HERBICIDE TOLERANCE IN IMIDAZOLINONE-RESISTANT WHEAT FOR WEED MANAGEMENT IN THE PACIFIC NORTHWEST U.S.A.

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Abstract: Winter wheat ranks high in importance as an agricultural crop in the Pacific Northwest states of Washington, Idaho, and Oregon. Winter annual grass weeds such as jointed goatgrass (*Aegilops cylindrica*), downy brome (*Bromus tectorum*), feral rye (*Secale cereale*), wild oat (*Avena fatua*) and Italian ryegrass (*Lolium multiflorum*) have the same life-cycle as winter wheat and are difficult to control in conventional wheat production systems. These weeds annually account for millions of dollars of lost wheat production and reduced quality (i.e. discount by impurities). There has been only moderate success in controlling winter annual grasses in wheat by utilizing multiple-year crop rotations with spring crops and fallow periods, and with chemical control. Selective herbicides have been available for chemical control of downy brome, Italian ryegrass, and wild oat. However, before use of imazamox herbicide with imidazolinone-resistant (CLEARFIELD*) wheat, there was no herbicide that could selectively control jointed goatgrass, feral rye, or volunteer cereals in winter wheat. The first commercial release of an imidazolinone-resistant winter wheat variety in the Pacific Northwest was made during the 2003 growing season. Plant breeders in the Pacific Northwest are continuing to develop imidazolinone-resistant winter wheat varieties adapted to a range of Pacific Northwest production regions

One issue of concern for wheat varietal development is that single-gene tolerance to imazamox in CLEARFIELD* varieties can sometimes show visible crop injury, and possibly, yield reductions in response to herbicide applications. Crop tolerance can vary with time of herbicide application relative to wheat stage of growth, environmental conditions that reduce the wheat plant's ability to metabolize imazamox and, possibly, with specific wheat varieties. A multiple year and location study was conducted to evaluate imazamox tolerance in CLEARFIELD* wheat lines being developed for Pacific Northwest production regions. Results indicate that during specific years and locations, single-gene CLEARFIELD* varieties differed slightly in their relative tolerance to imazamox. The most important determinant of crop tolerance was related to herbicide application rate and timing

Keywords: clearfield, herbicide tolerance

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INTRODUCTION

In the Pacific Northwest (PNW), winter annual grass weeds such as jointed goatgrass (*Aegilops cylindrica*), downy brome (*Bromus tectorum*), feral rye (*Secale cereale*), Italian ryegrass (*Lolium multiflorum*), and wild oat (*Avena fatua*) account for millions of dollars of lost wheat production and reduced quality through higher impurities. These weeds have the same, or very similar, life-cycles as winter wheat and are difficult to control in conventional wheat-fallow rotations. The development of winter wheat, marketed under the trade name CLEARFIELD*, provides growers with a new herbicide system for selective, in-crop control of previously uncontrolled jointed goatgrass (Ball et al. 1999), feral rye, volunteer cereals, and other winter annual grass weeds in winter wheat (Clemmer et al. 2004, Geier et al. 2004).

Imazamox (trade name Beyond® in the USA) is the herbicide used for weed control in the CLEARFIELD* wheat system. Imazamox is a broad-spectrum herbicide (grass and broadleaf weeds) that provides post-emergence and some in-season residual weed control. Imazamox, and similar, imidazolinone herbicides, inhibit the activity of the enzyme, acetolactate synthase (ALS), also known as acetohydroxyacid synthase (AHAS), which is necessary for the biosynthesis of the branched chain amino acids valine, leucine, and isoleucine (Anderson 1996). In response to an imazamox herbicide application, ALS is inhibited in susceptible plants depriving them of the previously mentioned, essential amino acids (Frihauf et al. 2005). This causes the eventual death of treated plants. The ALS enzyme is unique to bacterial and plant species and is not found in the animal kingdom. The gene providing herbicide resistance was derived through a sodium azide (pH 3) induced mutation of the French cultivar 'Fidel' (Newhouse et al. 1992). The mutation event resulted in an altered form of the ALS enzyme that is not inhibited by the herbicide at normal herbicide application rates. CLEARFIELD* varieties are non-genetically modified organisms (GMO), as no foreign DNA was introduced or inserted during the development process.

The first publicly-developed CLEARFIELD* winter wheat varieties released in the United States, were 'Above' (from Colorado State University) and 'AP502 CL' (marketed by AgriPro Wheat) in 2001. These two hard red varieties are best adapted to areas of the central Great Plains. In 2003, seed of the first CLEARFIELD* variety developed for the PNW was marketed to growers by General Mills. Since that time, two CLEARFIELD* varieties were developed and released by Oregon State University, and one by the University of Idaho. These have been rapidly adopted by growers in the PNW and acreage of publicly-developed CLEARFIELD* varieties are estimated to be approximately 400,000 acres for 2006. All winter wheat CLEARFIELD* varieties released in the U.S. to date are based on single-gene resistance to the herbicide.

An unknown in the development of CLEARFIELD* varieties was whether the altered ALS gene would respond similarly to herbicide applications in different genetic backgrounds. A further concern was possible interactions with environmental conditions, such as factors that impact herbicide applications, plant metabolism, and post-application plant stress. A three-year study was conducted to evaluate performance of CLEARFIELD* varieties under varying herbicide rates and application dates at two locations in Oregon.

MATERIALS AND METHODS

Field study sites were established near the Columbia Basin Agricultural Research Center in Pendleton, Oregon U.S.A (lat. 45° 43′ N, long. 118° 33′ W) and at a Research Center site near Moro, Oregon (lat. 45° 29′ N, long. 120° 43′W). Plots were established on land that had been fallowed the previous year. The 20-year average annual precipitation at the sites is 442 mm and 271 mm for Pendleton and Moro, respectively. Plots were established at typical commercial seeding rates and planting dates in autumn of 2002, 2003, and 2004. At both sites, evaluations of imazamox tolerance were made on soft white winter CLEARFIELD* varieties ORCF-101 and ORCF-102 from Oregon State University; Idaho 587 from the University of Idaho; ClearFirst™ from General Mills; and the soft red winter wheat line Cv. 9804 (aka FS-4), a derivative of the French variety Fidel. The line Cv. 9804 was the tolerance trait donor line for ORCF-101, ORCF-102, and Idaho 587 lines. The tolerance trait in the line ClearFirst was an independent mutation event. The tolerance trait resides on the *als1* locus (D-genome homeologue) in ORCF-101, ORCF-102, Idaho 587, and Cv. 9804. In the line ClearFirst, the trait resides on the *als2* locus (B-genome homeologue). Both traits have demonstrated comparable tolerance in field trials in the PNW (Dahmer, pers. comm.)

The herbicide treatment plan included an early treatment timing when wheat was in the four-leaf stage, and a second treatment timing when the wheat was in the 5–7 leaf stage with 1–2 tillers. Treatment dates varied with year and site depending on stage of wheat growth. The recommended commercial use rate of imazamox for CLEARFIELD* wheat ranges from 35 to 52.5 g active ingredient per ha (g a.i. ha^{-1}). The rates applied in these trials ranged from 35 to 105 g a.i. ha^{-1}, which included the recommended use rates and twice the recommended use rates. Treatments included a non-ionic surfactant at 0.25% v/v and a 32% UAN nitrogen solution at 2.5% v/v, applied either early (3–4 leaf wheat) or late (5–7 leaf wheat) post-emergence. Treatments at both sites were applied with a hand-held CO_2 pressured sprayer delivering 180 l ha^{-1} at 207 kPa. Individual plots were 1.5 by 4.5 m in a factorial design in 2003, and a split-plot design in 2004 and 2005 in which herbicide was the main factor and cultivar was the sub-factor, with 4 replications in blocks, each year. Visible crop injury (0 to 100%) was evaluated twice after the late herbicide application treatment. Evaluation times are denoted as being 30 and 60 days after late application treatment (DAT), but are approximate evaluation times after treatment, only. Wheat grain yield was taken each year by harvesting the entire 1.5 m by 4.5 m plot area with a plot combine. Yields were only obtained at Moro in 2005 due to production problems unrelated to the experiments. Kernel weights were measures as an indicator of grain quality in the 2003 through 2005 Pendleton trials.

Data from the two locations were analyzed separately over years using SAS GLM (SAS Institute, Inc. 2004). All analyses used the $p < 0.05$ level for tests of significance. The 2003 trial at Moro was abandoned due to poor stands and yield data was not collected at Moro in 2004. Kernel weight data was collected for Pendleton trials in 2004 and 2005. Mean squares from analysis of variance for crop injury, grain yield, and kernel weight are presented in Table 1.

RESULTS AND DISCUSSION

Significant herbicide injury was observed in the CLEARFIELD* varieties at the 105 g a.i. ha^{-1} rate at both Pendleton and Moro (Tables 2 and 3). This rate represents approximately 2 to 3 times the recommended label rate. Visual injury was minimal at the other herbicide rates, regardless of time of application. The crop injury that occurred due to herbicide application produced a transient stunting, apparent delay in crop growth, and slight chlorosis. These symptoms mostly disappeared over time. Evidence of this diminishing injury is apparent upon comparison of the 30 and 60 DAT injury evaluations (Tables 2 and 3). This crop injury did have some significantly effect on grain yield, only at the highest herbicide application rate, particularly at Pendleton (Table 3). However, crop injury did not appear to affect kernel weights averaged over a 2-year period at the Pendleton site (Table 3). Significant differences due to herbicide treatment could not be related to weed control differences since weed populations were relatively negligible in all years.

Significant interactions between variety and treatment existed for visual injury ratings at 30 DAT. Herbicide injury was most evident on the ClearFirst variety at Moro, and injury ratings increased as the application rate increased from 52.5 to 105 g a.i. ha^{-1}, particularly for the earlier application dates. Injury also was evident on the other varieties, but to a lesser degree than for ClearFirst (Tables 2 and 3). Except for the ClearFirst variety, the remaining CLEARFIELD* varieties, including the parent Cv. 9804 line, exhibited a similar response to increasing herbicide application rates each year. There was some evidence of variation in crop injury among varieties in individual years and location, but, with the exception of ClearFirst, there was no consistent pattern of response.

Results of analysis of grain yield indicated that, over test years and 2 locations, all varieties responded similarly to herbicide applications. There was some reduction in grain yield at the 105 g herbicide application rate at the Pendleton site (Table 3). Analysis of grain yield indicated a significant year by herbicide treatment interaction at Pendleton. However, no variety x herbicide treatment interaction occurred with respect to yield. In general, all varieties yielded higher at Pendleton than at Moro, due to greater precipitation at Pendleton. At Moro, ORCF-101 was the highest yielding cultivar over all years. At Pendleton ORCF-102 was the highest yielding cultivar. At both locations, Idaho 587 was the second highest yielding cultivar and had the greatest average yield across locations. In the untreated plots, grain yields of ORCF-101, ORCF-102, and Idaho 587 were comparable to that of the

Table 1. Mean squares from analysis of variance for crop injury at 30 and 60 days after application of treatments (DAT), grain yield, and kernel weight after imazamox treatments at Moro and Pendleton, Oregon, 2003–2005

	Moro Df	30 DAT	60 DAT	Yield[2]	Pendleton[1] Df	30 DAT	60 DAT	Yield	Kernel Wt
Year	1	139.4**	6.7		2	8148**	1779.7**	27521616**	514.9**
Rep	3	98.0**	22.0*	5775842**	3	110**	8.7	1625167*	1.3
Year x Rep	3	12.9	5.6		6	51	10.7	2537881	0.7
Cultivar	4	307.3**	9.6	42350448**	4	60**	7.8	15505990**	421.0*
Treatment	8	314.7**	145.3**	500685**	6	4921**	619.4**	4152744**	4.2
Cultivar x Treatment	32	38.9**	5.8	103857	24	34**	2.7	452510	1.2
Cultivar x Year	4	4.1	1.5		8	53**	3.6	2688959**	25.8**
Treatment x Year	8	32.1	9.1		12	2403**	447.2**	1730976**	19.9**
Cultivar x Treatment x Year	32	36.6**	2.6		48	29**	3.6	412877	2.4
Pooled error	264	19.8		185359	293	17	4	561390	3.9

*,** Significant at $P < 0.05$ and $P < 0.01$, respectively. Note: df for pooled error is 132 for grain yield at Moro.

[1] Pendleton seed yields are average of 3 years, and kernel weights are the average for 2 crop years.

[2] Moro yield is 2005 only.

Table 2. Mean crop injury and grain yield of imidazolinone resistant (IMI)-wheat varieties after imazamox application rates and timings. Moro, Oregon, 2004–2005

Treatment[1]	Cv. 9804			Idaho 587			ORCF-101			ORCF-102			ClearFirst			Treatment means		
	Injury		Yield[2]	Injury		Yield	Injury		Yield	Injury		Yield	Injury		Yield	Injury		Yield
	30 DAT	60 DAT		30 DAT	60 DAT		30 DAT	60 DAT		30 DAT	60 DAT		30 DAT	60 DAT		30 DAT	60 DAT	
	- - -%- - -		Kg ha^{-1}	- - -%- - -		Kg ha^{-1}	- - -%- - -		Kg ha^{-1}	- - -%- - -		Kg ha^{-1}	- - -%- - -		Kg ha^{-1}	- - -%- - -		Kg ha^{-1}
Untreated	0	0	2942	0	0	5464	0	0	5481	0	0	5027	0	0	4271	0	0	4637
35 Early	0	0	3009	0	1	5565	0	1	5599	0	0	5229	1	1	4304	0	0	4741
52.5 Early	0	0	2572	1	1	5296	1	1	5413	1	0	4876	8	1	4035	2	0	4439
105 Early	4	4	3127	8	6	5296	5	5	5649	6	6	4842	21	10	3665	9	6	4516
35 Late	0	0	2943	0	0	5716	0	0	5750	0	1	5094	1	0	4607	0	0	4822
52.5 Late	0	0	3111	3	0	5498	1	0	5918	0	0	5414	3	0	4287	1	0	4845
105 Late	2	2	3262	2	1	5229	2	1	5548	1	1	4943	6	1	4103	3	1	4617

[1] Yield is 2005 only.

Table 3. Mean crop injury, grain yield, and kernel weight of imidazolinone resistant (IMI)-wheat varieties after imazamox application rates and timings. Pendleton, Oregon, 2003–2005

Treatment[1]	Cv. 9804				Idaho 587				ORCF-101				ORCF-102				ClearFirst				Treatment Means			
	Injury		Kernel Wt.	Yield	Injury		Kernel Wt.	Yield	Injury		Kernel Wt.	Yield	Injury		Kernel Wt.	Yield	Injury		Kernel Wt.	Yield	Injury		Kernel Wt.	Yield
	30 DAT	60 DAT			30 DAT	60 DAT			30 DAT	60 DAT			30 DAT	60 DAT			30 DAT	60 DAT			30 DAT	60 DAT		
	---%---		-mg-	Kg ha^{-1}	---%---		-mg-	Kg ha^{-1}	---%---		-mg-	Kg ha^{-1}	---%---		-mg-	Kg ha^{-1}	---%---		-mg-	Kg ha^{-1}	---%---		-mg-	Kg ha^{-1}
Untreated	0	0	43	7583	0	0	44	7593	0	0	40	7157	0	0	42	8165	0	0	35	6691	0	0	41	7432
35 Early	1	1	44	7454	1	0	44	7398	1	0	41	7559	2	0	40	8088	2	1	33	6574	2	0	41	7398
52.5 Early	2	0	45	7685	3	2	45	7487	3	2	40	7414	5	1	42	7577	5	1	35	6823	4	1	41	7402
105 Early	21	7	45	7269	18	7	45	6839	23	8	40	6611	25	9	42	7392	27	7	36	6209	23	7	41	6874
35 Late	2	0	45	8048	1	0	45	7655	1	1	41	7515	1	0	41	8052	1	0	36	7112	1	0	41	7670
52.5 Late	4	1	44	7487	6	1	45	7672	3	1	40	7717	6	1	42	8089	5	1	36	6966	5	1	41	7578
105 Late	20	8	45	7241	19	7	45	7201	17	6	41	6670	21	8	42	8031	15	6	35	6627	19	7	42	7171

[1] Seed yields are average of 3 years, and kernel weights are the average for 2 crop years.

non-CLEARFIELD* check variety Stephens, which suggests that the *als1* gene does not have any negative effect on yield potential (data not shown).

Observations on crop injury, grain yield, and grain quality support a conclusion that herbicide tolerance from the *als1* locus tolerance trait (D-genome homeologue) among PNW breeding lines will likely be similar, so extensive, multi-year screening for herbicide tolerance during the varietal development process may not be necessary. The ClearFirst variety, with the herbicide tolerance trait residing on the *als2* locus (B-genome homeologue), is an exception. For this line, there was more herbicide injury at the high herbicide application rate, and seed yield and kernel weights were lower than with other, presumably better adapted lines, even when no herbicide was applied.

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