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Documentation and Control of Acetolactate Synthase-Resistant Barnyardgrass (*Echinochloa Crus-galli*) in Arkansas Rice

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DOCUMENTATION AND CONTROL OF ACETOLACTATE SYNTHASE-RESISTANT
BARNYARDGRASS (*ECHINOCHLOA CRUS-GALLI*) IN ARKANSAS RICE

DOCUMENTATION AND CONTROL OF ACETOLACTATE SYNTHASE-RESISTANT
BARNYARDGRASS (*ECHINOCHLOA CRUS-GALLI*) 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

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University of Arkansas
Bachelor of Science in Crop Management, 2009

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ABSTRACT

Barnyardgrass, the most problematic grass weed of Arkansas rice, was found resistant to propanil in 1990, and since then, it has evolved resistance to quinclorac and clomazone. Barnyardgrass is now believed to have evolved resistance to acetolactate synthase (ALS)-inhibiting herbicides. The goal of this research was to confirm and determine the level of resistance of the putative resistant biotype to the ALS-inhibiting herbicides imazethapyr, bispyribac, and penoxsulam and to develop herbicide programs for controlling ALS-, propanil-, quinclorac-, and clomazone-resistant barnyardgrass. The lethal dose needed to kill 50% of the putative ALS-resistant plants was higher than that of the susceptible biotype and greater than the field use rate of imazethapyr, bispyribac, and penoxsulam, indicating cross-resistance. The ALS-resistant biotype was also resistant to imazethapyr, propanil (photosystem II-inhibitor), and quinclorac (synthetic auxin). In the field, two applications of imazethapyr alone failed to control the ALS-resistant biotype (<43%); however, when imazethapyr was applied early postemergence followed by imazethapyr + fenoxaprop pre-flood, barnyardgrass control improved. When imazethapyr was applied twice following preemergence or delayed preemergence application of other herbicides, acceptable control was obtained with or without the addition of fenoxaprop pre-flood. Herbicide programs were developed that effectively controlled multiple-resistant biotypes, and some single-application programs consisting of three or four herbicides were as effective as multiple applications in providing season-long control.

This thesis is approved for recommendation
to the Graduate Council.

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Confirmation and Control of Acetolactate Synthase-Resistant Barnyardgrass in Arkansas

Barnyardgrass is the most problematic weed in Arkansas rice, infesting almost all Arkansas rice hectares and causing yield and quality reduction. Biotypes resistant to propanil, quinclorac, and clomazone exist. Intensive use of the acetolactate synthase (ALS)-inhibiting herbicides imazethapyr, penoxsulam, and bispyribac in imidazolinone-resistant (Clearfield) rice increases the risk of the evolution of ALS-resistant barnyardgrass. In 2009, imazethapyr failed to control barnyardgrass collected from a field in Arkansas following failure of the herbicide in 2008. A greenhouse experiment was conducted to confirm and document the level of resistance of the biotype against three ALS-inhibiting herbicides currently labeled in rice. The mortality of barnyardgrass evaluated in response to ten rates of imazethapyr, penoxsulam, and bispyribac applied at 1/32 to 32 times the labeled rate of rice for the resistant biotype and 1/128 to 4 times the labeled rate for a susceptible biotype. Control of the resistant biotype at the labeled rate of bispyribac was 10%, penoxsulam 0%, and imazethapyr 25%. Mortality of the susceptible biotype was 100% with all herbicides at the labeled rate. The dose needed to kill 50% of the resistant plants was 49 g ha⁻¹ of bispyribac, 254 g ha⁻¹ of penoxsulam, and 170 g ha⁻¹ of imazethapyr. For the susceptible biotype, bispyribac at 6 g ha⁻¹, penoxsulam at 10 g ha⁻¹, and imazethapyr at 12 g ha⁻¹ killed 50% of the treated plants. Based on these findings, it was confirmed that a barnyardgrass population has evolved cross-resistance to several ALS-inhibiting herbicides in rice culture in Arkansas. Furthermore, an experiment was conducted to determine if the ALS-resistant biotype was resistant to other mechanisms of action. The results indicate that propanil, a photosystem II inhibitor (PS II), and quinclorac, a synthetic auxin, failed to control the resistant biotype at the labeled rates.

Nomenclature: Barnyardgrass, *Echinochloa crus-galli* L. Beauv.

Introduction

Since the release of ALS-inhibiting herbicides, which include herbicides in the sulfonylurea, imidazolinone, sulfonylamino triazolopyrimidine, carbonyl triazolinone, and pyrimidinyl thiobenzoate groups, this mechanism of action has been used extensively to provide weed control in a wide array of crops (Tranel and Wright 2002). However, intensive use of these herbicides quickly led to the evolution of ALS-resistant weeds. In 1987, the first ALS-resistant weeds [prickly lettuce (*Lactuca serriola* L.) and kochia (*Kochia scoparia* (L.) Schrad.)] were documented (Mallory-Smith et al. 1990; Primiani et al. 1990). Since then, the number of resistant weed biotypes has increased at a faster rate than with any other herbicide mechanism of action, albeit the ALS-inhibiting herbicides are still an effective option for weed control in many crops globally (Heap 2012). Frequent use of ALS-inhibiting herbicides over large areas, limited use of other mechanisms of action in combination with these herbicides, and residual activity of these herbicides has contributed vastly to the evolution of resistance to this mechanism of action (Tranel and Wright 2002). By 1998, ALS-inhibiting herbicides had surpassed all other mechanisms of action for the number of resistant weeds and still today exceeds all other mechanisms of action.

Resistance to an ALS-inhibiting herbicide from one family sometimes confers resistance to ALS-inhibiting herbicides in different families. Herbicide resistance across chemical families within a mechanism of action is referred to as ‘cross-resistance’ (Herbicide Resistance Action Committee 2012). For example, a single-point mutation in the ALS enzyme may provide resistance to both the sulfonylurea and imidazolinone herbicide families. There are many different mutations and amino acid substitutions that can endow a plant with resistance to more than one family of ALS-inhibiting herbicides (HRAC 2012). A weed manifesting resistance to a

sulfonylurea herbicide may also manifest resistance to an imidazolinone herbicide. However, some resistant weed biotypes also possess resistance to other herbicide mechanisms of action, and this type of resistance is known as multiple-resistance (Hager and Refsell 2008; HRAC 2012). In the latter case, a plant resistant to an ALS-inhibiting herbicide may also be resistant to a photosystem II (PSII)-inhibiting herbicide or any other mechanism of action.

Barnyardgrass is the most problematic grass weed in Arkansas rice (Norsworthy et al. 2007). Herbicide-resistant barnyardgrass has been a problem for rice growers since the early 1990s when propanil-resistant barnyardgrass was reported (Baltazar and Smith 1994; Carey 1994). Propanil is a Group 7, PSII-inhibiting herbicide that was used on approximately 98% of the rice hectares in Arkansas by 1990 (Carey et al. 1995; Malik et al. 2010). Quinclorac, a Group 4, synthetic auxin commercialized in 1992, became the main option for barnyardgrass control following the evolution of propanil resistance; but quinclorac-resistant barnyardgrass had evolved by 1998 (Heap 2012; Lovelace et al. 2002; Lovelace et al. 2007; Malik et al. 2010; Talbert and Burgos 2007). Clomazone was commercialized in 2002 and is currently used on approximately 80% of the Arkansas rice hectares for the control of barnyardgrass (Dr. Charles Wilson Jr., personal communication). Repeated use of clomazone led to confirmation of a clomazone-resistant barnyardgrass biotype in 2008 (Norsworthy et al. 2008).

Because barnyardgrass has a history of evolving resistance to different mechanisms of action and because resistance to the ALS mechanism of action occurs frequently, the evolution of ALS-resistant barnyardgrass is inevitable. Resistance is especially expected in Clearfield rice [imidazolinone-resistant varieties that allow use of imazethapyr for barnyardgrass and red rice (*Oryza sativa*) control] (Norsworthy et al. 2007; Ottis et al. 2003; Steele et al. 2000; White and Hackworth 1999). The percentage of Clearfield rice hectares in Arkansas have increased each

year since release of this technology. Approximately 45% of rice in 2009 and 55% of rice in 2010 was planted to Clearfield varieties (Wilson et al. 2010). An increase in the use of Clearfield varieties has led to an increase in the use of the ALS-inhibiting herbicides imazethapyr and imazamox. Furthermore, penoxsulam and bispyribac are two additional ALS-inhibiting herbicides that are labeled for use in Arkansas rice for controlling barnyardgrass.

A field in Delaplaine, Arkansas, was planted in Clearfield rice in 2008, and multiple applications of imazethapyr failed to control existing barnyardgrass plants in the field. Plant samples from the field were received at the University of Arkansas Altheimer Laboratory for screening in the late fall of 2008. Compared to a susceptible standard, the sample appeared resistant to imazethapyr at the labeled rate of 70 g ai ha⁻¹.

The goals of this research were to 1) determine the level of imazethapyr resistance possessed by the resistant biotype compared to a susceptible biotype and 2) evaluate the putative resistant biotype for cross- and multiple-resistance. Dose response experiments were conducted to quantify the response of the two biotypes (resistant and susceptible) to imazethapyr and additional ALS-inhibiting herbicides. Conducting a dose response study allows one to understand the relationship between herbicide dose and plant response or the sensitivity of a weed to a particular herbicide (Seefeldt et al. 1995). The sigmoidal curves produced by these studies provide an estimate of herbicide efficacy on the particular species being tested (Seefeldt et al. 1995). The level of resistance to imazethapyr was established by determining the lethal dose needed to kill 50% (LD₅₀) of the imazethapyr-resistant and -susceptible barnyardgrass plants. Testing for cross- and multiple-resistance was conducted to determine if the resistant biotype was resistant to other families of ALS inhibitors (cross resistance) and to other commonly used grass herbicides with different mechanisms of action (multiple resistance).

Materials and Methods

Plant Material. Following failure in the field of repeat applications of imazethapyr at 70 g ha⁻¹, mature barnyardgrass plants were collected from a field in Delaplaine, Arkansas, and were sent to the University of Arkansas Altheimer Laboratory for further evaluation. The putative-resistant biotype was evaluated for control relative to a susceptible standard. The barnyardgrass seed that was used as a susceptible standard was purchased from a local weed seed supplier (Azlin Seed Company, 112 Lilac Dr., Leland, MS 38756). In order to verify a lack of resistance, without association to this experiment, the susceptible biotype was screened against many grass herbicides and expressed no resistance. After maturity, seeds were collected and planted in 45-by 60-cm plastic flats filled with potting mix (professional growing mix, LC1 Mix. Sun Gro Horticulture Distribution Inc., Bellevue, WA 98008), and flats were watered daily. The seeded flats were placed in the greenhouse with 33/20 C day/night temperatures and a 16-h photoperiod to stimulate germination. Cotyledon to one-leaf barnyardgrass seedlings were transplanted into 10-cm-diam pots containing potting mix (Professional Growing Mix, LC1 Mix. Sun Gro Horticulture Distribution Inc., Bellevue, WA 98008). The experiment/screening process was set up as a randomized complete block design (RCB) consisting of two runs of 20 resistant plants per treatment with 5 plants per replication and 4 replications per treatment. Imazethapyr was applied to three-leaf plants at 35, 70, 140, 280, and 560 g ha⁻¹ in a stationary spray chamber with a two-nozzle boom containing 800067 flat fan nozzles (Teejet Technologies, Springfield, IL 62703) calibrated to deliver 187 L ha⁻¹. Survival from these rates was 20, 16, 13, 9, and 7 plants, respectively. Seeds from plants surviving the 70 g ha⁻¹ in the screening experiment of the resistant biotype were collected at maturity and used for the subsequent experiments.

Dose Response. Seed from the imazethapyr-resistant and -susceptible biotypes were sown in separate 45- by 60-cm trays containing potting mix, and cotyledon to one-leaf seedlings were transplanted into 10-cm-diam pots containing potting mix.

The experimental design was a completely randomized design with 20 plants/rate/run. The experiment consisted of two runs of four replications with 10 rates each of imazethapyr, penoxsulam, and bispyribac ranging from 1/16 to 32 times (X) the labeled rate of each herbicide for the resistant biotype and 1/128 to 4X the labeled rate of each herbicide for the susceptible biotype. The labeled rates of the herbicides were: imazethapyr 70 g ha⁻¹, penoxsulam 35 g ai ha⁻¹, and bispyribac 22 g ai ha⁻¹. All barnyardgrass seedlings were three- to four-leaf (7 to 10 cm tall) when treated, and nonionic surfactant (NIS) (Induce, Helena Chemical Co., West Helena, AR 72390) at 0.25% v/v was added to imazethapyr, crop oil (Agri-Dex, Helena Chemical Co., West Helena, AR 72390) at 1% v/v was added to penoxsulam, and a nonionic spray adjuvant and deposition agent at 2.5% v/v (Dyne-a-Pak, Helena Chemical Co., Collierville, TN 38017) was added to bispyribac. A non-treated control was included. Treatments were applied in a stationary spray chamber with a boom with two flat fan 800067 nozzles calibrated to deliver 187 L ha⁻¹. After treatments were applied, the plants were returned to the greenhouse and supplied adequate amounts of water and nutrients for 30 d. Plant death (live or dead counts) was recorded 30 d after treatment (DAT). The lethal dose needed to kill 50 and 90% of each biotype (LD₅₀ and LD₉₅) along with confidence intervals (95%) was determined using PROC PROBIT in SAS 9.2 (SAS Institute Inc., Cary, NC).

Dry Weight. Biomass (green tissue) of living plants was harvested 30 DAT. Plants were clipped at the base at the soil surface and placed in heated drying chambers for seven days and were then

weighed, and dry weights were calculated for surviving plants. Dry weight reductions were averaged over plants within a run for each barnyardgrass biotype. Runs were considered random. For each barnyardgrass biotype, the proportion dry weight reduction relative to the nontreated control was fit to a logistic function where exponent was a quadratic function of the logarithm base 2 of the herbicide rate. The fitted model was used to obtain estimates of growth reduction by 50% (GR₅₀) and growth reduction by 90% (GR₉₀) on the log scale along with corresponding 95% confidence intervals. The estimates and confidence interval endpoints were back-transformed from the log scale to the herbicide rate scale. All analyses were carried out using SAS version 9.2.

Cross- and Multiple-Resistance. Response of the resistant biotype to other commonly used grass herbicides was evaluated in the greenhouse. The trial consisted of the imazethapyr-resistant and -susceptible biotypes used in the previous study. Both biotypes were seeded into individual 10-cm-diam pots containing a silt loam soil. Thirty seed were placed in each pot and watered adequately to stimulate germination. After emergence, seedlings were thinned to five plants per pot. Preemergence (PRE) applications were made immediately following planting before emergence, delayed preemergence (DPRE) applications were made 3 days after planting before emergence, and postemergence (POST) applications were applied to three-leaf (7- to 10-cm tall) barnyardgrass plants. The herbicides evaluated were the ALS-inhibiting herbicides (WSSA Group 2): imazethapyr at 70 and 106 g ha⁻¹, each applied PRE and POST, imazamox at 45 g ai ha⁻¹ applied POST, penoxsulam at 40 g ha⁻¹ applied POST, and bispyribac at 36 g ha⁻¹ applied POST; the synthetic auxin quinclorac (WSSA Group 4) at 560 g ai ha⁻¹ applied PRE and POST; the carotenoid biosynthesis inhibitor clomazone (WSSA Group 13) at 336 g ai ha⁻¹ applied PRE;

the fatty acid and lipid biosynthesis inhibitor thiobencarb (WSSA Group 8) at 4490 g ai ha⁻¹ applied DPRE; the mitotic inhibitor pendimethalin (WSSA Group 3) at 1120 g ai ha⁻¹ applied DPRE; the photosystem II inhibitors (PSII) atrazine (WSSA Group 5) at 2240 g ai ha⁻¹ applied POST and propanil (WSSA Group 7) at 4480 g ai ha⁻¹ applied POST; the acetyl CoA carboxylase inhibitors (ACCase) (WSSA Group 1) fenoxaprop at 120 g ai ha⁻¹ applied POST, cyhalofop at 314 g ai ha⁻¹ applied POST, and clethodim at 280 g ai ha⁻¹ applied POST; the photosystem I inhibitor (PSI) paraquat (WSSA Group 22) at 700 g ai ha⁻¹ applied POST; the 5-enolpyruval-shikimate-3-phosphate synthase (EPSPS) inhibitor glyphosate (WSSA Group 9) at 870 g ae ha⁻¹ applied POST; and the glutamine synthetase inhibitor glufosinate (WSSA Group 10) at 590 g ai ha⁻¹ applied POST. Herbicide rates and timing were applied according to recommendations in the University of Arkansas Weed and Brush Control Manual MP-44 (Scott et al. 2011). Nonionic surfactant at 0.25% v/v was added to POST applications of imazethapyr, quinclorac, fenoxaprop, paraquat, cyhalofop, and imazamox. Crop oil concentrate at 1% v/v was added to POST applications of penoxsulam, atrazine, clethodim, and propanil, and Dyne-a-Pak was added at 2.5% v/v to bispyribac. Plants were grown the same as the dose response study, and applications were made in a stationary spray chamber calibrated with a two-nozzle boom containing flat fan 80067 nozzles calibrated to deliver at 187 L ha⁻¹.

Barnyardgrass control was visually rated 14 and 21 DAT on a 0 to 100% scale, where 0 equals no control and 100 equals plant death. The experimental design was a completely randomized design with two runs of four replications of each herbicide and biotype combination. Barnyardgrass control was subjected to ANOVA. Data were analyzed using PROC MIXED in SAS.

Results and Discussion

Dose Response. For imazethapyr, penoxsulam, and bispyribac, the probability of barnyardgrass death for increasing rates of each herbicide for the resistant and susceptible biotypes are shown in Figures 1, 2, and 3. The LD₅₀ values for imazethapyr, penoxsulam, and bispyribac were 12, 10, and 6 g ha⁻¹ to control 50% of the susceptible plants, which is lower than the labeled rates of 70, 35, and 22 g ha⁻¹ for each herbicide, respectively. Based on the LD₉₅ values, greater than 95% mortality of the susceptible population is achieved at the labeled rates of imazethapyr, penoxsulam, and bispyribac which are 70, 35, and 22 g ha⁻¹, respectively.

The LD₅₀ value of imazethapyr for the resistant biotype was 170 g ha⁻¹, a 14-fold increase over the susceptible biotype (Figure 1). The LD₉₅ value of 1,715 g ha⁻¹ of imazethapyr was a 52-fold increase over the susceptible biotype. To achieve 95% mortality, imazethapyr had to be applied at 24 times the field use rate of 70 g ha⁻¹. It was predicted that imazethapyr needed to be applied at 25 g ha⁻¹ to achieve the GR₅₀ (Table 1), and a rate of 188 g ha⁻¹ of imazethapyr was needed for the GR₉₀ of the resistant biotype (Table 2).

For penoxsulam the LD₅₀ value for the resistant biotype was 25-fold greater than that of the susceptible biotype, which had an LD₅₀ of 254 g ha⁻¹ penoxsulam. Penoxsulam at 1192 g ha⁻¹ was needed to achieve 95% mortality of the resistant biotype, a 34-fold increase over the susceptible biotype and 33 times greater than the labeled use rate for penoxsulam (Figure 2). Furthermore, the GR₅₀ for penoxsulam was 37 g ha⁻¹ (Table 1), and the GR₉₀ was 169 g ha⁻¹, equivalent to almost a 5-fold difference in rate (Table 2).

The LD₅₀ value of bispyribac was 49 g ha⁻¹ for the resistant biotype, which was 9-fold greater than for the susceptible biotype (Figure 3). There was a 7-fold difference in the LD₉₅ value of the resistant compared to susceptible biotype and an almost 6-fold increase compared to

the labeled use rate. Based on the dose response curves, it was predicted that 12 g ha⁻¹ of bispyribac was needed to reduce growth 50% (Table 1), whereas 90% growth was reduced at a rate of 49 g ha⁻¹, indicating a 4-fold difference in herbicide rate (Table 2).

Response of the ALS-resistant biotype to the ALS-inhibiting herbicides imazethapyr, penoxsulam, and bispyribac shows a high level of resistance. Because of a high level of resistance to all three herbicides, increasing herbicide rate is not a feasible option for controlling this barnyardgrass biotype. Results of this research were similar to results of Nandula et al. (2010) and Riar et al. (2012) for other barnyardgrass accessions collected in Mississippi and Arkansas, where multiple applications of imazethapyr at the labeled rate failed to control barnyardgrass.

Barnyardgrass resistance is widespread throughout the world in sixteen countries (Heap 2012). Biotypes exist globally with resistance to acetyl-CoA carboxylase (ACC)-inhibiting (WSSA Group 1), ALS-inhibiting (WSSA Group 2), chloroacetamide (WSSA Group 15), dinitroaniline (WSSA Group 3), isoxazolidione (WSSA Group 27), thiocarbamate (WSSA Group 8), synthetic auxin (WSSA Group 4), and PSII-inhibiting (WSSA Group 7 urea and amide) herbicides. Barnyardgrass biotypes resistant to the ALS-inhibiting herbicides have been confirmed in Brazil, Turkey, China, South Korea, and Yugoslavia (Heap 2012). The resistance mechanism of the particular biotype in this experiment is unknown and would require further research; however, target-site resistance is commonly associated with resistance to ALS-inhibiting herbicides (Tranel and Wright 2002).

Cross- and Multiple-Resistance. *Imazethapyr/Imazamox/Penoxsulam/Bispyribac.*

Imazethapyr and imazamox represent the imidazolinone, penoxsulam the triazolopyrimidine, and

bispyribac the pyrimidinylthiobenzoate families of ALS-inhibiting herbicides (HRAC 2012). Each of these four herbicides failed to control the ALS-resistant barnyardgrass biotype as evidenced by no more than 45% control with any of them while the susceptible biotype was completely controlled by POST applications (Table 3). These findings along with those from the dose response experiments demonstrate that this barnyardgrass biotype exhibits cross-resistance to a range of ALS-inhibiting herbicides; albeit, the level of resistance differs by choice of herbicide.

Other Unique Mechanisms of Action Used in Rice for Residual Barnyardgrass Control.

Clomazone, thiobencarb, pendimethalin, and quinclorac comprise four different mechanisms of action that can be applied PRE or DPRE for control of barnyardgrass in rice (Scott et al. 2011). Clomazone is considered a base program for grass control in Arkansas (Norsworthy et al. 2007). Although two clomazone-resistant barnyardgrass biotypes have been found in Arkansas (Norsworthy et al. 2008), no other clomazone-resistant populations have been identified through the annual resistance screening program at the University of Arkansas (Dr. Jason Norsworthy, personal communication), indicating that clomazone resistance does not appear to be widespread. Additionally, the ALS-resistant biotype tested in the experiments reported here was not resistant to clomazone applied PRE (Table 3); hence, clomazone will still be an effective PRE option for control of this ALS-resistant barnyardgrass biotype in rice.

Thiobencarb and pendimethalin can be applied DPRE in rice for grass control. These herbicides have little POST activity but are effective for providing residual control of barnyardgrass and other annual grasses in rice. The ALS-resistant barnyardgrass biotype was not resistant to thiobencarb applied DPRE, with control of 92% at 21 DAT (Table 3). Although

control of the ALS-resistant biotype with pendimethalin DPRE was only 74% at 21 DAT, control did not differ from the 89% control of the susceptible biotype (Table 3). Therefore, thiobencarb and pendimethalin can still be used in fields with ALS-resistant barnyardgrass.

Quinclorac applied PRE controlled the ALS-resistant barnyardgrass 85% at 21 DAT (Table 3). However, control with quinclorac applied POST was only 43% at both 14 and 21 DAT. Quinclorac-resistant barnyardgrass generally shows a low level of resistance to PRE-applied quinclorac but is highly resistant to POST applications (Dr. Jason Norsworthy, personal communication). The ALS-resistant biotype appears less sensitive to quinclorac than the susceptible biotype, but additional dose response experiments would be needed to confirm resistance. The apparent reduced sensitivity of the ALS-resistant biotype to quinclorac would obviate the usefulness of quinclorac for barnyardgrass control in fields containing this population of ALS-resistant barnyardgrass. Multiple resistance in barnyardgrass, specifically resistance to propanil and quinclorac, is quite common in Arkansas (Norsworthy et al. 2012); hence, the inability of quinclorac to provide a high level of control of the ALS-resistant biotype is not surprising. However, further research will need to be conducted to determine if this particular ALS-resistant biotype is in fact resistant to quinclorac.

POST Options in Rice. Propanil is one of three well-recognized POST-applied herbicides in rice for grass control along with fenoxaprop and cyhalofop. Results of this research show that propanil effectively controlled the susceptible biotype 100% at both 14 and 21 DAT (Table 3); however, the resistant biotype was controlled only 2% 21 DAT, indicating that this ALS-resistant biotype likely exhibits multiple resistance to yet another mechanism of action.

Two acetyl CoA carboxylase (ACCase) inhibitors used for grass control POST in rice play an important role for propanil-, quinclorac-, and/or ALS-resistant barnyardgrass populations. At 21 DAT, fenoxaprop controlled both the ALS-resistant and –susceptible barnyardgrass biotypes at least 99% (Table 3). Although control of the ALS-resistant and –susceptible biotype with cyhalofop was only 79 and 92%, respectively, the control levels were not significantly different. Complete barnyardgrass control is often difficult to achieve with a single application of cyhalofop (Buehring et al. 2006). Also, the barnyardgrass plants in this experiment were not flooded, which is not typical in Arkansas rice culture, and according to the cyhalofop label (Anonymous 2012), the herbicide is most effective under flooded conditions. Because the ALS-resistant biotype does not appear to exhibit multiple resistance to cyhalofop or fenoxoprop, these herbicides can be applied as part of a weed control program in rice for control of this ALS-resistant barnyardgrass, and both herbicides would likely be salvage options in fields where ALS-inhibiting herbicides were ineffective.

Non-selective Herbicide Options. Three herbicides often used for desiccation (burndown) of vegetation to provide a weed-free planting bed are glyphosate, glufosinate, and paraquat, each with a unique mechanism of action. Currently, only glyphosate and paraquat are labeled for burndown use in rice, and neither glyphosate- nor glufosinate-resistant rice is presently marketed. Nevertheless, these herbicides are used widely in cropping systems for burndown control and in respective resistant crops. At 21 DAT, they all controlled the ALS-resistant resistant biotype 100% (Table 3). Any of these three herbicides could be used to control this ALS-resistant barnyardgrass biotype in preplant situations, and glyphosate and glufosinate could be used in-season for barnyardgrass control in glyphosate- or glufosinate-resistant crops.

Atrazine/Clethodim. Herbicide options in crops other than rice were tested for efficacy against ALS-resistant barnyardgrass. Atrazine, a Group 5 PSII-inhibiting herbicide, is used widely in corn (*Zea mays* L.) for control of some broadleaf weeds and grasses, and clethodim, a WSSA Group 1 ACCase-inhibiting herbicide, is used in broadleaf crops for POST grass control. Although barnyardgrass populations exist globally with resistance to Group 1 and 5 herbicides, atrazine and clethodim provided 100% control (Table 3) of both biotypes at 14 and 21 DAT and can be used as an effective means for barnyardgrass control in crops in which they are labeled.

Summary

With the increasing evolution of herbicide resistance by barnyardgrass over the past two decades and the extensive reliance on ALS-inhibiting herbicides in rice, the evolution of ALS-resistant barnyardgrass is not surprising (Bagavathiannan et al. 2012). An increase in Clearfield rice acreage, enabling the extensive use of ALS-inhibiting herbicides in rice, and the over use of these herbicides in other crops led to the ALS resistance of barnyardgrass. Barnyardgrass has now been documented to be resistant to four of seven mechanisms of action used in Arkansas rice today. In addition to resistance to ALS-inhibiting herbicides such as imazethapyr and penoxsulam, the ALS-resistant biotype is potentially resistant to the PSII inhibitor propanil and the synthetic auxin quinclorac if it is applied POST. Although the ALS-resistant biotype can be controlled with clomazone, clomazone-resistant barnyardgrass also exists. As these studies show, there are a number of herbicides that can be used to control ALS-resistant barnyardgrass. However, they should be applied as part of an integrated resistance-management program to avoid or delay further resistance issues.

Table 1. Dose needed for 50% growth reduction (GR₅₀) for ALS-resistant (R) and –susceptible (S) barnyardgrass biotypes with 95% upper (UCI) and lower (LCI) confidence intervals.

Herbicide	1X Rate g ai ha ⁻¹	Population	GR ₅₀ ^a ----- g ai ha ⁻¹ -----	UCI -----	LCI -----
Penoxsulam	35	R	36.6 a	29.8	44.1
Penoxsulam	35	S	1.1 f	0.8	1.4
Imazethapyr	70	R	24.8 b	20.9	29.6
Imazethapyr	70	S	3.8 d	3.3	4.2
Bispyribac	22	R	12.1 c	9.7	14.6
Bispyribac	22	S	1.5 e	1.3	1.7

^a GR₅₀ doses within a column followed the same letter are not statistical different at P ≤ 0.05.

Table 2. Dose needed for 90% growth reduction (GR_{90}) for ALS-resistant (R) and –susceptible (S) barnyardgrass biotypes with 95% upper (UCI) and lower (LCI) confidence intervals.

Herbicide	1X Rate g ai ha ⁻¹	Population	GR_{90} ^a ----- g ai ha ⁻¹ -----	UCI	LCI
Penoxsulam	35	R	168.9 b	121.8	237.3
Penoxsulam	35	S	6.6 de	4.6	10.1
Imazethapyr	70	R	187.6 a	129.8	316.5
Imazethapyr	70	S	11.8 d	9.5	14.9
Bispyribac	22	R	49.1 c	36.9	65.5
Bispyribac	22	S	5.9 e	4.7	7.6

^a GR_{90} doses within a column followed the same letter are not statistical different at $P \leq 0.05$.

Table 3. Percentage control of the acetolactate synthase-resistant (ALS-R) and -susceptible (ALS-S) barnyardgrass biotypes among different herbicides at 14 and 21 days after treatment (DAT).

Herbicide	Timing	Rate g ai ha ⁻¹	Barnyardgrass control ^a			
			14 DAT		21 DAT	
			ALS-S	ALS-R	ALS-S	ALS-R
			————— % —————			
Imazethapyr	PRE	70	83 a	25 b	82 a	23 b
Imazethapyr	PRE	107	88 a	33 b	90 a	35 b
Clomazone	PRE	36	100 a	100 a	100 a	100 a
Thiobencarb	DPRE	4490	99 a	90 a	99 a	92 a
Pendimethalin	DPRE	1120	87 a	72 a	89 a	74 a
Quinclorac	PRE	560	100 a	84 b	100 a	85 b
Imazethapyr	POST	70	100 a	8 b	100 a	11 b
Imazethapyr	POST	106	100 a	45 b	100 a	46 b
Penoxsulam	POST	40	100 a	13 b	100 a	15 b
Bispyribac	POST	36	100 a	39 b	100 a	43 b
Atrazine	POST	2243	100 a	100 a	100 a	100 a
Fenoxaprop	POST	120	100 a	98 a	100 a	99 a
Paraquat	POST	701	100 a	100 a	100 a	100 a
Cyhalofop	POST	314	92 a	77 a	92 a	79 a
Glyphosate	POST	870	100 a	100 a	100 a	100 a
Glufosinate	POST	590	100 a	100 a	100 a	100 a
Imazamox	POST	45	100 a	14 b	100 a	18 b
Clethodim	POST	280	100 a	100 a	100 a	100 a
Quinclorac	POST	560	100 a	43 b	100 a	43 b
Propanil	POST	4487	100 a	1 b	100 a	2 b

^a Means for each herbicide within each rating date and row with the same letter do not differ significantly according to Fisher's Protected LSD (0.05).

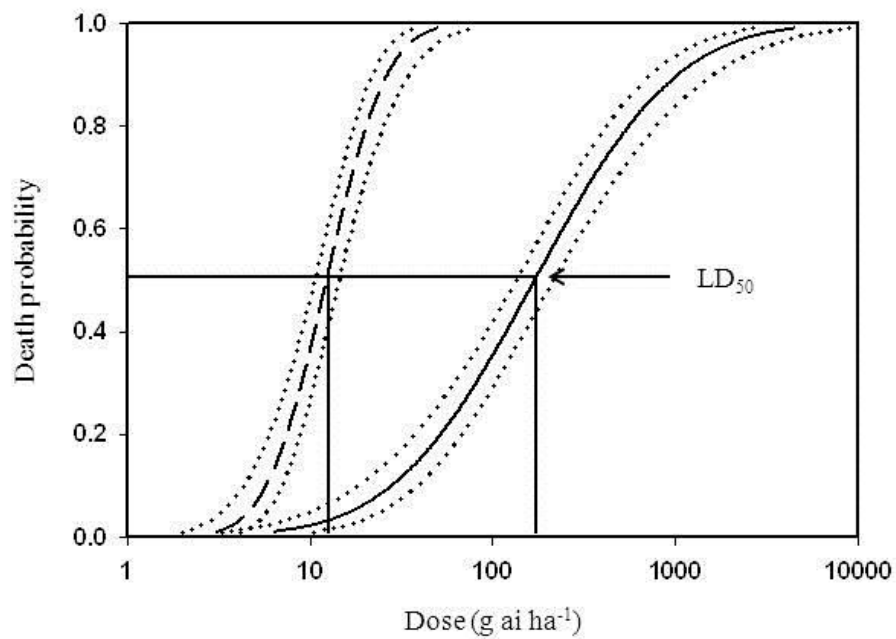


Figure 1. Probit analysis ($Y=a+bX+e$) with 95% confidence intervals (dotted lines) to predict the lethal dose (dashed line = susceptible and solid line = resistant) of imazethapyr needed to kill the ALS-resistant and -susceptible barnyardgrass biotypes.

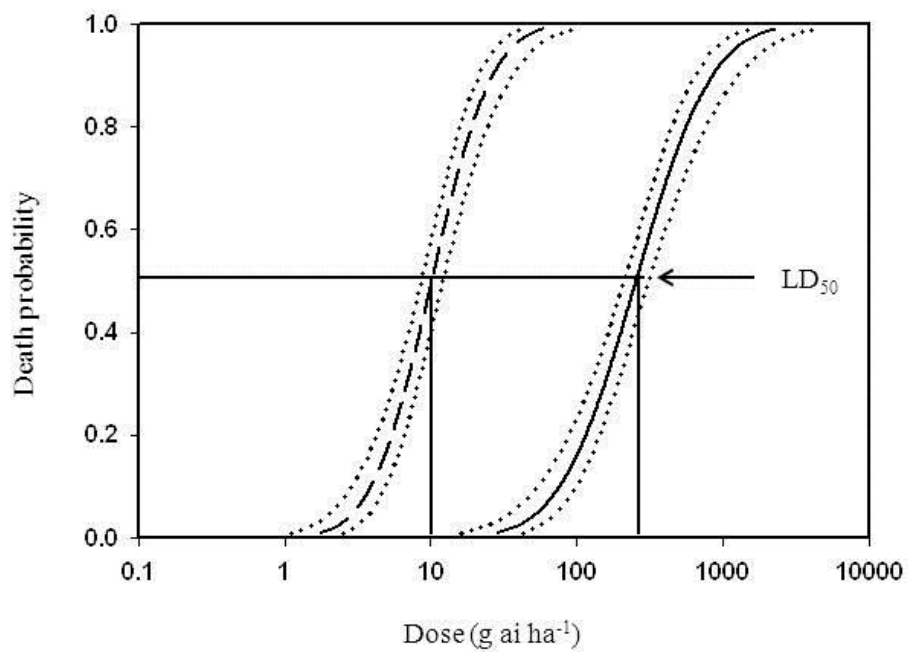


Figure 2. Probit analysis ($Y=a+bX+e$) with 95% confidence intervals (dotted lines) to predict the lethal dose (dashed line = susceptible and solid line = resistant) of penoxsulam needed to kill the ALS-resistant and -susceptible barnyardgrass biotypes.

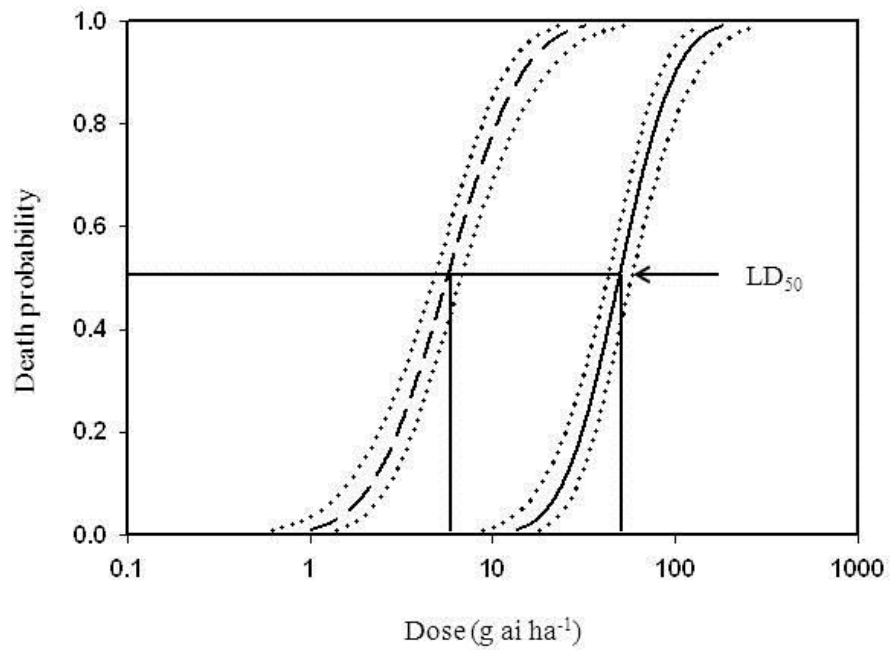


Figure 3. Probit analysis ($Y=a+bX+e$) with 95% confidence intervals (dotted lines) to predict the lethal dose (dashed line = susceptible and solid line = resistant) of bispyribac needed to kill the ALS-resistant and -susceptible barnyardgrass biotypes.

Program Approaches to Controlling Herbicide-Resistant Barnyardgrass (*Echinochloa crus-galli*) in Rice

Barnyardgrass, the most problematic weed of Arkansas rice, was first documented resistant to propanil in 1990, and since then, it has evolved resistance to quinclorac and clomazone. Most recently, barnyardgrass has evolved resistance to acetolactate synthase (ALS)-inhibiting herbicides. The goal of this research was to develop herbicide programs for controlling ALS-, propanil-, quinclorac-, and clomazone-resistant barnyardgrass. Multiple field trials were conducted over two growing seasons. In one trial, two applications of imazethapyr alone failed to control the ALS-resistant biotype (<43%); however, when imazethapyr was applied early postemergence followed by imazethapyr + fenoxaprop immediately prior to flooding (PREFLD), barnyardgrass control improved. When imazethapyr was applied twice following preemergence or delayed PRE applications of clomazone, quinclorac, pendimethalin, or thiobencarb, acceptable control was obtained with or without the addition of fenoxaprop PREFLD. Herbicide program costs associated with a standard multiple application program was compared to single application programs. Single-pass herbicide programs effectively controlled multiple-resistant biotypes, and some single application programs consisting of three or four herbicides were as effective as multiple applications in providing season-long control with less cost.

Nomenclature: Clomazone; fenoxaprop; imazethapyr; propanil; quinclorac; barnyardgrass, *Echinochloa crus-galli* (L.) Beauv.; rice, *Oryza sativa* L. 'CL151'.

Key words: Herbicide-resistant weeds, multiple resistance, weed control.

Introduction

Rice is an important aspect of Arkansas crop production, with the state producing approximately one-half of the total rice grown in the United States (Wilson and Branson 2005). The rice produced in Arkansas is dry-seeded and drilled, then flooded when rice is at the four- to six-leaf stage (Slaton 2001; Wilson and Branson 2005). The most troublesome weed in Arkansas rice is barnyardgrass, which infests almost all of the Arkansas rice hectares (Norsworthy et al. 2007). Barnyardgrass can cause as much as 80% yield loss in season-long competition with rice (Smith 1988), and a single plant located 40 cm from a rice plant can reduce rice yield by 27% (Stauber et al. 1991). Barnyardgrass also effectively scavenges for nitrogen (takes up to 80% available N) at the expense of rice, ultimately reducing yield (Holm et al. 1977). Barnyardgrass densities as low as 5 plants m⁻² cause annual economic losses (Smith 1988).

Barnyardgrass has always been considered a competitive weed in crop production, but its status of being a problem weed has increased recently because of herbicide resistance. In the early 1990s, propanil-resistant barnyardgrass was documented (Baltazar and Smith 1994; Carey et al. 1994). Propanil, a widely used rice herbicide for barnyardgrass control, inhibits photosystem II electron transport (Senseman et al 2007). Within a few days of application, plants show symptoms of chlorosis, eventually resulting in plant desiccation (Senseman et al. 2007). Propanil was labeled for use in rice in 1959 and was used repeatedly on approximately 98% of the Arkansas rice hectares through the early 1990s (Carey et al. 1995). The result of such sustained use was the evolution of propanil-resistant barnyardgrass, which was documented in Arkansas in 1990 (Carey et al. 1995). In 1992, quinclorac received a 24C label for control of

propanil-resistant barnyardgrass (Baldwin et al. 1996; Malik et al. 2010; Talbert et al. 1995, 1996).

Quinclorac inhibits the enzyme associated with cellulose biosynthesis, possibly leading to ethylene and cyanide production (Monaco et al. 2002). Treated broadleaf plants show symptoms of epinasty, stem swelling, bending, or leaf cupping/curling whereas chlorosis of new leaves often occurs in grasses (Senseman et al. 2007). In 1998, quinclorac-resistant barnyardgrass was reported in Arkansas, and by 1999, barnyardgrass was confirmed resistant to both propanil and quinclorac (Lovelace et al. 2002; Lovelace et al. 2007; Malik et al. 2010). With the confirmation of propanil-, quinclorac-, and propanil/quinclorac-resistant barnyardgrass, two herbicide mechanisms of action are no longer effective for controlling barnyardgrass in some fields (Lovelace et al. 2007; Malik et al. 2010).

Clomazone was labeled in rice in 2000 for control of propanil-, quinclorac-, propanil/quinclorac-, and susceptible barnyardgrass. Clomazone is currently applied to approximately 75 to 80% of rice in Arkansas (Dr. Charles Wilson Jr., personal communication) and 70 to 80% of the rice in Mississippi (Jason Bond, personal communication) and is still an effective herbicide for barnyardgrass control in most fields. A barnyardgrass population in Arkansas that survived a field application of clomazone in 2007 was later confirmed resistant to the herbicide (Norsworthy et al. 2008).

Historically, as barnyardgrass has evolved resistance to new mechanisms of action, new effective herbicides have become available to control barnyardgrass. In 2002, Clearfield™ rice was introduced into U.S. rice, with the varieties being a nontransgenic line bred from a rice plant that showed resistance to the imidazolinone class of herbicides that inhibit acetolactate synthase

(ALS). Inhibition of ALS leads to reduced production of the branched-chain amino acids isoleucine, leucine, and valine, causing meristematic tissue to become chlorotic with reddening of leaf veins in grasses, eventually leading to plant death (LaRossa and Schloss 1984; Senseman et al. 2007).

Imazethapyr, an imidazolinone herbicide, is effective on the two most troublesome weeds in Arkansas rice, barnyardgrass and red rice (*Oryza sativa* L.) (Masson et al. 2001; Norsworthy et al. 2007; Ottis et al. 2003; Steele et al. 2000). With the Clearfield technology being widely adopted throughout Arkansas rice production (65% of Arkansas hectares in 2011- Norsworthy, unpublished survey), an option exist for controlling propanil-, quinclorac-, and clomazone-resistant barnyardgrass. However, resistance to ALS-inhibiting herbicides occurs more frequently than with other herbicide mechanisms of action (Heap 2012), meaning the evolution of barnyardgrass with resistance to imazethapyr, which is commonly applied in Clearfield rice, along with other ALS-inhibiting herbicides that are effective on barnyardgrass such as penoxsulam and bispyribac was inevitable.

In the spring of 2008, a barnyardgrass population in a rice field near Delaplaine, Arkansas survived two applications of imazethapyr. That fall, a seed sample from surviving plants was collected and sent to the University of Arkansas to be screened for resistance. Resistance to imazethapyr was confirmed (Wilson et al. 2011), indicating that barnyardgrass in Arkansas rice has evolved resistance to four mechanisms of action. As resistance continues to evolve, herbicide options diminish and there is concern of increasing costs of controlling populations having multiple resistance. The objectives of this research were to develop: 1) herbicide programs for effectively controlling ALS-resistant barnyardgrass, 2) herbicide programs for controlling populations with resistance to propanil, quinclorac, clomazone, and ALS-inhibiting

herbicides, and 3) single-application herbicide programs for controlling propanil-, quinclorac-, clomazone-, and ALS-resistant barnyardgrass and determine the costs associated with each program.

Materials and Methods

General Procedures. Field experiments were conducted in 2009 and 2010 at the University of Arkansas Pine Bluff Research Station at Lonoke, AR, to evaluate programs for control of ALS-resistant barnyardgrass. The field was planted to soybean [*Glycine max* (L.) Merr.] the previous year for both growing seasons. Two additional experiments were conducted aimed at developing single and sequential herbicide programs for controlling propanil-, quinclorac-, clomazone-, and ALS-resistant barnyardgrass at Lonoke, AR, and Pine Tree, AR, in 2010. At Pine Tree, the field was fallow the previous year. Before planting at all sites and years, the fields were leveled then tilled with a field cultivator. Clearfield™ rice cultivar ‘CL151’ was drill-seeded at 79 seed m⁻¹ row on June 1, 2009 and 2010, at Lonoke and April 29, 2010, at Pine Tree with a nine-row drill with an 18-cm row spacing in 6-m-long plots. The soil texture at both locations was a Calhoun silt loam (fine-silty, mixed, active, thermic Typic Glossaqualfs) with a soil pH of 5.2 (Lonoke) and a soil pH of 6.7 (Pine Tree). Nitrogen was applied at 112 kg ha⁻¹ to the rice crop immediately before flooding at the 4- to 5-leaf stage and 50 kg ha⁻¹ at after flooding when rice was at the boot stage. Plots were maintained according to University of Arkansas pest and nutrient management recommendations (Slaton 2001). All experiments were flushed with irrigation following herbicide applications to ensure proper activation of each herbicide treatment at all locations. Weekly barnyardgrass control and rice injury ratings were taken throughout the growing season on a 0 to 100% scale, where 0 = no control or injury and 100 = complete control or crop death. Plots were harvested with a small-plot combine by cutting a 71 cm swath (four rows) from the

center of each plot. Rice was then adjusted to 12% moisture and yields calculated. Data were analyzed using ANOVA with the MIXED procedure in SAS. Means were separated using Fisher's protected LSD at a 5% level of significance.

Program Approaches for Controlling ALS-Resistant Barnyardgrass. The test was set up as a randomized complete block design (RCB) with four replications and a factorial arrangement of herbicide programs with and without the addition of PREFLD application of fenoxaprop.

Each plot consisted of ALS-resistant barnyardgrass sown in a row perpendicular to the rice rows in all four replications along with a natural population of susceptible barnyardgrass throughout the plot. Herbicides were applied at multiple timings: PRE (at planting), delayed PRE (DPRE) (5 to 7 d after planting), early POST (EPOST) (1- to 2-leaf rice stage), and pre-flood (PREFLD) (4- to 5-leaf rice stage immediately prior to flooding). Herbicide treatments consisted of 1) imazethapyr at 70 g ai ha⁻¹ EPOST and PREFLD 2) clomazone at 336 g ai ha⁻¹ PRE followed by (fb) imazethapyr at 70 g ha⁻¹ EPOST and PREFLD with and without fenoxaprop at 120 ai ha⁻¹ PREFLD 3) clomazone at 336 g ha⁻¹ + quinclorac at 560 g ai ha⁻¹ PRE fb imazethapyr at 70 g ha⁻¹ EPOST and PREFLD with and without fenoxaprop at 120 g ha⁻¹ PREFLD 4) pendimethalin at 1120 g ai ha⁻¹ + quinclorac at 560 g ha⁻¹ DPRE fb imazethapyr at 70 g ha⁻¹ EPOST and PREFLD with and without fenoxaprop at 120 g ha⁻¹ PREFLD 5) pendimethalin at 1120 g ha⁻¹ + thiobencarb at 4490 g ai ha⁻¹ DPRE fb imazethapyr at 70 g ha⁻¹ EPOST and PREFLD with and without fenoxaprop at 120 g ha⁻¹ PREFLD 6) quinclorac at 560 g ha⁻¹ + thiobencarb at 4490 g ha⁻¹ DPRE fb imazethapyr at 70 g ha⁻¹ EPOST and PREFLD with and without fenoxaprop at 120 g ha⁻¹ PREFLD 7) clomazone at 336 g ha⁻¹ + pendimethalin at 1120 g ha⁻¹ fb imazethapyr at 70 g ha⁻¹ EPOST and PREFLD with and without fenoxaprop at

120 g ha⁻¹ PREFLD. All plots were harvested at maturity for yield comparisons among herbicide programs. Formulations and manufacturers of all herbicide products can be found in Table 1.

Sequential Herbicide Applications for Controlling Multiple-Resistant Barnyardgrass. Field experiments were conducted in 2010 at the University of Arkansas Pine Bluff Research Station in Lonoke, AR, and at the University of Arkansas Pine Tree Branch Experiment Station (Pine Tree) in Pine Tree, AR. The experimental design was a randomized complete block with four replications.

Propanil-, quinclorac-, clomazone-, and ALS-resistant barnyardgrass populations were sown in individual rows perpendicular to the planted rice rows. The experiment consisted of five herbicide programs: 1) clomazone 336 g ha⁻¹ + quinclorac 560 g ha⁻¹ PRE followed by (fb) propanil at 4480 g ai ha⁻¹ + thiobencarb at 4480 g ha⁻¹ + bispyribac at 36 g ai ha⁻¹ PREFLD; 2) clomazone at 336 g ha⁻¹ + quinclorac at 560 g ha⁻¹ PRE fb propanil at 4480 g ha⁻¹ + thiobencarb at 4480 g ha⁻¹ + penoxsulam at 40 g ai ha⁻¹ PREFLD; 3) clomazone at 336 g ha⁻¹ + pendimethalin at 1120 g ha⁻¹ DPRE fb propanil at 4480 g ha⁻¹ + thiobencarb at 4480 g ha⁻¹ EPOST fb quinclorac at 560 g ha⁻¹ + fenoxaprop at 120 g ha⁻¹ + bispyribac at 36 g ha⁻¹ PREFLD; 4) pendimethalin at 1120 g ha⁻¹ + thiobencarb at 4480 g ha⁻¹ DPRE fb clomazone at 336 g ha⁻¹ + propanil at 4480 g ha⁻¹ EPOST fb quinclorac at 560 g ha⁻¹ + fenoxaprop at 120 g ha⁻¹ + bispyribac at 36 g ha⁻¹ PREFLD; 5) quinclorac at 560 g ha⁻¹ + pendimethalin at 1120 g ha⁻¹ DPRE fb clomazone at 336 g ha⁻¹ + propanil at 4480 g ha⁻¹ + thiobencarb at 4480 g ha⁻¹ EPOST fb fenoxaprop at 120 g ha⁻¹ + bispyribac at 36 g ai ha⁻¹ PREFLD, and a nontreated control.

A Single Herbicide Application for Controlling Multiple-Resistant Barnyardgrass. Field experiments were conducted in the 2010 growing season at the University of Arkansas Pine Bluff Research Station at Lonoke, AR, and at the University of Arkansas Pine Tree Branch Station near Pine Tree, AR. The experimental design was a randomized complete block with four replications. Yield data was subjected to Proc MIXED in SAS version 9.2.

Individual populations of propanil-, quinclorac-, clomazone-, and ALS-resistant barnyardgrass were sown in separate rows perpendicular to the rice rows. The experiment consisted of six single-application programs and two multiple-application programs for comparison with a nontreated control. Each herbicide program contained three or more herbicides applied once either DPRE or EPOST (one-leaf rice) in comparison to a standard, multiple-application program. Herbicides programs evaluated included: 1) clomazone at 336 g ha⁻¹ + quinclorac at 560 g ha⁻¹ + pendimethalin at 1120 g ha⁻¹ + thiobencarb at 4490 g ha⁻¹ DPRE, 2) clomazone at 336 g ha⁻¹ + propanil at 4480 g ha⁻¹ + thiobencarb at 4490 g ha⁻¹ + quinclorac at 560 g ha⁻¹ EPOST, 3) clomazone at 336 g ha⁻¹ + propanil at 4480 g ha⁻¹ + thiobencarb at 4490 g ha⁻¹ + quinclorac at 560 g ha⁻¹ + bispyribac at 36 g ha⁻¹ EPOST, 4) clomazone at 336 g ha⁻¹ + propanil at 4480 g ha⁻¹ + thiobencarb at 4490 g ha⁻¹ + quinclorac at 560 g ha⁻¹ + penoxsulam at 40 g ha⁻¹ EPOST, 5) clomazone at 336 g ha⁻¹ + propanil at 4480 g ha⁻¹ + thiobencarb at 4490 g ha⁻¹ + quinclorac at 560 g ha⁻¹ + imazosulfuron at 336 g ai ha⁻¹ EPOST, 6) clomazone at 336 g ha⁻¹ + quinclorac at 560 g ha⁻¹ + bispyribac at 36 g ha⁻¹ + fenoxaprop at 120 g ha⁻¹ + adjuvant (Dyne-a-Pak) at 2.5% v/v EPOST, 7) clomazone at 336 g ha⁻¹ PRE fb quinclorac at 560 g ha⁻¹ + propanil at 4480 g ha⁻¹ PREFLD, 8) clomazone at 336 g ha⁻¹ PRE fb imazethapyr at 105 g ha⁻¹ + non-ionic surfactant at 0.25% v/v EPOST fb imazethapyr at 105 g ha⁻¹ + non-ionic surfactant at 0.25% v/v PREFLD fb imazamox at 45 g ha⁻¹

+ non-ionic surfactant at 0.25% v/v postflood (PSTFLD) (14 days after flooding). Herbicide costs for each program were calculated using the herbicide prices published in the 2011 University of Arkansas MP44 Weed and Brush Control Manual (Scott et al. 2011). These prices were derived from local retailers throughout Arkansas to obtain an average price for each herbicide (Table 1). Application costs were obtained from custom applicators both ground and aerial.

Results and Discussion

Program Approaches for Controlling ALS-resistant Barnyardgrass. The ALS-resistant barnyardgrass density in the nontreated control plots was approximately 25 plants per m of row in 2009 and 2010. In both years, rice injury at all evaluations was <3% (data not shown). Due to the lack of treatment interactions with the year effect, barnyardgrass control was combined over years.

Herbicide programs consisting of two applications of imazethapyr alone or in combination with additional herbicides and application timings were evaluated as alternative programs for controlling ALS-resistant barnyardgrass in Clearfield rice. At 4 wk after planting (WAP), the PREFLD applications of imazethapyr + fenoxaprop had not been applied, and all programs provided 79% or greater control of the susceptible barnyardgrass biotype (Table 2). The ALS-resistant barnyardgrass was also effectively controlled by all programs at 4 WAP, except for imazethapyr alone which only provided 43% control.

Clomazone applied PRE fb imazethapyr applied EPOST or PREFLD with the addition of fenoxaprop PREFLD provided complete season-long control (100%) of ALS-resistant barnyardgrass, whereas without the addition of fenoxaprop, control was 92% (Table 2). When

pendimethalin, thiobencarb, clomazone, and quinclorac were applied DPRE in different combinations and were followed by imazethapyr EPOST and PREFLD, the ALS-resistant barnyardgrass was controlled $\geq 96\%$ at 10 WAP. The addition of fenoxaprop to any herbicide program improved control of the ALS-resistant barnyardgrass only when it was added to the two applications of imazethapyr alone. No improvement occurred when fenoxaprop was added to programs that contained herbicides other than imazethapyr due to the effectiveness of the other herbicides on barnyardgrass. PRE- or DPRE-applied clomazone, pendimethalin, thiobencarb, and quinclorac are all highly effective in controlling barnyardgrass (Malik et al. 2010; Norsworthy et al. 1999). Imazethapyr applied EPOST fb PREFLD was ineffective in controlling the ALS-resistant barnyardgrass (44%), but with the addition of fenoxaprop to imazethapyr at the PREFLD timing, control of the resistant barnyardgrass improved to 78% by late in the season.

The effectiveness of the evaluated programs on ALS-resistant barnyardgrass is due mainly to the use of an effective, non-ALS-inhibiting herbicide. Furthermore, multiple applications of imazethapyr continue to provide effective control of ALS-susceptible barnyardgrass. Similarly, Ottis et al. (2003) reported that two applications of imazethapyr provided effective season-long control of ALS-susceptible barnyardgrass. This research has shown that there are herbicide programs available for controlling ALS-resistant barnyardgrass in Arkansas rice fields. Programs containing additional mechanisms of action like clomazone, thiobencarb, and fenoxaprop and proper tank mixtures have been proven to provide control of barnyardgrass in previous research studies (Malik et al. 2010; Norsworthy et al. 1999; Ottis et al. 2003; Talbert et al. 1995). Effectiveness of any herbicide for weed control is highly dependent on timely application and proper weed identification in order to successfully control weed infestations in a salvage

situation or not. Yields were comparable across all herbicide-treated plots with no effects on rice quality (data not shown).

Sequential Herbicide Applications for Controlling Multiple-Resistant Barnyardgrass.

The five barnyardgrass biotypes that were planted emerged evenly throughout all plots at both locations. Rice also emerged evenly throughout all plots. Injury to rice of no more than 3% was seen in plots treated with clomazone or propanil (data not shown). For barnyardgrass control, there was a location by treatment interaction; therefore, the results are presented by location.

Barnyardgrass control is compared within biotype across herbicide programs for each location.

Lonoke. Preemergence (PRE) applications were applied June 1 at planting, and DPRE applications were made June 7, 6 days after planting. At 5 WAP, control with herbicide programs containing EPOST applications controlled all barnyardgrass populations 100% except for pendimethalin + thiobencarb DPRE fb clomazone + propanil EPOST, which controlled only 80% of the propanil-resistant biotype (Table 3). Control from programs without EPOST treatments ranged from 75 to 87%, significantly lower than those with EPOST treatments (Table 3). When DPRE applications were made, barnyardgrass and rice had emerged, and the herbicides that were applied DPRE are not as effective after barnyardgrass emerges. After the DPRE application failed to control the propanil-resistant biotype, the EPOST application of clomazone + propanil did not increase control substantially because clomazone provides more residual than POST control of emerged barnyardgrass (Taylor et al. 1996) and the particular biotype was resistant to propanil (Table 3). Furthermore, the tank mixture of thiobencarb + propanil did control the propanil-resistant biotype. Thiobencarb alone will not completely control emerged barnyardgrass but when in combination with propanil it will provide control of small propanil-

resistant barnyardgrass (Norsworthy et al. 1999). The resistant barnyardgrass in this trial was larger than that in the trial conducted by Norsworthy et al. (1999), which explains the lack of control with the thiobencarb + propanil combination. Although clomazone plus quinclorac applied PRE controlled the biotypes only 75 to 87% at 5 WAP, by 10 WAP PREFLD treatments had been applied and all populations were controlled 100% (data not shown).

Pine Tree. Herbicide programs containing a single PRE treatment provided significantly less control (81 to 87% control) compared to programs containing DPRE fb EPOST treatments (98 to 100% control) at 5 WAP across both susceptible and resistant biotypes (Table 3). However, >80% control of all resistant barnyardgrass biotypes at 5 WAP is still comparable to the other herbicide programs where two applications DPRE and EPOST had been applied. At 10 WAP, all herbicide programs controlled propanil-, quinclorac-, ALS-, and clomazone-resistant barnyardgrass biotypes 100% (data not shown).

Control at Pine Tree was similar to that at Lonoke, except that pendimethalin + thiobencarb DPRE fb clomazone + propanil EPOST controlled the propanil-resistant biotype at Pine Tree (Table 3). Reasoning for this was timely application of the DPRE treatment which was applied before the barnyardgrass emerged, and at the timing of the EPOST application there was no emerged propanil-resistant barnyardgrass; therefore, clomazone added more residual control prior to flooding.

Results signify that programs containing multiple mechanisms of action combined and applied at proper timing can control existing resistant barnyardgrass biotypes (Malik et al. 2010). Also, all resistant biotypes were controlled significantly less at 5 WAP after only a PRE application compared to those with the DPRE followed by an EPOST application. However, at

the end of the growing season, the PREFLD applications had been applied, and all programs provided complete control (data not shown).

Single Herbicide Applications for Controlling Multiple-Resistant Barnyardgrass. None of the treatments injured rice more than 5% and recovery was rapid (data not shown). Because of a location by treatment interaction, data are presented by location. Therefore, location was treated as fixed. Herbicide programs are compared within each individual barnyardgrass biotype.

Barnyardgrass control at Lonoke was generally less than that observed at Pine Tree for all biotypes (Table 4). A late planting date of June 1, 2010, at Lonoke provided warmer soil and air temperatures, increasing germination of both rice and barnyardgrass. Cooler temperatures at Pine Tree compared with the trial at Lonoke did not allow rapid growth of barnyardgrass or rice; therefore, when DPRE and EPOST treatments were applied, barnyardgrass was smaller and less dense, making it more susceptible to herbicide applications.

Multiple herbicides applied in a single application failed to provide complete control of the resistant biotypes at Lonoke. However, at Pine Tree, control did not differ among treatments and ranged from 95 to 100%. This is reflective of application timing. Treatments were more effective at the Pine Tree location where the DPRE applications were applied before emergence. These results show that to achieve complete or acceptable control a herbicide must be used at its recommended timing or its effectiveness decreases.

At Pine Tree, programs did not statistically differ within biotypes except for the susceptible biotype, which was controlled 98% with the EPOST treatment that included penoxsulam, and all other treatments controlled the susceptible barnyardgrass 100%. The biological significance of this is insignificant, however. Rice grain yield, which would mainly

be reflective of the level of control of the natural susceptible population, was numerically greatest when the multiple application timings were used to ensure season-long control (Table 5). The single-application DPRE program resulted in rice yields comparable to the multiple application program. At Lonoke, three of the EPOST programs did show significantly lower yields compared to the multiple application program (Table 5); however, barnyardgrass is most competitive to the rice crop early in the season causing up to 70% reduction in yield, the yield loss is likely a result of early-season interference from barnyardgrass, whereas the PRE application in the multiple timing program removed early-season weed interference (Ni et al. 2004; Slaton 2001; Smith 1974).

Yield data for Pine Tree showed the same trend as that of Lonoke (Table 5), but overall yields were higher and not significantly different among herbicide programs. However, the multiple application program did yield numerically higher than the single application programs, with the DPRE program being the next highest yielding treatment (Table 5). Furthermore, the two application program (PRE fb PREFLD) yielded similar to the single application programs (DPRE or EPOST) which were likely the result of only one herbicide being applied at the PRE timing compared to four or more for the single application. The two application treatment allowed for some barnyardgrass emergence early on before the PREFLD application resulting in potential yield reduction as seen with the early competition in the EPOST applications.

Herbicide Program Costs. In comparing control of different resistant and susceptible barnyardgrass biotypes, herbicide program costs were evaluated for those single application programs using the University of Arkansas Weed and Brush Control Manual MP-44 handbook (Scott et al. 2011). Herbicide costs in the MP-44 were obtained from local retailers. The cost of

each program is provided in Table 5 for both ground application and aerial application. The standard multiple application program that provided the highest yield and weed control cost was less than cost of some of the EPOST programs. The cost of the multiple application program was \$190.18 ha⁻¹ compared to the DPRE program at \$140.51 ha⁻¹, which was an effective program and comparable to the multiple-application program. The DPRE program was approximately \$50 ha⁻¹ less than the multiple-application program (Table 5).

After evaluating weed control programs, rice yield, and program costs, results from this research offer growers herbicide programs that will control resistant barnyardgrass as well as the economic value of each program. According to Norsworthy et al. (2007), consultants perceived that each resistant weed costs growers an additional \$65 ha⁻¹ compared to standard application costs. This is usually due to additional herbicides or reduction in yield/grain quality from incomplete weed control. Therefore, if a grower can save \$50 ha⁻¹ with a single application, it will provide more time and money for other agricultural expenses during the growing season and additional flexibility as a result of saved time. When a grower has extra time, it allows his operation to run more smoothly with the capability of maintaining larger operations of production crops as well. By treating fields with resistance management programs using multiple mechanisms of action, not only can it help deplete the seedbank of the resistant population, but it can also serve as a preventative measure for newly occurring resistant species.

Summary

When it comes to resistance management many factors play an important role: tillage, crop rotation, use of multiple herbicide mechanisms of action, avoid low rates, row spacing, plant population, proper weed identification, clean equipment, certified seed, and continuous

scouting of fields (Norsworthy et al. 2012). If these actions are taken resistance chances can be reduced if not eliminated.

The objectives of this research were to document ALS-resistant barnyardgrass and provide herbicide resistance management programs for all current resistant and susceptible barnyardgrass biotypes. Results showed that there are effective programs for managing resistant barnyardgrass in rice; however, these programs come at an added cost. Furthermore, if these programs are utilized, successful production practices can continue and will benefit in future years.

Table 1. Herbicide products used, production company, and cost^a.

Herbicide	Product Used	Company	Rate ha ⁻¹	Cost ha ^{-1a}
Imazethapyr	Newpath	BASF	70	\$39.77
Clomazone	Command 3M	FMC Corporation	336	\$29.16
Quinclorac	Facet	BASF	560	\$44.84
Pendimethalin	Prowl H20	BASF	1120	\$24.81
Thiobencarb	Bolero	Valent	4480	\$36.60
Fenoxaprop	Ricestar HT	Bayer Crop Science	120	\$73.66
Penoxsulam	Grasp	Dow AgroSciences	40	\$46.26
Bispyribac	Regiment	Valent	36	\$35.08
Propanil	Stam 4M	Dow AgroSciences	4480	\$61.71
Imazosulfuron	League	Valent	336	\$34.60
Imazamox	Beyond	BASF	45	\$53.28

^a Costs were derived using the University of Arkansas MP44 Weed and Brush Control manual which gives an average price from local retailers (Scott et al. 2010).

Table 2. Control of ALS-resistant (R) and -susceptible (S) barnyardgrass at 4 and 10 wk after planting (WAP) with herbicide programs alone with and without fenoxaprop (10 wk only) at the Pine Bluff Research Station in Lonoke, AR, averaged over 2009 and 2010.^a

Herbicide	Rate g ai ha ⁻¹	Timing	4 WAP ^c		10 WAP		
			ALS-S	ALS-R	ALS-S	ALS-R ^d	
					%		
					No fenoxaprop	Fenoxaprop	
Imazethapyr	70	EPOST ^a					
Imazethapyr	70	PREFLD	79c	43b	98a	36b	78a
Clomazone	336	PRE					
Imazethapyr	70	EPOST					
Imazethapyr	70	PREFLD	95ab	96a	100a	92a	100a
Clomazone	336	PRE					
Quinclorac	560	PRE					
Imazethapyr	70	EPOST					
Imazethapyr	70	PREFLD	97a	97a	100a	99a	99a
Pendimethalin	1120	DPRE					
Quinclorac	560	DPRE					
Imazethapyr	70	EPOST					
Imazethapyr	70	PREFLD	91b	91a	100a	96a	98a
Pendimethalin	1120	DPRE					
Thiobencarb	4490	DPRE					
Imazethapyr	70	EPOST					
Imazethapyr	70	PREFLD	96a	94a	100a	99a	100a
Quinclorac	560	DPRE					
Thiobencarb	4490	DPRE					
Imazethapyr	70	EPOST					
Imazethapyr	70	PREFLD	97a	94a	100a	99a	97a
Clomazone	336	DPRE					
Pendimethalin	1120	DPRE					
Imazethapyr	70	EPOST					
Imazethapyr	70	PREFLD	91b	89a	100a	97a	97a

^a Abbreviations: PRE, preemergence; DPRE, delayed preemergence; EPOST, early postemergence; PREFLD, immediately prior to flooding.

^b Means within a column for a specific WAP with the same letters do not differ significantly across programs for each biotype according to Fisher's LSD (0.05). For 10 WAP for the ALS-R barnyardgrass, all means can be compared with or without fenoxaprop.

^c Fenoxaprop had not been applied at the 4 WAP evaluation.

^d ALS, acetolactate synthase; ALS-S, susceptible biotype; ALS-R, resistant biotype.

Table 3. Control of susceptible barnyardgrass (Susc) and propanil- (Prop), quinclorac- (Quin), acetolactate synthase- (ALS), and clomazone- (Clom) resistant barnyardgrass 5 weeks after planting at Pine Bluff Research Station in Lonoke, AR, and Pine Tree Branch Station near Pine Tree, AR, 2010.^b

Treatments	Barnyardgrass control ^c										
	Lonoke					Pine Tree					
	Susc	Prop	Quin	ALS	Clom	Susc	Prop	Quin	ALS	Clom	
	%										
Clomazone + quinclorac fb propanil + thiobencarb + bispyribac	PRE ^a PREFLD	82b	84b	86b	80b	78b	83b	81b	83b	83b	84b
Clomazone + quinclorac fb propanil + thiobencarb + penoxsulam	PRE PREFLD	80b	85b	82b	83b	75b	87b	85b	86b	85b	81b
Clomazone + pendimethalin fb propanil + thiobencarb + quinclorac fb fenoxaprop + bispyribac	DPRE EPOST PREFLD	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a
Pendimethalin + thiobencarb fb clomazone + propanil fb quinclorac + fenoxaprop + bispyribac	DPRE EPOST PREFLD	100a	80b	100a	100a	100a	100a	100a	100a	100a	100a
Pendimethalin + quinclorac fb clomazone + propanil + thiobencarb fb fenoxaprop + bispyribac	DPRE EPOST PREFLD	100a	100a	100a	100a	100a	99a	98a	99a	98a	99a

^a Abbreviations: PRE, preemergence; DPRE, delayed preemergence; EPOST, early postemergence; PREFLD, pre-flood; WAP, wk after planting.

^b PREFLD treatments had not been applied.

^c Means with the same letters do not differ significantly within each barnyardgrass biotype according to Fisher's protected LSD (0.05).

Table 4. Single application programs for controlling susceptible (Susc) barnyardgrass and propanil- (Prop), quinclorac- (Quin), acetolactate synthase- (ALS), and clomazone- (Clom) resistant barnyardgrass 10 weeks after planting at the Pine Bluff Research Station in Lonoke, AR, and Pine Tree Branch Station in Pine Tree, AR, 2010.

Treatments	Timing ^a	Barnyardgrass control ^b									
		Lonoke					Pine Tree				
		Susc	Prop	Quin	ALS	Clom	Susc	Prop	Quin	ALS	Clom
		%									
Clomazone + quinclorac + pendimethalin + thiobencarb	DPRE	96ab	97ab	96a	94ab	96ab	100a	100a	100a	100a	100a
Clomazone + propanil + thiobencarb + quinclorac	EPOST	82b	79d	82ab	51d	89abc	100a	97a	98a	98a	100a
Clomazone + propanil + thiobencarb + quinclorac + bispyribac	EPOST	88ab	86bcd	95a	87b	95ab	100a	99a	100a	100a	100a
Clomazone + propanil + thiobencarb + quinclorac + penoxsulam	EPOST	82b	88a-d	86ab	78bc	74d	98b	97a	98a	100a	100a
Clomazone + propanil + thiobencarb + quinclorac + imazosulfuron	EPOST	84b	82cd	65b	74c	87bc	100a	100a	98a	100a	100a
Clomazone + quinclorac + bispyribac + fenoxaprop + Dyne-a-Pak	EPOST	88ab	88a-d	89a	84ab	89abc	100a	100a	95a	95a	100a
Clomazone fb quinclorac + propanil	PRE PREFLD	90a	93abc	79ab	71c	83cd	100a	98a	97a	96a	100a

Clomazone fb	PRE											
imazethapyr + NIS fb	EPOST											
imazethapyr + NIS fb	PREFLD											
imazamox + NIS	PSTFLD	99a	99a	99a	99a	99a	100a	96a	95a	100a	100a	

^a DPRE, delayed PRE; EPOST, early POST; PREFLD, pre-flood; PSTFLD, post-flood.

^b Means with the same letters do not differ significantly within each barnyardgrass biotype according to Fisher's protected LSD (0.05).

Table 5. Yield and costs of single-application programs for controlling herbicide-resistant barnyardgrass at the Pine Bluff Research Station at Lonoke, AR, and Pine Branch Station at Pine Tree, AR, 2010.

Treatments	Timing ^a	Yield ^b		Program cost ^c \$ ha ⁻¹
		Lonoke	Pine Tree	
Clomazone + quinclorac + pendimethalin + thiobencarb	DPRE	4,850 ab	5,650 a	140.51
Clomazone + propanil + thiobencarb + quinclorac	EPOST	4,490 abc	5,550 a	177.31
Clomazone + propanil + thiobencarb + quinclorac + bispyribac	EPOST	3,990 bc	5,400 a	212.09
Clomazone + propanil + thiobencarb + quinclorac + penoxsulam	EPOST	4,040 bc	5,050 a	223.57
Clomazone + propanil + thiobencarb + quinclorac + imazosulfuron	EPOST	3,640 c	5,150 a	211.91
Clomazone + quinclorac + bispyribac + fenoxaprop + Dyne-a-Pak	EPOST	4,540 ab	5,100 a	189.74
Clomazone fb quinclorac + propanil	PRE PREFLD	4,190 bc	5,150 a	145.71
Clomazone fb imazethapyr + NIS fb	PRE EPOST			
imazethapyr + NIS fb	PREFLD			
imazamox + NIS	PSTFLD	5,300 a	5,960 a	190.18

^a PRE, preemergence; DPRE, delayed preemergence; EPOST, early postemergence; PREFLD, pre-flood; PSTFLD, post-flood.

^b Means with the same letters do not differ significantly within each location according to Fisher's protected LSD (0.05).

^c Programs applied PRE, DPRE, EPOST, and PREFLD have a \$5.00 ground application fee and PSTFLD applications have a \$7.50 aerial application fee.

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