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### Evaluation of Acetochlor and other Very-Long-Chain Fatty Acid-Inhibiting Herbicides in Arkansas Rice

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Crop, Soil, and Environmental Science

by

### Michael E Fogleman University of Arkansas Bachelor of Science in Agricultural, Food, and Life Sciences, 2015

### August 2018 University of Arkansas

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This thesis is approved for recommendation to the Graduate Council.

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#### **ABSTRACT**

The prevalence of herbicide resistance and lack of effective management options for controlling problematic weeds such as barnyardgrass and weedy rice in Arkansas rice production has led to exploration of alternative herbicide sites of action (SOA). Very-long-chain fatty acid (VLCFA) inhibiting herbicides are not currently labeled for use in U.S. rice production but have been used with success in other row crops and in rice production in Asia. Based on preliminary research, rice tolerance and weed control were evaluated following various application timings and rates of acetochlor and pethoxamid, in addition to several other VLCFA-inhibiting herbicides. Rice tolerance to acetochlor was maximized when applied in a microencapsulated (ME) formulation at the 1-leaf growth stage. Rice also demonstrated adequate tolerance to ME acetochlor applied delayed-preemergence (DPRE); however, when activating rainfall was received soon after application, unacceptable rice injury was observed and is therefore not recommended. When properly activated, barnyardgrass control and rough rice yield was comparable between acetochlor-based herbicide programs and clomazone-based programs in Clearfield and Provisia rice systems. However, it should be noted that early-season barnyardgrass control and rough rice yields were generally higher following clomazone-based herbicide programs due to minimal rice injury and excellent barnyardgrass control in all environments. Both ME acetochlor and pethoxamid provided early-season control of weedy rice and other grass species when applied soon after planting. Although DPRE applications were the most effective for weed control, they pose extreme risk for rice injury and should be avoided. In contrast, weed control was slightly reduced by delaying applications to 1-leaf rice but risk for rice injury was also decreased. Winter-applied VLCFA-inhibiting herbicides caused tolerable injury to rice planted the following spring. Microencapsulated acetochlor and pyroxasulfone provided considerable

suppression of weedy rice for as long as seven weeks after planting, suggesting an alternative method for controlling weedy rice. Should ME acetochlor and pethoxamid be registered for use in U.S. rice production, they have potential to provide growers with an alternative SOA to combat herbicide resistance and control problematic weed species.

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

**Key words:** very-long-chain fatty acid-inhibiting herbicides, rice tolerance, herbicide-resistant weeds

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## **TABLE OF CONTENTS**



## **LIST OF TABLES**

## **Chapter 2**





Table 11. Average weed sizes in the nontreated plot at the time of application………………………80

Table 12. Rough rice yield following acetochlor- and clomazone-containing Clearfield herbicide programs…………………………………………………………………………………………81

Table 13. Rough rice yield following acetochlor- and clomazone-containing Provisia herbicide programs…………………………………………………………………………………………82

### **Chapter 4**





## **LIST OF FIGURES**

## **Chapter 2**







#### **Chapter 1**

#### **General Introduction and Review of Literature**

Rice (*Oryza sativa* L.) is the most consumed grain in the world and serves as the primary staple for more than 50% of the global population. In 2015 alone, 158,780,000 hectares of rice were produced worldwide, with 1,040,000 hectares produced in the United States (U.S.) and 525,000 hectares produced in Arkansas, the largest producer in the nation.

More than 485 million metric tons of rice was consumed worldwide in 2015. China and India are the current leading rice producers, providing 145 million metric tons and 100 million metric tons, respectively. These two countries alone produce more than 50% of the worlds' rice. The U.S. currently ranks as the 12th largest producer, providing more than 6 million metric tons in 2015 (USDA FAS 2016).

There are four major subspecies of rice produced worldwide: indica, japonica, aromatic, and glutinous. These four subspecies are further divided into different regions across the globe, based on favorable growing environments. Accounting for more than 75% of the global market, indica is the most common subspecies and is grown in tropical and subtropical regions. Conversely, japonica rice requires somewhat cooler regions, like the U.S., and accounts for 10% of the global market. Aromatic rice makes up 12 to 13% of the global market, sells at premium price, and is mostly grown in Thailand, India, and Pakistan. The remaining 2 to 3% of the global market belongs to glutinous rice, grown in Southeast Asia, and used in specialized dishes (USDA-ERS 2012).

#### **Rice Production Practices in Arkansas**

In any given year, most of the state's rice is grown on silt-loam or clay soils in the eastern Mississippi River Alluvial Plain region, with a small percentage of hectares remaining in the Arkansas, Ouachita, and Red River Valleys (Hardke 2012). Planting usually begins in late March and concludes near the first week of June, depending on the region. Roughly 80% of Arkansas rice is drill seeded, while 15% is broadcast dry seeded and the remaining 5% is broadcast waterseeded (Hardke and Wilson 2013). Planting method is largely dependent upon weather conditions and timing but can also be affected by availability of equipment.

Because rice grows vigorously in flooded environments, a delayed-flood system is ideal as it provides suppression of weeds and management of diseases and nutrients (Smith and Fox 1973). Depending on variety and planting date, the middle of August typically marks the beginning of harvest, which continues through October and into early November (Hardke and Wilson 2013).

#### **Weed Control**

Studies have shown that grain yield reductions from weed competition have ranged from 82% with red rice (*Oryza sativa* var. *sylvatica* L.), or weedy rice (*Oryza* spp.), to 10% with eclipta (*Eclipta prostrata* L.) (Smith 1988). For this reason, weed control is arguably the most important and often one of the most difficult obstacles for Arkansas rice producers. In the field, weeds compete directly with rice for resources such as sunlight, nutrients, and water. When not properly controlled, weeds can cause reduction in grain quality and grade, reduced harvest efficiency, and increase soil seedbank populations (Scott et al. 2013).

However, there are several ways of controlling weeds, using cultural, mechanical, chemical, and biological methods. The objective of most cultural methods is to increase the competitive nature of the rice plant. A plant's competitive nature can be increased through crop rotation, land preparation, variety selection, planting date and method, plant population, and irrigation (Ampong-Nyarko and Datta 1991). Crop rotation is arguably the most widely adopted method of cultural weed control. Research has shown that oftentimes, weeds that are difficult to control in rice can easily be controlled in another crop; thus, the logic behind crop rotation. Rotating a broadleaf crop with rice allows growers to use herbicides that are effective on grassy weeds which may be difficult to control in rice (Ampong-Nyarko and Datta 1991).

Mechanical weed control methods involve physically removing weeds from the soil surface using implements such as a harrow disk, field cultivator, or moldboard plow. When using tillage for weed control, it is important to allow as many weeds as possible to germinate prior to tilling, therefore destroying as many weeds as possible. However, in recent years, many growers in the state have adopted no-till or minimal tillage production practices, which require them to rely heavily on other methods of weed control such as chemical herbicides.

In today's modern crop-production systems, chemical herbicides are the common method of weed control. In most systems, growers implement a non-selective herbicide prior to planting (Scott et al. 2013), ensuring that the crop has an opportunity to germinate in a weed-free soil, giving it an early advantage against competitive weeds. Following planting, preemergence (PRE) herbicides are applied, providing residual weed control. This application must be made prior to crop and weed emergence to be effective, unless mixed with a contact herbicide. Despite burndown and residual herbicide applications, weeds are likely to emerge later in the growing season, requiring herbicides to be applied postemergence (POST). Preemergence herbicide

applications are generally made using ground equipment, prior to flooding or a significant rainfall event. However, most POST herbicide applications are made using aerial equipment, due to the lack of traction in flooded fields and risk of damage to levees when using ground equipment (Hardke et al. 2013). On average, Arkansas rice growers spend approximately \$100 million each year on weed control (Scott et al. 2013).

#### **Herbicide Resistance**

Modern agricultural practices, especially use of herbicides, have created a significant selection pressure, which has led to rapid evolution and resistance among weed populations. Among 254 species (148 dicots and 106 monocots), there are currently 494 unique cases of herbicide-resistant weeds in the world. Furthermore, weeds have evolved resistance to 163 different herbicides and to 23 of the 26 known MOAs (Heap 2018). Through repeated applications of non-lethal doses of the same herbicide MOA, weed species can evolve and ultimately survive by increasing the frequency of resistance alleles (Jasieniuk et al. 1996). Through adaptation and mutation, weed populations become increasingly competitive and more difficult to control over time.

Rice producers in Arkansas rely heavily on herbicides for weed control; however, the repeated use of chemicals with the same MOA has led to the selection of resistance among problematic weed populations (Carey et al. 1995). Barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.], yellow nutsedge (*Cyperus esculentus* L.), rice flatsedge (*Cyperus iria* L.), goosegrass [*Eleusine indica* (L.) Gaertn.], and weedy rice are common to Arkansas rice fields. Unfortunately, these weeds have also evolved resistance to commonly used herbicides such as

Group 1 [acetyl coenzyme A carboxylase (ACCase) inhibitors], Group 2 [acetolactate synthase (ALS) inhibitors), and Group 4 (synthetic auxins) (Heap 2018).

It has been more than two decades since the last herbicide MOA was developed for use in crops. In past decades, a new MOA became commercially available approximately every three years; however, consolidation among pesticide-producing companies and high cost associated with herbicide discovery and development have contributed to the major decline in the development of new chemistry (Duke 2012). Thus, efforts must be made to take advantage of the herbicide chemistries that we currently have to combat herbicide resistance issues in rice.

#### **Current Rice Herbicides**

The imidazolinone-resistant (Clearfield, BASF Corporation, Research Triangle Park, NC) rice technology, developed by an induced mutation of the seeds, was released for commercial production in 2002 (Croughan 1994). Intended for use in combination with ALS-inhibiting herbicides such as Newpath (imazethapyr) and Beyond (imazamox), the Clearfield technology was primarily developed to control weedy rice but also proved to be effective on barnyardgrass (Hardke 2012). Successful control of weedy rice and barnyardgrass in the mid-2000s resulted in this technology being planted across vast hectares in Arkansas and the Mississippi delta. Since its peak in 2011, Arkansas rice hectares planted in imidazolinone-resistant varieties have declined steadily from 64 to 45% in 2016 (Hardke 2016). Heavy reliance on ALS-inhibiting herbicides and failure to rotate with conventional crops, which were both advised against in the Clearfield system stewardship guidelines, quickly led to the development of ALS-resistant weedy rice (Burgos et al. 2008) and barnyardgrass (Riar et al. 2012). To combat resistant weeds in Clearfield systems today, growers rely on traditional herbicides such as quinclorac,

thiobencarb, cyhalofop, bispyribac, penoxsulam, propanil, and pendimethalin, often applying them in combination with ALS-inhibiting herbicides (Scott et al. 2013).

In response to ALS resistance, BASF has developed a new herbicide-resistant rice system, the Provisia Rice System, which is expected to be registered for commercial use in 2018. The anticipated technology will allow POST applications of quizalofop, an ACCase-inhibiting herbicide. Today, quizalofop and other ACCase-inhibiting herbicides are generally used for grass control in crops such as soybean [*Glycine max* (L.) Merr.], cotton (*Gossypium hirsutum* L.), sunflower (*Helianthus annuus* L.) and canola (*Brassica napus* L.) (Abit 1994). Like many other rice herbicides, quizalofop will be primarily used to control barnyardgrass and weedy rice.

#### **Barnyardgrass**

Barnyardgrass is a problematic weed in rice fields across North America and is the most important weed in Arkansas rice production (Norsworthy et al. 2007; Riar et al. 2013). This highly competitive weed infests most Arkansas rice, causing yield reduction, lodging, and poor grain quality. The fibrous root system of barnyardgrass allows it to grow vigorously in drill- or water-seeded rice (Talbert and Burgos 2007), removing as much as 80% of the available nitrogen (N) from the soil (Holm et al. 1991).

Native to Eurasia, barnyardgrass is a pale green, summer annual grass with glabrous leaves and leaf sheath and no ligule. Mature plants may be 1.5 to 2.0 m tall, with long, narrow leaves, and green to purple panicle inflorescences. The competitiveness of barnyardgrass may be attributed to its rapid development, ability to flower in a wide range of photoperiods, and ability to produce many small seeds per plant (Holm et al. 1977).

Today, barnyardgrass has evolved resistance to several rice herbicides including clomazone (Command), propanil (Stam), quinclorac (Facet), and ALS-inhibiting herbicides (Heap 2018). Based on its competitive nature and repeated ability to evolve herbicide resistance, it is imperative that cultural, mechanical, and chemical methods be implemented to maximize weed control.

#### **Weedy Rice**

The first documented case of weedy rice as a problematic weed occurred in 1846 in the Carolinas (Craigmiles 1978). Despite having many different scientific names, weedy rice is generally classified as *Oryza* spp. and is genetically similar to cultivated rice (Parker and Dean 1976). Although the two are quite similar, several characteristics distinguish weedy rice from cultivated rice; light green leaf color, tall stature, profuse tillering, and easily shattering seeds to name a few (Craigmiles 1978; Kwon et al. 1992).

In terms of nutritional value, weedy rice and cultivated rice are similar (Deosthale and Pant 1970). However, early seed shattering and low grain weight limit the amount of weedy rice harvested. Although weed seed contamination in harvested grains is a concern in terms of milling quality, competition for resources between weedy rice and cultivated rice during the growing season has a much greater impact on yield. The pigmentation of the pericarp in weedy rice is visually unappealing to consumers in packaged white rice; therefore, extra processing is required to eliminate the discolored grains. For producers, extra processing results in additional expenses and a loss in profit due to reduced grain grade (Dunand 1988).

A major concern with weedy rice lies in its resistance to ALS-inhibiting herbicides. Weedy rice populations, which can survive imidazolinone herbicides in Clearfield rice, are at

risk for developing resistance via hybridization with cultivated rice. Although reported to occur at <1% (Shivrain et al. 2009), hybridization can occur between weedy rice and cultivated rice when plants that escaped herbicide applications are exposed to pollen of cultivated rice. The two populations outcross to develop weedy rice containing the imidazolinone-resistant gene. Like other problematic weeds, effective management of ALS-resistant weedy rice requires the implementation of cultural, mechanical, and chemical methods including traditional and ALSinhibiting herbicides.

#### **Group 15 Herbicides**

Weed Science Society of America (WSSA) Group 15 herbicides are known as very-longchain fatty acid (VLCFA)- inhibitors and consist of four chemical families. The first and largest family is the chloroacetamide family. Popular herbicides such as acetochlor, metolachlor, and pethoxamid belong to this family. Diphenamid and napropamide are both herbicides that belong to the acetamide family, the second largest family, while pyroxasulfone has a family of its own, the pyrazole family. Oxyacetamide is the third chemical family in Group 15 and contains several other, less-common herbicides.

In plants, VLCFAs are fatty acids composed of an acyl chain of 20 or more carbons in length and are essential for many aspects of plant growth and development such as cell proliferation and tissue patterning (Roudier 2010). Group 15 herbicides are effective in managing weed populations because they inhibit VLCFAs and prevent cell division in developing shoots and roots of germinating weeds. Although some products can control smallseeded broadleaves, these herbicides are most effective on annual grasses (Lingenfelter 2016).

At this time, there are no Group 15 herbicides registered for weed management in U.S. rice production. Since the 1970s, an increase in reliance on PRE herbicides in Asian rice production has led many growers to implement applications of Group 15 herbicides such as butachlor and pretilachlor (Naylor 1996). However, from 1981 to 1991, the use of butachlor declined dramatically from 83% of rice hectarage to 36%, respectively (Kwon et al 1993). Today, butachlor and pretilachlor continue to be important tank-mix partners for POST herbicides in rice production across Asia (Woodburn 1993). The efficacy and relative safety of Group 15 herbicides in rice production have been successfully demonstrated in other countries for years. The time has come for Group 15 herbicides, specifically acetochlor, to be evaluated in U.S. rice production.

#### **Acetochlor**

Acetochlor is a Group 15 herbicide belonging to the chloroacetamide family. Currently, acetochlor is labeled for use in cotton, soybean, and grain sorghum [*Sorghum bicolor* (L.) Moench] and can be used in corn with the incorporation of a safener in the U.S. Acetochlor is generally applied PRE for control of annual grasses and small-seeded broadleaves. In the soil, the herbicide is primarily absorbed through seedling shoots and secondarily through seedling roots. Like other Group 15 herbicides, acetochlor controls plants by inhibiting VLCFAs and preventing cell division in roots and shoots of germinating weeds.

#### **Pyroxasulfone**

Pyroxasulfone is a VLCFA-inhibiting herbicide from the pyrazole family and is labeled for use in cotton, soybean, corn, and wheat (*Triticum aestivum* L.). Pyroxasulfone is generally applied PRE or early POST for control of common annual grasses and small-seeded broadleaves. Pyroxasulfone is absorbed through seedling roots and shoots of emerging weeds, where it inhibits VLCFAs and prevents cell division.

#### *S***-metolachlor**

Belonging to the chloroacetamide family, *S*-metolachlor is another Group 15, VLCFAinhibitor. The herbicide is labeled for use in several crops including cotton, soybean, peanut (*Arachis hypogaea*), and tomato (*Solanum lycopersicum* L.). Applied PRE or POST, *S*metolachlor controls a number of problematic grass weeds including barnyardgrass, weedy rice, crabgrass (*Digitaria* spp.), and broadleaf signalgrass [*Urochloa platyphylla* (Nash) R.D. Webster] in addition to several species of small-seeded broadleaf weeds. Like acetochlor and pyroxasulfone, roots and shoots of germinating weeds absorb *S*-metolachlor in soil solution. Once inside the plant, *S*-metolachlor prevents cell division by the inhibition of VLCFAs.

#### **Herbicide Formulation**

A herbicide's formulation is determined by the combination of active and inert (inactive) ingredients. Herbicides today are produced in a variety of formulations ranging from ready-touse dry granules to very finely ground solid material suspended in solution, often referred to as flowable. For the purposes of this research, emulsifiable concentrate (EC) and microencapsulated (ME) formulations were the main formulations used.

EC formulations generally contain a mixture of active ingredient (AI), a petroleum solvent, and an emulsifier that allows mixing with water. When mixed with water, the solution becomes "milky-white" and is ready to be applied. Oftentimes, EC formulations are ideal because they are non-abrasive and require minimal agitation because the solution will not readily settle out or separate. However, this formulation also has an increased phytotoxic hazard, may be

corrosive to application equipment, and is easily absorbed through the skin of humans and animals.

ME formulations are composed of one or more AI (molecules, solid/liquid particles), commonly referred to as the core, which is surrounded by a protective matrix of organic/inorganic polymer. Essentially, an ME herbicide is physically enclosed inside the shell, which protects it from degradation and allows the herbicide to be released slowly via molecular diffusion (Monsanto Technology, 2010). Although ME herbicides require constant agitation and may leave behind residues in application equipment, they provide long-term control and are very safe to handle.

When choosing between different formulations of a herbicide, one must consider cost and availability, application target (foliar vs. soil), selectivity and toxicity, and type of crop (Markus, 1996). For instance, EC formulations would be better suited for foliar targets, while ME formulations would be preferred for applications to bare soil.

### **Warrant**

Warrant is an ME formulation of acetochlor, produced and sold by Monsanto Company. The herbicide contains 360 grams/liter or 3.0 pounds/gallon of 2-chloro-N-ethoxymethyl-N-(2 ethyl-6-methylphenyl) acetamide. Applications of Warrant may be made prior to or at planting of labeled crops. Warrant must be applied to a weed-free soil or in a tank mixture with POST herbicides to control emerged weeds. Precipitation or irrigation is necessary to move the herbicide throughout the soil profile and into the weed germination zone. Depending on soil type, organic matter, and existing soil moisture, 1.25 to 2 cm of water is sufficient (Rao 2000). Warrant provides excellent control of small-seeded grasses and broadleaf weeds such as

barnyardgrass, weedy rice, broadleaf signalgrass, Palmer amaranth [*Amaranthus palmeri* (S.) Wats.], henbit (*Lamium amplexicaule* L.), and waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] (Anonymous 2016).

#### **Harness**

Harness is an EC formulation of acetochlor also produced and sold by Monsanto Company. The herbicide contains 840 grams/liter or 7.0 pounds/gallon of 2-chloro-Nethoxymethyl-N-(2-ethyl-6-methylphenyl) acetamide. Like Warrant, Harness will not control emerged seedlings alone. Therefore, applications must be made prior to weed seedling emergence or in a tank-mixture with a POST herbicide to control emerged weeds. The spectrum of control of Harness is like that of Warrant, since only the formulation differs between these products.

#### **Previous Research**

Preliminary studies have evaluated rice tolerance to various rates and application timings of VLCFA-inhibitors (Bararpour et al. 2012, 2013). Acetochlor (Warrant herbicide, Monsanto Company, St. Louis, MO) at 420, 840, and 1,260 g ai ha<sup>-1</sup>, S-metolachlor (Dual II Magnum, Syngenta Crop Protection LLC, Greensboro, NC) at 840 and 1,400 g ai ha<sup>-1</sup>, and pyroxasulfone (Zidua, BASF Corporation, Research Triangle Park, NC) at 50, 75, and 90 g ai ha<sup>-1</sup> were applied to rice at the spiking, 2-lf, and 4-lf growth stages. Regardless of growth stage at application, rice injury was <5% following the highest rate of acetochlor 5 weeks after treatment, and yields from all acetochlor treatments were comparable to the nontreated rice, except for the highest rate of acetochlor applied at the spiking growth stage. Unacceptable rice injury was observed following

most application rates and timings for *S*-metolachlor and pyroxasulfone, although rice injury generally decreased as application timing was delayed.

In addition, Godwin et al. (2017) conducted a series of experiments in 2015 and 2016 to evaluate tolerance parameters of rice to several different Group 15 herbicides applied PRE, delayed PRE (DPRE), at spiking, and early postemergence (EPOST). Results from these experiments also concluded that rice tolerance to VLCFA-inhibiting herbicides increases as application timing is delayed. Rice was most tolerant to applications of acetochlor and pethoxamid (FMC Corporation, Philadelphia, PA) when applied at the 1-to 4-leaf growth stage; however, soil moisture at or soon after application influenced phytotoxicity. Pethoxamid applied alone at 420 or 560 g ai ha<sup>-1</sup> controlled barnyardgrass 92 to 96% and increased efficacy of clomazone and imazethapyr, relative to either herbicide applied alone.

Due to minimal rice injury following early-season applications of acetochlor and pethoxamid, these VLCFA-inhibiting herbicides demonstrate potential to be incorporated into U.S rice production. When applied at the appropriate rates and rice growth stages, acetochlor and pethoxamid could provide growers with an alternative herbicide SOA to combat herbicide resistance and control problematic weeds. Studies in the following chapters were conducted to further evaluate rice tolerance and efficacy of acetochlor and pethoxamid in Arkansas rice systems.

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#### **Chapter 2**

# **Influence of Formulation and Rate on Rice Tolerance to Early-Season Applications of Acetochlor**

Lack of effective options for controlling herbicide-resistant weeds such as barnyardgrass has led to the exploration of alternative herbicide sites of action (SOA) in rice. Acetochlor (WSSA Group 15) is a very-long-chain fatty acid (VLCFA)-inhibiting herbicide used to control grass weed species in row crops and could potentially be effective when used in a rice herbicide program. Group 15 herbicides are not currently labeled for use in U.S. rice and limited research has been conducted on rice tolerance to acetochlor. Field experiments were conducted in 2016 and 2017 to determine the effects of acetochlor formulation and rate on rice tolerance. The experimental design was a three-factor randomized complete block with factors being A) formulation [microencapsulated (ME) as Warrant<sup>®</sup>; emulsifiable concentrate (EC) as Harness<sup>®</sup>], B) rate (1050 and 2100 g ai ha<sup>-1</sup>), and C) application timing (preemergence – PRE, delayed preemergence – DPRE, and early postemergence – EPOST). Overall, rice was more tolerant to the ME formulation of acetochlor than to the EC, likely due to the gradual release of acetochlor in the ME formulation and the potential for immediate absorption of acetochlor from the EC following rainfall. Differences in rainfall among experimental sites and years caused variation in acetochlor activation and influenced crop injury. In all environments, PRE applications of either formulation resulted in the greatest injury 2 WAT (59%), while injury following DPRE or EPOST applications was 25 to 32%. When ME acetochlor was applied EPOST, rough rice yield was 97% of nontreated rice or 9,020 kg ha<sup>-1</sup>, indicating that applications should be delayed until this stage to minimize crop damage and maximize yield.

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

**Key words:** Herbicide resistance, very-long-chain fatty acid-inhibiting herbicides, rice tolerance

#### **INTRODUCTION**

With over 639,000 ha of rice planted in 2016, Arkansas ranks first among U.S. riceproducing states. As a result, rice production plays a major role in the state economy, adding over \$995 million in revenue in 2016 (NASS 2016a,b). Weed control is typically a main concern for producers, as poor control can have negative effects on rice yield and grain quality, leading to profit losses. Smith (1988) demonstrated potential yield losses as high as 70% from infestations of barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] and 82% from red rice (*Oryza sativa* var. *sylvatica* L.) or weedy rice (*Oryza* spp.), two of the most problematic weeds of rice in the midsouthern U.S. (Norsworthy et al. 2007).

Beginning with propanil in the 1990's, barnyardgrass has become resistant to a number of herbicides which were once effective in Arkansas rice production systems, including several acetolactate synthase (ALS)-inhibiting herbicides, clomazone, and quinclorac (Heap 2018). To avoid reliance on the few remaining effective options, alternative herbicides targeting different sites of action (SOA) should be explored. A lack of new herbicide discovery in recent years has led to the evaluation of herbicides currently labeled in other crops for use in rice.

Very-long-chain fatty acid (VLCFA)-inhibiting herbicides (WSSA Group 15) are not currently labeled for use in U.S. rice but have been used successfully in Asian rice production (Chauhan 2012; Rao et al. 2007). This herbicide SOA includes the chloroacetamide, oxyacetamide, and pyrazole chemical families, which contain several herbicides used in U.S. row crops, including *S*-metolachlor, pyroxasulfone, acetochlor, and flufenacet. The Group 15 herbicides inhibit cell division in developing shoots and roots of annual grasses and small-seeded broadleaves but do not affect emerged species (Babczinski et al. 2011). Therefore, they should

be applied after rice germination but before weed germination. The weed control spectrum of these herbicides indicates that they have potential to control or suppress problematic weeds in the early stages of the growing season if crop tolerance can be established.

Palmer amaranth [*Amaranthus palmeri* (S.) Wats.], barnyardgrass, large crabgrass [*Digitaria sanguinalis* (L.) Scop.], goosegrass [*Eleusine indica* (L.) Gaertn.], and other annual grasses have been effectively controlled by acetochlor in corn (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.) (Cahoon et al. 2015; Janak and Grichar 2016; Krausz 2000; Riar et al. 2011). Furthermore, only five weeds have become resistant to this herbicide SOA world-wide, indicating a relatively low risk of resistance and providing growers with an alternative option to control resistant weeds in rice (Heap 2018). There are several commercialized formulations of acetochlor currently on the market, two of the more popular products being Warrant and Harness. Although both acetochlor formulations are produced and sold by Monsanto Company, they differ in several properties.

Warrant contains acetochlor at 360 g ai  $L^{-1}$  and is labeled for use in U.S. soybean [*Glycine max* (L.) Merr.], cotton, corn, and grain sorghum [*Sorghum bicolor* (L.) Moench]. Harness contains acetochlor at  $840g$  ai  $L^{-1}$  and is labeled for use in field corn, silage corn, sweet corn (*Zea mays* L. var. *saccharate*), popcorn (*Zea mays everta*), and several non-food perennial bioenergy crops. The active ingredient in Warrant is microencapsulated (ME) inside a shell-like matrix of organic and inorganic polymers, which protects against degradation and results in a slow, gradual release of acetochlor (Rao 2000). In contrast to Warrant, the active ingredient in Harness is blended with organic solvents and surfactants to form an emulsion when diluted in water and is readily available for plant uptake upon activation (Rao 2000). For the ME formulation of Warrant, the polymer shell will imbibe water from the soil and in turn release

acetochlor into the soil profile via diffusion. For the EC Harness formulation, the herbicide will simply desorb from soil colloids and enter the soil solution where it can be readily taken up by germinating plants.

As a soil-applied, PRE herbicide, a delayed, continuous release of herbicide through soil can offer longer residual weed control compared to a non-encapsulated formulation (Rao 2000). However, the length of residual activity is dependent upon several factors including herbicide rate, environmental conditions, soil characteristics, and perhaps most importantly, soil moisture (Carter 2000; Dhareesank et al. 2006; Jursik et al. 2015; Kotoula-Syka et al. 1997). Soil moisture influences herbicide absorption, translocation, and metabolism in plants and thus affects herbicide efficacy and crop phytotoxicity (Chauhan and Johnson 2011).

Godwin (2017) conducted several experiments in 2015 and 2016 to evaluate rice tolerance following early-season applications of various VLCFA-inhibiting herbicides. Pyroxasulfone, *S*-metolachlor, pethoxamid, and ME acetochlor were applied DPRE, at spiking, 1- to 2-leaf, and 3- to 4-leaf rice. Pyroxasulfone and *S*-metolachlor caused unacceptable levels of rice injury irrespective of rate or application timing and generally had negative effects on shoot density, height, maturity, and yield. In contrast, rice exhibited adequate tolerance to pethoxamid and acetochlor when application timings were delayed to the 1- to 4-leaf stage, although soil moisture relative to application timing affected rice injury, as demonstrated by Chauhan and Johnson (2011). Because there is limited research available on rice tolerance to acetochlor formulations, and none particularly on the EC formulation, experiments were conducted to determine the influence of acetochlor formulation and rate on rice tolerance to PRE, DPRE, and 1- to 2-leaf (EPOST) rice timings. In consideration of preliminary research, it was hypothesized

that rice would exhibit higher tolerance to ME acetochlor, low rates and delayed application timings.

#### **MATERIALS AND METHODS**

**Experimental Sites**. Experiments were conducted in 2016 on an Immanuel silt loam (Fine-silty, mixed, active, thermic Oxyaquic Glossudalfs) at the University of Arkansas Pine Bluff (UAPB) Farm near Lonoke, AR, and in 2017 on a Dewitt silt loam (fine, smectic, thermic typic Albaqualf) at the Rice Research and Extension Center (RREC) near Stuttgart, AR, and in both years on a Calloway silt loam (Fine-silty, mixed, active, thermic Aquic Fraglossudalfs) at the Pine Tree Research Station (PTRS) near Colt, AR (Table 1).

**Experimental Setup and Data Collection.** The experimental design for all trials was a threefactor factorial, randomized complete block with four replications. The first factor was formulation (ME as Warrant and EC as Harness), the second factor was rate [1050 (1X) and  $2100 (2X)$  g ai ha<sup>-1</sup> based on labeled rate for corn], and the third was application timing (PRE, DPRE approximately 5 days after planting, and EPOST at 1- to 2-leaf rice). A nontreated check was also included in the experiment for comparison. The imidazolinone-resistant (Clearfield™, BASF Corporation, Research Triangle Park, NC) cultivar 'CL151' was drill-seeded at 72 seeds  $m<sup>-1</sup>$  of row, with 18 cm between rows, in 1.8 by 5.2 m plots. Plots were maintained weed-free throughout the growing season using PRE-applied clomazone (Command herbicide, FMC Corporation, Philadelphia, PA) at 336 g ai ha<sup>-1</sup> at all locations, with postemergence (POST) applications of propanil (SuperWham<sup>TM</sup> herbicide, RiceCo LLC, Memphis, TN) at 4480 g ai ha<sup>-1</sup> and cyhalofop (Clincher<sup>TM</sup> herbicide, Dow AgroSciences, Indianapolis, IN) at 313 g ai ha<sup>-1</sup> + 1%  $v/v$  crop oil concentrate (COC) at both the UAPB and RREC and quinclorac (Facet  $L^{TM}$ herbicide, BASF Corporation, Florham Park, NJ) at 280 g ai ha<sup>-1</sup> and sequential applications of

imazethapyr (Newpath<sup>™</sup> herbicide, BASF Corporation) at 70 g ai ha<sup>-1</sup> + 0.25% v/v nonionic surfactant (NIS) both years at PTRS. All experiments were fertilized and otherwise managed according to University of Arkansas Extension recommendations (Hardke et al. 2012). Acetochlor treatments were applied using a CO<sub>2</sub>-pressurized backpack sprayer calibrated to deliver  $140$  L ha<sup>-1</sup>. Dates of planting, herbicide application, and harvest at each of the experimental sites in 2016 and 2017 are shown in Table 2.

Estimates of crop injury were assessed 2 weeks after treatment (WAT) and 4 weeks after flooding (WAF) on a scale of 0 to 100, with 0 being no injury and 100 being crop death. Shoot density per meter of row and rice height measurements of three random plants per plot were taken 3 weeks after EPOST applications at the PTRS and RREC in 2017 and converted to a percentage relative to the average of the nontreated at each location. Estimates of days delayed to 50% rice heading were collected weekly when 50% heading was observed in the nontreated. Rough rice yield was determined at physiological maturity using a small-plot combine and adjusted to 12% moisture.

**Statistical Analysis.** Data for some parameters were not found to be normally distributed, via a significant Shapiro-Wilk Test. Therefore, all data were subjected to analysis of variance as a three-factor factorial using the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc, Cary, NC) assuming beta distribution for rice injury, gamma distribution for relative shoot density and plant height, negative binomial distribution for delayed maturity, and lognormal distribution for relative yield (Gbur et al. 2012). To assess the behavior of acetochlor over a variety of environments, data were analyzed jointly, with replication nested within location and considered random effects in the model. Box and whisker plots were used to visually capture how the interaction of acetochlor formulation, application timing, and rate affected crop response across

the four environments. For crop responses in which the three-factor interaction was significant, mean separation based on Fisher's protected LSD  $(\alpha=0.05)$  was used to identify differences among means within the box and whisker plots.

#### **RESULTS AND DISCUSSION**

**Rainfall.** Amount and timing of rainfall in relation to acetochlor application varied among experimental locations and years (Figures 1-4). Performance of soil-applied herbicides is influenced by several factors, including soil characteristics and soil moisture (Curran 2001; Hartzler 2002). Because all experiments were conducted on silt loam soils with comparable organic matter and clay content, soil moisture is more likely to explain the differences in rice injury in 2016 and 2017 (Table 1). In 2016, rice was planted into relatively dry soil and received 1.4 and 0.9 cm of rainfall within 7 days after planting at the PTRS and UAPB sites, respectively (Figures 1, 2). In contrast, rice was planted into adequate moisture in 2017 and then received an additional 2.5 and 4.0 cm of rainfall within 7 days after planting at PTRS and RREC, respectively (Figures 3, 4).

Dry conditions prior to planting, in addition to marginal rainfall over the next 7 days, limited the availability of acetochlor in soil solution and resulted in reduced rice injury in 2016 (data not shown). In contrast, rainfall events occurred immediately prior to or following PRE and DPRE applications in 2017, which allowed acetochlor to rapidly desorb from soil colloids and enter soil solution, where it was absorbed by germinating rice. When VLCFA-inhibiting herbicides such as acetochlor are taken up by developing shoots and roots, they inhibit lipid biosynthesis in susceptible species and result in dead or permanently stunted plants, particularly when exposed at early growth stages (Shaner et al. 2014).

**Rice Response to Acetochlor Formulation, Timing, and Rate.** The response of all variables was influenced by at least one significant two-way interaction or main effect for all three factors (Table 3). Similar to findings by Godwin (2017), rice injury following early-season applications of ME acetochlor generally decreased as application timing was delayed. When averaged over main effects, rice was most tolerant to ME acetochlor  $(22%)$  at 1050 g ai ha<sup>-1</sup>  $(26%)$  when applied EPOST (16%), while the most severe injury to rice resulted from EC acetochlor (48%) applied PRE (61%) at  $2,100 \text{ g}$  ha<sup>-1</sup> (43%) at  $2 \text{ WAT}$  (Table 4).

In general, EC formulated acetochlor was more injurious to rice than ME acetochlor 2 WAT (Table 4; Figure 5). The increased injury is likely due to the rapid release of acetochlor from the EC formulation compared to a slower release from the ME formulation. In each case, rainfall or irrigation move herbicide below the soil surface and into soil solution where herbicide molecules can be adsorbed to soil colloids or absorbed by germinating seeds. However, in EC formulations, most of the applied herbicide is immediately available for uptake upon activation by moisture, whereas the polymer shell of a ME formulation offers protection from degradation processes and allows a slow release of acetochlor over time; therefore, for the EC formulation, more herbicide is available for absorption closer to application time, and at earlier growth stages, when rice is more vulnerable. Hence, rice was most tolerant to EPOST application timings, and tolerance generally increased as application timing was delayed.

Perhaps more importantly than the observed rice injury between the two rates, increased risk for rice injury was associated with the  $2100 \text{ g}$  ai ha<sup>-1</sup> rate of acetochlor, as indicated by larger box and whisker plots (Figure 5). The upper quartiles and extremes of these boxes represent the most damage observed by a given treatment, while the lower represent the contrary. Furthermore, the horizontal line inside the box represents the median for each treatment. At 2

WAT, there was considerable risk for  $>20\%$  injury from all treatment combinations, with the exception being EPOST applications of ME or EC acetochlor at 1050 g ai ha<sup>-1</sup> (Figure 5).

Trends remained consistent 4 WAF when the greatest injury resulted from applications of EC acetochlor, and injury generally decreased as application timing was delayed and at the lower acetochlor rate (Table 4; Figure 6). Regardless of formulation or application timing, the higher rate of acetochlor caused increased risk for injury relative to the lower rate. Generally, PRE and DPRE applications produced unacceptable rice injury  $(>25\%)$ , with the exception being when ME acetochlor was applied DPRE, where risk and observed injury were comparable to that of EPOST applications (Figure 6). At 4 WAF, rice was most tolerant to EPOST applications of either formulation (11 to 18% injury) or DPRE applications of ME acetochlor (21% injury).

Measurements of shoot density, relative to the nontreated, were influenced by an interaction of all three factors and corroborated the trends observed in estimates of rice injury (Table 3; Figure 7). Regardless of rate, relative shoot densities were highest when either formulation was applied EPOST or when ME acetochlor was applied DPRE. Relative shoot densities were unacceptable (<50%) for all other treatment combinations, suggesting that they should be avoided to prevent significant risk for crop loss. Rate seemed to have a greater effect at earlier application timings, as shoot densities were reduced by the higher rate of acetochlor when applied PRE or DPRE but not EPOST. The gradual release of ME acetochlor may allow it to be applied earlier (DPRE), whereas EC acetochlor should only be applied EPOST to minimize reduction in shoot density. In addition, the higher rate of ME acetochlor may be used to improve weed control without reducing rice shoot density, relative to the lower rate; however, increased risk for injury and reduction in shoot density may be associated with the higher rate (Figure 7).
Measurements of plant height, relative to the nontreated plots, followed similar trends as estimates of rice injury and relative shoot density (Figure 8). A main effect for all three factors influenced relative height where EC acetochlor, DPRE application timings, and the 2100 g ai ha<sup>-1</sup> rate caused the greatest reduction (Tables 3, 4). Contrary to injury estimates, there were minimal differences in relative height among most treatments, indicating that injury in these plots was mainly manifested as reduced shoot density (Figure 7).

A 1- to 10-day delay in days to 50% heading relative to the nontreated was often observed in association with damage to rice earlier in the year (Figure 9). Rice treated with ME acetochlor was delayed fewer days than rice treated with EC acetochlor. In addition, treatments that caused severe injury earlier in the season were delayed longer than treatments that did not experience early-season injury. For example, PRE application timings were responsible for severe rice injury 2 WAT and 4 WAF, and were also responsible for the greatest number of days delay in reaching 50% heading (Figures 5, 6, 9). Delays in rice heading are generally unfavorable for growers due to the cost of extending irrigation schedules and risk for crop damage from inclement weather later in the summer.

Rough rice yields generally increased, relative to the nontreated, as application timing was delayed and did not differ among EPOST applications, regardless of formulation or rate (Figure 10). The slow release of acetochlor in the ME formulation, in addition to delayed application timing, allowed rice to establish prior to being exposed to herbicide, which resulted in decreased early-season injury and ultimately increased yield. However, ME acetochlor applied DPRE yielded 58 to 93% of the nontreated, which was comparable to EPOST applications of either formulation which yielded 67 to 100% of the nontreated. The similarity in yields between ME acetochlor applied DPRE and either formulation applied EPOST suggests that the increased

rice tolerance to ME acetochlor would allow applications to be made slightly earlier than EPOST, which could bring value to weed control efforts. In addition to causing the highest visual rice injury 2 and 4 WAT, PRE applications caused the highest reduction in rice yield and should be avoided.

**Conclusions.** In the given environments, rice was most tolerant to applications of ME acetochlor at the EPOST timing. Regardless of application timing or rate, EC acetochlor caused greater crop injury and reduced shoot density and plant height, which ultimately delayed maturity and decreased yield. Increased rice tolerance to the ME formulation may be attributed to the gradual release of herbicide following an activating rainfall. Unlike the EC formulation, the polymer shell that protects acetochlor molecules in the ME formulation must imbibe water and break down for herbicide to be released. The elapsed time between application of ME acetochlor and herbicide absorption allows rice to develop uninhibited and even emerge before being exposed to lethal doses of herbicide, which reduces its susceptibility. Based on these experiments, acetochlor should be applied in an ME formulation at  $1050$  g ai ha<sup>-1</sup> to 1- to 2-leaf rice to minimize rice injury and maximize yield.

**Practical Implications.** Emulsifiable concentrate acetochlor resulted in unacceptable rice injury and is not a viable option for weed control in rice, even when applied EPOST. Conversely, ME acetochlor applied EPOST, even at the higher rate  $(2100 \text{ g ai ha}^{-1})$ , caused tolerable rice injury and indicates an opportunity for further evaluation as a component of rice weed control programs. These results coincide with those of Godwin (2017), and thus, rice tolerance to acetochlor increases as application timing is delayed. However, data presented here indicate that even when ME acetochlor is applied, increased risk for rice injury is associated with PRE and DPRE applications. Soil moisture at planting and near time of application can play a significant

role in the level of crop injury observed. For example, a PRE application of ME acetochlor that is activated >7 days after application would likely cause minimal rice injury. However, the same application followed by significant rainfall could be devastating to rice emergence and growth and therefore could never be recommended PRE or DPRE. Should ME acetochlor be granted a label for use in U.S. rice, it should be applied to 1- to 2-leaf rice at  $1050$  g ai ha<sup>-1</sup> to ensure adequate crop tolerance.

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# **APPENDIX**

|             | Soil properties |     |      |      |      |  |
|-------------|-----------------|-----|------|------|------|--|
| Location    | pH              | OM  | Sand | Silt | Clay |  |
|             |                 |     |      |      |      |  |
| <b>UAPB</b> | 5.6             | 1.3 | 14.2 | 78.1 | 14.0 |  |
| <b>RREC</b> | 6.0             | 1.8 | 8.4  | 71.4 | 20.2 |  |
| <b>PTRS</b> | 7.5             | 1.3 | 10.6 | 68.6 | 20.8 |  |

Table 1. Chemical and physical soil properties at experimental sites in 2016 and 2017<sup>a</sup>

<sup>a</sup> Abbreviations: UAPB, University of Arkansas Pine Bluff Farm near Lonoke, AR; RREC, Rice Research and Extension Center near Stuttgart, AR; PTRS, Pine Tree Research Station near Colt, AR

|                  | Dates of significance |            |             |              |         |  |  |  |
|------------------|-----------------------|------------|-------------|--------------|---------|--|--|--|
| Location         | Planting              | <b>PRE</b> | <b>DPRE</b> | <b>EPOST</b> | Harvest |  |  |  |
| <b>UAPB 2016</b> | May $18$              | May $19$   | May $23$    | May $31$     | Sept 20 |  |  |  |
| <b>PTRS 2016</b> | May 9                 | May $11$   | May $13$    | May $24$     | Sept 9  |  |  |  |
| <b>PTRS 2017</b> | May $16$              | May 17     | May 22      | May 30       | Sept 22 |  |  |  |
| <b>RREC 2017</b> | May 18                | May 19     | May 24      | June 2       | Sept 29 |  |  |  |

Table 2. Planting, herbicide application, and harvest dates for trials in 2016 and 2017.<sup>a</sup>

<sup>a</sup> Abbreviations: PRE, preemergence; DPRE, delayed preemergence; EPOST, early postemergence; UAPB, University of Arkansas Pine Bluff Farm near Lonoke, AR; PTRS, Pine Tree Research Station near Colt, AR; RREC, Rice Research and Extension Center near Stuttgart, AR



Table 3. Significance of P-values for factor main effects and interactions for rice injury, relative shoot density, relative plant height, days to 50% heading and rough rice yield averaged over site-years.<sup>a,b,c</sup>

<sup>a</sup> Abbreviations: Form, formulation; WAT, weeks after treatment; WAF, weeks after flooding

 $<sup>b</sup>$  Asterisks (\*) indicate significant treatment effects.</sup>

<sup>c</sup> Injury, 50% heading date, and yield are averaged over four locations. Shoot density and height are averaged over two locations.

|                      |                 |              | Injury          |              |                    |  |
|----------------------|-----------------|--------------|-----------------|--------------|--------------------|--|
| Factor               | 2 WAT           |              | 4 WAF           |              | Height             |  |
|                      | $\frac{0}{0}$ . |              | % of nontreated |              |                    |  |
| Form                 |                 |              |                 |              |                    |  |
| <b>ME</b>            | 22              | $\mathbf b$  | 21              |              | 83<br>a            |  |
| EC                   | 48              | a            | 48              |              | 76<br>$\mathbf b$  |  |
| Timing               |                 |              |                 |              |                    |  |
| <b>PRE</b>           | 61              | $\rm{a}$     | 52              |              | 82<br>a            |  |
| <b>DPRE</b>          | 30              | $\mathbf b$  | 41              |              | 74<br>$\mathbf b$  |  |
| <b>EPOST</b>         | 16              | $\mathbf{C}$ | 14              |              | 83<br>$\mathbf{a}$ |  |
| Rate                 |                 |              |                 |              |                    |  |
| 1X                   | 26              | $\mathbf b$  | 22              | $\mathbf b$  | 83<br>a            |  |
| 2X                   | 43              | $\rm{a}$     | 47              | $\rm{a}$     | 76<br>$\mathbf b$  |  |
| Form $\times$ Timing |                 |              |                 |              |                    |  |
| $ME \times PRE$      | 44              |              | 37              | $\mathbf b$  | 82                 |  |
| $ME \times DPRE$     | 18              |              | 21              | $\mathbf c$  | 82                 |  |
| $ME \times EPOST$    | 11              |              | 11              | $\mathbf{C}$ | 87                 |  |
| $EC \times PRE$      | 76              |              | 67              | a            | 81                 |  |
| $EC \times DPRE$     | 46              |              | 65              | a            | 68                 |  |
| $EC \times EPOST$    | 23              |              | 18              | $\mathbf{C}$ | 80                 |  |
| Form $\times$ Rate   |                 |              |                 |              |                    |  |
| $ME \times 1X$       | 16              |              | 15              |              | 88                 |  |
| $ME \times 2X$       | 28              |              | 28              |              | 79                 |  |
| $EC \times 1X$       | 38              |              | 31              |              | 79                 |  |
| $EC \times 2X$       | 59              |              | 66              |              | 74                 |  |
| Timing $\times$ Rate |                 |              |                 |              |                    |  |
| $PRE \times 1X$      | 52              |              | 36              |              | 83                 |  |
| $PRE \times 2X$      | 70              |              | 68              |              | 81                 |  |
| $DPRE \times 1X$     | 23              |              | 29              |              | 80                 |  |
| $DPRE \times 2X$     | 39              |              | 54              |              | 69                 |  |
| $EPOST \times 1X$    | 11              |              | 9               |              | 86                 |  |
| $EPOST \times 2X$    | 23              |              | 21              |              | 80                 |  |

Table 4. Influence of acetochlor formulation, application timing, and rate on rice injury and plant height.<sup>a,b,c,d</sup>

<sup>a</sup> Abbreviations: Form, formulation; ME, microencapsulated; EC, emulsifiable concentrate; PRE, preemergence; DPRE, delayed preemergence; EPOST, early postemergence; WAT, weeks after treatment; WAF, weeks after flooding

<sup>b</sup> Means within a column followed by the same lowercase letter are not different according to Fisher's protected LSD ( $\alpha$  = 0.05). Means for non-significant interactions for INJ 2 WAT and relative height are presented for informational purposes.

<sup>c</sup> Rice injury and relative height were averaged over four and two locations, respectively.

<sup>d</sup> Average height of nontreated plots was 19 cm.



Figure 1. Rainfall amount and dates at the Pine Tree Research Station (PTRS) near Colt, AR. Abbreviations: preemergence, PRE; delayed preemergence, DPRE; early postemergence, EPOST



Figure 2. Rainfall amount and dates at the University of Arkansas Pine Bluff (UAPB) Farm near Lonoke, AR. Abbreviations: preemergence, PRE; delayed preemergence, DPRE; early postemergence, EPOST



Figure 3. Rainfall amount and dates at the Pine Tree Research Station (PTRS) near Colt, AR. Abbreviations: preemergence, PRE; delayed preemergence, DPRE; early postemergence, EPOST



Figure 4. Rainfall amount and date at the Rice Research and Extension Center (RREC) near Stuttgart, AR. Abbreviations: preemergence, PRE; delayed preemergence, DPRE; early postemergence, EPOST



Figure 5. Percent rice injury 2 weeks after treatment averaged over site-years at the PTRS, RREC, and UAPB. Representation of the data using box and whisker plots provides an estimate of the variability in response of rice to the various treatments for the environments (sites and years) evaluated. Mean separation is not shown since the three-way interaction was non-significant. Abbreviations: PRE, preemergence; DPRE, delayed preemergence; EPOST, early postemergence; 1X, 1050 g ai ha<sup>-1</sup>; 2X, 2100 g ai  $ha^{-1}$ 



Figure 6. Percent rice injury 4 weeks after flooding averaged over site-years at the PTRS, RREC, and UAPB. Representation of the data using box and whisker plots provides an estimate of the variability in response of rice to the various treatments for the environments (sites and years) evaluated. Mean separation is not shown because the three-way interaction was non-significant. Abbreviations: PRE, preemergence; DPRE, delayed preemergence; EPOST, early postemergence; 1X, 1050 g ai ha<sup>-1</sup>; 2X, 2100 g ai  $ha^{-1}$ 



Figure 7. Percent shoot density relative to the nontreated averaged over site-years at the PTRS, RREC, and UAPB. The three-way interaction of formulation, application timing, and acetochlor rate was significant; hence, means of bars with the same letter are not significantly different according to Fisher's protected LSD ( $\alpha$ =0.05). Average shoot density for the nontreated was 46 shoots m<sup>-1</sup>. Abbreviations: PRE, preemergence; DPRE, delayed preemergence; EPOST, early postemergence; 1X, 1050 g ai ha<sup>-1</sup>; 2X, 2100 g ai  $ha^{-1}$ 



Figure 8. Percent plant height relative to the nontreated averaged over site-years at the PTRS, RREC, and UAPB. Representation of the data using box and whisker plots provides an estimate of the variability in response of rice to the various treatments for the environments (sites and years) evaluated. Mean separation is not shown because the three-way interaction was non-significant. Average plant height for the nontreated was 19 cm. Abbreviations: PRE, preemergence; DPRE, delayed preemergence; EPOST, early postemergence; 1X, 1050 g ai ha<sup>-1</sup>; 2X, 2100 g ai ha<sup>-1</sup>



Figure 9. Days delayed to 50% heading relative to the nontreated averaged over site-years at the PTRS, RREC, and UAPB. The threeway interaction of formulation, application timing, and acetochlor rate was significant; hence, means of bars with the same letter are not significantly different according to Fisher's protected LSD ( $α=0.05$ ). Abbreviations: PRE, preemergence; DPRE, delayed preemergence; EPOST, early postemergence; 1X, 1050 g ai ha<sup>-1</sup>; 2X, 2100 g ai ha<sup>-1</sup>



Figure 10. Percent yield relative to the nontreated averaged over site-years at the PTRS, RREC, and UAPB. The three-way interaction of formulation, application timing, and acetochlor rate was significant; hence, means of bars with the same letter are not significantly different according to Fisher's protected LSD ( $\alpha$ =0.05). Average yield of the nontreated was 9400 kg ha<sup>-1</sup>. Abbreviations: PRE, preemergence; DPRE, delayed preemergence; EPOST, early postemergence; 1X, 1050 g ai ha<sup>-1</sup>; 2X, 2100 g ai ha<sup>-1</sup>

## **Chapter 3**

## **Evaluation of Acetochlor in Mid-South Rice Herbicide Programs**

Today, few effective management strategies remain for controlling herbicide-resistant barnyardgrass in Midsouth rice production. Repeated use of the same herbicide site of action (SOA) is ineffective and may be overcome by targeting alternative SOA's. At relatively low risk for evolution of resistance, very-long-chain fatty acid (VLCFA)-inhibiting herbicides (WSSA Group 15) such as acetochlor are promising candidates for weed control in rice. Field experiments were conducted in 2016 and 2017 to evaluate the impact of acetochlor formulated as Warrant<sup>®</sup> on barnyardgrass control as part of a complete herbicide program in both imidazolinone- (Clearfield) and quizalofop- (Provisia) resistant rice systems. These studies were designed as a randomized complete block with a nontreated, weedy check included for comparison. In the Clearfield experiment, acetochlor at  $1,050$  or  $1470$  g ai ha<sup>-1</sup> or clomazone at 336 g ai ha<sup>-1</sup> was applied delayed preemergence (DPRE) 1) alone, 2) followed by imazethapyr at 70 g ai ha<sup>-1</sup> early-postemergence (EPOST), or 3) followed by imazethapyr EPOST followed by imazethapyr pre-flood (PREFLD). Herbicide treatments were identical in the Provisia experiment, with the exception being that quizalofop at 120 g ai ha<sup>-1</sup> was applied postemergence (POST) instead of imazethapyr. Rice injury was <15% following all DPRE applications in two of the three site-years; however, rainfall events shortly after application resulted in more severe rice injury 2 WAT in 2017, particularly following acetochlor applications (49 to 78%). Overall, clomazone-containing programs provided superior barnyardgrass control throughout the season and yielded higher than acetochlor-containing programs. Oftentimes, clomazone- and acetochlorcontaining programs were comparable within single- and multi-pass programs; however, the success of acetochlor-containing programs was more affected by rainfall and dependent on

POST herbicides. Should acetochlor be labeled for use in rice, it should be applied EPOST followed by sequential POST applications to provide weed control and yield comparable to standard programs used today.

**Nomenclature:** Acetochlor; clomazone; rice, *Oryza sativa* L.; barnyardgrass, *Echinochloa crusgalli* (L). Beauv.

**Key Words:** Herbicide resistance, very-long-chain fatty acid-inhibiting herbicides, acetochlor, clomazone, barnyardgrass, imazethapyr, quizalofop

## **INTRODUCTION**

Arkansas is the largest producer of rice in the United States, harvesting 615,800 hectares in 2016, or approximately 50% of the total U.S. rice production (USDA-ERS 2017). Most of the rice production is concentrated in the eastern region of the state on silt-loam soils (Hardke 2016). Typical production systems include direct-seeding, delayed-flooding, and heavy reliance on herbicides. Weed management efforts in this region are generally tailored toward controlling barnyardgrass, the most problematic weed in Arkansas rice (Norsworthy et al. 2013).

Barnyardgrass and other related *Echinochloa* species are extremely competitive with cultivated rice. Aggressive vegetative growth, extensive root system, and prolific seed production allow barnyardgrass to thrive in drill- or water-seeded rice systems (Holm et al. 1977; Talbert and Burgos 2007). If not properly managed, barnyardgrass infestations can cause yield losses of up to 70% (Smith 1988). Today, clomazone (WSSA Group 13) is the most frequently used preemergence (PRE) herbicide in rice for control of annual grasses and small-seeded broadleaves (Norsworthy et al. 2013). Overreliance on one herbicide site of action (SOA) increases risk for evolution of resistance and has led barnyardgrass to evolve resistance to several of the most common rice herbicides including quinclorac, propanil, clomazone, and acetolactate synthase (ALS)-inhibitors (Heap 2018).

Because there have been no commercialized herbicides with new SOAs in recent years, growers are limited in effective control options and often resort to traditional rice herbicides such as thiobencarb, cyhalofop, and pendimethalin (Scott et al. 2013). One method of combatting herbicide resistance is to use alternative SOAs or mix multiple SOAs, thereby reducing the risk for resistance while controlling species that may be resistant to one herbicide or the other

(Norsworthy et al. 2012). A lack of new herbicide chemistry has led to research evaluating existing herbicides with alternative SOAs in rice.

Very-long-chain fatty acid (VLCFA)-inhibiting herbicides are popular in U.S. row crop production but are not labeled for use in rice. VLCFA-inhibiting herbicides control annual grasses and small-seeded broadleaves by inhibiting root and shoot development during germination (Babczinski et al. 2012). Only five weed species worldwide are resistant to VLCFAinhibiting herbicides, indicating a lower risk for resistance relative to other rice herbicides (Heap 2018). Acetochlor, a VLCFA-inhibiting herbicide, is marketed as a microencapsulated (ME) formulation (Warrant®) and is currently labeled for use in soybean [*Glycine max* (L.) Merr.], corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), and grain sorghum [*Sorghum bicolor* (L.) Moench].

The ME formulation of acetochlor is advantageous from a crop safety and weed control standpoint in rice because the polymer coating allows a slow release of acetochlor into the soil profile after an activating rainfall (Rao 2000). The delayed release of herbicide allows time for rice to imbibe soil water during germination and grow uninhibited for a period following application. In addition, a gradual release of herbicide over time indicates that the ME formulation would offer longer residual control of targeted weeds relative to the emulsifiable concentrate (EC) formulation (Rao 2000). Acetochlor formulated as an EC allows herbicide to be immediately available for uptake by germinating shoots and roots, which would suggest that EC acetochlor would provide superior weed control. Krausz et al. (2000) found no differences in barnyardgrass or giant foxtail (*Setaria faberi* Herrm.) control between EC and ME acetochlor in corn, but Fogleman et al. (2018) reported unacceptable rice injury when applying the EC formulation. Furthermore, studies by Cahoon et al. (2015) demonstrated 84, 91, and 100%

control of Palmer amaranth [*Amaranthus palmeri* (S.) Wats.], large crabgrass [*Digitaria sanguinalis* (L.) Scop.], and goosegrass [*Eleusine indica* (L.) Gaertn.], respectively, using ME acetochlor in cotton.

From a rice weed control perspective, acetochlor would be most effective applied PRE to control resistant grasses such as barnyardgrass and red rice (*Oryza sativa* var. *sylvatica* L.) or weedy rice (*Oryza* spp.), which may be more difficult to control postemergence. However, a potential issue is presented in a two-year study by Godwin et al. (2017) who demonstrated that rice tolerance to acetochlor increases as application timing is delayed. In the first year of the study,  $\langle 2\%$  injury was observed 2 WAT when ME acetochlor at 1050 g ai ha<sup>-1</sup> was applied delayed preemergence (DPRE), at spiking, or to 1- to 2-leaf rice. However, when identical applications were followed by significant rainfall in the second year of the study, 89, 43, and 10% injury, respectively, resulted as application timing was delayed. These results indicate that acetochlor activity is highly dependent upon rainfall, and greater potential for rice injury exists at earlier application timings. Ideally, acetochlor would be applied prior to 1- to 2- leaf rice, as acetochlor has no postemergence activity and must be applied prior to emergence of targeted species. Perhaps the most effective option would be to mix acetochlor with a postemergence herbicide such as imazethapyr in Clearfield rice or quizalofop in Provisia rice to control emerged weeds.

Imidazolinone-resistant (Clearfield, BASF Corporation, Research Triangle Park, NC) rice was commercialized in 2002 to selectively control weedy rice and other weeds using ALSinhibiting chemistry (Burgos et al. 2008). The Clearfield system was quickly adopted and imidazolinone herbicides were heavily relied upon in Midsouth rice production. Failure to follow stewardship guidelines led to ALS-resistant weedy rice (Burgos et al. 2008) and barnyardgrass

(Riar et al. 2012), although the technology is still widely used today where weedy rice is prevalent.

Recently, quizalofop-resistant (Provisia, BASF Corporation, Research Triangle Park, NC) rice became commercially available to combat herbicide resistance issues in rice. The new technology enables POST applications of quizalofop, an acetyl coenzyme A carboxylase (ACCase)-inhibiting herbicide. With activity on annual and perennial grass weeds, the Provisia rice system has potential to be highly effective in areas where resistance to ALS-inhibiting herbicides limit the utility of Clearfield rice. Although ACCase-inhibiting rice herbicides have been shown to have a lower risk for resistance compared to ALS-inhibiting herbicides (Bagavathiannan et al. 2014), ACCase-resistant grasses, including barnyardgrass, have been documented (Heap 2018).

Considering the preliminary research conducted on rice tolerance and the efficacy of acetochlor in U.S. row crops, acetochlor has potential to be incorporated into existing rice herbicide programs to control or suppress problematic weeds by targeting an alternative SOA. These experiments were conducted to compare rice injury and weed control between acetochlorand clomazone-based herbicide programs in Clearfield and Provisia rice systems.

# **MATERIALS AND METHODS**

**Experimental Sites.** Field experiments were conducted on a Calloway silt loam (Fine-silty, mixed, active, thermic Aquic Fraglossudalfs) at the Pine Tree Research Station (PTRS) near Colt, Arkansas, and on a Dewitt silt-loam soil (Fine, smectitic, thermic Typic Albaqualfs) at the University of Arkansas Rice Research and Extension Center (RREC) near Stuttgart, Arkansas, in 2016 and 2017. Specific soil characteristics for each experimental site are presented in Table 3.

**Experimental Setup and Data Collection.** All experiments were designed as a randomized complete block with four replications and a nontreated check for comparison. In the Clearfield experiment, 'CL151' was planted at the PTRS in 2016 and 'CL172' was planted at the PTRS and RREC in 2017. In the Provisia experiment, the experimental variety 'HPHI2' was planted at all locations and years. Clearfield experiments were planted at 72 seeds m<sup>-1</sup> of row, while Provisia experiments were planted at 82 seeds  $m^{-1}$  row, with 18 cm between rows, into 1.8- by 5.2 mplots. A higher seeding rate was used in Provisia experiments to compensate for a lower germination rate.

Herbicide treatments were applied using a  $CO<sub>2</sub>$ -pressurized backpack sprayer calibrated to deliver 140 L ha<sup>-1</sup> through AIXR110015 (TeeJet Technologies, Wheaton, IL) nozzles on the dates indicated in Table 2. In Clearfield experiments, acetochlor (Warrant herbicide, Monsanto Company, St. Louis, MO) at  $1,050$  or  $1,470$  g ai ha<sup>-1</sup>, or clomazone (Command herbicide, FMC Corporation, Philadelphia, PA) at 336 g ai ha<sup>-1</sup> was applied delayed preemergence (DPRE) 1) alone, 2) followed by imazethapyr (Newpath herbicide, BASF Corporation, Florham Park, NJ) at 70 g ai ha<sup>-1</sup> or early-postemergence (EPOST), or 3) followed by imazethapyr EPOST followed by imazethapyr pre-flood (PREFLD). Acetochlor was applied at different rates to determine the impact on rice injury and barnyardgrass control. Imazethapyr was also applied EPOST and PREFLD without additional residual herbicides. A nonionic surfactant (NIS) was included at 0.25% v/v in treatments containing imazethapyr.

Herbicides, rates, and application timings in Provisia experiments were identical to those in Clearfield experiments except that quizalofop (Assure II herbicide, DuPont, Wilmington, DE) at 120 g ai ha<sup>-1</sup> replaced the imazethapyr applications. Crop oil concentrate (COC) was added to treatments containing quizalofop at 1% v/v. In all experiments, any treatment containing a PRE

herbicide mixed with or followed by a POST herbicide was considered a program. In each year, the experimental site was tilled prior to planting, and all DPRE applications were made to bare soil. Experiments were fertilized and otherwise managed according to University of Arkansas Extension recommendations (Hardke et al. 2012). Rough rice yield was determined at physiological maturity using a small-plot combine and adjusted to 12% moisture.

**Statistical Analysis.** All data were subjected to analysis of variance with herbicide programs as a single factor randomized complete block using the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc, Cary, NC). Analysis was performed assuming beta distribution for rice injury and barnyardgrass control and normal distribution for rough rice yield (Gbur et al. 2012). Due to considerable differences in environments, data were analyzed separately by location and year. Contrasts were conducted for each parameter to capture the overall trends between clomazoneand acetochlor-containing programs, and mean separation was performed for each parameter to compare individual treatments within an environment using Fisher's protected LSD ( $\alpha$ =0.05). Acetochlor or clomazone applied DPRE alone was not considered a herbicide program and was not included in contrast analysis for barnyardgrass control 6 WAF and rough rice yield. In addition, programs containing both acetochlor and clomazone were excluded from contrast analysis.

## **RESULTS AND DISCUSSION**

**Rainfall.** Efficacy of soil-applied herbicides, and crop injury, is dependent upon several factors, but perhaps most important are clay content, organic matter (OM), pH, and soil moisture (Curran 2001; Eberlein et al. 1984; Hartzler 2002). Although pH was slightly higher at the PTRS, clay content and OM were similar between the experimental sites (Table 1); however, amount and

timing of rainfall, in relation to DPRE applications, varied among experimental locations and years and likely explains the observed differences in rice injury (Figures 1-3). Because acetochlor and clomazone are taken up through roots and shoots of germinating seedlings, these herbicides typically require 1 to 2 cm of rainfall or irrigation for activation (Rao 2000). At the PTRS in 2016, DPRE applications were made to soil with adequate moisture 3 days after planting; however, an activating rainfall was not received until 11 days later (Figure 1). At the PTRS in 2017, DPRE herbicides were made 7 days after planting, which is slightly later than typical DPRE applications (3 to 5 days after planting), but still a practical application timing for growers with large acreage. An activating rainfall occurred two days after application at the PTRS in 2017; however, considering minimal injury, it is evident that rice seed had already imbibed sufficient soil water for germination prior to application (Figure 2). In contrast to relatively dry conditions at the PTRS, DPRE applications were made to moist soil four days after planting at the RREC, which continued to receive rainfall over the next seven days, causing water to accumulate in the test site (Figure 3).

At RREC, abundance of soil moisture at time of application followed by additional rainfall over the next several days likely moved herbicide down in the soil profile where rice was imbibing water and made herbicide immediately available for plant uptake in soil solution. High soil moisture increased absorption and translocation of both acetochlor and clomazone and resulted in increased phytotoxicity (Chauhan and Johnson 2011). Conversely, herbicides readily bind to soil colloids under dry conditions like those at the PTRS, meaning that limited herbicide was available in soil solution when rice was imbibing water during germination. The lack of activating rainfall during germination led to <15% injury at the PTRS in 2016 and 2017 (data not shown).

**Rice Injury.** In both Clearfield and Provisia experiments, rice injury 2 WADPRE was <15% for all treatments at the PTRS in 2016 and 2017 and therefore was excluded from analysis; however, injury was considerably higher at the RREC and is presented in Tables 3 and 4. Overall, rice injury was more severe following DPRE acetochlor than clomazone and was exacerbated by the higher rate. At 2 WADPRE, DPRE acetochlor treatments were responsible for 51 to 78% rice injury, while injury following clomazone treatments was 11 to 17%. Although rice had recovered by 2 WAF, injury remained relatively high following DPRE acetochlor compared to clomazone (Tables 3, 4). Contrast analysis between acetochlor and clomazone applications indicate that both rates of acetochlor caused more rice injury than clomazone ( $p \le 0.0001$ ); however, injury was slightly reduced when 1050 g ai ha<sup>-1</sup> was applied compared to 1470 g ai ha<sup>-1</sup> at 2 WADPRE (p= 0.0002, <0.0001) and 2 WAF (p <0.0001, or =0.0098) (Tables 5, 6).

**PREFLD Barnyardgrass Control in Clearfield and Provisia Systems.** When PREFLD barnyardgrass control was assessed, no PREFLD applications had been made, and therefore early-season barnyardgrass control was a result of DPRE and EPOST applications of acetochlor, clomazone, and other EPOST herbicides (Tables 7, 8). In all experiments, contrasts revealed that PREFLD barnyardgrass control increased when the higher rate of acetochlor was applied, except for the Provisia experiment at the PTRS in 2016 ( $p= 0.8521$ ); albeit, neither acetochlor rate provided PREFLD barnyardgrass control comparable to clomazone ( $p < .0001$  or  $= 0.0008$ ; Tables 5, 6). In the Provisia experiment at the PTRS in 2016, there was no difference in barnyardgrass control between the 1,050 and 1,470  $g$  ai ha<sup>-1</sup> rates of acetochlor, as each provided comparable control when applied DPRE (63 to 68%) or mixed with quizalofop EPOST (90 to 93%) (Table 8).

In all experiments, clomazone consistently provided the best barnyardgrass control. In contrast, acetochlor control was variable and was most efficacious at the RREC in 2017 where conditions remained wet early in the season (Tables 7, 8). When clomazone or acetochlor applications were delayed to EPOST and mixed with imazethapyr or quizalofop, barnyardgrass control was always improved relative to acetochlor DPRE alone but was not always improved relative to clomazone DPRE alone. In all experiments, there were no differences in PREFLD barnyardgrass control when acetochlor or clomazone was applied DPRE fb imazethapyr or quizalofop EPOST and when either residual herbicide was mixed with imazethapyr or quizalofop EPOST, suggesting that growers could achieve comparable control with one trip across the field rather than two, which could help mitigate application costs (Tables 7, 8).

**Barnyardgrass Control 6 WAF.** *Clearfield.* Although clomazone- and acetochlor-containing programs were generally comparable 6 WAF at the PTRS in 2016, contrasts indicated that clomazone-containing programs provided slightly better barnyardgrass control than acetochlorcontaining programs ( $p = 0.0030$ ). According to contrasts, barnyardgrass control was comparable at both locations in 2017, likely due to timely rainfall events early in the season  $(p= 0.3042,$ 0.4770, Table 5; Figures 2, 3). At the PTRS in 2016, clomazone-containing programs and acetochlor-containing programs at 1,470 g ai ha<sup>-1</sup> provided comparable barnyardgrass control ( $p=$ 0.1333), but control was slightly reduced when acetochlor was mixed with imazethapyr EPOST at the  $1,050$  g ai ha<sup>-1</sup> rate (Table 9).

Herbicide programs did not differ at the PTRS or RREC in 2017, as all programs provided >94% barnyardgrass control, regardless of residual herbicide used or imazethapyr application timings (Table 9). Similar to results shown by Ottis et al. (2003), imazethapyr EPOST fb imazethapyr PREFLD provided >96% control in all environments, indicating that

barnyardgrass populations in these experiments were not ALS-resistant. It should be noted that reliance on one herbicide or one herbicide SOA is not sustainable or recommended, as it places immense selection on the targeted population and can quickly lead to herbicide resistance (Jasieniuk et al. 1996; Norsworthy et al. 2012). In addition, there were no differences in barnyardgrass control between single-pass programs where acetochlor or clomazone was mixed with imazethapyr and applied EPOST, and two-pass programs where acetochlor or clomazone was applied DPRE fb imazethapyr PREFLD, further supporting the idea that growers could mitigate early-season application costs by reducing trips across the field without sacrificing barnyardgrass control when the DPRE treatment is activated (Table 9).

*Provisia.* Based on contrasts, clomazone-containing programs provided better barnyardgrass control than acetochlor-containing programs at the PTRS in  $2016$  ( $p= 0.0187$ ), but conversely, acetochlor-containing programs provided better control at the RREC in 2017 ( $p= 0.0084$ ), and there were no differences between programs at the PTRS in  $2017$  ( $p= 0.3264$ , Table 6). Although differences in barnyardgrass control between acetochlor- and clomazone-containing programs occurred by contrasts for PTRS in 2016 and the RREC in 2017, control was comparable within single- and multi-pass programs, respectively (Table 10). Two- and three-pass programs, however, did increase control relative to single-pass programs at the PTRS in 2016. Because single-pass programs provided barnyardgrass control comparable to that of multi-pass programs at both locations in 2017, the reduced control provided by single-pass programs at the PTRS in 2016 is likely due to higher density and larger size of barnyardgrass plants present at the time of EPOST application (Table 11). Furthermore, these findings emphasize the importance of timely herbicide applications in single-pass herbicide programs (Wilson et al. 2014).

**Rough Rice Yield**. *Clearfield.* Contrasts revealed there were no differences in yield between acetochlor- or clomazone-containing programs at the PTRS in 2016 ( $p= 0.3790$ ) or 2017 ( $p=$ 0.0742), although there were at the RREC ( $p \le 0.0001$ ; Table 5). At the RREC, clomazonecontaining programs yielded higher than acetochlor-containing programs, regardless of acetochlor rate ( $p < 0.0001$ ; Table 12). Because acetochlor- and clomazone-containing herbicide programs provided comparable barnyardgrass control 6 WAF, and interference from other species was minimal, differences in yield response is likely a function of early-season rice injury (Table 3). Similar to observations noted in barnyardgrass control 6 WAF, yields were comparable between single-pass programs where acetochlor was mixed with imazethapyr EPOST, and two-pass programs where acetochlor was applied DPRE fb imazethapyr PREFLD (Table 12). Generally, yield was maximized in three-pass herbicide programs where a residual herbicide was applied DPRE fb imazethapyr EPOST and PREFLD. Structuring a herbicide program in this manner provides residual early-season control followed by sequential POST applications, which controls weeds that emerge between applications possibly resulting in season-long control. In addition, rice in all herbicide treatments, including where acetochlor or clomazone was applied DPRE alone, yielded higher than the nontreated (Table 12).

It should be noted that in two of the three environments, the two-pass program where clomazone was applied DPRE fb acetochlor + imazethapyr EPOST yielded comparable to threepass programs where clomazone or acetochlor was applied DPRE fb imazethapyr EPOST and PREFLD. The true benefit of a program incorporating clomazone, acetochlor, and imazethapyr is the combination of three different herbicide SOAs, which reduces selection on any single herbicide and thereby reduces risk for resistance (Norsworthy et al. 2012).

*Provisia.* According to contrasts, clomazone-containing programs yielded higher than acetochlor programs at the PTRS in 2016 ( $p=0.0098$ ) and the RREC in 2017 ( $\lt 0.0001$ ; Table 6). At the PTRS in 2017, yields differed between clomazone and acetochlor programs only when the lower rate of acetochlor was used ( $p= 0.0115$ ; Table 6), although yields were comparable within single and multi-pass programs (Table 13). Yield did not differ within single-pass or three-pass programs at PTRS in 2017 (Table 13). Yields at the RREC in 2017 were higher following oneor two-pass clomazone-containing programs compared to acetochlor-containing programs, but yields were comparable within three-pass programs (Table 13). In all environments, yield was generally maximized by three-pass programs containing either clomazone or acetochlor, or twopass programs containing clomazone. Lack of differences between clomazone and acetochlor within three-pass programs are not surprising, as the POST applications of quizalofop at EPOST and PREFLD are likely to control any grass weeds that emerged following DPRE applications. Rice in plots treated with herbicide, including single applications of clomazone or acetochlor DPRE, yielded higher than the nontreated (Table 13).

**Practical Implications.** In both Clearfield and Provisia experiments, acetochlor DPRE caused more rice injury than clomazone (Tables 3, 4). When acetochlor DPRE went 7 to 10 days without an activating rainfall, minimal rice injury occurred; however, an activating rainfall soon after application resulted in severe rice injury. These results coincide with previous studies that demonstrated rice tolerance generally increases as application timing is delayed and can be influenced by rainfall (Fogleman et al. 2018). The increased rice injury following rainfall is a result of the herbicide being desorbed from soil colloids and absorbed as rice seed imbibes water during germination. In contrast, timing of rainfall seemed to have less effect on rice injury from DPRE clomazone than acetochlor. Because rainfall patterns are mostly unpredictable from

season to season, the increased risk for rice injury associated with DPRE acetochlor applications in rice is greater than most growers would likely tolerate.

Superior early-season barnyardgrass control provided by clomazone-based programs was expected, as clomazone is reported to be more effective than acetochlor on barnyardgrass, explaining why a single application of clomazone DPRE provided 75 to 86% barnyardgrass control at 6 WAF while acetochlor DPRE provided 47 to 65% control (Tables 9, 10; Scott et al. 2018). With clomazone considered the standard PRE herbicide in midsouthern US rice systems today, growers should refrain from abusing this alternate SOA to preserve its efficacy, as populations of clomazone-resistant barnyardgrass continue to increase in this geography (Norsworthy et al. 2008, 2013).

In many cases, barnyardgrass control PREFLD was lacking following acetochlor DPRE alone. However, when acetochlor DPRE was followed by POST applications at EPOST and/or PREFLD, control was generally comparable to clomazone-containing programs 6 WAF. It is possible that acetochlor is released from its microencapsulated formulation at a slower rate than clomazone, which suggests that more selection may be placed on POST herbicides such as imazethapyr and quizalofop when acetochlor is applied than when applying clomazone soon after planting.

Clomazone-containing programs generally caused less rice injury, provided superior barnyardgrass control, and yielded more than acetochlor-containing programs. However, it should be noted that within respective single- and multi-pass programs, acetochlor often provided barnyardgrass control and rice yield comparable to clomazone. In addition, some of the highest barnyardgrass control ratings and yields came from treatments where clomazone and

acetochlor were applied in the same program. The significance of these findings is that growers could prolong the efficacy of current herbicides by utilizing alternative SOAs without sacrificing weed control or yield. Should acetochlor be labeled for use in rice, growers could help preserve effective chemistry and delay the onset of herbicide resistance by incorporating this alternative into their current herbicide programs, while still achieving barnyardgrass control and yield comparable to that of standard programs used today.

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# **APPENDIX**

|             | Soil properties |     |      |      |      |
|-------------|-----------------|-----|------|------|------|
| Location    | pH              | OМ  | Sand | Silt | Clay |
|             |                 |     |      |      |      |
| <b>RREC</b> | 6.0             | 1.8 | 8.4  | 71.4 | 20.2 |
|             |                 |     |      |      |      |
| <b>PTRS</b> | 7.5             | 1.3 | 10.6 | 68.6 | 20.8 |

Table 1. Chemical and physical soil properties at experimental sites in 2016 and 2017.

<sup>a</sup> Abbreviations: RREC, Rice Research and Extension Center near Stuttgart, AR; PTRS, Pine Tree Research Station near Colt, AR; OM, organic matter

|                  | Dates of significance |             |              |          |          |  |
|------------------|-----------------------|-------------|--------------|----------|----------|--|
| Location         | Planting              | <b>DPRE</b> | <b>EPOST</b> | PREFLD   | Harvest  |  |
| <b>PTRS 2016</b> | May 9                 | May $13$    | May $25$     | June 16  | Sept 19  |  |
| <b>PTRS 2017</b> | Apr $13$              | Apr $20$    | May $16$     | May $30$ | Sept 18  |  |
| <b>RREC 2017</b> | May $18$              | May $23$    | June 2       | June 12  | Oct $10$ |  |

Table 2. Planting, herbicide application, and harvest dates for trials in 2016 and 2017.<sup>a</sup>

<sup>a</sup> Abbreviations: DPRE, delayed preemergence; EPOST, early postemergence; PREFLD, preflood; PTRS, Pine Tree Research Station near Colt, AR; RREC, Rice Research and Extension Center near Stuttgart, AR



Figure 1. Rainfall amount and dates at the Pine Tree Research Station (PTRS) near Colt, AR in 2016. Application dates and timings represent Clearfield and Provisia experiments. Abbreviations: DPRE, delayed preemergence; EPOST, early postemergence



Figure 2. Rainfall amount and dates at the Pine Tree Research Station (PTRS) near Colt, AR in 2017. Application dates and timings represent Clearfield and Provisia experiments. Abbreviations: DPRE, delayed preemergence; EPOST, early postemergence



Figure 3. Rainfall amount and dates at the Rice Research and Extension Center (RREC) near Stuttgart, AR in 2017. Application dates and timings represent Clearfield and Provisia experiments. Abbreviations: DPRE, delayed preemergence; EPOST, early postemergence



Table 3. Rice injury following acetochlor- and clomazone-containing herbicide programs in Clearfield rice at the Rice Research and Extension Center near Stuttgart, AR in 2017.<sup>a,b,c</sup>

<sup>a</sup> Abbreviations: WADPRE, weeks after DPRE; DPRE, delayed preemergence; WAF, weeks after flooding; clom., clomazone; acet., acetochlor; imaz., imazethapyr; EPOST, early postemergence; PREFLD, pre-flood

<sup>b</sup> Means within a column followed by the same lowercase letter are not different according to Fisher's protected LSD ( $\alpha$  = 0.05)

<sup>c</sup> Rice injury 2 WADPRE for programs that did not contain DPRE applications are represented by (-)



Table 4. Rice injury following acetochlor- and clomazone-containing herbicide programs in Provisia rice at the Rice Research and Extension Center near Stuttgart, AR in 2017.<sup>a,b,c</sup>

<sup>a</sup> Abbreviations: clom., clomazone; acet., acetochlor; quiz, quizalofop; WADPRE, weeks after DPRE; WAF, weeks after flooding; DPRE, delayed preemergence; EPOST, early postemergence; PREFLD, pre-flood

<sup>b</sup> Means within a column followed by the same lowercase letter are not different according to Fisher's protected LSD ( $\alpha$  = 0.05)

 $\textdegree$  Rice injury 2 WADPRE for programs that did not contain DPRE applications are represented by  $\textdegree$ 

Table 5. Significance of contrast statements between acetochlor- and clomazone-containing Clearfield herbicide programs.<sup>a,b,c</sup>









|                                   | Yield $(kg ha^{-1})$ |              |                    |              |                    |              |
|-----------------------------------|----------------------|--------------|--------------------|--------------|--------------------|--------------|
| Contrast                          | PTRS <sub>16</sub>   | <b>Means</b> | PTRS <sub>17</sub> | Means        | RREC <sub>17</sub> | Means        |
| Acetochlor high vs clomazone      | 0.7186               | 9600 vs 9700 | 0.2376             | 8400 vs 8600 | $< 0.0001*$        | 8700 vs 9600 |
| Acetochlor low vs acetochlor high | 0.4879               | 9400 vs 9600 | 0.5085             | 8200 vs 8400 | 0.4750             | 8600 vs 8700 |

Table 5. Significance of contrast statements between acetochlor- and clomazone-containing Clearfield herbicide programs.<sup>a,b,c</sup>

<sup>a</sup> Abbreviations: WADPRE, weeks after DPRE; DPRE, delayed preemergence; WAF, weeks after flooding; PREFLD, pre-flood; PTRS, Pine Tree Research Station near Colt, AR; RREC, Rice Research and Extension Center near Stuttgart, AR; acetochlor low, 1050 g ai ha<sup>-1</sup>; acetochlor high, 1470 g ai ha<sup>-1</sup>

<sup>b</sup> Acetochlor or clomazone applied alone is not considered an herbicide program; therefore, these were not included in contrast analysis for barnyardgrass 6WAF or yield. In addition, any program containing both acetochlor and clomazone was excluded from all contrast analysis.

<sup>c</sup> Significant P values ( $\alpha$ =0.05) are indicated by (\*)

Table 6. Significance of contrast statements between acetochlor- and clomazone-containing Provisia herbicide programs.<sup>a,b,c</sup>



|                                   | Yield $(kg ha^{-1})$ |              |                    |              |                    |               |
|-----------------------------------|----------------------|--------------|--------------------|--------------|--------------------|---------------|
| Contrast                          | PTRS <sub>16</sub>   | <b>Means</b> | PTRS <sub>17</sub> | Means        | RREC <sub>17</sub> | Means         |
| Acetochlor high vs clomazone      | 0.0825               | 8800 vs 9200 | 0.4386             | 9800 vs 9900 | $< 0.0001*$        | 9300 vs 10500 |
| Acetochlor low vs acetochlor high | 0.3856               | 8700 vs 8800 | 0.1702             | 9300 vs 9800 | 0.9089             | 9300 vs 9300  |

Table 6. Significance of contrast statements between acetochlor- and clomazone-containing Provisia herbicide programs.<sup>a,b,c</sup>

<sup>a</sup> Abbreviations: WADPRE, weeks after DPRE; DPRE, delayed preemergence; WAF, weeks after flooding; PREFLD, pre-flood; PTRS, Pine Tree Research Station near Colt, AR; RREC, Rice Research and Extension Center near Stuttgart, AR; acetochlor low, 1050 g ai ha<sup>-1</sup>; acetochlor high, 1470 g ai ha<sup>-1</sup>

<sup>b</sup> Acetochlor or clomazone applied alone is not considered an herbicide program; therefore, these were not included in contrast analysis for barnyardgrass 6WAF or yield. In addition, any program containing both acetochlor and clomazone was excluded from all contrast analysis

<sup>c</sup> Significant P values ( $\alpha$ =0.05) are indicated by (\*)



Table 7. Barnyardgrass control following acetochlor- and clomazone-containing Clearfield herbicide programs.<sup>a,b,c</sup>

<sup>a</sup> Abbreviations: PREFLD, pre-flood; PTRS, Pine Tree Research Station near Colt, AR; RREC, Rice Research and Extension Center near Stuttgart, AR; clom., clomazone; acet., acetochlor; imaz., imazethapyr; DPRE, delayed PRE; EPOST, early POST <sup>b</sup> Means within a column followed by the same lowercase letter are not different according to Fisher's protected LSD ( $\alpha$  = 0.05) <sup>c</sup> Barnyardgrass evaluations were made prior to PREFLD applications; however, for consistency, PREFLD application timings are shown in the treatment description



Table 8. Barnyardgrass control following acetochlor- and clomazone-containing Provisia herbicide programs.<sup>a,b,c</sup>

<sup>a</sup> Abbreviations: PREFLD, pre-flood; PTRS, Pine Tree Research Station near Colt, AR; RREC, Rice Research and Extension Center near Stuttgart, AR; clom., clomazone; acet., acetochlor; quiz., quizalofop; DPRE, delayed preemergence; EPOST, early postemergence

<sup>b</sup> Means within a column followed by the same lowercase letter are not different according to Fisher's protected LSD ( $\alpha$  = 0.05)

<sup>c</sup> Barnyardgrass evaluations were made prior to PREFLD applications; however, for consistency, PREFLD application timings are shown in the treatment description



Table 9. Barnyardgrass control following acetochlor- and clomazone-containing Clearfield herbicide programs.<sup>a,b</sup>

<sup>a</sup> Abbreviations: WAF, weeks after flooding; PTRS, Pine Tree Research Station near Colt, AR; RREC, Rice Research and Extension Center near Stuttgart, AR; clom., clomazone; acet., acetochlor; imaz., imazethapyr; DPRE, delayed PRE; EPOST, early POST; PREFLD, pre-flood

<sup>b</sup> Means within a column followed by the same lowercase letter are not different according to Fisher's protected LSD ( $\alpha$  = 0.05)



Table 10. Barnyardgrass control following acetochlor- and clomazone-containing Provisia herbicide programs.<sup>a,b</sup>

<sup>a</sup> Abbreviations: WAF, weeks after flooding; PTRS, Pine Tree Research Station near Colt, AR; RREC, Rice Research and Extension Center near Stuttgart, AR; clom., clomazone; acet., acetochlor; quiz., quizalofop; DPRE, delayed preemergence; EPOST, early postemergence; PREFLD, pre-flood

<sup>b</sup> Means within a column followed by the same lowercase letter are not different according to Fisher's protected LSD ( $\alpha$  = 0.05)

|                  | Barnyardgrass   |              |                 |               |  |  |  |
|------------------|-----------------|--------------|-----------------|---------------|--|--|--|
|                  |                 | <b>EPOST</b> |                 | <b>PREFLD</b> |  |  |  |
| Location         | Density         | Height       | Density         | Height        |  |  |  |
|                  | plants $m^{-2}$ | cm           | plants $m^{-2}$ | cm            |  |  |  |
| <b>PTRS 2016</b> |                 |              |                 |               |  |  |  |
| Clearfield       | 6               | $2 - 4$      | 10              | $4 - 10$      |  |  |  |
| Provisia         | 8               | $2 - 6$      | 11              | $4-9$         |  |  |  |
| <b>PTRS 2017</b> |                 |              |                 |               |  |  |  |
| Clearfield       | 4               | $2 - 4$      | 8               | $3-9$         |  |  |  |
| Provisia         | 5               | $1 - 3$      | 8               | $5 - 10$      |  |  |  |
| <b>RREC</b>      |                 |              |                 |               |  |  |  |
| Clearfield       | 7               | $2 - 5$      | 9               | $5-10$        |  |  |  |
| Provisia         | 6               | $2 - 3$      | 10              | $4 - 11$      |  |  |  |

Table 11. Average weed sizes in the nontreated plot at the time of application.<sup>a</sup>

<sup>a</sup> Abbreviations: EPOST, early postemergence; PREFLD, pre-flood; PTRS, Pine Tree Research Station near Colt, AR; RREC, Rice Research and Extension Center near Stuttgart, AR



Table 12. Rough rice yield following acetochlor- and clomazone-containing Clearfield herbicide programs.<sup>a,b</sup>

<sup>a</sup> Abbreviations: PTRS, Pine Tree Research Station near Colt, AR; RREC, Rice Research and Extension Center near Stuttgart, AR; clom., clomazone; acet., acetochlor; imaz., imazethapyr; PREFLD, pre-flood; DPRE, delayed preemergence; EPOST, early postemergence

<sup>b</sup> Means within a column followed by the same lowercase letter are not different according to Fisher's protected LSD ( $\alpha$  = 0.05)



Table 13. Rough rice yield following acetochlor- and clomazone-containing Provisia herbicide programs.<sup>a,b</sup>

<sup>a</sup> Abbreviations: PTRS, Pine Tree Research Station near Colt, AR; RREC, Rice Research and Extension Center near Stuttgart, AR; clom., clomazone; acet., acetochlor; quiz., quizalofop; DPRE, delayed preemergence; EPOST, early postemergence; PREFLD, preflood

<sup>b</sup> Means within a column followed by the same lowercase letter are not different according to Fisher's protected LSD ( $\alpha$  = 0.05)

#### **Chapter 4**

#### **Efficacy of Early-Season Applications of Acetochlor and Pethoxamid in Rice**

Limited options for controlling herbicide-resistant weedy rice and barnyardgrass in Arkansas rice has led to the exploration of alternative herbicide sites of action (SOA). Very long-chain fatty acid (VLCFA)-inhibiting herbicides have been used successfully in U.S. row crops and Asian rice production for control of annual grasses and small-seeded broadleaves but are not labeled for use in U.S. rice. Preliminary experiments have indicated adequate rice tolerance to acetochlor and pethoxamid; however, limited weed control information in rice systems is available. Field experiments were conducted in 2016 and 2017 to evaluate weed control with early-season applications of acetochlor and pethoxamid on weedy rice and annual grasses in rice. In separate experiments, microencapsulated acetochlor at 1050 and 1470 g ai ha<sup>-1</sup> or pethoxamid at 420 and 840 g ai ha<sup>-1</sup> was applied alone delayed preemergence (DPRE), at spiking, 1- to 2-leaf, and 3- to 4-leaf rice. In both years, injury less than 10 and 20% was observed for all acetochlor and pethoxamid treatments, respectively, 2 weeks after treatment (WAT). Both herbicides controlled barnyardgrass >92% and suppressed weedy rice 33 to 63% 2 WAT. Regardless of application timing or rate, acetochlor and pethoxamid reduced weedy rice density relative to the nontreated 4 WAT. Control of weedy rice, barnyardgrass, broadleaf signalgrass, and large crabgrass was maximized when either herbicide was applied DPRE or to spiking rice and generally decreased as application timing was delayed. Furthermore, control of weed species early in the season influenced rough rice yield, as the highest yields were harvested when acetochlor or pethoxamid was applied DPRE or at spiking. Residual control of annual grasses and suppression of weedy rice from early-season applications of acetochlor and pethoxamid indicate they could be valuable

in a season-long rice herbicide program while providing an alternative SOA to combat herbicideresistant weeds.

**Nomenclature:** Acetochlor; pethoxamid; rice, *Oryza sativa* L.; weedy rice, *Oryza sativa* var. *sylvatica* L.; barnyardgrass, *Echinochloa crus-galli* (L.) Beauv.; broadleaf signalgrass, *Urochloa platyphylla* (Nash) R.D. Webster; large crabgrass, *Digitaria sanguinalis* (L.) Scop.

**Key words:** Very long-chain fatty acid-inhibiting herbicides, herbicide-resistance, delayed preemergence

## **INTRODUCTION**

Red rice, also known as weedy rice, is one of the most problematic weeds in Arkansas rice production (Burgos et al. 2008). Weedy rice plants compete directly with cultivated rice for sunlight, nutrients, and water (Burgos et al. 2006). Shared morphological and physiological characteristics of rice and weedy rice make it almost impossible to discern the difference between them in the field, especially early in the season (Pantone and Baker 1991). However, weedy rice plants generally have a higher growth rate and are often taller and produce more tillers than cultivated rice (Diarra et al. 1985a, 1985b; Kwon et al. 1991; Smith 1988). In fact, previous research demonstrated that a single weedy rice plant per  $m<sup>2</sup>$  can reduce rice yield by 755 kg ha<sup>-1</sup> and has the competitive ability of four cultivated rice plants (Ottis et al. 2005; Pantone and Baker 1991).

Prior to the introduction of imidazolinone-resistant (Clearfield™ BASF Corporation, Research Triangle Park, NC) rice in 2002, weedy rice was mainly controlled using water seeding and crop rotation with soybean [*Glycine max* (L.) Merr.], corn (*Zea mays* L.), and grain sorghum [*Sorghum bicolor* (L.) Moench] (Burgos et al. 2008). The Clearfield™ technology was quickly adopted because it allowed growers to selectively control troublesome grasses such as weedy rice and barnyardgrass using acetolactate synthase (ALS)-inhibiting herbicides. In 2014, approximately 49% of Arkansas rice acreage was planted to Clearfield™ rice (Hardke 2014), although that percentage has decreased slightly in recent years. In the mid-2000s, extensive use of ALS inhibitors such as imazethapyr and imazamox, in addition to poor adherence to stewardship guidelines, quickly led to resistance among several weed populations. To date, 11 species have confirmed resistance to the ALS site of action (SOA) in Arkansas, including weedy rice, barnyardgrass, junglerice [*Echinochloa colona* (L.) Link], yellow nutsedge (*Cyperus* 

*esculentus* L.), rice flatsedge (*Cyperus iria* L.), and Palmer amaranth [*Amaranthus palmeri* (S.) Wats.] (Heap 2018). However, the natural hybridization and resulting outcrossing between weedy rice and cultivated rice is largely responsible for the increase in ALS-resistant weedy rice populations (Shivrain et al. 2007).

Aggressive growth habit, extensive root system, and prolific seed production contribute to the extreme competitiveness of barnyardgrass in rice (Holm et al. 1977; Talbert and Burgos 2007). Barnyardgrass infestations can cause up to 70% yield loss if not properly managed (Smith 1988). Beginning with propanil in 1990, barnyardgrass has since become resistant to seven different herbicides among four SOA including: propanil (Weed Science Society of America [WSSA] Group 7), clomazone (WSSA Group 13), quinclorac (WSSA Group 4), and ALSinhibitors imazethapyr, bispyribac, imazamox, and penoxsulam (WSSA Group 2) (Heap 2018). In a 2011 survey of crop consultants in Arkansas and Mississippi, 58% of respondents reported populations of herbicide-resistant barnyardgrass in fields they scouted, indicating widespread resistance (Norsworthy et al. 2013).

The repetitive use of the same herbicide SOA has been shown to quickly lead to herbicide resistance. When the same SOA is repeatedly targeted, frequency of resistance alleles increases in the population as a function of selection pressure, thereby reducing herbicide efficacy and limiting control options (Jasieniuk et al. 1996). However, the evolution of resistance among problematic weeds such as barnyardgrass and weedy rice may be delayed by rotating and mixing different herbicide SOAs (Norsworthy et al. 2012). Since there have been no new SOA discovered in recent years, there is a need to explore alternative herbicides that may be used to delay resistance and control resistant weeds in rice.

Very long-chain fatty acid (VLCFA)-inhibiting herbicides such as *S*-metolachlor, acetochlor, and pyroxasulfone are used in row crops for control of annual grasses and smallseeded broadleaves (Krausz 2000; Nurse et al. 2011; Zemolin et al. 2014); however, VLCFAinhibiting herbicides are not labeled for U.S rice production. In contrast, pretilachlor and butachlor, also VLCFA-inhibitors, are common in Asian rice production and have been used to control grass species such as barnyardgrass, Chinese sprangletop (*Leptochloa chinensis* L.), and knotgrass (*Paspalum distichum* L.) (Chauhan et al. 2013; Mutnal et al. 1998). These soil-applied herbicides are primarily absorbed through seedling shoots and roots where they inhibit cell development and cell division by interfering with protein synthesis (Anonymous 2017).

Acetochlor is a widely-used VLCFA-inhibitor belonging to the chloroacetamide family. Currently labeled for use in U.S. corn, cotton, soybean, and grain sorghum, acetochlor is generally applied preemergence for control of annual grasses and small-seeded broadleaves. Warrant (Monsanto Company, St. Louis, MO) is a microencapsulated (ME) formulation of acetochlor in which herbicide molecules are protected from degradation processes by a porous, polymer shell (Rao 2000). When exposed to soil moisture, the polymer shell dissolves and allows a slow release of acetochlor, which can prolong residual activity and influence weed control and crop tolerance (Rao 2000).

The efficacy of ME acetochlor on target weeds such as barnyardgrass and weedy rice has been reported in several row crops (Cahoon et al. 2015; Janak and Grichar 2016; Krausz et al. 2000; Riar et al. 2011) and wet-seeded rice (Eleftherohorinos and Dhima 2002). Studies conducted by Godwin et al. (2017) evaluated tolerance of drill-seeded rice to 630 and 1050 g ai ha<sup>-1</sup> of ME acetochlor applied DPRE, and at the spiking, 1- to 2-leaf, and 3- to 4-leaf rice stages. Results from these experiments indicated that rice tolerance to acetochlor generally increased as

application timing was delayed, and that minimal crop injury occurred when acetochlor was applied at the 1- to 2-leaf stage or later. Additionally, increased risk may be associated with PRE or DPRE applications of acetochlor, as dry conditions at application followed by heavy rains activated the herbicide simultaneously with rice germination and resulted in rice injury.

Pethoxamid (FMC Corporation, Philadelphia, PA) is a new VLCFA-inhibitor currently being developed for use in corn, cotton (*Gossypium hirsutum* L.), soybean, canola (*Brassica napus* L.), sunflower (*Helianthus annuus* L.), and rice in the U.S. and Canada. Similar to other chloroacetamides such as acetochlor and metolachlor, pethoxamid is a soil-applied herbicide with activity on annual grasses and small-seeded broadleaves (Dhareesank et al. 2005). In preliminary studies, pethoxamid has shown initial promise, with high levels of barnyardgrass control and rice tolerance. Godwin et al. (2017) reported less than 5% rice injury and no yield reduction when pethoxamid was applied DPRE and at the spiking and 1- to 2-leaf growth stages. Doherty et al. (2015) also evaluated rice injury and weed control following pethoxamid applications to spiking rice. There were no differences in control (97 to 99%) of barnyardgrass, Amazon sprangletop [*Leptichloa panicoides* (J. Presl) A.S. Hitchc.], or eclipta (*Eclipta prostrata* L.) 26 days after application (DAA) when pethoxamid at 420 or 560 g ai ha<sup>-1</sup> was applied alone or in combination with imazethapyr, clomazone, quinclorac, or pendimethalin. In addition, no injury was observed following any treatment.

With only five weeds having resistance to VLCFA-inhibiting herbicides worldwide, there is relatively low risk for resistance compared to rice herbicides used today (Heap 2018). The ability of acetochlor and pethoxamid to control weedy rice, barnyardgrass, and other problematic species in row crops, in addition to the preliminary assessments of tolerance in drill-seeded rice, indicate that these herbicides may be used successfully in Midsouth rice production. By targeting

an alternative SOA, acetochlor and pethoxamid may help delay the onset of resistance while providing high levels of weed control and minimizing crop injury. Because limited research has been conducted on these particular VLCFA-inhibitors in rice, experiments were conducted to evaluate the ability of acetochlor and pethoxamid to provide early-season weed control in drillseeded rice. It was hypothesized that rice will be most tolerant at the 1- to 4-leaf growth stages; however, the best weed control will result from DPRE and spiking application timings.

## **MATERIALS AND METHODS**

**Experimental Sites**. All experiments were conducted on a Calloway silt loam (Fine-silty, mixed, active, thermic Aquic Fraglossudalfs) at the Pine Tree Research Station (PTRS) near Colt, AR.

**Experimental Setup and Data Collection.** Clearfield cultivar 'CL151' was planted on May 11, 2016, and 'CL172' was planted on May 16, 2017 at 72 seeds  $m^{-1}$  of row, with 18 cm between rows, in 1.8 by 5.2 m plots. To mimic the beginning of a standard rice herbicide program, preemergence applications of clomazone (Command herbicide, FMC Corporation, Philadelphia, PA) were applied to both experiments at 336 g ai ha<sup>-1</sup>. Experiments were fertilized and otherwise managed according to University of Arkansas Extension recommendations (Hardke et al. 2012). Herbicide treatments were applied using a  $CO<sub>2</sub>$ -pressurized backpack sprayer calibrated to deliver 140 L ha<sup>-1</sup> at 276 kPa. In each year, rice injury and weed control were evaluated 2 and 4 weeks after treatment (WAT) on a scale of 0 to 100, with 0 being no control or injury and 100 being complete control or crop death. In 2017, the number of weedy rice plants per  $m^2$  in each plot was counted 2 and 4 WAT. Plots were harvested on September 15, 2016, and September 19, 2017, using a small-plot combine, and weight of rice grain was adjusted to 12% moisture for determining rough rice yield.

**Acetochlor Experiment.** Acetochlor (Warrant herbicide, Monsanto Company, St. Louis, MO) was applied at 1050 (low) and 1470 (high) g ai ha<sup>-1</sup> at the DPRE, spiking, 1- to 2-leaf, and 3- to 4-leaf timings. Herbicide applications were made as follows: DPRE on May 16, 2016, and May 22, 2017; spiking growth stage on May 25, 2017; 1- to 2-leaf rice on May 25, 2016, and May 30, 2017; 3- to 4-leaf rice on June 2, 2016, and June 7, 2017. Spiking applications were not made in 2016.

**Pethoxamid Experiment.** Pethoxamid (FMC Corporation, Philadelphia, PA) was applied at 420 (low) and 840 (high) g ai ha<sup>-1</sup> at the DPRE, spiking, 1- to 2-leaf, and 3- to 4-leaf timings. Herbicide applications were made as follows: DPRE on May 16, 2016, and May 22, 2017; spiking growth stage on May 25, 2017; 1- to 2-leaf rice on May 25, 2016, and May 30, 2017; 3 to 4-leaf rice on June 2, 2016, and June 7, 2017. Spiking applications were not made in 2016.

**Statistical Analysis.** The yield data were found to be normally distributed, via a non-significant Shapiro-Wilk Test; however, all other parameters were analyzed assuming beta distribution (Gbur et al. 2012). All data were analyzed as a two-factor factorial randomized complete block using the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc., Cary, NC). The first factor being application timing: delayed preemergence (DPRE), spiking, 1- to 2-leaf, and 3-to 4-leaf rice; the other being herbicide rate: low and high. A weedy check plot was included in both experiments for comparison. Due to inconsistency of weed species between experimental locations, barnyardgrass, broadleaf signalgrass, and large crabgrass control was reported for 2016, while weedy rice control was reported for 2017. For these reasons, rice injury and rough rice yield were analyzed and reported separately by year. Weedy rice counts  $m<sup>2</sup>$  were converted to proportions of the average of the nontreated for each experiment and year, respectively, and presented as a percent reduction relative to the nontreated check. Analysis of variance indicated

no significant interactions between factors in any experiment and therefore only main effects are presented. All means were separated using Fisher's protected LSD ( $\alpha$ =0.05).

# **RESULTS AND DISCUSSION**

**Rice Injury and Weed Control Using Acetochlor.** In both years, a main effect of application timing influenced rice injury 2 WAT ( $p = 0.0015, 0.0040$ ). As also reported in similar studies (Godwin et al. 2017), rice injury to acetochlor, averaged over rate, generally decreased as application timing was delayed although no treatment caused more than 10% injury (Table 1). The increased injury from earlier application timings was that rice was probably absorbing higher concentrations of herbicide in the soil solution during germination, resulting in more growth inhibition relative to 1- to 4-leaf applications when plants were established prior to herbicide application.

Although weedy rice pressure varied within the experimental area, achievement of high levels of control from all treatments was not expected, as drill-seeded rice has shown adequate tolerance to some application timings evaluated in this experiment (Godwin et al. 2017). The challenge, of course, is finding an application timing that minimizes rice injury while maximizing suppression of weedy rice. Main effects of application timing and rate influenced weedy rice control at 2 WAT ( $p \le 0.0001$  and  $p = 0.0218$ ) and 4 WAT ( $p \le 0.0001$  and  $p =$ 0.0097, respectively). DPRE, spiking, and 1- to 2-leaf applications provided comparable control 2 WAT; however, by 4 WAT, control was better following DPRE than 1- to 2-leaf applications, although spiking treatments were comparable to both (Table 1). Weedy rice control averaged over acetochlor rate decreased when applications were delayed until 3- to 4-leaf rice 2 WAT and 1- to 2-leaf rice 4 WAT. Similarly, DPRE, spiking, and 1- to-2 leaf application timings averaged over acetochlor rates reduced weedy rice density 2 WAT, but there were no differences among applications by 4 WAT. All treatments reduced weedy rice density relative to the nontreated (data not shown), which averaged 4 weedy rice plants per  $m^2$  six weeks after planting.

Very-long-chain fatty acid-inhibitors are primarily absorbed through emerging shoots and secondarily through roots; therefore, plants beyond the seedling stage will still absorb herbicide through roots, but translocation to shoots is limited and thus efficacy is decreased as application timing is delayed (Senseman 2007). The limited translocation to shoots and resulting reduced efficacy of VLCFA-inhibitors when absorbed through roots could explain why 1- to 2-leaf applications were comparable to DPRE and spiking applications in some instances, while 3- to 4 leaf applications were not. In general, the lack of control from the 3- to 4-leaf application timing is likely due to the presence of emerged weedy rice plants at application, which would not be controlled by acetochlor, as it has little or no effect on emerged seedlings (Babczinski et al. 2012). When averaged across timings, the higher rate of acetochlor increased weedy rice control. In addition, increased rates would likely have more impact at DPRE than EPOST application timings due to aforementioned absorption characteristics.

Main effects of both application timing and rate influenced barnyardgrass, broadleaf signalgrass, and large crabgrass control 2 WAT (see Tables 1 and 2 for p-values). Nontreated plots averaged 5, 3, and 4 plants per  $m<sup>2</sup>$  for barnyardgrass, broadleaf signalgrass, and large crabgrass, respectively, 6 weeks after planting. Overall, control ratings for all species followed trends similar to those observed in weedy rice, in that control generally decreased as application timing was delayed but increased with rate. Averaged across rates, acetochlor DPRE provided ≥89% control of all species 2 WAT; however, control was reduced when applications were delayed to 1- to 2-leaf or 3- to 4-leaf rice.

For all species evaluated, the best control was observed following acetochlor applied DPRE or at 1470 g ai ha<sup>-1</sup>, when averaged over acetochlor rate and application timing, respectively. In contrast, weed control was reduced when acetochlor applications were delayed to 3- to 4-leaf timings or applied at the lower rate. It should be noted that acetochlor applied alone at any timing is not a herbicide program and should not be relied upon to provide season-long control. No postemergence herbicides were applied in these experiments; however, in a seasonlong program with herbicides such as fenoxaprop, imazethapyr, and quizalofop, postemergence herbicides could be used where appropriate to control plants that escaped acetochlor activity (Scott et al. 2018; Buehring et al. 2006).

Overall, rough rice yield followed patterns similar to those observed in weed control; yield decreased as application timing was delayed (Table 2). Treatments that provided superior weed control also had higher rice yields than those that did not. Thus, rice yields were generally higher following the high rate of acetochlor and were maximized at the DPRE and spiking timings. In addition, rice in all treated plots yielded higher than in the nontreated (Table 2).

**Rice Injury and Weed Control Using Pethoxamid.** Rice injury 2 WAT was influenced by the main effects of application timing and rate, with injury generally decreasing at the lower rate and as application timing was delayed (Table 3). Although injury did not exceed 20% for any treatment in either year, rice injury observed in these experiments was slightly higher than reported by Godwin et al. (2017) on a similar soil. Nonetheless, 20% rice injury 2 WAT is not particularly concerning, as all plots recovered to <5% injury by 4 WAT (data not shown). Generally, 1 to 2 cm of rainfall is required to activate VLCFA-inhibiting herbicides (Rao 2000); however, Dhareesank et al. (2006) demonstrated pethoxamid phytotoxicity to rice increases with soil moisture. Increased rice injury in this experiment can be attributed to rainfall events prior to

and just after application, which increased pethoxamid availability in soil while rice was germinating (Figures 1 and 2).

Application timing and rate affected weedy rice control with pethoxamid 2 and 4 WAT (Table 3). The highest weedy rice control was achieved by DPRE and spiking treatments 2 WAT; however, 1- to 2-leaf timings provided comparable control 4 WAT. Generally, weedy rice control decreased as application timing was delayed past spiking (2 WAT) or 1- to 2-leaf timings  $(4$  WAT), and when the 420 g ai ha<sup>-1</sup> rate was used. The value of pethoxamid to reduce weedy rice density at 6 weeks after planting relative to nontreated rice should be noted for all treatments, even though differences among timings were not observed.

Barnyardgrass and broadleaf signalgrass populations in this experiment were similar to those in the acetochlor experiment, averaging four and two plants per  $m<sup>2</sup>$  in the nontreated plots, respectively. At 2 WAT, barnyardgrass and broadleaf signalgrass control was influenced only by the main effect of application timing ( $p < 0.0001$ ); however, by 4 WAT a main effect of both application timing and rate was observed (Table 4). Similar to trends in rice injury and weedy rice control, barnyardgrass and broadleaf signalgrass control with pethoxamid decreased as application timing was delayed and at the lower rate. Pethoxamid applied DPRE controlled barnyardgrass 93 and 78% at 2 and 4 WAT, respectively, while broadleaf signalgrass was controlled 81% and 65%, respectively. Main effects of application timing and rate influenced rice yield in 2016 and 2017 (Table 4). Although there were no differences between DPRE and 1 to 2-leaf applications in 2016 and DPRE and spiking applications in 2017, yield generally decreased as application timing was delayed, likely due to higher weed interference in plots treated at later growth stages. Pethoxamid applied DPRE yielded 1000 and 1,300 kg ha<sup>-1</sup> more than pethoxamid at the 3- to 4-leaf stage in 2016 and 2017, respectively, highlighting the

importance applying VLCFA-inhibiting herbicides prior to weed emergence. Additionally, all pethoxamid treatments, regardless of rate or application timing, yielded higher than the nontreated, demonstrating the value of residual grass control with pethoxamid.

**Practical Implications.** Minimal rice injury, combined with some weedy rice suppression and control of barnyardgrass, broadleaf signalgrass, and large crabgrass in these experiments indicate that acetochlor and pethoxamid could be extremely valuable in providing residual grass control prior to flooding rice. In both experiments, weedy rice and annual grass control decreased as application timing was delayed, with DPRE and spiking timings being the most efficacious. In addition, weed control and rough rice yield increased when the higher rate of either herbicide was used, with little to no increase in crop injury. The decreased control from 3- to 4-leaf rice application timings support the importance of applying chloroacetamides such as acetochlor and pethoxamid prior to weed emergence. However, previous research also demonstrates the ability of VLCFA-inhibiting herbicides to cause significant rice injury when applied at the PRE or DPRE timing, warranting caution when applying prior to rice emergence (Fogleman 2018). The results of these experiments lead to the suggestion that acetochlor or pethoxamid be applied after rice emergence but by the 1- to 2-leaf rice growth stage to maximize weed control and minimize rice injury.

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## **APPENDIX**



Table 1. Rice injury, weedy rice control, reduction of weedy rice density, and barnyardgrass control following early season applications of acetochlor.<sup>a,b,c,d</sup>



<sup>a</sup> Abbreviations: WAT, weeks after treatment; DPRE, delayed preemergence; BYG, barnyardgrass

<sup>b</sup> At 6 weeks after planting, average weedy rice and barnyardgrass density in the nontreated plot was approximately 4 and 5 plants per  $m^2$ , respectively.

 $\epsilon$  Spiking treatments were not made in 2016, therefore rice injury and BYG control were not recorded as indicated by (-).

<sup>d</sup> Means within a column followed by the same lowercase letter are not different according to Fisher's protected LSD at ( $\alpha$  = 0.05). Significant P-values are indicated by (\*)



Table 2. Control of broadleaf signalgrass, large crabgrass, and rough rice yield following early season applications of acetochlor.a,b,c,d

<sup>a</sup> Abbreviations: WAT, weeks after treatment; DPRE, delayed preemergence; BLSG, broadleaf signalgrass; LCG, large crabgrass  $b$  At 6 weeks after planting, broadleaf signalgrass and large crabgrass density in the nontreated plot averaged 3 and 4 plants per  $m^2$ , respectively. Rough rice yield in the nontreated averaged 2700 and 4500 kg ha<sup>-1</sup> in 2016 and 2017, respectively.

 $\degree$  Spiking treatments were not made in 2016; therefore BLSG, LCG, and rough rice yield were not recorded as indicated by (-).

<sup>d</sup> Means within a column followed by the same lowercase letter are not different according to Fisher's protected LSD at ( $\alpha$  = 0.05). Significant P values are indicated by (\*)



Table 3. Rice injury, weedy rice control and reduction of weedy rice density following early-season applications of pethoxamid.<sup>abcd</sup>

<sup>a</sup> Abbreviations: WAT, weeks after treatment; DPRE, delayed preemergence

 $b$  6 weeks after planting, average weedy rice density in the nontreated plot was approximately 4 plants per m<sup>2</sup>.

<sup>c</sup> Spiking treatments were not made in 2016, therefore rice injury was not recorded as indicated by (-).

<sup>d</sup> Means within a column followed by the same lowercase letter are not different according to Fisher's protected LSD at ( $\alpha$  = 0.05). Significant P values are indicated by (\*)



Table 4. Control of barnyardgrass, broadleaf signalgrass and rough rice yield following early season applications of pethoxamid.a,b,c,d

<sup>a</sup> Abbreviations: WAT, weeks after treatment; DPRE, delayed preemergence; BYG, barnyardgrass; BLSG, broadleaf signalgrass <sup>b</sup> 6 weeks after planting, average barnyardgrass and broadleaf signalgrass density in the nontreated plot was approximately 4 and 2 plants per  $m^2$ , respectively. Rough rice yield in the nontreated averaged 1600 and 5600 kg ha<sup>-1</sup> in 2016 and 2017, respectively.  $\overline{c}$  Spiking treatments were not made in 2016, therefore BYG, BLSG, and rough rice yield were not recorded, as indicated by (-). <sup>d</sup> Means within a column followed by the same lowercase letter are not different according to Fisher's protected LSD at ( $\alpha$  = 0.05). Significant P values are indicated by (\*)



Figure 1. Rainfall amount and dates at the Pine Tree Research Station (PTRS) near Colt, AR in 2016. Application dates and timings represent acetochlor and pethoxamid experiments. Abbreviations: delayed preemergence, DPRE; LF, leaf



Figure 2. Rainfall amount and dates at the Pine Tree Research Station (PTRS) near Colt, AR in 2017. Application dates and timing represent acetochlor and pethoxamid experiments. Abbreviations: delayed preemergence, DPRE; LF, leaf

#### **Chapter 5**

#### **Efficacy of Winter-Applied Residual Herbicides on Weedy Rice Control in Rice**

Outside of crop rotation, there are limited options for controlling weedy rice in cultivated rice. Herbicides such as imazethapyr have been successful in controlling weedy rice in imidazolinoneresistant (Clearfield) rice since commercialization in 2002; however, imidazolinone-resistant weedy rice has been documented and continues to increase throughout the southern U.S. rice growing region. Very-long-chain fatty acid (VLCFA)-inhibiting herbicides are not currently labeled for use in U.S. rice production but have been successfully used in Asian rice production and in U.S. row crops for control of annual grasses and small-seeded broadleaves. An experiment was initiated in the fall of 2016 and continued in the spring of 2017, to evaluate rice tolerance and weedy rice control following fall-applied VLCFA-inhibiting herbicides. A splitplot design was used for the experiment, with the whole-plot factor being winter condition (flooded or non-flooded), and the split-plot factors being herbicide and rate [acetochlor at 1050 and 2100 g ai ha<sup>-1</sup>, dimethenamid-P at 525 and 1050 g ai ha<sup>-1</sup>, pethoxamid at 420 and 840 g ai ha<sup>-</sup> <sup>1</sup>, pyroxasulfone at 205 and 410 g ai ha<sup>-1</sup>, and *S*-metolachlor at 1070 and 2140 g ai ha<sup>-1</sup>]. Rice injury did not exceed 11% 7 days after emergence (DAE) for any treatment evaluated. Generally, herbicides performed better under aerobic (non-flooded) winter conditions and when applied at the higher rate. Overall, acetochlor and pyroxasulfone provided the highest levels of weedy rice control 3, 5, and 7 weeks after planting (WAP). Dimethenamid-P and *S*-metolachlor provided control comparable to acetochlor and pyroxasulfone 3 WAP but generally were not comparable 5 and 7 WAP. Acetochlor, pyroxasulfone, and dimethenamid-P also reduced weedy rice density 48 to 69% relative to nontreated plots 5 WAP, highlighting the length of residual control provided by these herbicides. Based on this research, acetochlor and pyroxasulfone applied in the fall provides some weedy rice control the subsequent spring with minimal injury to drill-seeded rice.

**Nomenclature:** weedy rice, *Oryza* spp.; rice, *Oryza sativa* L.; acetochlor, dimethenamid-P, pethoxamid, pyroxasulfone, *S*-metolachlor

**Key words:** Very-long-chain fatty acid-inhibiting herbicides, weedy rice, fall-applied herbicides, rice tolerance

#### **INTRODUCTION**

Weedy rice (also referred to as red rice) is one of the most problematic weeds of U.S. rice production and was ranked as the fourth most problematic weed in Arkansas rice, behind barnyardgrass [*Echinochloa crus-galli* (L). Beauv.], sprangletops (*Leptochloa* spp.), and Northern jointvetch [*Aeschynomene virginica* (L.) B.S.P.] (Norsworthy et al. 2013). Direct competition with cultivated rice during the season, and seed contamination at harvest reduce grain yield and grain quality (Diarra et al. 1985; Kwon et al. 1991; Ottis et al. 2005); thus, weedy rice has been classified as a noxious weed in the U.S. Similar physiological and morphological features of weedy rice and cultivated rice make it extremely difficult to differentiate between them early in the season, and impossible to selectively control in rice fields (Pantone and Baker 1991). Prior to the  $21<sup>st</sup>$  century, weedy rice was controlled using water-seeding techniques and crop rotation with alternative crops such as soybean [*Glycine max* (L.) Merr.], corn (*Zea mays*  L.), and grain sorghum [*Sorghum bicolor* (L.) Moench] (Burgos et al. 2008).

In 2002, a non-transgenic, imidazolinone-resistant rice (Clearfield BASF Corporation, Research Triangle Park, NC) was commercialized, which allowed over-the-top use of imazethapyr, an acetolactate synthase (ALS)-inhibiting herbicide, in rice for control of weedy rice. The Clearfield technology has been successful, providing 95 to 100% control of weedy rice, and can be combined with other rice herbicides such as propanil or quinclorac for excellent postemergence control of other grass and broadleaf weeds (Avila et al. 2005; Levy et al. 2006; Ottis et al. 2004; Steele at al. 2002).

Despite high levels of weedy rice control, it is inevitable that escapes occur in the field, regardless of herbicide, due to various environmental, biological, and application factors. Weedy rice escapes are particularly problematic because flowering often occurs simultaneously in

weedy rice and cultivated rice; thus, when weedy rice is not controlled, herbicide-resistance genes can be transferred into weedy rice, creating herbicide-resistant weedy rice (Shivrain et al. 2007). Although the outcrossing rate is reported to be low (0.109 to 0.434%), the fecundity of weedy rice could easily turn a few escapes into an infestation of herbicide-resistant plants in a commercial field (Burgos et al. 2007). In addition to outcrossing concerns, the repetitive use of imidazolinone herbicides in the early to mid-2000s placed significant selection on weed populations, increasing the frequency of resistance alleles, and ultimately leading to herbicide resistance (Jasieniuk et al. 1996). Stewardship guidelines focused on mitigating resistance by minimizing escapes, crop rotation, and use of alternative herbicides were effective in most cases but exacerbated the issue when not followed. Today, 11 species have evolved resistance to ALSinhibiting herbicides in Arkansas including weedy rice, barnyardgrass, junglerice [*Echinochloa colona* (L.) Link], and Palmer amaranth [*Amaranthus palmeri* (S.) Wats.] (Heap 2018). The evolution of herbicide resistance to ALS and other herbicide SOAs in problematic rice weeds has limited effective control options.

Very-long-chain fatty acid (VLCFA)-inhibiting herbicides are currently labeled in U.S. row crop production for control of annual grasses and small-seeded broadleaf weeds (Knowles 1998). Very-long-chain fatty acid-inhibitors (WSSA Group 15) are soil-residual herbicides that are absorbed primarily by shoots and secondarily through roots of germinating seedlings, where cell development is inhibited by interfering with protein synthesis (Anonymous 2017). Group 15 herbicides such as *S*-metolachlor, pyroxasulfone, and acetochlor provide weedy rice control in U.S. row crops but are not currently labeled for use in rice.

Khodayari et al. (1987) demonstrated the efficacy of VLCFA-inhibitors on weedy rice control in soybean. When applied preplant incorporated, metolachlor at  $2.2$  kg ai ha<sup>-1</sup> or alachlor

at 3.6 kg ai ha<sup>-1</sup> provided >90% control of weedy rice. Zemolin et al. (2014) evaluated susceptible and herbicide-resistant weedy rice control in soybean using preemergence (PRE) and early postemergence (EPOST) applications of *S*-metolachlor at 768, 1152, and 1680 g ai ha<sup>-1</sup>. Without the addition of glyphosate, EPOST applications of *S*-metolachlor were not effective; however, *S*-metolachlor applied PRE provided 74 to 84% and 53 to 64% control of weedy rice 28 days after application in the first and second year of the study, respectively. In addition, there was no significant effect of weedy rice biotype, indicating that imidazolinone-susceptible and imidazolinone-resistant weedy rice populations were equally sensitive to *S*-metolachlor.

From 1997 to 1999, studies were conducted in Greece to evaluate PRE applications of alachlor  $(2.40 \text{ kg}$  ai ha<sup>-1</sup>), dimethenamid  $(1.44 \text{ kg}$  ai ha<sup>-1</sup>), metolachlor  $(2.50 \text{ kg}$  ai ha<sup>-1</sup>), and acetochlor  $(1.54 \text{ kg}$  ai ha<sup>-1</sup>) on weedy rice control in water-seeded rice. All herbicide treatments caused a significant reduction in weedy rice stems and panicles, relative to the nontreated; however, alachlor, metolachlor, and acetochlor provided the best weedy rice control at 92%, while dimethenamid provided slightly less control at 84% (Eleftherohorinos and Dhima 2002).

Although previous studies have indicated adequate crop tolerance and weedy rice control using VLCFA-inhibiting herbicides in row crops and water-seeded rice, there has been limited research in drill-seeded rice. Earlier studies on VLCFA-inhibitor use in drill-seeded rice by Godwin et al. (2017) led to the conclusion that rice is adequately tolerant to acetochlor and pethoxamid; however, application timing and rate greatly influence crop injury. In addition, PRE and DPRE applications of acetochlor and pethoxamid can be used to control weedy rice and annual grasses in drill-seeded rice (Fogleman et al. 2018; Godwin et al. 2017); however, these application timings also pose significant risk to growers, as severe crop injury can result from rainfall soon after application.

In addition, studies by Bond et al. (2014) demonstrated that fall-applied residual herbicides can provide superior glyphosate-resistant (GR) Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) control, compared to fall tillage. Herbicides evaluated in this study included clomazone, flumioxazin, pendimethalin, pyroxasulfone, *S*-metolachlor, and trifluralin. Most treatments provided adequate control of GR Italian ryegrass 100 DAT; however, control generally decreased at 140 and 180 DAT. By 180 DAT, clomazone, pyroxasulfone, *S*metolachlor, and incorporated pendimethalin or trifluralin provided >83% control. The study concluded that fall-applied *S*-metolachlor, clomazone, and pyroxasulfone ultimately provided the best control of GR Italian ryegrass.

A similar study by Lawrence et al. (2018) evaluated the effect of fall-applied clomazone, pyroxasulfone, *S*-metolachlor, and trifluralin on rice growth and yield. At 14 DAE, rice seedling density and height were negatively affected by all herbicides, except clomazone. Averaged across pyroxasulfone rate (170 and 340 g ai  $ha^{-1}$ ), rice injury and shoot density was 37 and 72% of the nontreated, respectively, 14 DAE. Similarly, rice injury and relative shoot density in plots treated with *S*-metolachlor (1420 and 2840 g ai ha<sup>-1</sup>) was 30 and 73%, respectively. By 28 DAE, rice injury from the lower rate of pyroxasulfone and *S*-metolachlor had declined to 17 and 9%, respectively. Regardless of application rate, plots treated with pyroxasulfone or *S*-metolachlor yielded >90% of the nontreated and did not differ from clomazone-treated plots when clomazone was applied at the lower rate.

Winter flooding of rice fields is a common practice in the southern U.S. rice-producing region for habitat conservation and hunting of local and migratory waterfowl (Eadie et al. 2008). Not only does flooding facilitate habitat management, but benefits growers by reducing viability of weed seed, decreasing soil erosion, and promoting decomposition of rice straw (Anders et al.

2008; Manley et al. 2005). In addition, waterfowl that find refuge in flooded fields are reported to enhance straw decomposition through trampling (Manley et al. 2005) and even feed on waste rice, suggesting that weed seed populations could be diminished, although this has not been proven (Brogi et al. 2015; Suh 2014).

Limited options for controlling weedy rice in rice, fecundity of escaped plants, and longevity of weedy rice seed in the soil seedbank has led to the exploration of other means of control. Due to the prevalence of weedy rice and inability for growers to control weedy rice in non-Clearfield rice systems, growers could potentially reduce population size in the soil seedbank and limit weedy rice emergence early-season by applying residual herbicides in the fall. Thus, an experiment was conducted to evaluate the efficacy of flooded and non-flooded fallapplied VLCFA-inhibiting herbicides on weedy rice control the following spring.

### **MATERIALS AND METHODS**

**Experimental Site.** A field experiment was initiated in September 2016 on a Calloway silt loam (Fine-silty, mixed, active, thermic Aquic Fraglossudalfs) at the Pine Tree Research Station (PTRS) near Colt, AR. The soil at the PTRS was representative of rice fields in Arkansas with a pH of 7.5, 1.3% organic matter, 10.6% sand, 68.6% silt, and 20.8% clay.

**Experimental Setup and Data Collection.** The experiment was conducted as split-plot design with the whole-plot factor being flooded or non-flooded winter conditions, and the split-plot being a factorial arrangement of herbicide and rate in a randomized complete block. A weedy, nontreated and a weed-free nontreated was included in all four replications for comparison of rice injury and weedy rice control. In 2016, cultivar 'XL753' was mixed with weedy rice and sown at 33 seed  $m^{-1}$  of row. To ensure adequate weedy rice populations in the subsequent spring,

cultivated rice and weedy rice were allowed to compete throughout the growing season without any herbicide applications. At maturity, the area was mowed to disperse seeds across the soil surface and then the remaining foliage was burned approximately 7 days later.

Fall herbicide applications were made on September 28, 2016, using a  $CO<sub>2</sub>$ -pressurized backpack sprayer calibrated to deliver 140 L ha<sup>-1</sup> through AIXR 110015 (TeeJet Technologies, Wheaton, IL) nozzles. Acetochlor (Warrant herbicide, Monsanto Company, St. Louis, MO) was applied at 1050 and 2100 g ai ha<sup>-1</sup>, dimethenamid-P (Outlook herbicide, BASF Corporation, Research Triangle Park, NC) at 525 and 1050 g ai ha<sup>-1</sup>, pethoxamid (FMC Corporation, Philadelphia, PA) at 420 and 840 g ai ha<sup>-1</sup>, pyroxasulfone (Zidua herbicide, BASF Corporation, Research Triangle Park, NC) at 205 and 410 g ai ha<sup>-1</sup>, and S-metolachlor (Dual II Magnum herbicide, Syngenta Crop Protection LLC, Greensboro, NC) at 1070 and 2140 g ai ha<sup>-1</sup>. Plots that received flooding treatments were flooded on November 8, 2016, and remained flooded until they were drained on February 10, 2017.

Flooded and non-flooded bays were treated with glyphosate (Roundup PowerMA $X^{\circledast}$ II, Monsanto Company, St. Louis MO) at 1.26 kg ae ha<sup>-1</sup> on March 29, 2017, to control winter annuals. Aside from this application, plots were left undisturbed from herbicide application in the fall until planting in the spring. 'CL172' was drill-seeded using a no-till drill on April 6, 2017, at 72 seeds  $m^{-1}$  of row into 1.8- by 6.1-m plots. Immediately following planting, clomazone (Command herbicide, FMC Corporation, Philadelphia, PA) at 336 g ai ha<sup>-1</sup> + glyphosate at 1.26 kg ae ha<sup>-1</sup> was applied to the entire experiment to simulate the beginning of a standard rice herbicide program in Arkansas.

Visual estimates of rice injury and shoot density  $m^{-1}$  of row were recorded 7 and 28 DAE, respectively, while visual estimates of weedy rice control and density per  $m<sup>2</sup>$  were evaluated 3, 5, and 7 WAP. Rice injury and weedy rice control were recorded on a scale of 0 to 100, with 0 being no injury or control, and 100 being complete crop death or control. Weedy rice densities were determined by counting the number of plants in two  $1-m^2$  quadrats in each plot and then calculating the mean. To ensure accurate assessments of weedy rice emergence, the posterior 1.5 m of each plot was treated with a glyphosate-soaked roller after each evaluation. Because no postemergence treatments were evaluated in this experiment, plots were not harvested at maturity due to excessive weediness.

**Statistical Analysis.** Data were tested for normality using the Shapiro-Wilk Test. All data were subjected to analysis of variance as a split-plot design using the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc., Cary, NC) assuming beta distribution for rice injury, weedy rice control, rice shoot density, and weedy rice density (Gbur et al. 2012). Rice shoot densities were converted to proportions of the average density in weed-free nontreated plots within each replication and presented as a percent of the nontreated. Weedy rice densities were converted to proportions of the average density in weedy nontreated plots within each replication and presented as percent reduction from the nontreated. Means were separated using Fisher's protected LSD ( $\alpha$ =0.05).

#### **RESULTS AND DISCUSSION**

**Rice Injury.** Main effects of winter condition, herbicide, and application rate influenced rice injury but no interactions among factors were observed (Table 1). None of the treatments evaluated resulted in >11% rice injury 7 DAE (Table 2), and no injury was observed thereafter (data not shown). When averaged over herbicide and rate, rice injury was greatest under nonflooded winter conditions. Likewise, when averaged over winter condition and rate, rice injury was greatest following acetochlor, dimethenamid-P, and pyroxasulfone applications and when averaged over winter condition and herbicide, the higher rate exacerbated rice injury (Table 2). Although rice injury was statistically different within whole-plot and split-plot factors, it should be noted that values differed by only one to five percentage points, which is not particularly concerning given the scale. Lawrence et al. (2018) also observed rice injury following fall applications of pyroxasulfone, although to a greater extent. Reduced pyroxasulfone injury in the current study is likely due to the fact that herbicide applications were made in late September, in comparison to early November in the Lawrence et al. (2018) study, which may have allowed more herbicide degradation prior to planting rice.

A three-way interaction influenced rice shoot density 28 DAE (p= 0.0072) (Table 1). Shoot densities in non-flooded plots that were treated with the low rate of dimethenamid-P, pyroxasulfone, acetochlor, or either rate of *S*-metolachlor did not differ from the flooded or nonflooded nontreated plots, which averaged 51 and 52 plants  $m^{-1}$  of row, respectively (data not shown). In contrast, shoot density was approximately 66% of the nontreated in plots that received the high rate of acetochlor and were flooded after application (Table 3). In general, shoot densities were higher under non-flooded winter conditions and when herbicides were applied at the low rate; however, shoot densities were comparable between low and high rates within some herbicides. Considering the low levels of rice injury overall, it is likely that rice seed continued to emerge after injury was evaluated 7 DAE, which would explain why rice injury and shoot density were both greater in non-flooded winter conditions. In addition, rice in those plots that were injured initially likely compensated for stand loss and stunted growth by producing more tillers (Yoshida 1981).

Increased rice injury in plots that were not flooded in the winter was expected, as chloroacetamide herbicides are known to break down more rapidly in anaerobic than aerobic soils (Loor-Vela et al. 2003). In flooded environments, water fills air spaces between colloids in the soil profile, thus forcing air out and producing anaerobic conditions. Furthermore, the rate of decomposition and herbicide persistence in the field is influenced by several factors including chemical and physical soil characteristics, herbicide properties, application rate, and temperature (Kotoula-Syka et al. 1997; Loor-Vela et al. 2003). Since the herbicides in this experiment were subject to the same environmental conditions, we can infer that the differences observed in rice injury are mainly a function of herbicidal properties (Table 4) and application rates. The objectives of this study were not to compare herbicide properties and persistence in flooded and non-flooded soils, but perhaps those herbicides that caused more rice injury degrade more slowly than others, or at least were less affected by degradation under the given conditions.

**Weedy Rice Control.** A significant three-way interaction influenced weedy rice control at 3 (p= 0.0177) and 5 WAP (p= 0.0009), while only main effects of winter condition, herbicide, and application rate influenced weedy rice control 7 WAP (Table 1). Overall, the greatest control of weedy rice was observed soon after planting and generally declined with time. Although herbicide degradation processes generally decrease under cooler air and soil temperatures (Curran 2001), complete weedy rice control was not expected since degradation is still likely to occur to some extent in the 210 days between application and the first evaluation of weedy rice. However, given the current state of herbicide-resistant weedy rice in the southern U.S., any PRE control or suppression of weedy rice would be considered advantageous.

At 3 WAP, weedy rice control ranged 31 to 65% (Table 3). In general, herbicides provided better weedy rice control when they were applied at the higher rate and were not

flooded in the winter. Within non-flooded winter conditions, the higher rates of dimethenamid-P and pyroxasulfone or either rate of acetochlor provided the best weedy rice control. The higher rate of acetochlor provided the best control under flooded winter conditions; however, both application rates of dimethenamid-P and pyroxasulfone provided control comparable to the lower rate of acetochlor (Table 3). Although weedy rice control was generally reduced in plots treated with *S*-metolachlor or pethoxamid, control was more comparable to acetochlor, dimethenamid-P, and pyroxasulfone under non-flooded than flooded winter conditions.

Overall, weedy rice control decreased from 3 WAP to 5 WAP. Similar to observations 3 WAP, control was generally improved in plots that were not flooded after application in the fall; however, there were no differences in control between flooded and non-flooded winter conditions when the higher rate of pethoxamid or either rate of pyroxasulfone was applied. Acetochlor distinguished itself as the superior herbicide, providing the best overall control 5 WAP at the high rate when not flooded in the winter (Table 3). Weedy rice control from acetochlor applications ranged from 45 to 56%, and 30 to 40% in non-flooded and flooded winter conditions, respectively. Pyroxasulfone was statistically comparable to acetochlor in flooded winter conditions; however, weedy rice control was reduced, relative to acetochlor, in non-flooded winter conditions. By 5 WAP, *S*-metolachlor, and pethoxamid, were noticeably losing efficacy, especially in plots that were flooded in the winter.

At 5 WAP a main effect of herbicide was observed for weedy rice reduction ( $p= 0.0053$ ), where acetochlor, pyroxasulfone, and dimethenamid-P provided greatest reduction in population density relative to the flooded and non-flooded nontreated, which averaged 16 and 23 plants per m<sup>2</sup>, respectively (Table 5). Although there were no interactions among factors, these results

coincide with visual observations of weedy rice control, where acetochlor, pyroxasulfone and dimethenamid-P were generally the most efficacious herbicides (Table 3).

By 7 WAP, all herbicides continued to lose efficacy. Although there were no significant interactions among factors, weedy rice control was influenced by main effects of all three factors, and overall trends remained consistent (Table 1). When averaged across herbicide and rate, plots that were not flooded in the winter controlled weedy rice better than those that were flooded ( $p= 0.0051$ ). In addition, acetochlor continued to provide the highest level of weedy rice control at 28%, while control from pyroxasulfone was slightly reduced at 19%, and dimethenamid-P, pethoxamid, and *S*-metolachlor controlled weedy rice <11%. Nonetheless, it should be noted that all treatments were still providing some level of weedy rice suppression approximately 230 days after applications were made in the winter. The extended residual control from acetochlor and pyroxasulfone is likely due to herbicidal properties. In particular, acetochlor was applied as Warrant, a microencapsulated formulation. In the microencapsulated formulation, herbicide molecules are protected from degradation processes by a polymer shell, which slowly releases herbicide after absorbing water and thus offers longer residual control (Rao 2000).

The reason that herbicides in this study provided control of weedy rice but did not injure cultivated rice in the same manner is not clear. One explanation could be that weedy rice seeds absorbed high concentrations of herbicide over the winter months, and growth became inhibited in the spring when temperatures were conducive for germination. Future studies should identify the survival of weedy rice populations after being exposed to VLCFA-inhibiting herbicides under temperatures that promote dormancy followed by temperatures conducive for germination to further understand observations of this study.

**Practical Applications.** The results of this experiment indicate that acetochlor, dimethenamid-P, pethoxamid, pyroxasulfone, and *S*-metolachlor can be applied in the fall after harvest with minimal injury to cultivated rice the following spring. However, none of these herbicides are currently labeled for use in rice production, and all have recommended plant-back intervals that exceed the ~6-month period evaluated in this experiment (Scott et al. 2018). Although <12% rice injury was observed for all treatments, plots that were not flooded after application in the fall were injured more than those that were flooded, and higher injury was associated with acetochlor, dimethenamid-P, and pyroxasulfone, especially when applied at the higher rate (Table 2).

Treatments that caused the highest rice injury generally also provided the highest levels of weedy rice control throughout the season. Overall, acetochlor and pyroxasulfone were the most effective herbicides evaluated in this experiment, although dimethenamid-P, pethoxamid, and *S*-metolachlor provided comparable early-season control under non-flooded winter conditions when applied at the higher rate. At later evaluations, pethoxamid and *S*-metolachlor were often responsible for the lowest levels of weedy rice control and appeared to lose efficacy more quickly than the other herbicides.

It should be noted that the results of this experiment are based on one year of data; therefore, it is imperative that this experiment be repeated under similar conditions to confirm or refute the observations made thus far. Nonetheless, data presented here indicate the potential for fall-applied VLCFA-inhibitors to provide rice growers with an alternative means of managing weedy rice in cultivated rice. Should VLCFA-inhibiting herbicides be labeled for use in U.S. rice, fall applications to fields with severe weedy rice infestations or where populations of imidazolinone-resistant weedy rice are known to exist should improve in-season control.

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# **APPENDIX**

Table 1. Significance of P-values for interactions and main effects of winter condition, herbicide, and application rate on rice injury and shoot density, and weedy rice control.<sup>a,b</sup>



<sup>a</sup> Abbreviations: DAE, days after emergence; WAP, weeks after planting



Table 2. Rice injury as influenced by main effects of winter condition, herbicide, and rate.<sup>a,b</sup>

<sup>a</sup> Abbreviations: DAE, days after emergence; low, acetochlor (1050 g ai ha<sup>-1</sup>), dimethenamid-P (525 g ai ha-1 ), pethoxamid (420 g ai ha-1 ), pyroxasulfone (205 g ai ha-1 ), *S*-metolachlor (1070 g ai ha<sup>-1</sup>); high, acetochlor (2100 g ai ha<sup>-1</sup>), dimethenamid-P (1050 g ai ha<sup>-1</sup>), pethoxamid (840 g ai ha<sup>-1</sup>), pyroxasulfone (410 g ai ha<sup>-1</sup>), *S*-metolachlor (2140 g ai ha<sup>-1</sup>)

<sup>b</sup> Means within a factor followed by the same letter are not significantly different according to Fisher's protected LSD  $(\alpha=0.05)$ 



Table 3. Relative shoot density and weedy rice control as influenced by interactions between winter condition, herbicide, and rate.<sup>a,b,c</sup>

<sup>a</sup> Abbreviations: DAE, days after emergence, WAP, weeks after planting; low, acetochlor (1050 g ai ha<sup>-1</sup>), dimethenamid-P (525 g ai ha<sup>-1</sup>), pethoxamid (420 g ai ha<sup>-1</sup>), pyroxasulfone (205 g ai ha<sup>-1</sup>), *S*-metolachlor (1070 g ai ha<sup>-1</sup>); high, acetochlor (2100 g ai ha<sup>-1</sup>), dimethenamid-P (1050 g ai ha<sup>-1</sup>), pethoxamid (840 g ai ha<sup>-1</sup>), pyroxasulfone (410 g ai ha<sup>-1</sup>), *S*-metolachlor  $(2140 \text{ g ai ha}^{-1})$ 

 $<sup>b</sup>$  Means within a column followed by the same letter are not significantly different according</sup> to Fisher's protected LSD  $(α=0.05)$ 

 $\rm c$  Shoot density in the flooded and non-flooded nontreated plots averaged 51 and 52 plants m<sup>-1</sup>, respectively

| Herbicide      | Half-life | Water solubility | $K_{oc}$    |
|----------------|-----------|------------------|-------------|
|                | days      | $mg L^{-1}$      | mL $g^{-1}$ |
| acetochlor     | 12        | 223              | 200         |
| dimethenamid-P | 20        | 1174             | 155         |
| pethoxamid     | 15        | 400              | 154         |
| pyroxasulfone  | 16-26     | 3.49             | 57-114      |
| S-metolachlor  | $15 - 25$ | 488              | 200         |

Table 4. Half-life, water solubility, and  $K_{\infty}$  values for evaluated herbicides in soil.

Source: U.S. EPA

 $K_{oc}$  = soil organic carbon sorption coefficient

| Factor         | Weedy rice 5 WAP    |  |
|----------------|---------------------|--|
|                | % reduction         |  |
| Herbicide      |                     |  |
| acetochlor     | 69a                 |  |
| dimethenamid-P | 48 ab               |  |
| pethoxamid     | 36 h                |  |
| pyroxasulfone  | 60.<br><sub>a</sub> |  |
| S-metolachlor  | $\mathbf{b}$<br>26. |  |
|                |                     |  |

Table 5. Percent weedy rice reduction relative to the nontreated as influenced by herbicide.<sup>a,b,c</sup>

<sup>a</sup> Abbreviations: WAP, weeks after planting

<sup>b</sup> Means followed by the same letter are not significantly different according to Fisher's protected LSD  $(\alpha=0.05)$ 

<sup>c</sup> Weedy rice density in the flooded and non-flooded nontreated plots averaged 16 and 23 plants per  $m^2$ , respectively



Table 6. Weedy rice control 7 weeks after planting as influenced by winter condition, herbicide, and rate. $a,b$ 

<sup>a</sup> Abbreviations: WAP, weeks after planting; low, acetochlor (1050 g ai ha<sup>-1</sup>), dimethenamid-P (525 g ai ha-1 ), pethoxamid (420 g ai ha-1 ), pyroxasulfone (205 g ai ha-1 ), *S*-metolachlor (1070 g ai ha<sup>-1</sup>); high, acetochlor (2100 g ai ha<sup>-1</sup>), dimethenamid-P (1050 g ai ha<sup>-1</sup>), pethoxamid (840 g ai ha<sup>-1</sup>), pyroxasulfone (410 g ai ha<sup>-1</sup>), *S*-metolachlor (2140 g ai ha<sup>-1</sup>)

<sup>b</sup> Means within a factor followed by the same letter are not significantly different according to Fisher's protected LSD  $(\alpha=0.05)$