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Distribution and Control of Herbicide-Resistant Italian Ryegrass (Lolium perenne L. ssp. multiflorum Lam. Husnot) in Arkansas

James Walker Dickson
University of Arkansas, Fayetteville

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DISTRIBUTION AND CONTROL OF HERBICIDE-RESISTANT ITALIAN RYEGRASS (*Lolium perenne* L. ssp. *multiflorum* Lam. Husnot) IN ARKANSAS

DISTRIBUTION AND CONTROL OF HERBICIDE-RESISTANT ITALIAN RYEGRASS (*Lolium perenne* L. ssp. *multiflorum* Lam. Husnot) IN ARKANSAS

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

By

James W. Dickson
University of Arkansas at Monticello
Bachelor of Science in Agriculture, 2006

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University of Arkansas

ABSTRACT

Italian ryegrass populations have evolved resistance to herbicides that producers rely on for weed control both in wheat and burn-down. The objectives of this research were to: test populations of Italian ryegrass from across Arkansas for resistance to glyphosate, diclofop, pinoxaden, and pyroxsulam; determine if there were any differences in control of 12 glyphosate-resistant populations in relation to glyphosate rate or application timing; determine the level of glyphosate resistance in one selected population versus a susceptible standard and a previously discovered glyphosate-resistant population; and determine the best options for controlling Italian ryegrass prior to planting crops. A total of 215 population samples were tested. On average 17% of the samples were resistant to glyphosate, 95% were resistant to diclofop, 64% were resistant to pyroxsulam, and 12% were resistant to pinoxaden. A few were resistant to all four chemistries tested. Control of glyphosate-resistant populations was improved with the high rate of glyphosate at the three- to four-tiller growth stage; however, results for individual populations were variable. When averaged across populations, no rate or timing of glyphosate controlled these resistant populations greater than 62%. One population was found to be 23 times more tolerant to glyphosate than a susceptible standard. Three field experiments were conducted for Italian ryegrass control in the spring, in no-till production in the fall, and following fall tillage. Herbicide applications in the spring were unsuccessful, especially when glyphosate is not an option. Even when postemergence (POST) treatments visually controlled ryegrass at least 80%, enough ryegrass residue remained that would cause problems with spring tillage, planting, and overall crop stand establishment. In the fall-tilled study, the residual herbicides flumioxazin plus *S*-metolachlor, *S*-metolachlor, clomazone, and pyroxasulfone applied immediately following fall tillage reduced Italian ryegrass biomass by 83 to 95% at 200 days after treatment.

This thesis is approved for recommendation
to the Graduate Council.

Thesis Director:

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Dr. Robert C. Scott

Thesis committee:

____

Dr. Nilda R. Burgos

____

Dr. Edward E. Gbur

____

Dr. Robert K. Bacon

____

Dr. Jason P. Kelley

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Distribution and Control of Herbicide-Resistant Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum* Lam. Husnot) in Arkansas

James W. Dickson

Introduction

Italian ryegrass is the most troublesome weed in Arkansas wheat production and has become increasingly difficult to control with glyphosate in spring burn-down applications. Complaints of glyphosate failing to control Italian ryegrass arose in southeast Arkansas in 2007 and have continued through 2012. These glyphosate failure complaints, coupled with commonly used herbicides in wheat failing to control Italian ryegrass, prompted a statewide screening of Arkansas's Italian ryegrass populations in 2009 for resistance to commercial use rates of glyphosate, diclofop, pinoxaden, and pyroxsulam. Furthermore, the level of glyphosate resistance in two glyphosate-resistant Italian ryegrass populations was evaluated. Three field studies were also conducted to evaluate several preemergence and postemergence herbicide options for control of Italian ryegrass in the spring, in the fall in a no-till production system, and in the fall, following fall tillage.

Chapter 1

Confirmation of Glyphosate-resistant Italian Ryegrass (*Lolium perenne* ssp. *multiflorum*) in Arkansas

James W. Dickson, Robert C. Scott, Nilda R. Burgos, Reiofeli A. Salas, and Kenneth L. Smith

In 2007, populations of Italian ryegrass in Arkansas were observed surviving applications of glyphosate under field conditions in Southeast Arkansas. At least 10 reports of Italian ryegrass escaping glyphosate applications followed in subsequent years in Arkansas. These were unconfirmed reports of resistance from county agents, consultants and farmers. The objectives of this research were to confirm resistance to glyphosate in a suspected resistant population collected in 2007 (Desha 2007) and determine the level of resistance of a putative glyphosate-resistant population collected in 2009, both from Desha County, Arkansas. Other objectives were to determine the resistance frequency in these populations, to determine if the 2009 population was also acetolactate synthase (ALS) or acetyl-CoA carboxylase (ACCase-resistant), and to determine the effect on plant size as it relates to dose-response to glyphosate. The Desha 2007 population exhibited a low level of resistance to glyphosate. The estimated glyphosate dose that would control this population 50% was 1260 g ae ha$^{-1,}$ compared with 190 g ae ha^{-1} for the susceptible check. In 2009, a population of Italian ryegrass (Des03) was identified that survived a glyphosate application of 1740 g ae ha^{-1} made in the field, which is twice the commercial use rate for glyphosate. Dose-response experiments determined that an estimated 3890 g ae ha^{-1} glyphosate was required to obtain 50% biomass reduction of Des03; this was 23 times that of the susceptible standard. Neither growth stage nor glyphosate rate evaluated affected the level of resistance observed in the Des03 population. This population was determined to be over 70% resistant at the levels reported. In addition to glyphosate, Des03 was also resistant to diclofop, a commonly used wheat herbicide in Arkansas and other areas. As a result, alternative management strategies for Italian ryegrass are currently being explored.

Nomenclature: Glyphosate; Italian ryegrass, *Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot.

Key Words: Herbicide resistance, multiple resistance, resistance level

Italian ryegrass is one of the most troublesome weeds in Arkansas winter wheat (*Triticum aestivum L.*) production and has recently been determined to be resistant to glyphosate in Mississippi, creating difficulties with spring burn-down applications (Nandula et al. 2007). Options for burn-down other than glyphosate are limited; this can result in crops being planted into less than weed-free seed beds. In studies conducted from 1981 to 1983, wheat grain yields were reduced an average of 4.2% for every 10 Italian ryegrass plants m^{-2}, primarily due to reduced crop tillering (Liebl and Worsham 1987). In another study, wheat yields were reduced up to 92% when Italian ryegrass densities approached 400 plants m^{-2} (Hashem et al. 1998). Before chemical control, Italian ryegrass control options were limited to cultural practices such as crop rotation and fallowing fields. With the introduction of diclofop in the 1970s, Italian ryegrass could be chemically controlled in wheat. Diclofop is a herbicide that kills susceptible plants by inhibiting ACCase, the enzyme catalyzing the first committed step in *de novo* fatty acid synthesis (Burton et al. 1989; Focke and Lichtenthaler 1987).

In 1987, a population of Italian ryegrass in Oregon was confirmed resistant to diclofop (Betts et al. 1992; Stranger and Appleby 1989). To date, diclofop-resistant Italian ryegrass has been documented in at least 14 wheat-producing states including Arkansas (Kuk et al. 2000, 2008). Relatively new herbicides are now labeled for Italian ryegrass control in Arkansas wheat including pinoxaden (ACCase inhibitor), mesosulfuron and pyroxsulam, which are acetolactate synthase (ALS) inhibitors. The target of ALS inhibitors is a key enzyme in the biosynthesis of the branched-chain amino acids isoleucine, leucine, and valine (LaRossa and Schloss 1984; Senseman 2007). Five of the 25 diclofop-resistant populations from Arkansas tested by Kuk et al. (2008) were also resistant to pinoxaden. One of the 36 Italian ryegrass populations tested was resistant to mesosulfuron, but not to diclofop (Kuk et al. 2008).

Recently in Arkansas, Italian ryegrass has been difficult to control with commonly used herbicides in spring burn-down programs (personal observations by the authors). In Arkansas, growers usually begin spring burn-down applications in March and continue into May depending on weather conditions and cropping system. By March, Italian ryegrass growth can range from three-leaf to jointing stage. Because of its broad-spectrum, short plant-back intervals and price, most spring burn-down applications in Arkansas contain glyphosate. Glyphosate kills weeds by inhibiting 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase (Amrhein et al. 1980), which produces EPSP from shikimate-3-phosphate and phosphoenolpyruvate in the shikimic acid pathway (Coruzzi and Last 2000). Typically failures of Italian ryegrass control with glyphosate have been attributed to differences among individuals of the treated populations, suboptimal temperatures, and other environmental conditions that reduce glyphosate efficacy at the time of application (Martin and Slack 2005). However, several instances of glyphosate-resistant ryegrass (*Lolium* spp.) have been reported worldwide (Nandula et al 2007, Perez-Jones et al. 2005 and Powles et al. 1998).

The first reported instance of glyphosate resistance in *Lolium* spp. was in a population of rigid ryegrass (*Lolium rigidum* Gaudin) in Australia (Powles et al. 1998). Glyphosate resistance has been confirmed in a population of Italian ryegrass in Chile (Perez and Kogan 2003) and in three states in the United States: Oregon (Perez-Jones et al. 2005), Mississippi (Nandula et al. 2007), and California (Jasieniuk et al. 2008). Instances of Italian ryegrass populations surviving glyphosate applied in the spring in Mississippi and Arkansas as well as other southern states are increasing at an alarming rate.

The objectives of this research were to determine the level of glyphosate resistance in two populations of Italian ryegrass from Arkansas that were discovered in 2007 and 2009, to determine the effect of application timing and rate on the level of resistance observed, to observe the frequency of the resistant trait in the 2009 population, and to determine if this population was resistant to other commonly used Italian ryegrass herbicides.

Materials and Methods

2007 Dose Response Study. In 2007, some Arkansas growers reported instances of Italian ryegrass escaping glyphosate application during spring burn-down. Some escapes from one such field in Desha County were collected, allowed to mature in a greenhouse, and seeds were collected for whole-plant bioassays. Italian ryegrass seeds were planted in 15-cm-diameter pots filled with commercial potting medium (Sunshine Mix®, Sun Gro Horticulture Inc., Bellevue, WA 98008), at a rate of 25 seeds per pot. The pots were placed in trays, sub-irrigated with adequate water to soak the potting soil, and kept in the greenhouse at 14-h days. Day length was achieved with natural lighting supplemented by metal halide lamps. Day/night temperatures were maintained at 30/25 C. A commercial source of seed was used as a susceptible population.

Four-leaf seedlings were treated with glyphosate doses of 0, 200, 405, 810, 1625 and 3250 g ae ha^{-1} in the first run. In the second run, a higher rate of 6500 g ae ha^{-1} was added for the putative resistant population. The experimental units (pots) were arranged in a completely randomized design with five and four replications in the first run and second run, respectively. Glyphosate (potassium salt formulation) was applied in 187 L ha^{-1} spray volume in a spray chamber fitted with a motorized boom with flat fan 800067 nozzles (TeeJet 800067 flat-fan nozzle, Spraying Systems Co., Wheaton, IL 60189).

Visual estimates of Italian ryegrass control were recorded 14 and 21 d after treatment (DAT) on a scale of 0 to 100, with 0 equal to no Italian ryegrass control, and 100 equal to complete Italian ryegrass control. Maximum control was observed 21 DAT and only these data are presented. The number of survivors (those plants with new growth) in each experimental unit was also recorded and the percent mortality was calculated based on the number of plants treated. Percentage of mortality data were square-root transformed to partially correct for nonnormal data distribution. Data were modeled with a sigmoid, three-parameter, logistic regression equation (Equation 1) in SigmaPlot v.9(Sigma Plot, Jandel Scientific, Point Richmond, CA 94804):

$$Y = \mathrm{a}/(1 + [x/x_{\mathrm{o}}]^{\mathrm{b}}) \qquad [1]$$

2009 Dose Response Study. Glyphosate-dose-response experiments were conducted on a population of Italian ryegrass that survived field applications of glyphosate in 2009 (Des03), and a glyphosate-susceptible Italian ryegrass population obtained commercially (Pennington Seed Co., Madison, GA 30650) in 2009 (Com01). Seeds of these two populations were planted into flats 50 cm long by 25 cm wide by 5 cm deep filled with commercial potting medium. The flats were kept in a greenhouse with 12-h days and 24/18 C day/night temperatures. Day length was shortened and temperatures were lowered for this study to promote tillering for the timing study. Following emergence, individual two-leaf seedlings were transplanted into 15-cm pots filled with commercial potting medium.

Treatments included: an untreated check and 15 rates of glyphosate starting at 9 and doubling with each treatment up to 35,900 g ae ha^{-1}. Glyphosate rates evaluated in this study corresponded to 1/96 to 42 times the commercial rate of 870 g ae ha^{-1}. MON 78623 (potassium salt of glyphosate supplied by Monsanto Co., St. Louis, MO) was applied with 0.25% nonionic surfactant (NIS). Glyphosate treatments were applied with a CO_2-pressurized backpack sprayer equipped with 110015 flat fan nozzles (TeeJet 110015 flat-fan nozzle, Spraying Systems Co., Wheaton, IL 60189) calibrated to deliver a spray volume of 140.5 L ha^{-1}. Experimental units were arranged in a completely randomized design with four replications, and the experiment was repeated in time.

At 28 DAT, plants were cut at the soil surface and fresh weights were measured and recorded. Fresh-weight data were converted to a percentage of the untreated check and regression analysis was conducted using Sigma Plot. This calculation resulted in the effective "reduction in biomass". The reduction in biomass with increasing glyphosate rate was modeled with a sigmoid, three parameter, logistic function (Equation 1). These data were used to calculate relative tolerance to glyphosate, including the rates needed to provide at least 50% control or biomass reduction (GR_{50} values).

Effect of Application Timing on the Response of Des03 to Glyphosate. A third experiment was conducted to determine whether the size of Italian ryegrass at the time of application could affect efficacy and the levels of resistance to glyphosate observed in these populations. This experiment was comprised of three factors, evaluated in a split-split plot experimental design. Treatments included Italian ryegrass population (Des03 and Com01) as main plot, glyphosate dose (0, 870 and 1740 g ae ha^{-1}) as subplot, and growth stage at herbicide application (three- to four-leaf and three- to four-tiller) as sub-subplot. Composite seed samples of Des03 and COM01 were seeded into flats, 50 cm long by 25 cm wide by 5 cm deep filled with commercial potting medium. Two flats were included per treatment and the experiment was repeated in time. The flats were kept in the greenhouse with 12-h days and 24/18 C day/night temperatures.

Following emergence, Italian ryegrass was thinned to approximately 40 plants per flat. This resulted in approximately 160 plants from each population exposed to each herbicide treatment. Plants in each flat were counted prior to treatment. Applications were made using a CO_2-pressurized backpack sprayer and a hand-held boom equipped with 110015 flat fan nozzles calibrated to deliver a spray volume of 141 L ha^{-1}. Visual estimates of Italian ryegrass control were recorded 21 DAT. Final stand count in each flat was recorded 28 DAT to calculate percent mortality following herbicide treatment. Data were analyzed using SAS JMP software (SASJMP8 statistical software, SAS Institute, Cary, NC 27513) and means were separated using Fisher’s protected LSD at P=0.05.

Frequency of Glyphosate-Resistant Plants in Des03 Population. The proportion of resistant Italian ryegrass plants in the Des03 population was determined during the previously described application timing experiment, which was conduct at the Lonoke Agricultural Center, near Lonoke, AR. Resistance frequency was also determined at the Arkansas Agricultural Research and Extension Center, near Fayetteville, AR, using 80 Italian ryegrass seedlings, grown from a composite seed sample of the Des03 population. Once tillering occurred, each seedling was separated into two clones and transplanted into separate pots. One week after transplanting, the shoots were clipped at 5 cm height and allowed to regrow to approximately 15 cm. Plants were watered daily and fertilized with MiracleGro complete fertilizer (MiracleGro, The Scott's Co., Marysville, OH 43041) every two weeks. At the target regrowth stage, one set of clones was treated with 2240 g ae ha^{-1} glyphosate to discriminate between resistant (R) and susceptible (S) plants. The duplicate set was used as the corresponding nontreated check. Percentage mortality was calculated at 28 DAT by simply dividing the number of survivors by the total number of plants tested, mortality was based on the appearance or absence of new growth.

Resistance of Des03 to ACCase Inhibitor Herbicides Used in Wheat. This experiment was established using the same techniques outlined for the glyphosate application timing experiment. The herbicide treatments included the commercial rates of diclofop, pinoxaden, and glyphosate. At the three- to four-leaf stage, flats of Italian ryegrass representing Des03 and Com0,1 were treated with diclofop at 1125 g ai ha^{-1} plus crop oil concentrate at 1.2 L ha^{-1} and pinoxaden at 60 g ai ha^{-1}. Because these samples were part of a larger survey, glyphosate was also included at 870 g ae ha^{-1}. The experimental design was a split-split plot with population as the mainplot, herbicide rate (treated vs. nontreated) as subplot, and Italian ryegrass growth stage at application as the sub-subplot. Data were collected as in the glyphosate timing experiment and analyzed using SAS JMP software and means were separated using Fisher's protected LSD at P=0.05.

Results and Discussion

2007 Dose Response. At the lowest glyphosate dose evaluated, all resistant plants survived and all susceptible plants died (Figure 1). At the next highest dose, only a few resistant plants were killed. The estimated glyphosate dose to achieve 50% control of the susceptible Italian ryegrass population was 190 g ae ha^{-1} (Figure 1). The putative resistant population from Desha County, AR, collected in 2007 required 1260 g ae ha^{-1} glyphosate for 50% control, which was 6.6 times the rate required for 50% control of the susceptible standard population. The amount of glyphosate that would cause 50% mortality in the resistant population was 1040 g ae ha^{-1}, which was 7.8 times that of the susceptible population (134 g ae ha^{-1})(Figure 2). These data indicate that over 50% of the Italian ryegrass plants in this population would survive a commercial application of glyphosate at 840 g ae ha^{-1}. The grower was notified of this and his field has subsequently been managed to prevent further proliferation of this population. This included destroying the existing population with tillage and with tillage the next fall. This also prompted closer monitoring of Italian ryegrass populations for potential resistance to glyphosate. Italian ryegrass occurs commonly in Arkansas wheat, roadsides, and fallow fields. It is believed to be spread primarily by mechanical means during wheat harvest and by tillage equipment. In addition, it is also often spread as a pasture grass or as soil cover for roadsides and industrial sites. Often populations can be observed creeping in from roadsides and field edges where it may be left uncontrolled. However, at that time, no other fields had this level of survival from glyphosate applications. Increasing complaints of Italian ryegrass escapes in the following season precipitated a statewide sampling of Italian ryegrass populations in 2009 and this survey was continued into 2010.

2009 Dose Response. Preliminary screening of the 2009 statewide collection of Italian ryegrass revealed one population, also from Desha County, which was not controlled by the commercial rate of glyphosate. The glyphosate dose required for 50% control of the glyphosate-susceptible population (Com01) was 171 g ae ha^{-1} (Figure 3). This was very similar to the does required for

50% control of the susceptible population in the 2007 experiment. The suspected resistant population (Des03) required 3880 g ae ha^{-1} glyphosate for 50% control, which was 23 times that of the susceptible population. This 23-fold glyphosate resistance level is much higher than the three-fold resistance level reported by Nandula et al. (2007) and the 7.8-fold level obtained for the Desha 2007 population, although this calculation is relative to the susceptible standard used. Although the glyphosate-resistant population from Mississippi was reported to have reduced mobility of glyphosate in the plant (Nandula et al. 2007), it is possible, because of the observed increase in tolerance/resistance, that the Des03 population in Arkansas exhibits a different mechanism of resistance. The mechanism of resistance is currently being evaluated in the Des03 population. It may also be a simple function of a higher frequency of glyphosate resistance in this population.

Because the GR_{50} values of the susceptible populations used in 2007 and 2009 were very similar, the large difference in resistance index values for Desha 2007 and Des03 were indicative of an increasing glyphosate resistance problem in Italian ryegrass. It is very likely that more glyphosate-resistant Italian ryegrass populations will be discovered. This may also be a function of increasing frequency in resistant populations across the state. Increased efforts are now underway to develop Italian ryegrass management strategies for fall and spring.

Effect of Application Timing on the Response of DES03 to Glyphosate. Glyphosate rate (one to two times the commercial rate) did not affect Italian ryegrass control, regardless of population or growth stage at application (Table 1). This experiment further confirmed the resistance of Des03 to glyphosate. Although the susceptible standard population was controlled at least 90% at both growth stages, Des03 was controlled 26% or less following application at both growth stages. Control of the susceptible population and lack of control of the Des03 population was similar whether glyphosate was applied at the three- to four-leaf stage or at three- to four-tiller stage. The frequency of glyphosate-resistant individuals in the Des03 Italian ryegrass population was 71%, averaged over application timing and glyphosate rate (data not shown).

A common rationalization for Italian ryegrass escaping glyphosate is that Italian ryegrass was too big (already tillering) at the time of application. However, for the timings evaluated, this research indicates that control of glyphosate-resistant Italian ryegrass populations cannot be overcome by earlier timings and/or higher rates.

Frequency of Glyphosate-Resistant Plants in Des03 Population. The frequency of glyphosate-resistant Italian ryegrass in the subset of Des03 seeds tested in Fayetteville was 91%, in response to the 2240 kg ha^{-1} rate of glyphosate only (data not shown). Of the 80 plants treated, 73 survived. This high frequency of glyphosate-resistant individuals in the Des03 subset mirrors the high level of resistance in this population. This high occurrence of resistant biotypes can only lead to a more rapid spread of glyphosate-resistant Italian ryegrass in this area.

The loss of glyphosate for Italian ryegrass control will increase the cost of herbicide burn-down programs in no-till crop production. The addition of other herbicides or sequential applications may be needed for complete control. Additionally, tillage may be required in some cases, affecting soil conservation practices.

Resistance of Des03 to ACCase Inhibitor Herbicides Used in Wheat. The Des03 population was determined to be resistant to diclofop, with control of only 70% control exhibited at the commercial rate (Table 2). This level of control is not acceptable and causes economic problems for growers. The Des03 population was not significantly controlled (only 9%) by glyphosate in this trial. This occurrence of multiple resistance in Des03 is not surprising, considering the widespread resistance to diclofop among Italian ryegrass populations in Arkansas (Kuk et al. 2000; 2008). Selection for resistance to glyphosate occurred in populations already resistant to diclofop.   About 20% of the diclofop-resistant Italian ryegrass in Arkansas is cross-resistant to pinoxaden (Kuk et al. 2008); however, Des03 is still susceptible to pinoxaden (Table 2).

This is the first reported case of Italian ryegrass with a high level of resistance to glyphosate in Arkansas. A herbicide-resistance screening program was begun in 2009 to identify other populations of glyphosate-resistant Italian ryegrass in Arkansas, and more populations of glyphosate-resistant Italian ryegrass have been discovered (research is ongoing). Coupled with the widespread resistance to diclofop and the rapidly increasing resistance problems to ALS inhibitors used in wheat, management of Italian ryegrass in wheat and during burn-down is becoming very complicated. Wheat growers are quickly running out of chemical weed-control options. Italian ryegrass control often involves the use of burn-down herbicide programs that are becoming more complex and often involve sequential applications of other less-effective herbicides, fall followed by spring programs, and tillage. This pest is known to creep in from the field edges; it can also spread easily though harvest equipment and seed. Lack of herbicide rotation has also led to these resistance problems. Rapid adoption of alternative control measures, including fallowing some fields and adoption of practices to prevent the spread of this pest are needed to stop further evolution of glyphosate-resistant populations of Italian ryegrass in the midsouthern United States.

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Table 1. Effect of growth stage and application rate on glyphosate efficacy for control of two populations of Italian ryegrass in greenhouse experiments conducted at Lonoke, Arkansas.

	Italian ryegrass control, 28 days after treatment			
	Three- to four leaf stage		Three- to four -tiller	
Population	866 g ha^{-1}	1732 g ha^{-1}	866 g ha^{-1}	1732 g ha^{-1}
	- - - - - - - - - - - - - - - - - - % -			
Com01	91	98	85	95
Des03	9	14	13	26
$LSD_{0.05}$	---------------------------------25------------------------------------			

Table 2. Control of glyphosate-susceptible (Com01) and glyphosate-resistant (Des03) Italian ryegrass populations with glyphosate and ACCase herbicides, used in wheat, in greenhouse experiments at Lonoke, Arkansas.

Population	Italian Ryegrass control, 28 days after treatment		
	Pinoxaden	Diclofop	Glyphosate
	- - - - - - - - - - - - - - - - - -	%	- - - - - - - - - - - - - - - - - -
Com01	96	89	91
Des03	96	70	9
LSD $_{0.05}$	----------------------------	15	-------------------------------

[a] Herbicide application rates: Pinoxaden = 60 g ai ha^{-1}, diclofop = 1125 g ai ha^{-1} , and glyphosate = 870 g ae ha^{-1}. Treatments were applied at three-to four leaf growth stage.

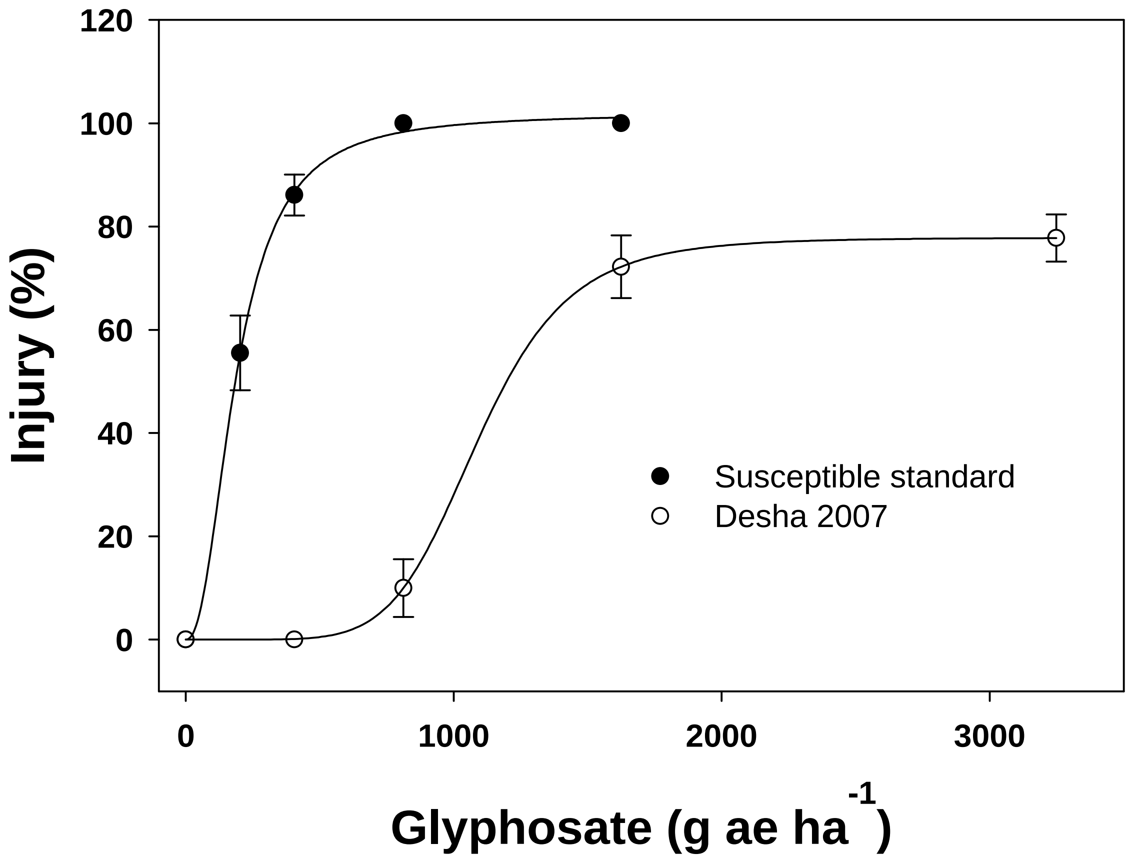

Figure 1. Visual estimates of injury (%) for Italian ryegrass ‘Desha 2007’ population and a susceptible standard, 21 d after glyphosate treatment. Each data point is the average of two experiments, with nine replications. Error bars are standard errors of the mean. The response to glyphosate of both populations was best described with a sigmoidal, three-parameter, logistic-regression function, with an estimated 50% control at 1260 g ae ha^{-1} for Desha 2007 and 190 g ae ha^{-1} for the susceptible standard.

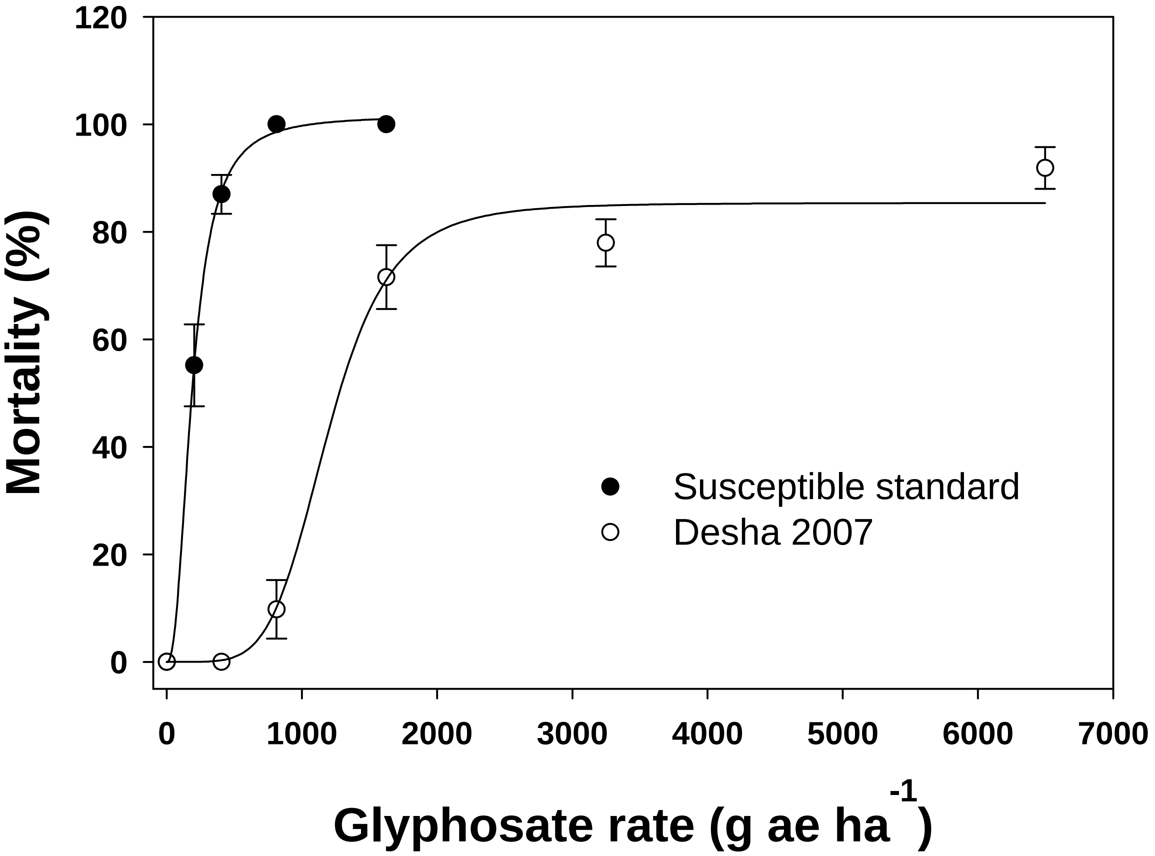

Figure 2. Mortality (%) evaluation of Italian ryegrass ‘Desha 2007’ population and a susceptible standard, 21 d after glyphosate treatment. Each data point is the average of two experiments, with nine replications. Error bars are standard errors of the mean. The response of Desha 2007 and the susceptible standard to glyphosate was best described with a three-parameter, logistic regression function. Desha 2007 had an estimated 50% mortality at 1040 g ae ha^{-1}. The susceptible standard had an estimated 50% mortality at 134 g ae ha^{-1}.

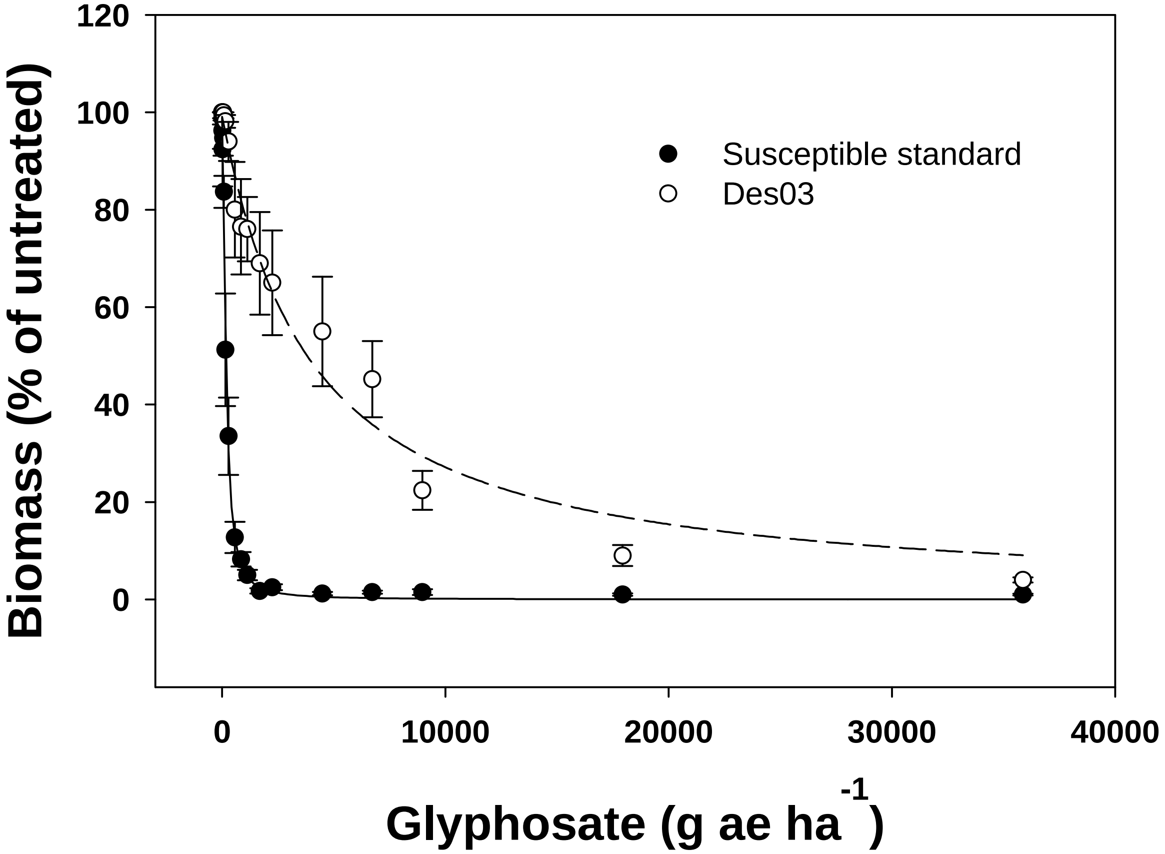

Figure 3. Shoot biomass reduction (%) of Italian ryegrass ‘Des03’ population and a susceptible standard, 28 d after glyphosate treatment. Each data point is the average of two experiments with two replications per experiment. Error bars were standard errors of the mean. Data were best described with a three-parameter, logistic-regression function. Des03 had an estimated 50% injury or visual biomass reduction (GR_{50}) value of 3886 g ae ha^{-1} glyphosate. The susceptible standard had an estimated GR_{50} value of 171 g ae ha^{-1} glyphosate.

Chapter 2

Distribution of Herbicide-Resistant Italian Ryegrass (*Lolium perenne* ssp. *multiflorum*) in Arkansas

James W. Dickson

Italian ryegrass is the most troublesome weed in wheat production in Arkansas and other states across the southern U. S., and has recently developed resistance to glyphosate in row crop production. Increasing complaints from producers about commonly used herbicides failing to control Italian ryegrass prompted a statewide sampling and testing of ryegrass to glyphosate, diclofop, pinoxaden, and pyroxsulam in 2009. Mature Italian ryegrass panicles were collected from 215 sites. Twenty-two of the 215 population samples were obtained from various retailers as commercial ryegrass seed. Seed from these population samples were grown in the greenhouse and treated at the 3- to 4-leaf stage with glyphosate 867 g ae ha^{-1}, diclofop 1122 g ai ha^{-1}, pinoxaden 60 g ai ha^{-1}, and pyroxsulam 18 g ai ha^{-1}. Stand counts before and 28 d after treatment (DAT) were conducted to obtain the percent survivors from each population sample. Twelve populations that survived a glyphosate application in the field were also treated with glyphosate 867 g ae ha^{-1}at 3- to 4-tiller growth stage, and 1734 g ae ha^{-1} at 3- to 4-leaf and 3- to 4-tiller growth stages to determine if there were any differences between application rates or timings. Thirty-seven population samples were resistant to glyphosate, 205 were resistant to diclofop, 25 were resistant to pinoxaden, and 137 were resistant to pyroxsulam. Furthermore, 18 population samples were resistant to diclofop, pinoxaden, and pyroxsulam, but not to glyphosate. All 37 population samples that were resistant to glyphosate were also resistant to diclofop, pinoxaden, or pyroxsulam, with two populations resistant to all four herbicides tested. Fourteen commercial samples were resistant to diclofop, one was resistant to pyroxsulam, and six were resistant to both diclofop and pyroxsulam. There was a significant improvement in glyphosate-resistant Italian ryegrass control (38% survivors) with 1734 g ae ha^{-1} applied to 3- to 4-tiller Italian ryegrass, compared to 55 to 58% survivors resulting from 867 g ae ha^{-1} applied to 3- to 4-leaf and 3- to 4-tiller Italian ryegrass; however, the results for individual populations was variable, indicating that Arkansas' Italian ryegrass populations are diverse and may currently be evolving into biotypes with higher levels of glyphosate resistance. The loss of these key herbicides for Italian ryegrass control in Arkansas severely limits the options available for controlling this rapidly-evolving weed both in burn-down and in-season in wheat.

Nomenclature: Diclofop, glyphosate, pinoxaden, pyroxsulam; Italian ryegrass, *Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot.

Key words: Glyphosate resistance, diclofop resistance, pinoxaden resistance, pyroxsulam resistance, herbicide resistance.

Italian ryegrass is the most troublesome weed in wheat production in Arkansas (Bararpour et al. 2003), Georgia, Kentucky, North and South Carolina, and Tennessee (Webster 2008). Liebl and Worsham (1987) reported wheat grain yield reductions of 4.2 % for every10 plants m^{-2}. Wheat grain yields can be reduced by as much as 92% when Italian ryegrass densities are as high as 400 plants m^{-2} (Hashem et al. 1998). These wheat yield reductions caused by competition with Italian ryegrass have been attributed to reduced crop tillering (Liebl and Worsham 1987), interception of up to 68% of photosynthetically active radiation during the boot stage in wheat due to Italian ryegrass' taller height and greater leaf area index (Hashem et al. 1998), and lodging and contaminating wheat grain at harvest with weed seed (Justice et al. 1994).

Since its introduction in the early 1980s, diclofop has been used extensively for the control of Italian ryegrass in winter-wheat production (Kuk et al. 2000). Diclofop is an aryloxypropionate (AOPP) herbicide which inhibits the enzyme acetyl-CoA carboxylase (ACCase), the enzyme catalyzing the first committed step in *de novo* fatty acid synthesis (Burton et al. 1989; Focke and Lichtenthaler 1987; Senseman 2007). The widespread use of diclofop, however, has led to numerous reports of diclofop-resistant Italian ryegrass. To date, six countries worldwide, including nine states in the United States, have reported diclofop-resistant Italian ryegrass (Heap 2012). Another ACCase herbicide labeled in Arkansas for Italian ryegrass control in wheat is pinoxaden. Pinoxaden is in a different chemical family, phenylpyrazolin, than diclofop (Senseman 2007). Since the introduction of pinoxaden in 2006, three countries worldwide, Argentina, Chile, and the United States, have reported pinoxaden-resistant Italian ryegrass (Heap 2012). In the United States, pinoxaden-resistant Italian ryegrass has been reported in Arkansas (Heap 2012; Kuk et al. 2008), North Carolina (Chandi et al. 2011), and in the Palouse region in Idaho and Washington (Rauch et al. 2010). Alternative herbicides with different modes of action, including acetolactate synthesis (ALS) inhibitors, are available to Arkansas wheat producers for Italian ryegrass control, these include: premixes of chlorsulfuron plus either metsulfuron or flucarbazone, mesosulfuron, imazamox, and pyroxsulam. The target of ALS herbicides is a key enzyme in the biosynthesis of the branched-chain amino acids isoleucine, leucine, and valine (LaRossa and Schloss 1984; Senseman 2007) However, in addition to the ACCase inhibitors, ALS-resistant Italian ryegrass has also been reported in Arkansas (Kuk et al. 2000; 2008; Heap 2012). In 2000, Kuk reported the first ryegrass population in Arkansas that was resistant to both diclofop and an ALS-herbicide, chlorsulfuron (2000).

Glyphosate is the most common burn-down product for fallow and preplant ryegrass control, and overall winter vegetation desiccation, in Arkansas. Glyphosate kills weeds by inhibiting 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase (Amrhein et al. 1980), which produces EPSP from shikimate-3-phosphate and phosphoenolpyruvate in the shikimic acid pathway (Senseman 2007). Italian ryegrass has become increasingly difficult to control with glyphosate in Arkansas (Dickson et al. 2011), as well as in Mississippi (Nandula et al. 2007) because of the development of glyphosate resistant biotypes of Italian ryegrass. The first reported instance of glyphosate resistance in ryegrass was with a population of rigid ryegrass (*Lolium rigidum* Gaud.) in Australia (Powles et al. 1998). Glyphosate resistance has since been found in populations of Italian ryegrass in Chile (Perez and Kogan 2003), Argentina, Brazil, Spain (Heap 2012), and in four states in the U.S. including Oregon (Perez-Jones et al. 2005), Mississippi (Nandula et al. 2007), California (Jasieniuk et al. 2008), and Arkansas (Heap 2012; Dickson et al. 2011).

The occurrence of ryegrass biotypes that are resistant to multiple herbicide families represents a significant threat to southern soft red winter wheat production. In addition, the presence of these biotypes increases the likelihood that populations of ryegrass found to be resistant to glyphosate may already be resistant to some alternative chemistry, making fallow and burn-down options for this pest increasingly limited.

The objectives of this research were to survey the occurrence and levels of glyphosate, diclofop, pinoxaden and pyroxsulam resistance in Arkansas populations of Italian ryegrass, and to evaluate the effect of rate and timing of glyphosate on glyphosate-resistant populations.

Materials and Methods

Mature panicles of Italian ryegrass were harvested from a 4 m^2 area at various locations throughout Arkansas in June 2009. Many of these population samples survived various herbicide treatments, while others were harvested from randomly selected sites. Only one location per site was sampled. When possible, global positioning system coordinates and production practices, including herbicide programs, were recorded on an information sheet for each sample. Samples were placed into one of five categories based on where they were collected: wheat field, row crop, glyphosate-burn-down survivor, commercially obtained, or other situation. Each population sample and information sheet was placed in paper bags and transported to the Lonoke Agricultural Research and Extension Center, near Lonoke, AR, for processing. A total of 215 population samples were obtained. Seeds from each population sample were threshed by hand and placed in envelopes. These envelopes were stored in a freezer at 0 C until initiation of experiments. Twenty-two of the population samples were purchased from various retailers across Arkansas that sell annual ryegrass for pasture seeding, erosion control, and various other purposes. Upon examination, all of the commercial samples that were supplied with a seed tag originated from Oregon.

Herbicide screening study. One day prior to experiment initiation, each envelope of seed was removed from the freezer and allowed to thaw at room temperature. A small scoop of seeds was sown into plastic trays measuring 25 cm wide by 25 cm long by 5 cm deep filled with a commercial potting medium (Sunshine Mix®, Sun Gro Horticulture Inc., Bellevue, WA 98008) and watered daily. The target seeding rate for each tray was 40 seeds per tray. This number of plants prevented overcrowding in each tray and allowed for adequate spray coverage. The trays were kept in the greenhouse with 12-h days and 24/18 C day/night temperatures. Stand counts were recorded when Italian ryegrass seedlings reached the three-leaf growth stage. Four trays from each population sample were treated with respective herbicides, resulting in approximately 160 plants from each population being exposed to each herbicide treatment. Glyphosate (867 g ae ha^{-1}), diclofop plus a crop oil concentrate (1122 g ai ha^{-1}+ 1% v/v), pinoxaden (60 g ai ha^{-1}), and pyroxsulam plus a non-ionic surfactant (18 g ai ha^{-1} + 0.05% v/v) were applied when Italian ryegrass reached the three-leaf growth stage. Herbicide applications were made using a CO_2 pressurized backpack sprayer equipped with TeeJet 110015 flat-fan nozzles (TeeJet 110015 flat-fan nozzle, Spraying Systems Co., Wheaton, IL 60189) calibrated to deliver 140 L ha^{-1} at 276 kPa. A preliminary run of this experiment identified a commercial ryegrass sample that was susceptible to all four herbicides, and served as a herbicide-susceptible population each time the experiment was conducted. A nontreated check for each population sample was included as well. Stand counts of surviving Italian ryegrass plants were recorded, 28 DAT. The number of surviving plants after treatment was divided by the number of plants before treatment and multiplied by 100 to obtain the percentage of surviving plants after treatment. The data presented are the percent survivors of the total sampled from each population following each herbicide application (28 DAT). At this time, for all herbicides evaluated, it was apparent which plants were dead versus alive or if re-growth was going to occur, in which case the plant was considered alive.

Population by glyphosate rate by growth stage study. Twenty-seven of the population samples were collected from Italian ryegrass plants that survived at least one application of glyphosate in the field. These 27 population samples were included in the herbicide screening study, and were investigated further. These population samples were planted and maintained with the same methods as previously described in the herbicide screening study. Fifteen of these population samples germinated poorly and adequate sample size could not be obtained; therefore, these 15 population samples were excluded from this study. Glyphosate at 867 g ae ha^{-1} and 1734 g ae ha^{-1} was applied at the three-leaf and 3-tiller growth stages to determine if there were any differences in glyphosate efficacy among these populations in relation to glyphosate rate or growth stage at the time of glyphosate application. Herbicide applications were made the same way as described in the herbicide screening study. This experiment was conducted as a split-split plot design with population sample as main plot, glyphosate rate as subplot, and growth stage at application as sub-subplot. The experiment consisted of two replications (each plastic tray of approximately 40 plants as a replicate) and was repeated once. The data presented are the percent of survivors, at 28 DAT.

Results and Discussion

Herbicide screening study. Almost half of all Italian ryegrass-populations sampled, 101 of 215, were harvested from wheat fields (Table 1). Of these 101 population samples, 98 were resistant to diclofop; furthermore 205 of the total 215 population samples were resistant to diclofop, including 20 of the 22 commercial population samples tested. Excluding the commercial populations, 1 to 100% of individuals per population survived the commercial rate of diclofop, with an average survivor frequency of 31% (Table 2). This high number of diclofop-resistant populations is not surprising. Previously, Kuk et al. (2000; 2008) reported that most of their Arkansas samples tested were resistant to diclofop. However, at that time only one population was found to have multiple resistance to an ALS-inhibiting herbicide, chlorsulfuron.

Although pinoxaden and diclofop are both ACCase inhibitors, pinoxaden has a novel chemical structure that alters its efficacy (Boeger et al. 2006; Ellis et al. 2010). While 205 samples in this survey were found to be resistant to diclofop, only 25 were resistant to pinoxaden (Table 1). In addition, the median level of resistance was much higher with respect to diclofop (26%) than to pinoxaden (3%) (Table 2). Even though the frequency of resistance to pinoxaden was relatively low in this study, it is a cause for concern. Introduced in 2006, pinoxaden has become a major tool for resistant ryegrass management in Arkansas and across the mid-south, especially where populations of ryegrass are already resistant to diclofop and ALS herbicides. Of the 193 population samples from Arkansas, 105 were resistant to diclofop and pyroxsulam, three were resistant to both diclofop and pinoxaden, and 18 were resistant to diclofop, pinoxaden, and pyroxsulam (Table 3). In general, the populations with resistance to these three wheat herbicides had highly variable frequencies of resistance; however, Whi03 had 79% or greater resistance to all three wheat herbicides. Pyroxsulam is the most recently introduced ALS wheat herbicide, receiving registration in 2008 (Anonymous 2008). In 2008, Kuk et al. discovered one Italian ryegrass population that was 33 times more resistant to mesosulfuron than a susceptible population. This ALS-resistant population had not been exposed to ALS herbicides in the three years prior to collection; therefore Kuk et al. (2008) concluded that resistance to mesosulfuron and other ALS herbicides within the same chemical family (sulfonylurea) and of another chemical family (imidazolinone) already existed in this population. The 130 population samples from Arkansas that were found to be resistant to pyroxsulam (Table 1) were more than likely already resistant to ALS herbicides, before pyroxsulam was available for use in Arkansas.

Twenty-one out of 22 commercial samples tested were resistant to either diclofop, pyroxsulam, or both herbicides (Appendix 1). Fourteen were resistant to diclofop, one was resistant to pyroxsulam, and six were resistant to both diclofop and pyroxsulam. No commercial sample was resistant to glyphosate or pinoxaden. For 18 of these populations resistant to diclofop, there were six percent survivors or below; however, for two populations, Com13 and Com22 there were 12 and 55% survivors, respectively. Com22 also had 3% survivors, following the pyroxsulam treatment. All of the other commercial samples resistant to pyroxsulam had 8% survivors or less. This indicates that diclofop and, to a lesser degree, pyroxsulam resistance is present in some of the commercially available ryegrass in Arkansas, which will contribute to the spread of herbicide-resistant populations. Ryegrass is a popular pasture grass, is used for soil stabilization following construction and, to a lesser degree, as a winter lawn grass in Arkansas. Research has shown that pollen from *Lolium* spp. can travel up to 3000 m (Busi et al. 2008); therefore, if these commercial populations are allowed to flower, herbicide resistance from these populations will more than likely be spread to neighboring populations.

A total of 37 populations of ryegrass were found with some level of resistance to glyphosate (Table 1). The average number of resistant plants was 31% and ranged from 1 to 94% (Table 2), although, the median level of resistance to glyphosate was 7% (Table 3). Of the 37 glyphosate-resistant populations in Arkansas, 12 were also resistant to diclofop, three were also resistant to pyroxsulam (Appendix 1), 19 were also resistant to diclofop and pyroxsulam, one was also resistant diclofop and pinoxaden, one was resistant to pinoxaden and pyroxsulam, and two were resistant to all four herbicides tested; however, resistance to pinoxaden was 3% or less in the populations resistant to all four herbicides (Table 3).

Figures 1-4 illustrate the geographic distribution of the population samples by category. This excludes the commercial samples as well as any samples where GPS coordinates were not provided (see Appendix 1). For diclofop (Figure 2), pinoxaden (Figure 3), and pyroxsulam (Figure 4) distribution patterns appear to be fairly random across the sampled areas. These areas indicate major wheat producing counties in Arkansas. At least for pinoxaden and pyroxsulam, there are both resistant and non-resistant populations interspersed throughout the state and in the areas sampled. However, most glyphosate resistant samples are focused in counties along the Mississippi River, with the exception of one population in the SW corner of the state (Figure 1).

Glyphosate resistance has previously been reported to a much greater degree in Mississippi than in Arkansas (Bond and Nandula 2011). The distribution pattern in Figure 1 indicates somewhat of a concentration of glyphosate-resistant samples in counties bordering Mississippi; in addition, these samples were loosely taken from counties near major bridges at Greenville, MS; Helena, AR; and West Memphis, AR. This could possibly explain the pattern of concentrations of glyphosate-resistant populations found in this survey; however, a more detailed sampling process would be needed of the areas directly connected to bridges to make this assumption.

Population by glyphosate rate by growth stage study. Averaged across population, there was a significant improvement in glyphosate-resistant Italian ryegrass control (38% survivors) with 1734 g ae ha^{-1} applied to 3- to 4-tiller Italian ryegrass, compared to 55 to 58% survivors resulting from 867 g ae ha^{-1} applied to 3- to 4-leaf and 3- to 4-tiller Italian ryegrass (Table 4). This data indicates that as plant size increases, a higher dose of glyphosate is required for increased control; however, even at the higher rate, on average 38 to 56% of the individuals in these populations survived and would have produced seed.

Not all population samples responded the same to increasing glyphosate rate or application timing (Table 5). When the glyphosate rate was doubled, percent survivors decreased for seven population samples but remained the same or increased for five population samples; furthermore, when applications were delayed until Italian ryegrass was three- to four-tiller, percent survivors increased for four populations but remained the same or decreased for eight population samples (Table 5). These different responses among the glyphosate-resistant populations indicate the occurrence of highly variable levels of glyphosate resistance present in Arkansas ryegrass populations. It could also be an indication that populations are currently dynamically evolving to higher levels of resistance. For example, when averaged over two rates or averaged over two timings, the effect of glyphosate on ryegrass samples varied between the original screening (Appendix 1) and the results of this study (Table 5).

As a result of this work, one population, Des03, was evaluated further and required 3886 g ae ha^{-1} (4.5 times the labeled use rate) just to obtain 50% biomass reduction (Dickson et al. 2011). This rate of glyphosate was in fact 23 times the rate required for 50% biomass reduction in a known susceptible population of ryegrass. Previous research with glyphosate resistance in *Lolium* spp. has indicated the possibility of multiple mechanisms of resistance including reduced glyphosate translocation to the shoot meristem, target site mutations (Preston et al. 2009) and EPSPS gene amplification (Salas et al. 2012).

This research indicates that while Italian ryegrass control increased with increasing glyphosate rates, on average, over one third of the plants survived to produce seed. Increasing glyphosate rates will not overcome resistance in these populations. In addition, the continued use of higher rates of glyphosate on populations with low levels of resistance will inevitably lead to more resistance. Currently, Arkansas ryegrass populations contain individuals that are resistant to ACCase, ALS, and EPSPS inhibiting herbicides. Cross- and multiple-resistant populations have been found. In addition, resistance to some of the newest chemistry available for ryegrass control is emerging. For the majority of the populations, no fitness penalty associated with herbicide resistance appears to be present. Fifteen of the population samples harvested following glyphosate-application failures exhibited very poor germination and therefore were omitted from the population by glyphosate rate by application timing study. Further research on these population samples may reveal fitness penalties or delayed germination (increased dormancy) associated with glyphosate resistance; however, this poor germination of glyphosate-resistant populations is contrary to the results of research by Nandula et al. (2009). Nandula et al. found that a glyphosate-resistant population had higher germination compared to a glyphosate-susceptible population (2009).

The loss of the key herbicides evaluated in this research as effective tools for Italian ryegrass control will negatively impact crop-production systems in Arkansas. There are only two herbicides with different modes of action than ACCase and ALS inhibitors available to producers in Arkansas for Italian ryegrass in winter wheat: flufenacet (inhibitor of very-long-chain fatty acid biosynthesis) and pendimethalin (microtubule-assembly inhibitor). These two herbicides only control ryegrass before emergence, and rely on rainfall or overhead irrigation for activation in the soil (Anonymous 2010; 2007). There are no postemergence applied herbicides available in Arkansas that are not ACCase or ALS inhibitors (Scott et al. 2012). In fields infested with ACCase- and ALS-resistant Italian ryegrass populations, producers may not have the option to plant winter wheat, thus losing a source of income. Furthermore, the loss of glyphosate as a tool for Italian ryegrass control prior to planting spring crops will only complicate weed control by increasing input costs and, in some cases, result in crops being planted into residues that can negatively affect yields. Control with herbicides other than glyphosate is often inconsistent, and may leave some Italian ryegrass that will spread glyphosate resistance to neighboring populations. The situation that this research has revealed in Arkansas is very similar to the situation with rigid ryegrass in Australia in which widespread occurrence of multiple- and cross-herbicide resistant *Lolium* spp. is common and results in severe economic loss (Heap 2012). The Australian situation prompted one researcher to report: “Overreliance on glyphosate to the exclusion of other weed management practices is the prime cause of glyphosate resistance evolving in these species” (Preston et al. 2009). Alternatives to glyphosate and herbicides in general, for Italian ryegrass control in Arkansas need to be explored.

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Table 1. Total number of resistant populations by category.

Origin	Population samples	Resistant population samples[a]			
		Glyphosate	Diclofop	Pinoxaden	Pyroxsulam
Wheat	101	11	98	17	68
Row crop	28	4	27	1	22
Glyphosate burn-down survivor	23	15	19	2	15
Commercial	22	0	20	0	7
Other	33	7	33	3	21
N/A[b]	8	0	8	1	4
Total	215	37	205	25	137

[a] A resistant population in this table means at least one percent survival of a population with the commercial dose of glyphosate (867 g ae ha^{-1}), diclofop (1122 g ai ha^{-1}), pinoxaden (60 g ai ha^{-1}), or pyroxsulam (18 g ai ha^{-1}).

[b] Abbreviation used, N/A: no information available.

Table 2. Survival (%) of resistant Italian ryegrass population samples to four herbicides[a].

Herbicide	Observations	Survivor Range	Survivor Mean	Survivor Median
		%	%	%
Glyphosate	37	1 to 94	28	7
Diclofop	185	1 to 100	31	26
Pinoxaden	25	1 to 79	13	3
Pyroxsulam	130	1 to 92	26	10

[a] Commercial samples were not included in this analysis.

Table 3. Population samples and the respective percent survivors, 28 days after treatment, of each population that is resistant to three or four of the four herbicides evaluated.

Population	County	Source[a,b]	% Survivors			
			Glyphosate	Diclofop	Pinoxaden	Pyroxsulam
Chi04	Chicot	Other	1	3	2	0
Chi05	Chicot	Burndown	2	10	0	22
Chi06	Chicot	Other	3	4	0	1
Chi09	Chicot	Other	3	3	0	8
Cri02	Crittenden	Wheat	1	5	0	80
Cri03	Crittenden	Row crop	2	2	0	90
Cri06	Crittenden	Wheat	38	0	1	28
Des07	Desha	Burndown	77	9	0	12
Des09	Desha	Burndown	72	21	0	5
Des10	Desha	Burndown	94	52	0	7
Des14	Desha	Burndown	76	8	0	6
Des15	Desha	Row crop	23	15	0	17
Des18	Desha	Wheat	6	26	0	85
Des19	Desha	Other	10	9	0	1
Des20	Desha	Burndown	67	13	0	4
Des21	Desha	Wheat	7	3	0	13
Fau03	Faulkner	Other	0	2	1	11
Gre02	Greene	N/A	0	84	2	74
Gre06	Greene	Row crop	0	71	3	24
Laf03	Lafayette	Wheat	16	40	0	10
Laf06	Lafayette	Wheat	0	55	4	48
Laf07	Bossier[c]	Burndown	1	24	0	1
Lon02	Lonoke	Wheat	0	80	14	57
Lon05	Lonoke	Wheat	0	73	8	66
Lon06	Lonoke	Wheat	0	7	2	1
Lon10	Lonoke	Other	0	39	67	89
Phi01	Phillips	Burndown	4	3	0	33
Phi02	Phillips	Burndown	3	28	0	57
Phi03	Phillips	Burndown	6	10	0	8
Poi21	Poinsett	Wheat	0	89	21	91
Poi22	Poinsett	Wheat	0	63	13	65
Poi23	Poinsett	Wheat	0	73	3	86
Stf03	St. Francis	Wheat	0	34	1	17
Stf06	St. Francis	Wheat	0	80	1	5
Whi01	White	Wheat	0	64	8	52
Whi03	White	Wheat	0	91	79	87
Whi04	White	Wheat	0	82	26	57
Whi16	White	Wheat	0	74	28	77
Whi17	White	Wheat	0	84	33	76
Des04	Desha	Other	84	13	2	6
Des05	Desha	Burndown	56	14	3	20

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[a] Row crop includes corn, cotton, grain sorghum, rice, and soybean. Other includes all other environments in which Italian ryegrass was harvested.

[b] Abbreviation used, N/A: no information available.

[c] Laf 07 was harvested in Bossier P+arish, Louisiana.

Table 4. Percent survivors of 12 glyphosate-resistant Italian ryegrass populations averaged over glyphosate rate and application timing.

	Survivor	
Application timing	867 g ae ha^{-1}	1734 g ae ha^{-1}
	———————%———————	
3 Leaf	58	56
3 Tiller	55	38
LSD (0.05)	---------------------6---------------------	

Table 5. Percent survivors of glyphosate-resistant Italian ryegrass populations averaged over two glyphosate rates and two application timings.

	Survivor			
	Glyphosate rate		Application timing	
Population	867 g ae/ha	1734 g ae/ha	3 leaf	3 tiller
	%			
DES01	83	52	60	75
DES02	67	43	51	59
DES03	80	63	64	78
DES05	49	57	64	43
DES06	15	83	38	60
DES07	65	40	76	29
DES09	80	78	94	64
DES10	73	29	74	28
DES14	70	77	86	62
DES20	45	35	80	0
DES24	24	1	0	25
PHI08	30	8	0	38
LSD (0.05)	----------15----------		----------15----------	

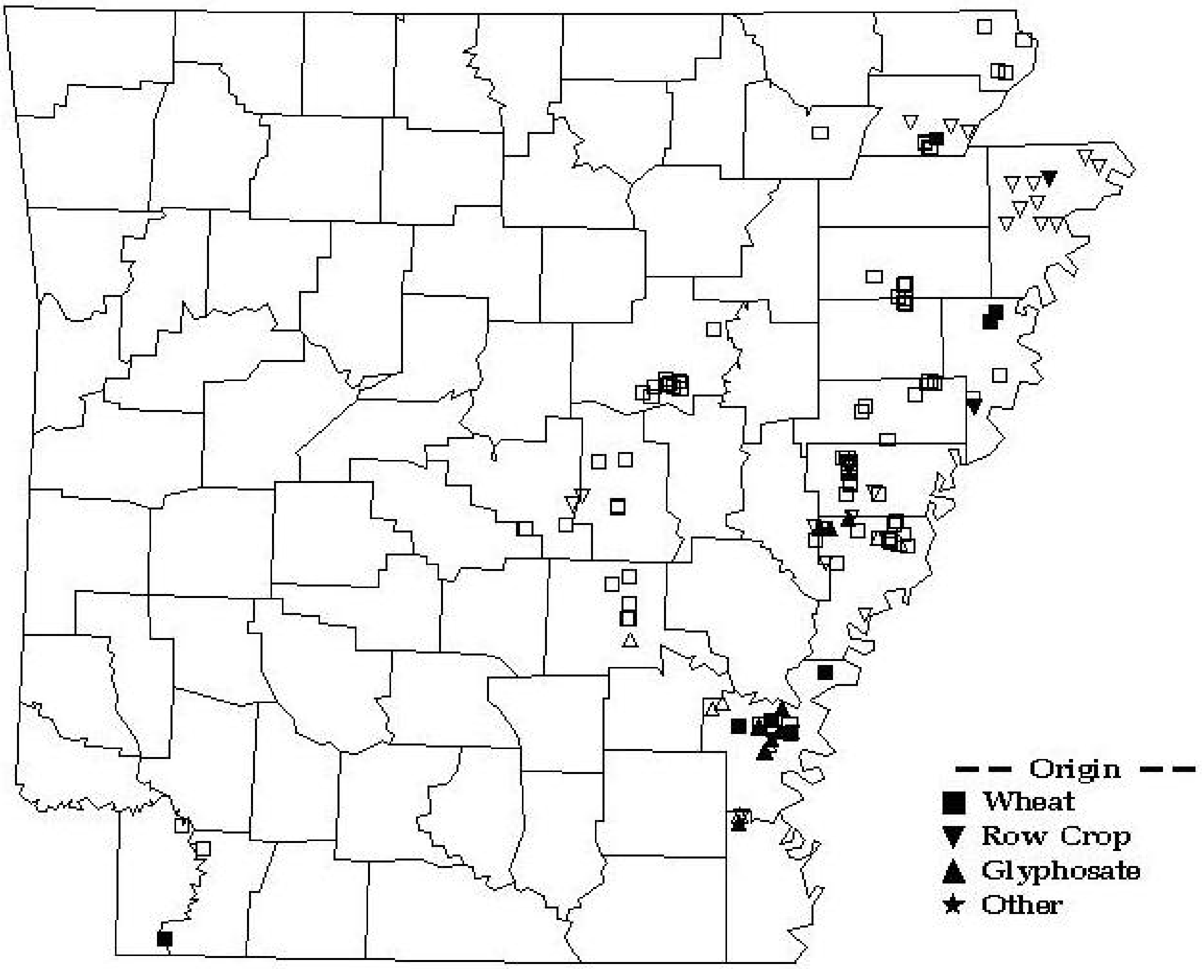

Figure 1. Distribution of glyphosate-resistant Italian ryegrass populations in Arkansas (solid shapes); blank shapes are locations of glyphosate-susceptible populations.

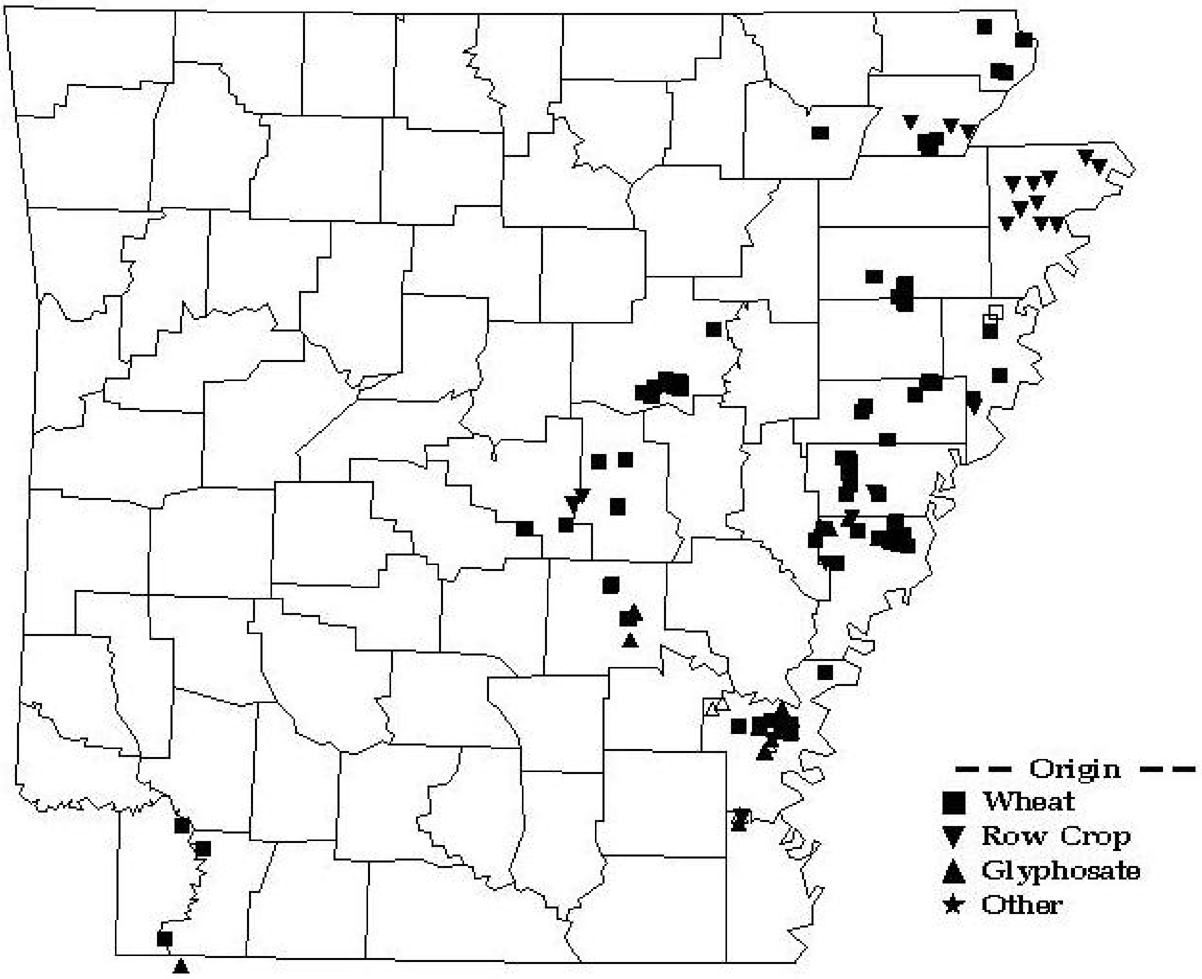

Figure 2. Distribution of diclofop-resistant Italian ryegrass populations in Arkansas (solid shapes); blank shapes are locations of diclofop-susceptible populations.

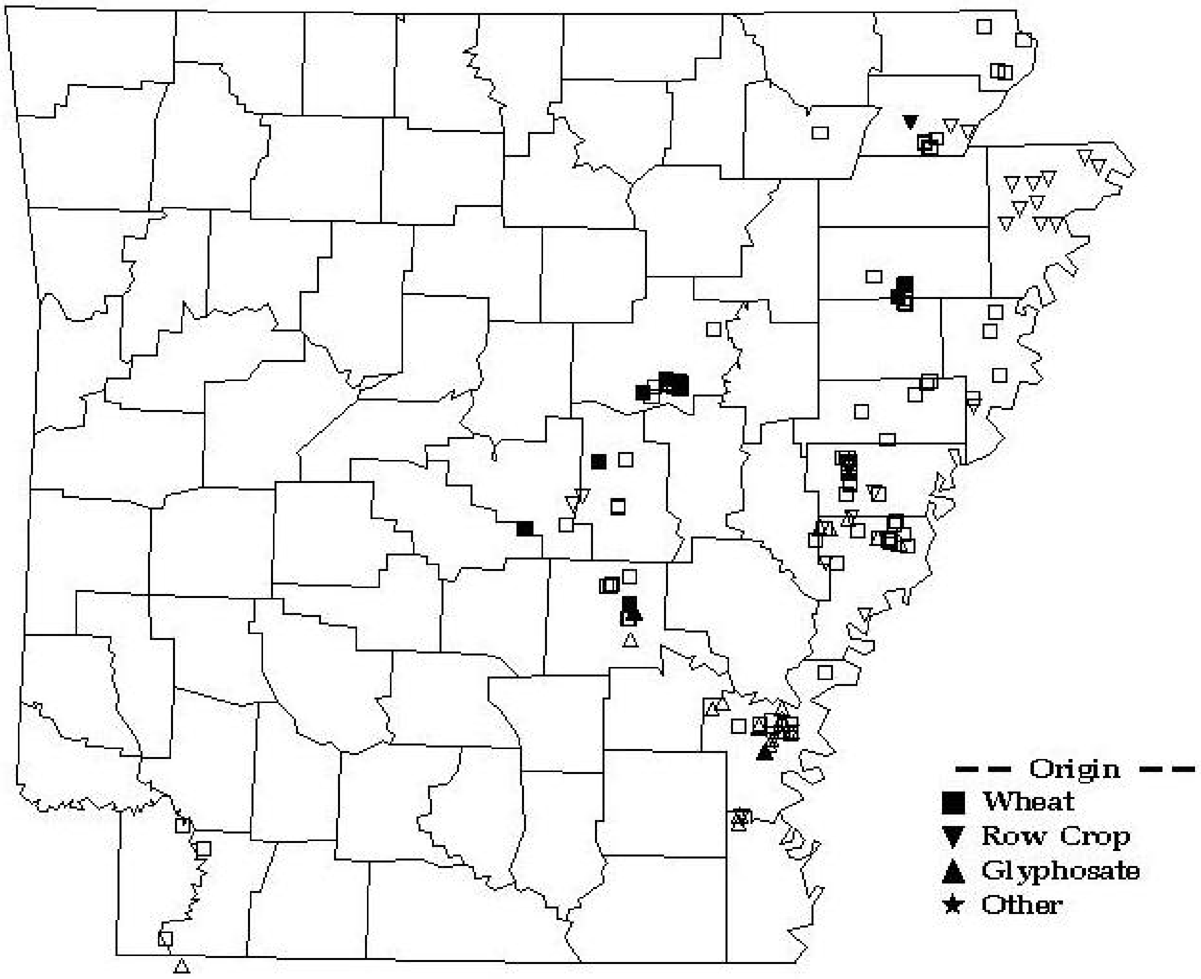

Figure 3. Distribution of pinoxaden-resistant Italian ryegrass populations in Arkansas (solid shapes); blank shapes are locations of pinoxaden-susceptible populations.

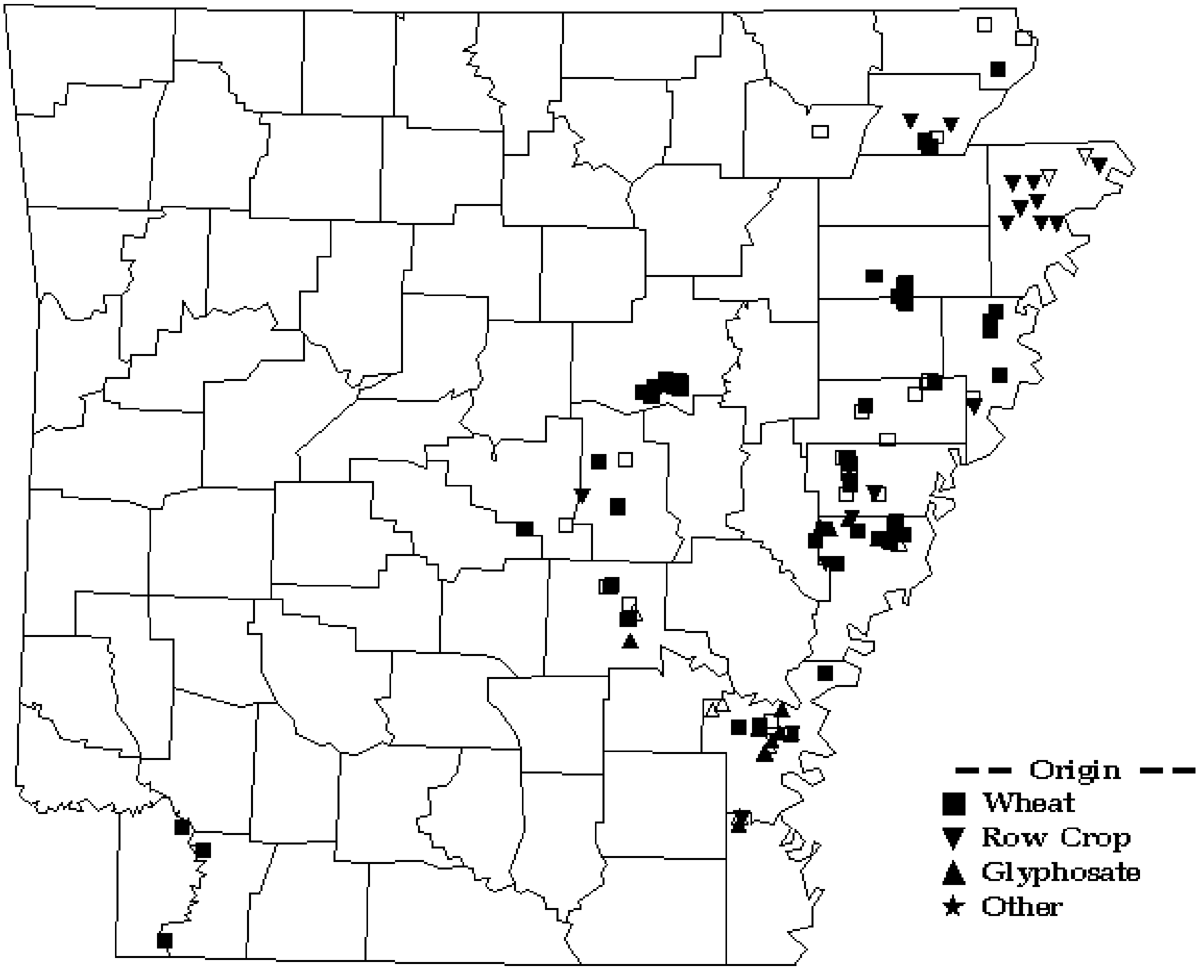

Figure 4. Distribution of pyroxsulam-resistant Italian ryegrass populations in Arkansas (solid shapes); blank shapes are locations of pyroxsulam-susceptible populations.

Appendix

Appendix 1. Population sample information and percent survivors of each population sample, 28 days after treatment.

			% Survivors			
Population	County	Source[a]	Glyphosate	Diclofop	Pinoxaden	Pyroxsulam
Chi01	Chicot	Row crop	0	0	0	0
Chi02	Chicot	Other	15	5	0	0
Chi03	Chicot	Row crop	0	9	0	7
Chi04	Chicot	Other	1	3	2	0
Chi05	Chicot	Burndown	2	10	0	22
Chi06	Chicot	Other	3	4	0	1
Chi07	Chicot	Other	1	1	0	0
Chi08	Chicot	Other	0	3	0	1
Chi09	Chicot	Other	3	3	0	8
Cla01	Clay	Wheat	0	18	0	0
Cla02	Clay	Wheat	0	23	0	6
Cla03	Clay	Wheat	0	100	0	0
Cla04	Clay	N/A[b]	0	20	0	0
Cla05	Clay	Wheat	0	6	0	1
Cla06	Clay	Other	0	70	0	0
Cla07	Clay	Other	0	15	0	0
Cri01	Crittenden	Wheat	0	12	0	0
Cri02	Crittenden	Wheat	1	5	0	80
Cri03	Crittenden	Row crop	2	2	0	90
Cri04	Crittenden	Wheat	0	5	0	55
Cri05	Crittenden	Wheat	17	0	0	62
Cri06	Crittenden	Wheat	38	0	1	28
Cro01	Cross	Wheat	0	26	0	55
Des01	Desha	Burndown	78	8	0	N/A
Des02	Desha	Burndown	62	2	0	N/A
Des03	Desha	Burndown	69	7	0	N/A
Des04	Desha	Other	84	13	2	6
Des05	Desha	Burndown	56	14	3	20
Des06	Desha	Burndown	0	0	0	0
Des07	Desha	Burndown	77	9	0	12
Des08	Desha	Row Crop	62	10	0	0
Des09	Desha	Burndown	72	21	0	5
Des10	Desha	Burndown	94	52	0	7
Des11	Desha	Burndown	4	0	0	29
Des12	Desha	Wheat	0	7	0	2
Des13	Desha	Wheat	67	16	0	0
Des14	Desha	Burndown	76	8	0	6
Des15	Desha	Row crop	23	15	0	17
Des16	Desha	Wheat	7	7	0	0
Des17	Desha	Wheat	0	3	0	1
Des18	Desha	Wheat	6	26	0	85

Des19	Desha	Other	10	9	0	1
Des20	Desha	Burndown	67	13	0	4
Des21	Desha	Wheat	7	3	0	13
Des22	Desha	Burndown	0	0	0	0
Des24	Desha	Burndown	0	0	0	0
Fau01	Faulkner	Wheat	0	23	0	75
Fau03	Faulkner	Other	0	2	1	11
Gre01	Greene	N/A	0	67	0	20
Gre02	Greene	N/A	0	84	2	74
Gre03	Greene	Wheat	0	11	0	2
Gre04	Greene	Wheat	0	11	0	4
Gre06	Greene	Row crop	0	71	3	24
Gre08	Greene	Wheat	0	34	0	3
Gre09	Greene	N/A	0	17	0	5
Gre10	Greene	Row crop	0	22	0	1
Gre11	Greene	N/A	0	62	0	0
Gre12	Greene	Wheat	3	45	0	0
Gre13	Greene	Row crop	0	38	0	2
Gre14	Greene	Wheat	0	28	0	49
Jac02	Jackson	Wheat	0	39	0	2
Jac03	Jackson	Wheat	0	46	0	0
Jac04	Jackson	Wheat	0	73	0	13
Jac05	Jackson	Wheat	0	17	0	2
Jac06	Jackson	Wheat	0	20	0	0
Jac07	Jackson	Wheat	0	42	0	1
Jac08	Jackson	Other	0	19	0	1
Jac09	Jackson	Other	0	19	0	0
Jac10	Jackson	Wheat	0	72	1	0
Jac11	Jackson	Wheat	0	33	0	0
Jac12	Jackson	Other	0	33	0	0
Jac13	Jackson	Other	0	8	0	13
Jac14	Jackson	Other	0	21	0	0
Jac15	Jackson	N/A	0	5	0	0
Jac16	Jackson	Wheat	0	15	0	0
Jac17	Jackson	Wheat	0	27	0	0
Jef01	Jefferson	Burndown	0	43	0	69
Jef03	Jefferson	Wheat	0	1	0	1
Jef04	Jefferson	Wheat	0	3	0	11
Jef05	Jefferson	Wheat	1	4	0	0
Jef06	Jefferson	Wheat	1	1	0	0
Jef07	Jefferson	Other	0	1	0	2
Jef08	Jefferson	Other	0	5	0	2
Jef09	Jefferson	Wheat	0	1	2	0
Jef10	Jefferson	Other	0	1	0	0
Jef11	Jefferson	Burndown	0	8	2	0

Jef12	Jefferson	Row crop	0	1	0	1
Jef13	Jefferson	Wheat	0	3	0	0
Jef14	Jefferson	Wheat	0	0	0	2
Jef15	Jefferson	Other	0	37	0	0
Laf01	Lafayette	Wheat	0	34	0	27
Laf03	Lafayette	Wheat	16	40	0	10
Laf04	Lafayette	Wheat	0	83	0	29
Laf05	Lafayette	Burndown	0	11	0	17
Laf06	Lafayette	Wheat	0	55	4	48
Laf07	Bossier[c]	Burndown	1	24	0	1
Laf08	Lafayette	Wheat	0	27	0	16
Law01	Lawrence	Wheat	0	72	0	0
Lee01	Lee	Other	0	45	0	0
Lee02	Lee	Row crop	0	56	0	92
Lee03	Lee	Wheat	0	52	0	21
Lee04	Lee	Row crop	0	31	0	1
Lee05	Lee	Wheat	0	21	0	3
Lee06	Lee	Wheat	0	5	0	0
Lee07	Lee	Wheat	0	42	0	2
Lee08	Lee	Row crop	0	34	0	9
Lee09	Lee	Wheat	0	41	0	3
Lee11	Lee	Wheat	0	21	0	0
Lee12	Lee	Wheat	0	13	0	0
Lee13	Lee	Row crop	0	6	0	0
Lee14	Lee	Wheat	0	25	0	0
Lee15	Lee	Wheat	0	37	0	0
Lee16	Lee	Wheat	0	30	0	0
Lon01	Lonoke	Other	0	28	0	2
Lon02	Lonoke	Wheat	0	80	14	57
Lon03	Lonoke	Other	0	3	0	0
Lon04	Lonoke	Other	0	37	0	14
Lon05	Lonoke	Wheat	0	73	8	66
Lon06	Lonoke	Wheat	0	7	2	1
Lon07	Lonoke	Wheat	0	28	0	0
Lon08	Lonoke	Wheat	0	8	0	0
Lon09	Lonoke	Wheat	0	22	0	3
Lon10	Lonoke	Other	0	39	67	89
Mis01	Mississippi	Row crop	0	54	0	73
Mis02	Mississippi	Row crop	0	41	0	3
Mis03	Mississippi	Row crop	0	39	0	2
Mis04	Mississippi	Row crop	4	22	0	0
Mis05	Mississippi	Row crop	0	31	0	0
Mis06	Mississippi	Row crop	0	24	0	8
Mis07	Mississippi	Row crop	0	20	0	10
Mis08	Mississippi	Row crop	0	10	0	33

Mis09	Mississippi	Row crop	0	13	0	10
Mis10	Mississippi	Row crop	0	23	0	77
Mon01	Monroe	Wheat	0	35	0	4
Mon02	Monroe	Row crop	0	37	0	6
Mon03	Monroe	Row crop	0	43	0	6
Mon04	Monroe	Other	0	52	0	84
Phi01	Phillips	Burndown	4	3	0	33
Phi02	Phillips	Burndown	3	28	0	57
Phi03	Phillips	Burndown	6	10	0	8
Phi04	Phillips	Other	0	11	0	17
Phi05	Phillips	Wheat	0	36	0	3
Phi06	Phillips	Wheat	0	49	0	8
Phi07	Phillips	Wheat	0	34	0	73
Phi08	Phillips	Burndown	0	34	0	17
Phi09	Phillips	Wheat	0	48	0	33
Phi10	Phillips	Wheat	0	26	0	4
Phi12	Phillips	Wheat	0	25	0	1
Phi13	Phillips	Wheat	0	65	0	3
Phi15	Phillips	Other	0	11	0	0
Phi16	Phillips	Wheat	0	14	0	3
Phi17	Phillips	Wheat	0	9	0	4
Phi18	Phillips	Wheat	0	5	0	18
Phi19	Phillips	Burndown	0	21	0	0
Phi20	Phillips	Row crop	0	5	0	2
Phi21	Phillips	Wheat	0	2	0	3
Poi16	Poinsett	N/A	0	44	0	69
Poi17	Poinsett	Wheat	0	60	0	84
Poi18	Poinsett	Wheat	0	35	0	0
Poi19	Poinsett	Wheat	0	43	0	17
Poi20	Poinsett	Wheat	0	45	0	8
Poi21	Poinsett	Wheat	0	89	21	91
Poi22	Poinsett	Wheat	0	63	13	65
Poi23	Poinsett	Wheat	0	73	3	86
Pul01	Pulaski	Row crop	0	20	0	10
Pul02	Pulaski	Row crop	0	35	0	1
Pul03	Pulaski	Other	0	50	0	28
Pul04	Pulaski	Other	0	60	0	10
Pul05	Pulaski	Wheat	0	31	0	0
Pul06	Pulaski	N/A	0	66	0	0
Stf01	St. Francis	Wheat	0	6	0	0
Stf02	St. Francis	Wheat	0	31	0	0
Stf03	St. Francis	Wheat	0	34	1	17
Stf04	St. Francis	Wheat	0	48	0	0
Stf05	St. Francis	Wheat	0	48	0	0
Stf06	St. Francis	Wheat	0	80	1	5

Stf07	St. Francis	Wheat	0	11	0	0
Stf08	St. Francis	Wheat	0	45	0	0
Whi01	White	Wheat	0	64	8	52
Whi02	White	Wheat	0	52	0	51
Whi03	White	Wheat	0	91	79	87
Whi04	White	Wheat	0	82	26	57
Whi07	White	Wheat	0	17	0	1
Whi08	White	Wheat	0	34	0	2
Whi09	White	Wheat	0	83	0	88
Whi10	White	Wheat	0	56	0	47
Whi11	White	Wheat	0	48	0	55
Whi12	White	Other	0	57	0	2
Whi13	White	Other	0	26	0	2
Whi14	White	Wheat	0	36	0	52
Whi15	White	Other	0	66	0	46
Whi16	White	Wheat	0	74	28	77
Whi17	White	Wheat	0	84	33	76
Com01	OR[d]	Commercial	0	6	0	0
Com06	OR	Commercial	0	3	0	0
Com07	OR	Commercial	0	1	0	0
Com08	OR	Commercial	0	5	0	0
Com09	OR	Commercial	0	5	0	0
Com10	OR	Commercial	0	6	0	0
Com11	OR	Commercial	0	5	0	0
Com12	OR	Commercial	0	0	0	3
Com13	OR	Commercial	0	12	0	0
Com14	OR	Commercial	0	3	0	0
Com15	OR	Commercial	0	2	0	4
Com16	OR	Commercial	0	0	0	0
Com17	OR	Commercial	0	1	0	8
Com18	OR	Commercial	0	3	0	4
Com19	OR	Commercial	0	1	0	5
Com20	OR	Commercial	0	1	0	0
Com21	OR	Commercial	0	2	0	0
Com22	OR	Commercial	0	55	0	3
Com23	OR	Commercial	0	2	0	0
Com24	OR	Commercial	0	2	0	0
Com26	OR	Commercial	0	2	0	0
Com27	OR	Commercial	0	1	0	2

[a] Row crop includes corn, cotton, grain sorghum, rice, and soybean. Other includes all other environments in which Italian ryegrass was harvested.

[b] Abbreviation used, N/A: no information available.

[c] Laf 07 was harvested in Bossier parish, Louisiana.

[d] All commercial samples were purchased from retailers in Arkansas but originated from Oregon.

Chapter 3

Control of Glyphosate-Resistant Italian Ryegrass (*Lolium perenne* ssp. *multiflorum*)

James W. Dickson

Populations of Italian ryegrass have been documented as being resistant to glyphosate in the Midsouth, including Arkansas, and are difficult to control with common burn-down practices. Three field experiments were conducted in 2009 and repeated in 2010 to evaluate several herbicides for Italian ryegrass control in the spring, in no-till production in the fall, and following fall tillage. In the spring burn-down study, herbicides were applied in April to Italian ryegrass that was 50 cm. In the no-till study, herbicides were applied in November to 3- to 4- leaf Italian ryegrass. In the fall-tillage study, preemergence (PRE) herbicides were applied in November 2009 and 2010 immediately following fall tillage, and postemergence (POST) herbicides were applied when Italian ryegrass reached the 3-leaf to 2-tiller stage of growth in April 2010 and 2011. Glyphosate at 1734 (twice the labeled use rate) and 3468 g ae ha^{-1} alone, or glyphosate at 867 or 1734 g ae ha^{-1} plus clethodim at 68 or 136 g ai ha^{-1} applied in April to 50-cm-tall Italian ryegrass, reduced ryegrass biomass an average of 80% compared the nontreated check, 49 d after treatment (DAT). Seventy percent biomass reduction was the best control achieved from treatments applied in April that did not contain glyphosate. Of treatments applied in the fall in the no-till system, glyphosate plus *S*-metolachlor or pyroxasulfone reduced Italian ryegrass biomass by 78 and 100%, respectively, 200 DAT. In the fall-tilled study, the residual herbicides flumioxazin plus *S*-metolachlor, *S*-metolachlor, clomazone, and pyroxasulfone applied immediately following fall tillage reduced Italian ryegrass biomass by 83 to 95% at 200 DAT. Herbicide application in the spring when Italian ryegrass is 50 cm tall is an unsuccessful practice, especially when glyphosate is not an option. Even when POST treatments visually controlled

ryegrass at least 80%, enough crop residue or biomass remained that would cause problems with spring tillage, planting and overall stand establishment. A more successful practice, in this research, was applying residual herbicides in the fall, following tillage.

Nomenclature: Clethodim, clomazone, flumioxazin, glyphosate, pyroxasulfone, *S*-metolachlor; Italian ryegrass, *Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot.

Key words: Biomass, ryegrass, fall tillage, no-till, residual herbicides, glyphosate resistance, spring burn-down.

Italian ryegrass is a winter-annual grass commonly found throughout North America (Anonymous 2012). In Arkansas, it typically germinates in the fall and flowers the following spring, but it can also emerge in the spring. Italian ryegrass does not require vernalization to induce flowering (Heide 1994); therefore, plants that emerge in the early spring have the capacity to flower and produce viable seed the same season.

Italian ryegrass is not only a troublesome weed in Arkansas wheat; it also creates difficulties when planting corn, cotton, rice, soybean, and sorghum in the spring. Italian ryegrass grows in dense bunches that can be comprised of 280 or more tillers (Bararpour et al. 2003). When densities are high, Italian ryegrass has the effect of a winter cover crop that can outcompete other weed species. When Italian ryegrass populations are this dense, however, it creates large amounts of biomass that interfere with crop production and can make both chemical and mechanical control difficult. In research evaluating Italian ryegrass and other species as a cover crop, 69% of desiccated Italian ryegrass residue was still remaining 9 WAP, resulting in reduced soybean stand, shorter plants, and reduced yield (Reddy 2001). When planted into

Italian ryegrass residue, rice seedlings were reduced by 25%, plant height reduced by 33%, maturity was delayed 7 to 12 d, and yield was reduced by 15% (Bond 2012). The residue from Italian ryegrass alone can make planting and stand establishment more difficult under no-till production practices.

Over the past 6 yr in Arkansas, Italian ryegrass has become increasingly difficult to control with glyphosate, the most commonly used herbicide in Arkansas for killing winter vegetation prior to planting crops. The first reported instance of glyphosate resistance was with a population of rigid ryegrass (*Lolium rigidum* Gaud.) in Australia (Powles et al. 1998). Glyphosate resistance has since been found in populations of Italian ryegrass in Chile (Perez and Kogan 2003), Argentina, Brazil, Spain (Heap 2012), and in four states in the U.S. including Oregon (Perez-Jones et al. 2005), Mississippi (Nandula et al. 2007), California (Jasieniuk et al. 2008), and Arkansas (Heap 2012; Dickson et al. 2011). At least 33 glyphosate-resistant Italian ryegrass populations in 12 counties in Mississippi have since been discovered (Bond and Nandula 2011). In 2007, producers in southeast Arkansas began reporting that several Italian ryegrass plants survived applications of glyphosate in the spring. Also in 2007, a population was confirmed to be resistant to glyphosate by researchers at the University of Arkansas at Fayetteville, AR, (Heap 2012; Dickson et al. 2011). In the following years, reports of Italian ryegrass surviving glyphosate applications increased. A statewide screening of Italian ryegrass populations conducted in 2009 revealed at least one population in Arkansas that was 23 times more resistant to glyphosate than a susceptible standard (Dickson et al. 2011). In addition, over 40 other populations were found with enhanced tolerance to glyphosate (Dickson et al. 2012).

The occurrence of glyphosate-resistant Italian ryegrass is only expected to increase. With the loss of glyphosate as an effective tool for Italian ryegrass control prior to planting, other options need to be explored. The objectives of this research were to evaluate several herbicides for Italian ryegrass control in the spring, in a no-till situation in the fall, and following fall tillage.

Materials and Methods

Three field experiments (spring burn-down, fall tilled, and fall no-till) were initiated in November 2009 and repeated in 2010 at the Newport Research Station, near Newport, Arkansas, on a Beulah fine sandy loam soil (coarse-loamy, mixed, active, thermic Typic Dystrochrepts) with a pH of 5.6. A naturalized population of glyphosate-susceptible Italian ryegrass was present at the research site. The experimental design was a randomized complete block with four replications. Plots were 2 m wide and 6 m long with 1.5-m alleys separating replications. Herbicide applications were made using a CO_2-pressurized backpack sprayer equipped with TeeJet 110015 flat-fan nozzles (TeeJet 110015 flat-fan nozzle, Spraying Systems Co., Wheaton, IL 60189) calibrated to deliver 93 L ha^{-1} at 165 kPa. Visual ratings of percentage Italian ryegrass control relative to an untreated check (UTC) were recorded at various intervals. Visual ratings were based on a scale of 0 to 100, with 0 being no Italian ryegrass control, and 100 being complete control. In 2009, the spring burn-down study was conducted in two areas at the Newport station, separated by 800 m. There were no differences among means of treatments between the two studies in 2009; therefore, this study was repeated only once in 2010.

Herbicide treatments for the spring burn-down study (Table 1) were applied when Italian ryegrass was approximately 50 cm tall in April 2010 and 2011. Visual ratings of percent Italian ryegrass control relative to an UTC were recorded 17 and 42 days after treatment (DAT). The herbicide treatments for the fall no-till study (Table 2) were applied at the three- to four-leaf stage of Italian ryegrass in November of 2009 and 2010. Visual ratings of Italian ryegrass control were recorded 14, 72, and 121 DAT. For the fall tillage study, the preemergence (PRE) herbicide treatments (Table 3) were applied immediately following tillage in November 2009 and 2010. The postemergence (POST) herbicides (Table 3) were applied when Italian ryegrass reached the three-leaf to two-tiller stage of growth in April 2010 and 2011. Visual ratings of Italian ryegrass control were recorded 30 and 110 d after the PRE (DAPR) treatments were applied and 30 and 40 d after the POST (DAPO) treatments were applied. A broadcast application of 2,4-D at 1076 g ai ha^{-1} was applied 3 wk prior to biomass harvest to kill any broadleaf weeds in the fall no-till and fall tillage studies.

A swath from the center of each plot measuring 1 m wide and 6 m long was harvested on May 25, 2010, and June 9, 2011, using a sickle-bar mower (Troy-Built Sickle Bar Mower, Troy-Built LLC, Cleveland, OH 44136). Weights of the above-ground biomass from this harvested swath were recorded to obtain whole-plot fresh weight. A subsample from each harvested swath was weighed to obtain the sub-sample fresh weight and placed in paper bags for transportation. Each subsample was oven dried at 50 C for 36 h and re-weighed to obtain the sub-sample dry weight. The sub-sample dry weight was divided by the sub-sample fresh weight and multiplied by 100 to obtain percent dry matter for each subsample. Each sub-sample dry matter value was multiplied by its respective whole-plot fresh-weight and divided by 100 to obtain dry weight of each plot. The equation 100(1-(plot dry weight/average UTC dry weight)) yielded the percentage biomass reduction for each plot in relation to the UTC.

All data were analyzed by ANOVA using the Mixed Procedure in Statistical Analysis Systems software (Version 9.1, SAS Institute Inc., Cary, NC). Means were separated using Fisher's protected least significance difference (LSD) test at the 5% level of probability.

Results and Discussion

Spring Burn-down Study. Year as a factor was not significant when comparing means for each treatment; therefore, all data were averaged across years. At 49 DAT, glyphosate at 1734 g ae ha^{-1} (twice the labeled use rate for glyphosate) and 3468 g ae ha^{-1} and all treatments with glyphosate plus clethodim resulted in the highest Italian ryegrass biomass reduction (77 to 83%) (Table 1). Italian ryegrass control with these treatments were high (87 to 96%); however, as much as 23% biomass remained in these plots at 49 DAT (Table 1), which creates difficulties in planting and establishing crops. The population of Italian ryegrass evaluated in this study was susceptible to glyphosate; given its large size at the time of application, the level of control achieved with glyphosate was expected to be less than 100%. However, control of this glyphosate-susceptible Italian ryegrass population was 83% or less even with glyphosate applied at the labeled use rate for burn-down applications. The same treatment is not expected to control glyphosate-resistant Italian ryegrass. Clethodim alone at 136 g ai ha^{-1} and glufosinate alone at 820 g ai ha^{-1} reduced the biomass of Italian ryegrass by 52% only. However, when glufosinate and clethodim at 136 g ai ha^{-1} were applied together, biomass reduction of Italian ryegrass was increased to 70%. Paraquat applied alone or in combination with metribuzin or diuron reduced Italian ryegrass biomass by only 66 to 68%. Initially treatments containing paraquat or glufosinate controlled ryegrass 79-89 % at 17 DAT; however, by 42 DAT these ratings had dropped to 47-64%. Italian ryegrass control at 42 DAT for treatments without glyphosate were

76% or less (Table 1), and further reflected the lack of Italian ryegrass control with these herbicides, at this timing.

This study demonstrates the difficulty in controlling large (50 cm tall) Italian ryegrass. It also indicates the level of control that might be expected with alternative herbicides besides glyphosate. Many producers in Arkansas face this problem when timing their burn-down programs. Producers typically wait on other problem weeds to emerge so that they can make only one herbicide application prior to planting the desired crop. Waiting for other weeds to emerge allows Italian ryegrass to grow big and become more difficult to control. This study simulates the situation that producers face, and shows that a single herbicide application is not adequate to control Italian ryegrass late in the spring. Lins et al. (2007) evaluated three rates of glyphosate for Italian ryegrass cover crop desiccation, referred to as annual ryegrass in the text, and concluded that "no timing or rate of glyphosate was effective for complete control or removal of an annual ryegrass cover crop." Another problem encountered when ryegrass is not treated when small is the amount of residue that remains once Italian ryegrass actually dies. This residue interferes with planting operations, reduces seed-to-soil contact for crops, and reduces sunlight penetration to the soil surface, thereby also reducing crop emergence. In research evaluating Italian ryegrass as a winter cover crop, Italian ryegrass residues remaining after desiccation at planting reduced southern pea stand by 42% and reduced yield (Burgos and Talbert 1996). Controlling Italian ryegrass this late in the spring is especially difficult when the populations are resistant to glyphosate. In fact, no treatments in this trial provided acceptable "commercial" levels of control or reduction in biomass, and further evaluation of sequential POST options is needed.

Fall No-Till Study. Because differences between years were not significant, all data were averaged across years. Italian ryegrass control was 84% or less for all herbicides at 14 DAT, but increased to 84 to 100 %, 72 DAT (Table 2), indicating that all herbicides killed the first flush of Italian ryegrass in the fall. However, most of the POST-applied herbicide treatments that did not contain a residual herbicide reduced Italian ryegrass biomass by only 53% or less, 200 DAT, leaving almost 50% of the biomass which could interfere with planting crops. The POST-applied herbicide treatments that did not contain a residual herbicide that reduced Italian ryegrass by 69% or better were clethodim at both rates and glufosinate plus clethodim at 136 ai ha^{-1} (Table 2). In this study, clethodim applied alone at both rates reduced Italian ryegrass biomass 100% at 200 DAT. Grass crops such as rice, corn, and sorghum may not be planted until 30 d after a clethodim application (Anonymous 2006) because of the risk of crop injury from clethodim residues in the soil; however, clethodim is not expected to control Italian ryegrass for 200 days. In the first and second years of this study, a buttercup (*Ranunculus* sp.) species and Carolina geranium (*Geranium carolinianum*), respectively, had emerged just prior to herbicide application. Because clethodim does not control broadleaf weeds, these two species quickly established a canopy that prevented further emergence of Italian ryegrass in the plots treated with clethodim alone. Where clethodim was successful in controlling Italian ryegrass, a follow-up burn-down treatment with a broadleaf herbicide would have been needed for no-till production. All other herbicide treatments controlled these broadleaf species and allowed for subsequent flushes of Italian ryegrass to emerge. Glufosinate plus clethodim at 136 g ai ha^{-1} reduced Italian ryegrass biomass by 69% at 200 DAT, which was similar to the control achieved with residual herbicides applied in November. The only other herbicide treatments that provided an acceptable (70% or more) reduction in Italian ryegrass biomass at 200 DAT were tank mixtures containing glyphosate plus pyroxasulfone (100% biomass reduction), *S*-metolachlor (78%), and *S*-metolachlor plus flumioxazin (73%) or clomazone (72%). Pyroxasulfone is a potent inhibitor of very-long-chain fatty acid biosynthesis and is categorized within the K3 group of herbicides with *S*-metolachlor and others (Tanetani et al. 2009). Products containing pyroxasulfone alone and in combination with other herbicides are expected to be available to producers in 2012 (Anonymous 2011a; 2011b; 2012b). Italian ryegrass biomass reduction (100%) with glyphosate plus pyroxasulfone was significantly higher than that with any other treatment.

Acceptable levels of Italian ryegrass control were achieved in this study. Pyroxasulfone, *S*-metolachlor, and clomazone provided control of Italian ryegrass all winter and spring; however, glyphosate was applied with these herbicides to kill the ryegrass that had already emerged in the fall. Glyphosate-resistant Italian ryegrass cannot be successfully controlled with glyphosate alone. Furthermore, the amount of Italian ryegrass biomass remaining at 200 DAT was as high as 28% for treatments deemed acceptable, which can still interfere with planting and establishing a crop; therefore, alternative POST herbicides will be needed to kill emerged glyphosate-resistant Italian ryegrass. In this study, paraquat applied alone provided 84 and 100% Italian ryegrass control at 14 and 72 DAT, respectively (Table 2). Paraquat may be tank mixed with clomazone and *S*-metolachlor (Anonymous 2008) and is an alternative for controlling emerged Italian ryegrass. In some cases, Italian ryegrass stands may be very dense and/or too large for a single application of paraquat to be effective. In such instances, two applications of paraquat may be needed for adequate control of Italian ryegrass (Bond 2012). Another option for controlling Italian ryegrass that has emerged in the fall prior to residual-herbicide applications is tillage. However, this may result in subsequent flushes of ryegrass if no residual herbicide is applied following tillage.

Fall Tillage Study. Year as a factor was not significant; therefore, data were averaged over years. In this study, the addition of flumioxazin to *S*-metolachlor applied PRE increased Italian ryegrass control compared to *S*-metolachlor alone (Table 3). *S*-metolachlor and flumioxazin applied alone in the fall reduced Italian ryegrass biomass by 83 and 15%, respectively, at 200 DAT. When these two herbicides were combined, Italian ryegrass biomass reduction was increased to 95%. Pyroxasulfone and clomazone reduced Italian ryegrass biomass 95 and 94%, respectively, 200 DAT.

Tillage in the fall effectively killed the Italian ryegrass that emerged in the fall, and the POST treatments were not needed until April the following years. Visual estimates of Italian ryegrass were fair to good for most of the POST treatments 30 DAT. For example, a 2X rate of glyphosate alone, treatments containing the 1X rate of glyphosate (867 g ha^{-1}) plus the high rate of clethodim, or a 2X rate of glyphosate plus both rates of clethodim provided 74 to 83% reduction of Italian ryegrass biomass (Table 3). The addition of *S*-metolachlor or rimsulfuron to a 1X rate of glyphosate significantly improved Italian ryegrass control, compared to a 1X rate of glyphosate alone. Glyphosate alone at 867 g ha^{-1} reduced Italian ryegrass biomass by only 66%. Italian ryegrass biomass reduction was increased to 79 and 84% with the addition of *S*-metolachlor or rimsulfuron, respectively. Rimsulfuron, which is labeled in Arkansas only for use in field corn or early burn-down, partially controls Italian ryegrass when applied POST and can provide suppression of further flushes of ryegrass (Anonymous 2007).

Paraquat alone or combined with metribuzin reduced Italian ryegrass biomass by only 55 and 59%, respectively. When paraquat was combined with diuron, Italian ryegrass biomass reduction increased to 70% at 40 DAT; however, the visual estimates of Italian ryegrass control did not reflect this improvement. Previous research has shown that the addition of a Photosystem II herbicide, such as diuron or metribuzin, to paraquat increases the control of Italian ryegrass compared to paraquat applied alone. Griffin et al. (2004) reported that when diuron was added to paraquat, Italian ryegrass control was increased by 15 to 17% compared to paraquat applied alone. Eubank et al. (2011) reported a 19% increase in Italian ryegrass control when diuron was added to paraquat, compared to paraquat applied alone. In the fall no-till study, a 17% increase in Italian ryegrass biomass reduction was observed. When diuron was applied with paraquat in the fall tillage study, biomass was reduced 17% more than with paraquat applied alone at 200 DAT (Table 2).

Italian ryegrass is difficult to control with single herbicide applications in the spring when it is at an advanced growth stage. This research suggests that a program approach starting with *S*-metolachlor plus flumioxazin, *S*-metolachlor alone, clomazone, or pyroxasulfone applied in the fall after tillage are the best options for controlling Italian ryegrass. This PRE program could then be followed up by various POST options as needed.

The occurrence of glyphosate-resistant ryegrass in Arkansas and other southern states will have a major financial impact on grower production practices. All the alternative control options outlined in this work are more costly than a simple early spring burn-down application of glyphosate alone. The potential impact on no-till production practices is also a concern. However, these data indicate that with a program approach and diligent effort, glyphosate-resistant ryegrass populations can be controlled with existing, or soon to be labeled, herbicide options.

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Table 1. Ryegrass control averaged over three experiments as affected by herbicides applied in the spring to 50-cm-tall Italian ryegrass.

		Ryegrass control		
		Visual estimate		Biomass reduction[d]
Treatment	Rate[a]	17 DAT[b]	42 DAT	49 DAT
		%		
Glyphosate	867	76	76	68
Glyphosate	1734	88	87	80
Glyphosate	3468	96	95	80
Glyphosate + clethodim	867 + 68	90	86	77
Glyphosate + clethodim	867 + 136	90	90	80
Glyphosate + clethodim	1734 + 68	96	96	83
Glyphosate + clethodim	1734 + 136	96	95	80
Clethodim[c]	68	64	61	41
Clethodim	136	70	76	52
Glufosinate	820	81	52	52
Paraquat	700	79	47	66
Paraquat + metribuzin	700 + 105	85	50	68
Paraquat + diuron	700 + 565	88	48	66
Glufosinate + clethodim	820 + 68	87	51	60
Glufosinate + clethodim	820 + 136	89	64	70
LSD (0.05)		5	6	8

[a] Glyphosate rates are g ae ha^{-1}: all other herbicide rates are g ai ha^{-1}.

[b] DAT, days after treatment.

[c] Crop oil concentrate included at 1%v/v with clethodim; non-ionic surfactant at 0.25% v/v included with paraquat.

[d] The equation 100(1-(plot dry weight/average UTC dry weight)) yielded the percentage biomass reduction for each plot in relation to the UTC.

Table 2. Ryegrass control averaged over 2 yr of a no-till system as affected by herbicides applied in the fall to three- to four-leaf Italian ryegrass.

		Ryegrass control			
		Visual estimate			Biomass reduction[c]
Treatment	Rate[a]	14 DAT[b]	72 DAT	121 DAT	200 DAT
		%			
Glyphosate	867	59	100	73	41
Glyphosate	1734	54	100	71	40
Glyphosate + clethodim	867 + 68	57	100	78	52
Glyphosate + clethodim	867 + 136	56	100	83	49
Glyphosate + clethodim	1734 + 68	54	100	75	38
Glyphosate + clethodim	1734 + 136	56	100	83	52
Clethodim[d]	68	28	96	76	100
Clethodim	136	28	96	79	100
Glufosinate	820	67	84	51	22
Paraquat	700	84	100	67	36
Paraquat + metribuzin	700 + 105	81	95	58	20
Paraquat + diuron	700 + 565	81	100	83	53
Glufosinate + clethodim	820 + 68	73	97	67	48
Glufosinate + clethodim	820 + 136	69	98	70	69
Glyphosate + *S*-metolachlor	867 + 1068	60	100	92	78
Glyphosate + flumioxazin	867 + 71	66	98	84	54
Glyphosate + pendimethalin	867 + 1118	60	99	68	35
Glyphosate + rimsulfuron	867 + 35	59	100	98	69

Glyphosate + diuron	867 + 565	40	100	85	45
Glyphosate + flumioxazin + *S*-metolachlor	867 + 71 + 1068	83	99	92	73
Glyphosate + clomazone	867 + 841	59	100	100	72
Glyphosate + pyroxasulfone	867 + 165	58	100	98	100
LSD (0.05)		19	4	9	18

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[a] Glyphosate rates are g ae ha$^{-1;}$ all other herbicide rates are g ai ha^{-1}.

[b] DAT, days after treatment.

[c] The equation 100(1-(plot dry weight/average UTC dry weight)) yielded the percentage biomass reduction for each plot in relation to the UTC.

[d] Crop oil concentrate included at 1%v/v with clethodim; non-ionic surfactant at 0.25% v/v included with paraquat.

Table 3. Ryegrass control averaged over 2 yr as affected by herbicides applied following fall tillage and before ryegrass emergence (PRE) in the fall and when ryegrass had three to four leaves (POST) in the spring.

			Ryegrass control				
			Visual estimate				Biomass reduction[c]
Treatment	Rate[a]	Timing	30 DAPR[b]	110 DAPR	30 DAPO	40 DAPO	40 DAPO
			%				
Flumioxazin + *S*-metolachlor	71 + 1068	PRE	84	98	71	80	95
S-metolachlor	1068	PRE	76	92	61	64	83
Flumioxazin	71	PRE	63	56	0	0	15
Pendimethalin	1118	PRE	43	6	0	0	14
Clomazone	841	PRE	94	100	89	89	94
Pyroxasulfone	165	PRE	82	97	89	84	95
Glyphosate	867	POST	–[d]	–	81	42	66
Glyphosate	1734	POST	–	–	93	48	78
Glyphosate + clethodim	867 + 68	POST	–	–	86	44	69
Glyphosate + clethodim	867 + 136	POST	–	–	92	49	74
Glyphosate + clethodim	1734 + 68	POST	–	–	94	53	82
Glyphosate + clethodim	1734 + 136	POST	–	–	95	67	83
Clethodim	68	POST	–	–	65	20	38
Clethodim	136	POST	–	–	74	42	73
Glufosinate	820	POST	–	–	53	39	50
Paraquat	700	POST	–	–	68	30	55

Paraquat + metribuzin	700 + 105	POST	–	–	64	31	59
Paraquat + diuron	700 + 565	POST	–	–	65	44	70
Glufosinate + clethodim	820 + 68	POST	–	–	68	15	44
Glufosinate + clethodim	820 + 136	POST	–	–	68	28	56
Glyphosate + *S*-metolachlor	867 + 1068	POST	–	–	90	49	79
Glyphosate + flumioxazin	867 + 71	POST	–	–	88	48	70
Glyphosate + pendimethalin	867 + 1118	POST	–	–	81	38	58
Glyphosate + rimsulfuron	867 + 35	POST	–	–	91	61	84
Glyphosate + diuron	867 + 565	POST	–	–	82	46	68
Glyphosate + flumioxazin + *S*-metolachlor	867 + 71 + 1068	POST	–	–	91	48	76
LSD (0.05)			6	12	11	16	11

[a] Glyphosate rates are g ae ha^{-1}; all other herbicide rates are g ai ha^{-1}.

[b] Abbreviations: DAPR, days after PRE application timing; DAPO, days after POST application timing.

[c] The equation 100(1-(plot dry weight/average UTC dry weight)) yielded the percentage biomass reduction for each plot in relation to the UTC.

[d] "–": treatments had not been applied.

Distribution and Control of Herbicide-Resistant Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum* Lam. Husnot) in Arkansas

James W. Dickson

Conclusion

This research confirms that several Italian ryegrass populations in Arkansas are resistant to glyphosate, diclofop, pinoxaden, and pyroxsulam. Of the 215 Italian ryegrass populations sampled, 205 were resistant to diclofop, 137 were resistant to pyroxsulam, 25 were resistant to pinoxaden, and 37 were resistant to glyphosate. Several populations were resistant to more than one of these herbicides. One of the glyphosate-resistant populations was discovered to be 23 times more resistant to glyphosate than a susceptible population sample. The results presented from the field studies suggest that Italian ryegrass can best be controlled with preemergence applications of *S*-metolachlor, *S*-metolachlor plus flumioxazin, clomazone, or pyroxasulfone applied in the fall following fall tillage. Applying herbicides in the fall for spring-weed control is not a common practice in Arkansas, but appears to be the best option for successfully controlling Italian ryegrass before planting spring crops.