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Resistance to ACCase and ALS inhibitors in *Lolium perenne* ssp. *multiflorum* in the United States

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ABSTRACT

Lolium perenne ssp. multiflorum (Italian ryegrass) is a major weed problem in wheat production. Being an obligate outcrossing species, it has a high propensity to evolve resistance to herbicides. This study was conducted to determine the level of resistance and resistance patterns of Italian ryegrass populations in the United States to ACCase- (diclofop and pinoxaden) and ALS (imazamox, mesosulfuron, and pyroxsulam) inhibitors. Dose-response bioassays were conducted on 47 populations from suspect herbicide-resistant fields in the southern United States (Arkansas, Mississippi, Georgia, North Carolina, South Carolina,Virginia), Kansas, and Washington collected between 2008 and 2011 from fields suspected of resistance to ALS inhibitors. Eighty-seven percent of the populations were resistant to diclofop. Eight of the diclofop-resistant populations (25%) were also resistant to pinoxaden. Thirty-eight diclofop-resistant populations (81%) were resistant to at least one ALS inhibitor. Thirty-nine populations (83%) were resistant to mesosulfuron, 38 of which were also resistant to pyroxsulam. All mesosulfuron-resistant populations tested were cross-resistant to imazamox and pyroxsulam. Cross-resistance patterns to ALS inhibitors differed. Of 45 populations with resistance to ACCase or ALS inhibitors, 35 (78%) had multiple resistance to both modes of action. The majority of diclofop-resistant L. perenne populations can be controlled with pinoxaden, but widespread resistance to pinoxaden can evolve soon if it is intensively used. A comprehensive weed management approach prior to planting will be critical in managing L. perenne in wheat and preventing or delaying resistance evolution.

Nomenclature: Italian ryegrass, *Lolium perenne* ssp. *multiflorum*; diclofop; imazamox; mesosulfuron; pinoxaden; pyroxsulam; ACCase herbicides; ALS herbicides.

***Keywords*:** ACCase resistance, ALS resistance, herbicide resistance pattern

Lolium perenne ssp. *multiflorum* (Lam) Husnot (Italian ryegrass) is a major weed problem in wheat production areas in the United States. High infestation of Italian ryegrass can reduce wheat yield up to 92% (Hashem *et al.* 1998). The commercialization of diclofop in 1980 enabled the control of Italian ryegrass in wheat fields (Stanger and Appleby, 1989). Diclofop is an aryloxyphenoxypropanoate (AOPP) herbicide that inhibits acetyl coenzyme-A carboxylase (ACCase), an enzyme necessary for fatty acid biosynthesis (Burton *et al.*, 1989; Delyé ,2005). Although diclofop has controlled *L. perenne* historically, its repeated use has selected for resistant populations, in less than 10 years of use (Stanger and Appleby, 1989). In Arkansas, diclofop-resistant *L. perenne* was first documented in 1998 (Kuk *et al.*, 2000). Since then, diclofop-resistant *L. perenne* has been reported in 10 states in the United States and in six other countries (Heap, 2012).

Since the discovery of diclofop-resistant *L. perenne*, another ACCase inhibitor (pinoxaden) and some ALS (acetolactate synthase) inhibitors were introduced in wheat to control *L. perenne*. Thus, alternative herbicides now include imazamox (in ALS-resistant wheat), mesosulfuron, pinoxaden, and pyroxsulam (Dickson *et al.*, 2011). Imazamox, mesosulfuron, and pyroxsulam are ALS inhibitors belonging to imidazolinone, sulfonylurea, and triazolopyrimidine sulfonamide families, respectively (DeBoer *et al.*, 2011; Hand *et al.*, 2002; Kuk *et al.*, 2008). Acetolactate synthase (EC4.1.3.18) is the first enzyme in the biosynthesis pathway of the branched-chain amino acids isoleucine, valine, and leucine (Umbarger, 1978). To date, populations of *L. perenne* have evolved resistance to multiple ACCase- and ALS inhibitors. The evolution of resistance to ALS inhibitors among weedy species occurs quickly, relative to other herbicide modes of action (Tranel and Wright 2002). For example, an ecotype of *Alopecurus myosuroides* (Huds.) in the United Kingdom had evolved resistance to chlorsulfuron in 1984, after only two years of commercialization (Moss 1987). To date,

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there are at least 127 weed species with resistance to ALS-inhibiting herbicides (Heap, 2012).

Mesosulfuron and pinoxaden were registered in 2004 and 2005, respectively, to manage diclofop-resistant *L. perenne* in wheat (USA EPA 2004; USA EPA 2005). However, resistance to mesosulfuron was reported in Arkansas one year before its commercialization (Kuk and Burgos 2007), and shortly after, also in Texas (Ellis *et al.* 2008). Such populations were presumed to have been preselected for resistance to ALS inhibitors by other ALS herbicides that have been historically used preplant (PPL) or preemergence (PRE) in wheat such as chlorsulfuron and metsulfuron. The first confirmed mesosulfuron-resistant *L. perenne* from Arkansas was also resistant to other ALS inhibitors, such as chlorsulfuron, imazamox, and sulfometuron, but not to the ACCase herbicide diclofop (Kuk and Burgos 2007). Many diclofop-resistant *L. perenne* populations are also resistant to other herbicides (Kuk *et al.* 2008; Eleni *et al.* 2000; Holtum and Powles 1991). Kuk *et al.* (2008) reported that of 25 diclofop-resistant populations from Arkansas, five (20%) were cross-resistant to pinoxaden. A diclofop-resistant population from North Carolina was also found resistant to pinoxaden (Ellis *et al.* 2010). Of the populations tested from Idaho and Washington, 27% were resistant to both ACCase- and ALS inhibitors (Rauch *et al.* (2010). Likewise, *Lolium rigidum* (Gaudin) populations from Israel exhibiting multiple resistance to ACCase- and ALS inhibitors were reported by Matzrafi and Rubin (2012). In the western Australian wheat belt, 64% of 441 *L. rigidum* populations were confirmed resistant to both ACCase- (diclofop) and ALS (sulfometuron) herbicides (Owen *et al.* 2007). Multiple resistance of diclofop-resistant *L. perenne* to mesosulfuron, imazamox, and pinoxaden was reported in 2008, but included only populations from Arkansas (Kuk *et al.* 2008). So far, *L. perenne* resistance to pyroxsulam, the most recent ALS herbicide introduced for weed control in wheat, is already confirmed in North Carolina (Chandi *et al.* 2011). Within the same time frame, evaluations for resistance to pyroxsulam were also being conducted on *L. perenne* populations from Arkansas and other states in the United States.

In the context of this paper, cross-resistance pertains to resistance of a species to two or more herbicides with the same mode of action, after having been exposed to only one of the herbicides. Multiple resistance refers to resistance of a species to two or more herbicides with different modes of action due to more than one mechanism of resistance (Rauch *et al.* 2010). Evaluation of resistance patterns in *L. perenne* is necessary to determine alternative management programs. The objectives of this study were to confirm and assess cross-resistance patterns to ACCase- (diclofop and pinoxaden) and ALS (mesosulfuron, imazamox, pyroxsulam) inhibitors in *L. perenne* populations from suspect fields in the southern United States, Kansas (central US), and Washington (Pacific Northwest).

MATERIALS AND METHODS

Plant materials

Composite seed samples of 47 *L. perenne* populations suspected of resistance to ACCase- and ALS inhibitors were collected from Arkansas, Georgia, Kansas, Mississippi, North Carolina, South Carolina, Virginia, and Washington between 2008 and 2011 (Fig. 1 and Table 1). Of these, the largest group (18 populations) was from Arkansas. A commercial *L. perenne* accession was used as the susceptible standard (SS).

Evaluation of resistance patterns to ACCase inhibitors

Seeds were sown in 11-cm pots filled with commercial potting mixture (Sunshine Mix®, Sun Gro Horticulture Inc., Bellevue, WA 98008). Seedlings were thinned to five plants per pot 1 wk after emergence. Plants were watered daily and fertilized with MiracleGro complete fertilizer (MiracleGro, The Scott's Co., Marysville, OH 43041) every 2 wk to ensure optimum growth. Bioassays were conducted in August to November of the years 2008 through 2011 with natural daylength of 10 to 13 h. Seedlings were kept in the greenhouse with day temperatures (across years) ranging from 22 to 35 C and night temperatures ranging from 21 to 25 C. Herbicide treatments, dose ranges, and populations sprayed are listed in Table 2.

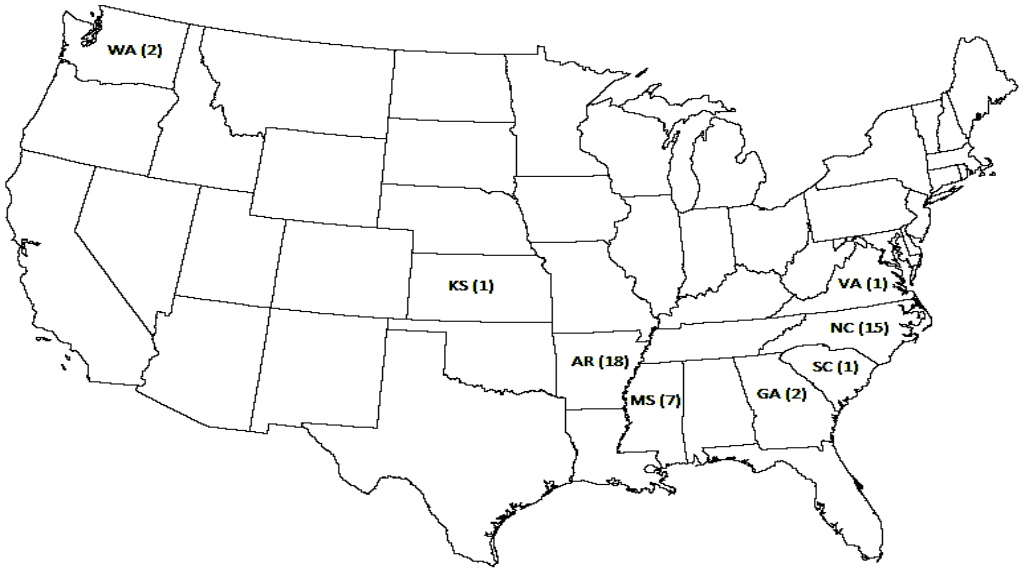

Fig. 1: Distribution of *Lolium perenne* ssp. *multiflorum* populations used in this research. The majority of populations (44) were collected from the southern US (AR, MS, GA, SC, NC, and VA). Two populations were from the Pacific Northwest (Washington State) and one population was from the central US (Kansas). The number of populations tested from each state is enclosed in parenthesis.

Table 1: *Lolium perenne* ssp. *multiflorum* (Italian ryegrass) populations tested for resistance to ACCase- and ALS inhibitors

Population code	Year collected	County and State[a]
08-AR-01	2008	Phillips, AR
08-AR-02	2008	Lawrence, AR
08-AR-03	2008	Cross, AR
08-AR-04	2008	Cross, AR
08-AR-05	2008	Cross, AR
08-AR-06	2008	Prairie, AR
08-AR-07	2008	Prairie, AR
08-AR-08	2008	Prairie, AR
08-AR-09	2008	Poinsett, AR
08-AR-10	2008	Craighead, AR
08-AR-11	2008	Arkansas, AR
08-AR-12	2008	Arkansas, AR
09-GA-01	2009	Hart, GA
09-MS-01	2009	Bolivar, MS
09-MS-03	2009	Bolivar, MS
09-MS-05	2009	Washington, MS
09-MS-06	2009	Washington, MS
09-MS-07	2009	Washington, MS
09-MS-08	2009	Washington, MS
09-NC-01	2009	Pasquotank, NC
09-NC-02	2009	Pasquotank, NC
09-NC-03	2009	Union, NC
09-NC-04	2009	Iredell, NC
09-NC-05	2009	Union, NC
10-GA-01	2010	Hart, GA
10-KS-01	2010	Montgomery, KS
10-NC-01	2010	Rowan, NC
10-NC-02	2010	Iredell, NC
10-SC-01	2010	Oconee, SC
10-VA-01	2010	Pittsylvania, VA
11-AR-01	2011	White, AR
11-AR-02	2011	White, AR
11-AR-03	2011	White, AR
11-AR-04	2011	White, AR
11-AR-05	2011	White, AR
11-AR-06	2011	White, AR
11-MS-01	2011	Tate, MS
11-NC-01	2011	Tyrell, NC
11-NC-02	2011	Tyrell, NC
11-NC-03	2011	Union, NC
11-NC-04	2011	Union, NC
11-NC-05	2011	Mecklenburg, NC
11-NC-06	2011	Union, NC
11-NC-07	2011	Stanly, NC
11-NC-08	2011	Union, NC
11-WA-A	2011	Walla Walla, WA
11-WA-D	2011	Walla Walla, WA

Note: [a]AR = Arkansas, GA = Georgia, MS = Mississippi, NC = North Carolina, KS = Kansas, SC = South Carolina, VA = Virginia, WA = Washington

Table 2: *Lolium perenne* ssp. *multiflorum* populations, herbicides, and dose ranges that were used in the bioassays

Population[a]	Herbicide dose ranges[b,c] (g ha^{-1})				
	Diclofop	**Imazamox**	**Mesosulfuron**	**Pinoxaden**	**Pyroxsulam**
08-AR-01	0 to 4480	0 to 143	0 to 58	NT	0 to 72
08-AR-02	0 to 4480	0 to 143	0 to 58	NT	0 to 72
08-AR-03	0 to 4480	0 to 143	0 to 58	NT	0 to 72
08-AR-04	0 to 4480	0 to 143	0 to 58	NT	0 to 72
08-AR-05	0 to 4480	0 to 143	0 to 58	NT	0 to 72
08-AR-06	0 to 4480	0 to 143	0 to 58	NT	0 to 72
08-AR-07	0 to 4480	0 to 143	0 to 58	NT	0 to 72
08-AR-08	0 to 4480	0 to 143	0 to 58	NT	0 to 72
08-AR-09	0 to 4480	0 to 143	0 to 58	NT	0 to 72
08-AR-10	0 to 4480	0 to 143	0 to 58	NT	0 to 72
08-AR-11	0 to 4480	0 to 143	0 to 58	NT	0 to 72
08-AR-12	0 to 4480	0 to 143	0 to 58	NT	0 to 72
09-GA-01	0 to 1680	NT	0 to 29	0 to 120	0 to 36
09-MS-01	0 to 1680	NT	0 to 29	0 to 120	0 to 36
09-MS-03	0 to 1680	NT	0 to 29	0 to 120	0 to 36
09-MS-05	0 to 1680	NT	0 to 29	0 to 120	0 to 36
09-MS-06	0 to 1680	NT	0 to 29	0 to 120	0 to 36
09-MS-07	0 to 1680	NT	0 to 29	0 to 120	0 to 36
09-MS-08	0 to 1680	NT	0 to 29	0 to 120	0 to 36
09-NC-01	0 to 1680	NT	0 to 29	0 to 120	0 to 36
09-NC-02	0 to 1680	NT	0 to 29	0 to 120	0 to 36
09-NC-03	0 to 1680	NT	0 to 29	0 to 120	0 to 36
09-NC-04	0 to 1680	NT	0 to 29	0 to 120	0 to 36
09-NC-05	0 to 1680	NT	0 to 29	0 to 120	0 to 36
10-GA-01	0 to 1680	NT	0 to 29	0 to 120	0 to 36
10-KS-01	0 to 1680	NT	0 to 29	0 to 120	0 to 36
10-NC-01	0 to 1680	NT	0 to 29	0 to 120	0 to 36
10-NC-02	0 to 1680	NT	0 to 29	0 to 120	0 to 36
10-SC-01	0 to 1680	NT	0 to 29	0 to 120	0 to 36
10-VA-01	0 to 1680	NT	0 to 29	0 to 120	0 to 36
11-AR-01	0 to 1680	NT	0 to 29	0 to 120	0 to 36
11-AR-02	0 to 1680	NT	0 to 29	0 to 120	0 to 36
11-AR-03	0 to 1680	NT	0 to 29	0 to 120	0 to 36
11-AR-04	0 to 1680	NT	0 to 29	0 to 120	0 to 36
11-AR-05	0 to 1680	NT	0 to 29	0 to 120	0 to 36
11-AR-06	0 to 1680	NT	0 to 29	0 to 120	0 to 36
11-MS-01	0 to 1680	NT	0 to 29	0 to 120	0 to 36
11-NC-01	0 to 1680	NT	0 to 29	0 to 120	0 to 36
11-NC-02	0 to 1680	NT	0 to 29	0 to 120	0 to 36
11-NC-03	0 to 1680	NT	0 to 29	0 to 120	0 to 36
11-NC-04	0 to 1680	NT	0 to 29	0 to 120	0 to 36
11-NC-05	0 to 1680	NT	0 to 29	0 to 120	0 to 36
11-NC-06	0 to 1680	NT	0 to 29	0 to 120	0 to 36
11-NC-07	0 to 1680	NT	0 to 29	0 to 120	0 to 36
11-NC-08	0 to 1680	NT	0 to 29	0 to 120	0 to 36
11-WA-A	0 to 1680	NT	0 to 29	0 to 120	0 to 36
11-WA-D	0 to 1680	NT	0 to 29	0 to 120	0 to 36

Note: [a]AR = Arkansas, GA = Georgia, MS = Mississippi, NC = North Carolina, KS = Kansas, SC = South Carolina, VA = Virginia, WA = Washington, [b]NT = not tested, [c]Recommended dose (g a.i. ha^{-1}): diclofop = 840; mesosulfuron = 15 ; pinoxaden = 60; pyroxsulam = 18

All populations tested in 2008 were from Arkansas. These were tested for resistance to diclofop to continue monitoring the spread of diclofop resistance in the state. At that time, the frequency of cross-resistance to diclofop and pinoxaden was just determined (Kuk *et al.* 2008) therefore, bioassays

were focused on testing for resistance to ALS inhibitors (see next section), which was of more recent occurrence (Kuk and Burgos 2007). At the three- to four-leaf stage, the 2008 populations were sprayed with 0, 560, 1120, 2242, and 4480 g ai ha^{-1} diclofop (Hoelon®, Bayer Crop Science, Research Triangle Park, NC 2770) which correspond to 0, 0.5, 1, 2, and 4X the manufacturer's recommended dose (Anon., 2003; Scott *et al.*, 2012). The rest of the populations were tested with up to 2X the recommended dose. Cross-resistance to pinoxaden was assayed on the populations collected between 2009 and 2011; doses used were 0, 30, 60, and 120 g ai ha^{-1} (Axial XL®, Syngenta Crop Protection, Inc., Greensboro, NC 27419), or up to 2X the recommended dose. Because of the large number of populations tested, the dose ranges used were intended only to confirm resistance and obtain some idea of the resistance level. The data were not intended to estimate the amount of herbicide needed to achieve practical levels of weed control, i.e. the amount of herbicide needed to provide 90% (GR_{90}) or 95% (GR_{95}) weed control.

Diclofop was applied with 1.0% (v/v) crop oil concentrate (Agri-Dex® crop oil concentrate, Helena Chemical Co., Collierville, TN 38017) and pinoxaden with 0.7% (v/v) methylated rapeseed oil based adjuvant (Adigor® adjuvant, Syngenta Crop Protection, Inc., Greensboro, NC 27409) (Anonymous 2003; Anonymous 2010). Herbicide treatments were applied in a spray cabinet, using a motorized boom equipped with flat fan nozzles (TeeJet spray nozzles, Spraying Systems Co., Wheaton, IL 60189) delivering 187 L ha^{-1} at 241 kPa. The experiment was conducted in a completely randomized design with four replications. Each herbicide bioassay was conducted as a separate experiment. The experiments were conducted twice. Visible injury was evaluated at 4 wk after treatment (WAT) relative to the non-treated control using a 0 to100 % scale, with 0 as no control and 100 as complete control. Injury pertains to the overall visible negative effects of the herbicide on the plant including chlorosis, stunting, and total desiccation. Populations were categorized based on visible injury at 4 WAT at 1X dose, except for diclofop which was based at 1.5X dose: 0 to 20% control as highly resistant (HR), 21 to 60% control as moderately resistant (MR), 61 to 80% as slightly resistant (SR), and 81 to 100% control as susceptible (S).

Evaluation of resistance patterns to ALS inhibitors

Populations in 2008 were treated with up to 4X the recommended dose of mesosulfuron (Osprey®, Bayer CropScience, Research Triangle Park, NC 27709), pyroxsulam (Powerflex®, Dow AgroSciences, LLC, Indianapolis, IN 46268) and imazamox (Beyond®, BASF Corp., Research Triangle Park, NC 27709). Herbicide doses (g ai ha^{-1}) were 0, 7, 15, 29, and 58 for mesosulfuron; 0, 9, 18, 36, and 72 for pyroxsulam; and 0, 18, 36, 72, and 143 for imazamox. The recommended doses are 36, 15, and 18 g ha^{-1} for imazamox, mesosulfuron, and pyroxsulam, respectively. Testing for cross-resistance to ALS inhibitors among populations collected between 2009 and 2011 was focused on two herbicides; imazamox was excluded because herbicide-resistant (Clearfield®) wheat comprise only a small proportion of US wheat and resources for large-scale testing were limited. Thus, the 2009-2011 populations were treated with up to 2X the labeled dose of mesosulfuron (0, 7, 15, and 29 g ha^{-1}) and pyroxsulam (0, 9, 18, and 36 g ha^{-1}). The SS was treated with up to the 1X dose only. A 1.0% (v/v) methylated seed oil (MES-100™, Drexel Chemical Co., Memphis, TN 38113) was included with mesosulfuron. A 1.25% (v/v) crop oil concentrate (Agri-Dex® crop oil concentrate, Helena Chemical Co., Collierville, TN 38017) was included with pyroxsulam. Visible injury was evaluated at 4 WAT. Experiments were conducted twice. Other procedures were the same as described in the previous section.

Resistance levels to ACCase- and ALS inhibitors in populations with different resistance patterns

This experiment included populations 09-NC-01, 09-NC-04, and 09-NC-05, representing different herbicide resistance patterns. Population 09-NC-01 is resistant to mesosulfuron; 09-NC-04 is resistant to diclofop, mesosulfuron, and pyroxsulam; 09-NC-05 is resistant to diclofop, mesosulfuron, pyroxsulam, and pinoxaden. A dose-response assay was conducted to evaluate the respective levels of resistance to diclofop, pinoxaden, mesosulfuron, and pyroxsulam. Seedlings were thinned to 10 plants per pot 5 d after emergence. Herbicide doses, ranging from 0 to 8X of the recommended doses of each herbicide, were applied to the selected populations at the three- to four-leaf stage. The SS was sprayed with up to 2X of the labeled dose. The experiment was conducted in a completely randomized design with four replications. Recommended adjuvants were used. At 4 WAT, the plants were cut at the soil surface, dried for 48 h, and weighed. The experiments were conducted twice. All other procedures were the same as in the previous section.

Biomass data were expressed as percentages of the mean of the nontreated control to standardize comparisons among populations. Statistical analysis showed that the experiment run-by-herbicide dose interaction and the main effect of experiment run were not significant. Therefore, data were combined over the two experiment runs. ANOVA also showed that the SS population-by-year interaction and the main effect of year were not significant, thus, data for SS were combined across years.

Regression analysis in Sigma Plot v. 12 (Jandel Scientific, Point Richmond, CA 94804) was done on the resistance pattern evaluation experiments for the 2008 populations which included five herbicide doses. Resistance pattern evaluation experiments for 2009, 2010 and 2011 populations which contained less than five herbicide doses were analyzed as completely randomized design in JM Pro v.10. The populations were then placed in resistance categories based on their responses to the labeled herbicide dose at 4 WAT.

Regression analyses for the dose-response experiments (09-NC-01, 09-NC-04, and 09-NC-05) were performed in Sigma Plot v. 12. Biomass reduction and visible injury data at 4 WAT with increasing herbicide doses were modeled with either a three-parameter sigmoidal (equation 1) or logistic (equation 2) regression functions.

$$Y = a/[1+ e^{-((x-xo)/b)}] \quad [1]$$

$$Y = a/[(1 + (x/x_o)^b] \quad [2]$$

The amount of herbicide needed to reduce aboveground weight by 50%, or to incur 50% injury (GR_{50}) was obtained from regression equations in Sigma Plot v. 12 using the injury ratings for 2008 Arkansas and 2011 Washington populations and biomass reduction data for the 09-NC-01, 09-NC-04, and 09-NC-05 populations. Herbicide resistance levels (R/S ratios) were estimated from the GR_{50} of the resistant population relative to the GR_{50} of the SS.

RESULTS AND DISCUSSION

Resistance to ACCase inhibitors

The selection for resistance to ACCase inhibitors in *L. perenne* in the United States started with diclofop (Stanger and Appleby 1989). Of the 47 populations tested in this research, from various states, only seven (15%) could be considered susceptible to diclofop; the rest had different levels of resistance (Tables 3 and 4). All populations from Arkansas were diclofop-resistant (Table 3).

Table 3: GR_{50}[a] values and resistance levels to diclofop among 2008 *Lolium perenne* ssp. *multiflorum* populations from Arkansas, USA.

Population[b]	Regression equation	R^2	GR_{50}	SE[c]	R/S[d]
			g ha^{-1}		
SS[e]	$Y = 99/[1+ e^{-((x-0.46)/0.059)}]$	0.99	458	1.15	-
08-AR-01	$Y = 73/[1+ e^{-((x-3.69)/1.44)}]$	0.96	>4480	5.17	>10
08-AR-02	$Y = 89/[(1 + (x/1.22)^{-3.28}]$	0.99	1313	3.72	3
08-AR-03	$Y = 72/[1+ e^{-((x-3.77)/1.21)}]$	0.99	>4480	2.97	>10
08-AR-04	$Y = 73/[1+ e^{-((x-3.20)/0.99)}]$	0.99	3955	2.78	9
08-AR-05	$Y = 136/[(1 + (x/3.53)^{-1.08}]$	0.99	2144	1.63	5
08-AR-06	$Y = 14/[(1 + (x/1.03)^{-5.64}]$	0.98	>4480	1.21	>10
08-AR-07	$Y = 29/[(1 + (x/1.85)^{-2.45}]$	0.99	>4480	1.27	>10
08-AR-08	$Y = 5.43/[(1 + (x/2.91)^{-1.84}]$	0.99	>4480	0.23	>10
08-AR-09	$Y = 72/[1+ e^{-((x-1.38)/0.50)}]$	0.99	1785	3.85	4
08-AR-10	$Y = 13.1/[1+ e^{-((x-1.14)/0.039)}]$	0.99	>4480	0.63	>10
08-AR-11	$Y = 82/[1+ e^{-((x-2.05)/0.88)}]$	0.97	2450	7.13	5
08-AR-12	$Y = 63/[1+ e^{-((x-0.66)/0.31)}]$	0.94	1085	9.27	2

Note: [a]GR_{50} is the herbicide concentration that reduced shoot growth by 50% based on visual evaluation at 4 WAT. The recommended dose is 840 g a.i. ha^{-1}. [b]Susceptible and putative resistant populations had five diclofop doses ranging from 0 to 4X of the recommended dose. [c]SE = standard error of the estimate. [d]R/S (resistant/susceptible) ratios were calculated based on GR_{50} values of populations relative to the susceptible standard. [e]Susceptible standard population.

Sixty-seven percent of the populations from Arkansas required >4480 g ha^{-1} diclofop to reduce plant growth by 50% (GR_{50}) compared with 458 g ha^{-1} for the SS. The recommended dose in wheat is between 560 and 1120 g ha^{-1} (Scott *et al.* 2012); for this research we used 840 g ha^{-1} as the 1X dose. It is believed that resistance to diclofop in *L. perenne* occurs in all wheat-producing counties in Arkansas (Kuk *et al.* 2008) and this is supported by a recent statewide survey (Jim Dickson, Arkansas Cooperative Ext. Service, unpublished data). Based on the dose response assays, the resistant populations were 2- to >10-fold more resistant to diclofop than the SS. The low-level (2-fold) resistance entailed using 1085 g ha^{-1} diclofop to reduce biomass by 50%. This implies that many survivors can be expected from a commercial application of diclofop to this population in the field, which increase the resistance frequency and cause significant economic loss. Of the thirty seven populations tested with up to 1680 g ha^{-1} diclofop, collected between 2009 and 2011, 5 were HR, 14 were MR, 8 were SR, and 7 were susceptible (Table 4).

Table 4: Response of 2009-11 *Lolium perenne* ssp. *multiflorum* populations from the United States to ACCase-inhibitors diclofop and pinoxaden

Population	Visible injury, 4 WAT (%)			
	Diclofop		Pinoxaden	
	840	1680[a]	60	120[a]
09-GA-01	29	63	100	100
09-MS-01	19	34	100	100
09-MS-03	10	25	100	100
09-MS-05	30	31	100	100
09-MS-06	29	79	100	100
09-MS-07	25	36	100	100
09-MS-08	25	61	100	100
09-NC-01	18	19	100	100
09-NC-02	28	36	100	100
09-NC-03	6	6	20	20
09-NC-04	12	14	100	100
09-NC-05	16	20	60	64
10-GA-01	48	56	39	66
10-KS-01	90	96	100	100
10-NC-01	45	50	45	54
10-NC-02	71	79	98	100
10-SC-01	74	81	100	100
10-VA-01	75	87	99	100
11-AR-01	37	39	94	95
11-AR-02	44	45	98	100
11-AR-03	53	78	100	100
11-AR-04	40	50	73	85
11-AR-05	52	64	91	96
11-AR-06	7	20	94	100
11-MS-01	82	94	100	100
11-NC-01	97	100	100	100
11-NC-02	100	100	100	100
11-NC-03	91	99	100	100
11-NC-04	4	24	61	83
11-NC-05	37	54	95	96
11-NC-06	43	48	98	98
11-NC-07	65	78	100	100
11-NC-08	29	39	92	92
11-WA-A	30[b]	47[c]	49	61
11-WA-D	29[b]	33[c]	58	74
SS[b]	90	98	100	-
$LSD_{0.05}$[c]				
Herbicide dose[d]	2		1	
Herbicide dose ×population[e]	13		7	

Note: [a]1X doses for diclofop and pinoxaden are 840 and 60 g a.i. ha^{-1}, respectively. [b]Susceptible standard. [c]Main effect of population was not significant. [d]Fisher's Protected LSD to compare herbicide dose means averaged over populations. [e]LSD to compare population means at the same or different herbicide dose.

Populations with high level of resistance may harbor more than one resistance mechanisms, most likely target site mutation and enhanced metabolism (Tardif and Powles 1994). *L. perenne* has also evolved resistance to diclofop in Brazil, Chile, France, Italy, United Kingdom, and in nine states in the United States (Heap 2012). The frequency of herbicide-resistant populations in this research is higher than the actual distribution of resistant populations across Arkansas or the rest of the United States because these samples were collected from fields reporting control failures with diclofop.

Because of the increasing number of diclofop-resistant *L. perenne* populations, diclofop is no longer a viable option for wheat weed control. Pinoxaden, an ACCase-inhibiting herbicide belonging to the phenylpyrazoline family (Hofer *et al*. 2006), was then introduced as an alternative graminicide for

wheat (Dickson *et al.* 2011). Pinoxaden has the same mode of action as the other AOPP herbicides but with a novel chemical structure (Hofer *et al.* 2006). It is effective in controlling the majority of ACCase (diclofop)-resistant populations. In using pinoxaden, growers need to consider the field history and monitor the field closely because of the potential cross-resistance with diclofop. In Arkansas, about 20% of diclofop-resistant *L. perenne* populations in Arkansas are cross-resistant to pinoxaden (Kuk *et al.* 2008).

Because cross-resistance to ACCase inhibitors in Arkansas *L. perenne* populations collected up to 2005 was already investigated (Kuk *et al.* 2008), it was decided to wait a few more years to retest for cross-resistance to pinoxaden in Arkansas samples. However, samples from other states collected between 2009 and 2011 were all tested for cross-resistance to diclofop and pinoxaden. Of these, eight were resistant to pinoxaden with *L. perenne* control ranging from 20 to 73% at the recommended dose, all of which were also resistant to diclofop (Table 4). Four of these pinoxaden-resistant populations were from North Carolina (09-NC-03, 09-NC-05, 10-NC-01, 11-NC-04), 2 from Washington (11-WA-A and 11-WA-D), 1 from Georgia (10-GA-01), and 1 from Arkansas (11-AR-04). The frequency of cross-resistance to ACCase inhibitors was 28% (8 of 28 diclofop-resistant populations), slightly higher than that reported by Kuk *et al.* (2008) for Arkansas. To date, resistance to pinoxaden is confirmed in populations from Arkansas (Kuk *et al.*, 2008), Idaho (Rauch *et al.*, 2010), Louisiana (Ellis *et al.*, 2010), North Carolina (Chandi *et al.*, 2011), and Washington (Rauch *et al.*, 2010). The four pinoxaden-resistant populations from North Carolina reported in this paper is an indicator that pinoxaden-resistant *L. perenne* in North Carolina may be spreading.

Resistance to pinoxaden in *L. perenne* was already reported in Chile in 2006 and Israel in 2007 (Heap 2012). Before the commercialization of pinoxaden, resistance to pinoxaden in *A. myosuroides* was already detected in France (Petit *et al.*, 2010). These grass populations have been exposed to selection pressure from other ACCase inhibitors, including diclofop. Pinoxaden has been commercially used only since 2006, at least 25 yr from the introduction of diclofop (Hofer *et al.*, 2006). Diclofop and pinoxaden inhibit the same enzyme; thus, selection pressure from diclofop could predispose *Lolium* populations to pinoxaden resistance (Kuk *et al.* 2008). As in any other herbicides, intensive use of pinoxaden, and allowing survivors to set seed would result in the evolution of resistant populations.

Resistance patterns to ALS inhibitors

Traditionally, ALS inhibitors such as chlorsulfuron plus metsulfuron and tribenuron are used PRE and postemergence (POST), respectively, in wheat cropping systems in the USA. The widespread occurrence of diclofop-*resistant L. perenne* has ushered in the POST ALS-inhibitor mesosulfuron (Kuk and Burgos 2007). However, similar to the case of pinoxaden, a population collected a year before the commercialization of mesosulfuron, was confirmed mesosulfuron-resistant in Arkansas (Kuk and Burgos 2007).

Table 5: GR_{50}[a] values and resistance levels to mesosulfuron in 2008 *Lolium perenne* ssp. *multiflorum* populations from Arkansas, USA

Population[b]	Regression equation	R^2	GR_{50}	SE[c]	*R/S*[d]
			g ha^{-1}		
SS[e]	$Y = 100[1+ e^{-((x-0.0073)/0.015)}]$	0.99	7.3	0.90	-
08-AR-01	$Y = 67/[(1 + (x/0.023)^{-2.61}]$	0.98	34.7	4.63	5
08-AR-02	$Y = 133[1+ e^{-((x-0.016)/0.82)}]$	0.99	8.8	2.15	1
08-AR-03	$Y = 40[1+ e^{-((x-0.030)/0.0057)}]$	0.99	>58.2	0.24	>8
08-AR-04	$Y = 102/[(1 + (x/0.023)^{-1.24}]$	0.99	>58.2	0.22	>8
08-AR-05	$Y = 96[1+ e^{-((x-0.0058)/0.0008)}]$	0.99	5.9	3.32	1
08-AR-06	$Y = 8.76/[(1 + (x/0.039)^{-2.23}]$	0.99	>58.2	0.17	>8
08-AR-07	$Y = 21.3/[(1 + (x/0.013)^{-7.82}]$	0.99	>58.2	1.24	>8
08-AR-08	$Y = 12.3/[(1 + (x/0.012)^{-1.36}]$	0.99	>58.2	0.70	>8
08-AR-09	$Y = 37[1+ e^{-((x-0.0027)/0.0084)}]$	0.98	>58.2	3.39	>8
08-AR-10	$Y = 23/[(1 + (x/0.0096)^{-3.45}]$	0.99	>58.2	1.05	>8
08-AR-11	$Y = 30/[(1 + (x/0.016)^{-0.0070}]$	0.98	>58.2	2.51	>8
08-AR-12	$Y = 69[1+ e^{-((x-0.032)/0.017)}]$	0.99	48.3	9.35	7

Note: [a]GR_{50} is the herbicide concentration that reduced shoot growth by 50% based on visual evaluation at 4 WAT. The recommended dose for mesosulfuron is 15 g a.i. ha^{-1}. [b]Susceptible and putative resistant populations had five mesosulfuron doses ranging from 0 to 4X of the recommended dose. [c]SE = standard error of the estimate. [d]R/S (resistant/susceptible) ratios were calculated based on GR_{50} values of populations relative to the susceptible standard. [e]Susceptible standard population.

Of the populations tested in this recent research, 83% exhibited resistance to mesosulfuron. The amount of mesosulfuron causing 50% injury to the SS is only 7.3 g ha^{-1}, which is equivalent to one-half the recommended dose (Table 5). Among the 2008 Arkansas populations, 10 out of 12 were resistant to the 15 g ha^{-1} recommended dose of mesosulfuron with 5-fold to >8-fold resistance relative to the SS (Table 5). Eight of these resistant populations could not be controlled by 58.2 g ha^{-1}, which is more than 4X the recommended dose. Among the 35 populations collected from 2009 to 2011, 29 were resistant (≤ 80% control) to mesosulfuron (Table 6). Of these mesosulfuron-resistant populations, two were SR, 16 were MR, and 11 were HR. Thirty-six of the 41 diclofop-resistant populations collected from 2008 to 2011 were also resistant to mesosulfuron (Tables 3 to 6).

Table 6: Response of *Lolium perenne* ssp. *multiflorum* populations from the United States to ALS-inhibitors mesosulfuron and pyroxsulam

Population	Visible injury, 4 WAT (%)					
	Mesosulfuron			Pyroxsulam		
	7	15[a]	29	9	18[b]	36
09-GA-01	13	11	13	13	12	12
09-MS-01	19	30	36	28	44	48
09-MS-03	20	21	30	13	15	18
09-MS-05	11	16	25	10	18	20
09-MS-06	19	21	25	11	19	20
09-MS-07	21	28	28	16	18	23
09-MS-08	15	19	20	16	26	26
09-NC-01	8	8	13	55	96	96
09-NC-02	74	100	100	92	95	99
09-NC-03	19	20	23	11	19	21
09-NC-04	20	25	25	10	29	29
09-NC-05	21	24	33	24	46	49
10-GA-01	53	71	73	49	68	73
10-KS-01	34	43	50	31	38	52
10-NC-01	27	33	34	40	48	74
10-NC-02	31	36	44	32	31	51
10-SC-01	51	57	57	54	57	66
10-VA-01	87	97	100	79	89	100
11-AR-01	13	13	40	13	25	30
11-AR-02	11	13	34	25	29	38
11-AR-03	25	28	40	25	35	36
11-AR-04	19	19	24	20	26	36
11-AR-05	35	38	51	13	36	38
11-AR-06	21	26	36	23	23	38
11-MS-01	1	10	30	11	13	20
11-NC-01	39	73	73	65	73	85
11-NC-02	91	100	99	64	90	96
11-NC-03	90	96	99	96	100	100
11-NC-04	8	24	39	23	28	28
11-NC-05	13	15	36	5	28	38
11-NC-06	23	23	31	25	29	34
11-NC-07	20	30	44	16	49	61
11-NC-08	16	20	44	21	23	23
SS[c]	91	99	100	91	100	100
$LSD_{0.05}$[d]						
Herbicide dose[e]	2			2		
Herbicide dose X population[f]	11			12		

Note: [a]Recommended dose for mesosulfuron in g a.i. ha^{-1}. [b]Recommended dose for pyroxsulam in g a.i. ha^{-1}. [c]Susceptible standard. [d]Main effect of population was not significant. [e]Fisher's Protected LSD to compare herbicide dose means averaged over populations. [f]LSD to compare population means at the same or different herbicide dose.

Three diclofop-susceptible populations (10-KS-01, 11-MS-01, and 11-NC-01) were poorly controlled at the recommended dose of mesosulfuron (15 g ha^{-1}). Doubling the recommended mesosulfuron dose did not control the putative resistant *L. perenne* populations (Table 6). The high frequency of mesosulfuron-resistant populations is expected because the majority of samples were collected from wheat fields where mesosulfuron applications failed. Earlier, resistance to mesosulfuron in Idaho and Washington was found in 27% of the populations tested (Rauch *et al.* 2010). Rauch *et al.* (2010) also reported that 27% of the populations they tested exhibited multiple resistance to ALS- and ACCase inhibitors. Multiple resistance to diclofop and mesosulfuron was also observed in North Carolina, and Texas (Chandi *et al.* 2011). The first mesosulfuron-resistant populations documented in Arkansas (Kuk and Burgos 2007) and Texas (Ellis *et al.* 2008) did not have multiple resistance to diclofop. Although the level of resistance to mesosulfuron differed among the populations tested, it appeared that diclofop-resistant *L. perenne* evolved resistance to mesosulfuron quickly, or that the ALS-resistant allele already existed prior to the use of mesosulfuron. For example, population 08-AR-02 in this study was first exposed to mesosulfuron in 2008, the same year it was sampled, and was confirmed resistant (Salas *et al.* 2010). Mesosulfuron-resistant populations may have been selected for with other ALS inhibitors such as chlorsulfuron and metsulfuron that were previously used PRE in wheat. The use of mesosulfuron to control diclofop-resistant ryegrass could accelerate the selection of resistance alleles. Results imply that the utility of mesosulfuron for Italian ryegrass management is diminished by the high frequency of mesosulfuron-resistant populations.

Imazamox, an ALS herbicide belonging to the imidazolinone chemistry, is used to manage weeds in imidazolinone-tolerant (Clearfield®) wheat in several states in the United States including Oklahoma, Colorado, Oregon, Idaho, Washington, Kansas, and Nebraska (Bond *et al.* 2005). *L. perenne* is naturally susceptible to imazamox. However, some *L. perenne* populations are already resistant to imazamox, even in locations where imazamox had not been used previously, because of cross-resistance to other ALS inhibitors such as mesosulfuron (Kuk *et al.* 2007). The SS was completely controlled by the recommended dose of imazamox (Table 7). Ten of 12 populations from Arkansas in 2008 were resistant to the 36 g ha^{-1} labeled dose of imazamox, with GR_{50} values ranging from 37 to >143 g ha^{-1}. The most resistant (08-AR-06) population requires more than 4X the recommended dose to achieve 50% control. These 10 imazamox-resistant populations were the same populations resistant to mesosulfuron (Tables 5 and 7). Resistance to imazamox was reported earlier in 11% of the *L. perenne* populations from Idaho and Washington (Rauch *et al.* 2010).

A similar resistance pattern was observed with pyroxsulam, a new triazolopyrimidine sulfonamide herbicide that provides selective POST grass and broadleaf weed control in wheat (DeBoer *et al.* 2011). Eighty-one percent of the populations from suspect fields were resistant to pyroxsulam. In the 2008 Arkansas populations, 10 were resistant to the 18 g ha^{-1} recommended dose of pyroxsulam with GR_{50} values of 14 to more than 72 g ha^{-1} (Table 8). The amount of herbicide needed to control the SS by 50% was 7 g ha^{-1} pyroxsulam which is one-half the recommended dose. Based on GR_{50}, the 10 resistant populations were 2- to 10-fold more resistant than the SS. These pyroxsulam-resistant populations were also resistant to mesosulfuron and imazamox (Table 5 and 8). The low level resistance of 08-AR-08 (2-fold) is still an economic concern because the population cannot be controlled 100% by the recommended dose. Populations collected between 2009 and 2011 from suspect fields in other states were also mostly not controlled by pyroxsulam. Of the 35 populations, only seven (09-NC-01, 09-NC-02, 10-VA-01, 11-NC-02, 11-NC-03, 11-WA-A, and 11-WA-D) were controlled >80% at the 1X dose (Table 6). Of the 28 pyroxsulam-resistant populations, 2 were SR, 19 were MR, and 7 were HR.

Related research have shown that cross-resistance occurs between sulfonylureas and imidazolinones such as observed in *Lactuca sativa* L. (Mallory-Smith *et al.* 1990) and redroot *Amaranthus retroflexus* L. (Sibony *et al.* 2001). The occurrence of cross-resistance between these two ALS-inhibitor families is expected to be infrequent because imidazolinone herbicides have a different binding configuration at the ALS active site compared with sulfonylurea herbicides (McCourt *et al.* 2006; Rauch *et al.* 2010). However, data accumulated thus far show that cross-resistance to these two chemistries is common. It appears that the binding sites of mesosulfuron and imazamox in the ALS enzyme are in close enough proximity such that when a plant is resistant to one it is also resistant to the other. The bioassays demonstrated that whenever there was control failure in the field, often the population already has resistant ALS alleles. The relatively high frequency of cross-resistance to mesosulfuron and pyroxsulam in the southern states and in Kansas (Table 6) should be noted because these herbicides are the two most recent weed control options in wheat, with pyroxsulam being registered for wheat in 2008 (US EPA 2008).

Table 7: GR_{50}[a] values and resistance levels to imazamox among 2008 *Lolium perenne* ssp. *multiflorum* populations from Arkansas, USA

Population[b]	Regression equation	R^2	GR_{50}	SE[c]	R/S[d]
			g ha^{-1}		
SS[e]	$Y = 100[1+ e^{-((x-0.017)/0.0031)}]$	0.99	17.3	0.51	-
08-AR-01	$Y = 64[1+ e^{-((x-0.10)/0.028)}]$	0.98	136.6	3.95	8
08-AR-02	$Y = 87[1+ e^{-((x-0.020)/0.0072)}]$	0.99	22.4	5.04	1
08-AR-03	$Y = 98[1+ e^{-((x-0.039)/0.021)}]$	0.94	39.5	13.04	2
08-AR-04	$Y = 68[1+ e^{-((x-0.084)/0.032)}]$	0.99	117.0	3.93	7
08-AR-05	$Y = 98[1+ e^{-((x-0.014)/0.0014)}]$	0.99	13.6	2.60	1
08-AR-06	$Y = 37[1+ e^{-((x-0.049)/0.020)}]$	0.99	>143.4	2.40	>8
08-AR-07	$Y = 68[1+ e^{-((x-0.044)/0.017)}]$	0.99	61.4	3.76	4
08-AR-08	$Y = 62[1+ e^{-((x-0.023)/0.010)}]$	0.98	37.0	5.75	2
08-AR-09	$Y = 86[1+ e^{-((x-0.055)/0.0079)}]$	0.98	57.0	0.59	3
08-AR-10	$Y = 152/[(1 + (x/0.21)^{-0.99}]$	0.99	103.0	2.20	6
08-AR-11	$Y = 331[1+ e^{-((x-0.19)/0.051)}]$	0.98	98.0	7.68	6
08-AR-12	$Y = 61[1+ e^{-((x-0.056)/0.021)}]$	0.98	86.0	5.58	5

Note: [a]GR_{50} is the herbicide concentration that reduced shoot growth by 50% based on visual evaluation at 4 WAT. Recommended imazamox dose is 36 g a.i. ha^{-1}. [b]Susceptible and putative resistant populations had five imazamox doses ranging from 0 to 4X of the recommended dose. [c] SE = standard error of the estimate. [d] R/S (resistant/susceptible) ratios were calculated based on GR_{50} values of populations relative to the susceptible standard. [e]Susceptible standard population.

Table 8: GR_{50}[a] values and resistance levels to pyroxsulam among 2008 *Lolium perenne* ssp. *multiflorum* populations from Arkansas, USA

Population[b]	Regression equation	R^2	GR_{50}	SE[c]	R/S[d]
			g ha^{-1}		
SS[e]	$Y = 99[1+ e^{-((x-0.0069)/0.0007)}]$	0.99	7	1.44	-
08-AR-01	$Y = 71[1+ e^{-((x-0.014)/0.0042)}]$	0.99	18	2.40	3
08-AR-02	$Y = 100/[(1 + (x/0.0082)^{-2.16}]$	0.99	8	1.77	1
08-AR-03	$Y = 86/[(1 + (x/0.018)^{-4.42}]$	0.99	20	5.43	3
08-AR-04	$Y = 24/[(1 + (x/0.012)^{-1.70}]$	0.99	>72	1.44	>10
08-AR-05	$Y = 99/[(1 + (x/0.0069)^{-3.56}]$	0.99	7	0.75	1
08-AR-06	$Y = 61[1+ e^{-((x-0.063)/0.017)}]$	0.99	>72	1.26	>10
08-AR-07	$Y = 57[1+ e^{-((x-0.030)/0.016)}]$	0.94	62	7.19	9
08-AR-08	$Y = 76[1+ e^{-((x-0.013)/0.0020)}]$	0.99	14	0.63	2
08-AR-09	$Y = 52[1+ e^{-((x-0.026)/0.0073)}]$	0.99	52	2.46	7
08-AR-10	$Y = 40[1+ e^{-((x-0.027)/0.014)}]$	0.91	>72	6.23	>10
08-AR-11	$Y = 112/[(1 + (x/0.039)^{-1.33}]$	0.96	33	9.13	5
08-AR-12	$Y = 39[1+ e^{-((x-0.0071)/0.0022)}]$	0.91	>72	7.57	>10

Note: [a]GR_{50} is the herbicide concentration that reduced shoot growth by 50% based on visual evaluation at 4WAT. Recommended pyroxsulam dose is 18 g a.i. ha^{-1}. [b]Susceptible and putative resistant populations had five pyroxsulam doses ranging from 0 to 4X of the recommended dose. [c]SE = standard error of the estimate. [d] R/S (resistant/susceptible) ratios were calculated based on GR_{50} values of populations relative to the susceptible standard. [e]Susceptible standard population.

More weed species are resistant to ALS-inhibitors than any other herbicide group (Heap 2012). The high frequency of weed populations resistant to ALS inhibitors can be attributed to extensive use of these herbicides, the high selection pressure they exert, and the many resistance-conferring mutations in the *ALS* gene (Tranel and Wright 2002). Cross-resistance to ALS herbicides, particularly to sulfonylureas and imidazolinones, had been reported in *L. rigidum*, *Sisymbrium orientale* L., *A. retroflexus*, *Xanthium strumarium* L., *Kochia scoparia* (L.) Schrad, *Ambrosia artemisiifolia* L., and *Ambrosia trifida* L. (Boutsalis *et al.* 1999; Foes *et al.* 1999; Patzoldt *et al.* 200l; Patzoldt and Tranel 2002; Primiani *et al.* 1990; Sibony *et al.* 2001; Woodworth *et al.*1996;Yu *et al.* 2008). The magnitude of resistance to different ALS herbicides varies widely depending on the location of substitutions in the *ALS* gene (Tranel and Wright 2002). *L. perenne* populations exhibiting cross-resistance to

mesosulfuron, imazamox, and pyroxsulam may exhibit target-site mutation in the *ALS* gene. It is also possible that these populations can detoxify the herbicide rapidly. The mechanisms of resistance to ALS herbicides in these populations are yet to be verified.

Resistance levels to ACCase- and ALS inhibitors in selected *L. perenne* populations with different herbicide resistance patterns

Three populations were chosen to represent the following resistance patterns: (1) 09-NC-01, resistant to diclofop and mesosulfuron; (2) 09-NC-04, resistant to diclofop, mesosulfuron, and pyroxsulam; and (3) 09-NC-05, resistant to diclofop, mesosulfuron, pyroxsulam, and pinoxaden. This experiment was intended to help design follow-up experiments on population adaptation to weed management. A population resistant to diclofop and mesosulfuron was chosen because mesosulfuron was the first POST, ALS inhibitor used to manage diclofop-resistant *L. perenne* in wheat. Pattern #2 was chosen because this is common owing to the widespread occurrence of diclofop-resistant populations and the eventual resistance to the alternative POST, ALS herbicides mesosulfuron and pyroxsulam. Pattern #3 is an extreme case of resistance to four herbicide families and is the ultimate threat to crop production. Population 09-NC-01, determined HR to diclofop, had 33-fold resistance to mesosulfuron (Table 9). Population 09-NC-04 was only SR to diclofop (R/S=3), had 71-fold resistance to mesosulfuron, was cross-resistant to pyroxsulam, but was more resistant to the former than the latter. Population 09-NC-05 was highly resistant to diclofop (R/S=18); requiring an estimated 5X the recommended diclofop dose to achieve 50% control. It has low level (2-fold) of cross resistance to pinoxaden. It is also highly resistant to mesosulfuron (18-fold) with low level (3-fold) cross resistance to pyroxsulam. The herbicide physiology in this population is different between the two ACCase inhibitors (diclofop and pinoxaden) and the ALS inhibitors (mesosulfuron and pyroxsulam). Also, it should be noted that *L. perenne* populations are in their early stages of selection with these recent herbicides.

Table 9: GR_{50}[a] values and resistance levels to diclofop, mesosulfuron, pinoxaden, and pyroxsulam in selected *Lolium perenne* ssp. *multiflorum* populations from the United States, representing different resistance patterns

Population	Herbicide[b]	Regression equation	R^2	GR_{50}	SE[c]	R/S[d]
09-NC-01	mesosulfuron	$Y = 88/[1+ e^{-((x-29.3)/0.0245)}]$	0.82	36	16.29	33
09-NC-04	diclofop	$Y = 94/[(1 + (x/875)-4.57]$	0.99	899	1.15	3
	mesosulfuron	$Y = 105/[1+ e^{-((x-80.8)/0.0301)}]$	0.95	78	8.86	71
	pyroxsulam	$Y = 196/[(1 + (x/1606.3)-0.2636]$	0.99	28	2.36	6
09-NC-05	diclofop	$Y = 60/[(1 + (x/1155)-1.01]$	0.98	5432	2.58	18
	mesosulfuron	$Y = 63/[(1 + (x/10.8)^{-2.16}]$	0.97	20	5.68	18
	pinoxaden	$Y = 98/[1+ e^{-((x-28.0)/0.0471)}]$	0.99	28	0.21	2
	pyroxsulam	$Y = 80/[(1 + (x/5.2)^{-0.5559}]$	0.99	13	1.54	3
SS[e]	diclofop	$Y = 110/[(1 + (x/347)^{-1.28}]$	0.93	304	7.87	-
	mesosulfuron	$Y = 97/[(1 + (x/1.7)^{-2.48}]$	0.99	1	0.68	-
	pinoxaden	$Y = 96/[1+ e^{-((x-11.5)/0.0010)}]$	0.99	12	1.14	-
	pyroxsulam	$Y = 97/[1+ e^{-((x-4.3)/0.0006)}]$	0.99	4	3.75	-

Note: [a]GR_{50} is the herbicide concentration that reduced shoot biomass by 50% based on shoot dry weights, 4 wk after treatment. Recommended doses are 840, 15, 60, and 18 g a.i. ha^{-1} for diclofop, mesosulfuron, pinoxaden, and pyroxsulam, respectively. [b]Six herbicide doses ranging from 0 to 8X of the recommended dose were used for the resistant populations. The susceptible standard population was sprayed with six doses of herbicide from 0 to 2X of the recommended dose. [c]SE = standard error of the estimate. [d]R/S (resistant/susceptible) ratios were calculated based on GR_{50} values of resistant populations relative to the susceptible standard. [e]Susceptible standard population

These populations may possess two or more mechanisms that confer resistance to a single herbicide or class of herbicides. An ACCase- and ALS-resistant *L. rigidum* population from Australia (VLR69) harbors multiple resistance mechanisms, including a resistant ACCase, a resistant ALS, and enhanced herbicide metabolism (Preston *et al.* 1996). Multiple resistance to ACCase- and ALS herbicides in two resistant Australian *L. rigidum* populations is due to enhanced herbicide metabolism mediated by cytochrome P450 monooxygenase (Yu *et al.* 2009). Preliminary investigation on populations 09-NC-03 and 09-NC-04 suggests that cytochrome P450-mediated enhanced metabolism play a role in their resistance to ACCase-and ALS inhibitors and that other mechanisms may also be involved (Salas 2012).

In summary, 47 populations were tested for ACCase- and ALS- resistance. Almost 90% were resistant to the ACCase herbicide diclofop, which reflects the fact that diclofop was the first major selector of these populations. Most of the populations were controlled by the ACCase herbicide pinoxaden; however, 25% of diclofop-resistant populations were cross-resistant to pinoxaden (Table 10). Most of the diclofop-resistant populations in this research were also resistant to other herbicides with the same or different modes of action as was reported by others (Cocker *et al.* 2001; Eleni *et al.* 2000; Holtum and Powles 1991; Kuk *et al.* 2000; Kuk *et al.* 2008). Of 45 populations with resistance to ACCase or ALS inhibitors, 35 (78%) had multiple resistance to both herbicide modes of action. Of the 41 diclofop-resistant populations, 38 were resistant to at least one ALS inhibitor. Thirty-nine populations were resistant to mesosulfuron, 38 of which were also resistant to pyroxsulam. Of the 12 populations tested for resistance to imazamox, 10 were resistant to the labeled dose. Broad resistance to ALS inhibitors mesosulfuron, pyroxsulam, and imazamox was detected in all the nine populations tested with all these herbicides. Resistance to both ACCase- and ALS inhibitors in Italian ryegrass populations is a serious problem to wheat growers in the United States.

Considering the resistance patterns that are observed, pinoxaden is still an alternative herbicide for *L. perenne* control. It is effective on most of the populations; however, uninterrupted use of pinoxaden should be discouraged because of the 25% tendency of diclofop-resistant *L. perenne* to be cross-resistant to pinoxaden. *Lolium* infestation can be managed by having a full tillage program following the first period of fresh growth of *L. perenne* followed by a POST herbicide such as pinoxaden. Another program approach is a PRE application of chlorsulfuron/metsulfuron in the fall followed by POST herbicides (Scott *et al.* 2012). It is also necessary to vary the POST herbicide across growing seasons to delay the evolution of resistance. ALS herbicides, mesosulfuron and pyroxsulam, can still be used to diversify weed control in fields where no resistance to these herbicides had been observed. In Arkansas, ALS- and diclofop-resistant *L. perenne* in wheat fields is managed with the application of a pre-mix of flufenacet plus metribuzin at the one- to two-leaf wheat stage, followed by pinoxaden and pendimethalin at four-leaf to one-tiller *Lolium*. Other than the flufenacet plus metribuzin mixture and pendimethalin, all other herbicides currently registered for *L. perenne* control in wheat are either ACCase- or ALS inhibitors (Scott *et al.* 2012). Currently, a new PRE grass-selective herbicide, pyroxasulfone, an inhibitor of very long chain fatty acids (Tenetani *et al.* 2009) is being evaluated for potential use in wheat. Pyroxasulfone provides excellent control of *L. perenne* and is safe on wheat (Walsh *et al.* 2011; Scott *et al.* 2012). Resistance to multiple herbicides and limited herbicide options for *L. perenne* control in wheat emphasize the need for diversified, integrated weed management approach to reduce the reliance on herbicides, prolong the sustainability of herbicides and to delay, if not prevent, the evolution of herbicide-resistant weeds. Diversification entails integrating agronomic approaches (tillage, crop rotation, planting densities, timing of planting, cultivar choices, etc.) to improve the overall results (Hofer *et al.* 2006; Rossig *et al.* 2004).

Table 10: Summary of ACCase- and ALS-inhibiting herbicide resistance patterns among *Lolium perenne* ssp. *multiflorum* populations from the United States

Population[a]	Diclofop	Pinoxaden	Imazamox	Mesosulfuron	Pyroxsulam
08-AR-01	>10[b]	NT[c]	8	5	3
08-AR-02	3	NT	1	1	1
08-AR-03	>10	NT	2	>8	3
08-AR-04	9	NT	7	>8	>10
08-AR-05	5	NT	1	1	1
08-AR-06	>10	NT	>8	>8	>10
08-AR-07	>10	NT	4	>8	9
08-AR-08	>10	NT	2	>8	2
08-AR-09	4	NT	3	>8	7
08-AR-10	>10	NT	6	>8	>10
08-AR-11	5	NT	6	>8	5
08-AR-12	2	NT	5	7	>10
09-GA-01	SR[e]	S[d]	NT	HR[g]	HR
09-MS-01	MR[f]	S	NT	MR	MR
09-MS-03	MR	S	NT	MR	HR
09-MS-05	MR	S	NT	HR	HR
09-MS-06	SR	S	NT	MR	HR
09-MS-07	MR	S	NT	MR	HR
09-MS-08	SR	S	NT	HR	MR
09-NC-01	HR	S	NT	HR	S
09-NC-02	MR	S	NT	S	S
09-NC-03	HR	HR	NT	HR	HR
09-NC-04	3	S	NT	71	6
09-NC-05	18	2	NT	18	3
10-GA-01	MR	MR	NT	SR	SR
10-KS-01	S	S	NT	MR	MR
10-NC-01	MR	MR	NT	MR	MR
10-NC-02	SR	S	NT	MR	MR
10-SC-01	S	S	NT	MR	MR
10-VA-01	S	S	NT	S	S
11-AR-01	MR	S	NT	HR	MR
11-AR-02	MR	S	NT	HR	MR
11-AR-03	SR	S	NT	MR	MR
11-AR-04	MR	SR	NT	HR	MR
11-AR-05	MR	S	NT	MR	MR
11-AR-06	HR	S	NT	MR	MR
11-MS-01	S	S	NT	HR	HR
11-NC-01	S	S	NT	SR	SR
11-NC-02	S	S	NT	S	S
11-NC-03	S	S	NT	S	S
11-NC-04	MR	R	NT	MR	MR
11-NC-05	MR	S	NT	HR	MR
11-NC-06	MR	S	NT	MR	MR
11-NC-07	SR	S	NT	MR	MR
11-NC-08	MR	S	NT	HR	MR
11-WA-A	>10	SR	NT	1	1
11-WA-D	>10	SR	NT	1	1

Note: [a]AR = Arkansas; GA = Georgia; KS = Kansas; MS = Mississippi; NC = North Carolina; VA = Virginia; WA = Washington. [b]Numbers are the R/S ratios based on GR_{50} values estimated from dose response assays. [c]NT = not tested. [d]S = susceptible; 80 – 100% control at recommended dose. [e]SR = slightly resistant; 61 – 80% control at recommended dose. [f]MR = moderately resistant; 21 – 60% control at recommended dose. [g]HR = highly resistant; 0 – 20% control at recommended dose.

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