure within SAS version 9.4 with a beta distribution

[e] P-values were generated using the fit model platform of JMP Pro 16.1

Table 3. Influence of herbicide choice, herbicide rate, and fenclorim seed treatment on rice injury, height, shoots, and rough rice yield from a weed control and a tolerance trial conducted in 2020 and 2021 at the Pine Tree Research Station near Colt, AR.

	Study type and year combined						Year combined	
	Rice injury		Height[a]		Shoots[b]		Yield[c]	
	14 DAE[d]	28 DAE	14 DAE	28 DAE	14 DAE	28 DAE	Tolerance	Weed control
Herbicide choice	-------- % --------		-------------------------------- % of nontreated --------------------------------------					
Acet	5 B[e]	6	93	96 A	96	108	104	86 A
Pyrox	44 A	43	68	83 B	61	80	82	76 B
Herbicide rate								
Low[f]	17 B	19	84	93 A	85	96	98	88 A
High	32 A	30	77	86 B	73	92	87	75 B
Fenclorim								
Without	26	28 A	80	89	78	92	87 B	72 B
With	23	21 B	81	90	80	95	99 A	90 A
Herbicide choice × herbicide rate								
Acet × low	3	3 D	93 A	97	96 A	102 A	102 AB	94
Acet × high	8	8 C	94 A	95	97 A	113 A	106 A	78
Pyrox × low	31	39 B	75 B	89	73 B	89 B	95 B	81
Pyrox × high	56	47 A	60 C	78	49 C	69 C	70 C	72

[a] Nontreated height averaged 9.4 and 14.9 cm 14 and 28 days after emergence, respectively

[b] Nontreated shoot density averaged 44 and 48 shoots m^{-1} of row 14 and 28 days after emergence, respectively

[c] Nontreated yield averaged 5,410 and 4,370 kg ha^{-1} for the tolerance and weed control studies, respectively

[d] Abbreviations: DAE, days after emergence; Acet, acetochlor; Pyrox, pyroxasulfone; NS, not significant

[e] Means within a column for each effect level not containing the same letter are different according to Fisher's protected LSD (α=0.05). If the interaction of herbicide and rate was significant, letters were not placed on the main effects of herbicide and rate.

[f] low and high represent 2,100 and 4,200 g ai ha^{-1} of acetochlor and 237 and 475 g ai ha^{-1} of pyroxasulfone, respectively

Table 4. Influence of herbicide choice and herbicide rate on weedy rice control and density at 14 and 28 days after rice emergence.

	Weedy rice control				Weedy rice shoot density			
	14 DAE[a]		28 DAE		14 DAE[b]		28 DAE[c]	
	2020	2021	2020	2021	2020	2021	2020	2021
Herbicide	---------------------- % ----------------------				---------------- % of nontreated ----------------			
Acet	29 B[d]	5 B	30	0 B	80 A	95 A	56 A	86 A
Pyrox	66 A	87 A	68	55 A	35 B	18 B	27 B	14 B
Rate								
Low[f]	47	25 B	47	7	64 A	48	48 A	43 A
High	48	51 A	50	12	43 B	35	33 B	28 B
Herbicide × Rate								
Acet × low	25	3	21 B	0	90	98	68	98
Acet × high	35	7	38 B	0	72	92	51	75
Pyrox × low	59	77	61 A	41	46	23	34	19
Pyrox × high	72	93	73 A	69	26	13	22	11

[a] Abbreviations: DAE, days after emergence; Acet, acetochlor; Pyrox, pyroxasulfone; NS, not significant

[b] Weedy rice shoot density averaged in the nontreated 51 and 236 m^{-2} for 2020 and 2021, respectively

[c] Weedy rice shoot density averaged in the nontreated 103 and 350 m^{-2} for 2020 and 2021, respectively

[d] Means within a column for each effect level not containing the same letter are different according to Fisher's protected LSD (α=0.05). If the interaction of herbicide and rate was significant, letters were not placed on the main effects of herbicide and rate

[e] low and high represent 2,100 and 4,200 g ai ha^{-1} of acetochlor and 237 and 475 g ai ha^{-1} of pyroxasulfone, respectively

General Conclusions

The need for new sites of action for rice producers to control problematic weeds is a challenge across the mid-southern United States. The addition of acetochlor to current rice herbicide programs would provide an alternative site of action for rice producers to control problematic weeds such as barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.], Amazon sprangletop [*Diplachne panicoides* (J. Presl) McNeill], weedy rice (*Oryza sativa* L.), and potentially Palmer amaranth (*Amaranthus palmeri* S. Watson). With the addition of the herbicide safener fenclorim as a seed treatment, rice injury can be reduced and the variability of crop tolerance with delayed preemergence applications of acetochlor can be mitigated. Not only does fenclorim safen applications of acetochlor, some beneficial effects from the fenclorim seed treatment alone have been observed and will be studied further.

Based on the results from the fall-applied experiments, fenclorim does not provide a substantial safening response for pyroxasulfone. Additionally, adequate rice tolerance was not observed with either rate of pyroxasulfone, and fall-applied acetochlor provided inconsistent weedy rice control from one year to the next. Though in-season applications and fall applications were not directly compared, more consistent control of weedy rice should be expected with delayed preemergence applications of acetochlor in rice.

Weedy rice and barnyardgrass control from acetochlor were improved with earlier applications and increasing rates of acetochlor. However, in 2021, rice did not tolerate preemergence-applied acetochlor, and in both years, the crop did not tolerate any application timing of acetochlor at 1,890 g ai ha^{-1}. Furthermore, the inclusion of the fenclorim seed treatment provided improved rice tolerance without reducing herbicide efficacy, indicating that the safening effects provided to the crop were not reciprocated to the weeds. Based on this research and previous literature, the optimum application timing for weed control appears to be delayed preemergence, but the fenclorim seed treatment must be utilized to provide adequate rice tolerance.

The optimum fenclorim seed treatment rate appears to be 2.5 g kg^{-1} of seed. Below this rate, rice tolerance to acetochlor was inconsistent and the rate of 5.0 g kg^{-1} provided no additional benefit. Additionally, < 24% injury was observed across all cultivars with both acetochlor and fenclorim, indicating the feasibility of fenclorim to provide a safening response across a wide variety of cultivars. Lastly, ≤ 24% injury was observed with acetochlor and fenclorim under adverse rice growing conditions within a growth chamber, demonstrating the ability of this safener to perform in conditions typical of early rice planting dates across the U.S.

Currently, acetochlor is not labeled for rice production in the U.S. With a fenclorim seed treatment, rice producers could potentially apply a microencapsulated formulation of acetochlor to provide an alternative site of action for weed control, while maintaining adequate rice tolerance. However, more site years and further experimentation is required to confirm or refute these results. Additionally, future studies need to evaluate rice tolerance with the fenclorim seed treatment to acetochlor applied to heavy clay soils and alternative chloroacetamide herbicides.