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ACCase-Inhibitor Herbicide Resistance in Downy Brome (*Bromus tectorum*) in Oregon

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In spring 2005, a downy brome population with possible resistance to fluazifop-P, an acetyl-CoA carboxylase (ACCase) inhibitor (group 1) herbicide was found in a commercial creeping red fescue seed production field, near La Grande, OR, where fluazifop-P had been used to control downy brome repeatedly over 7 yr. Greenhouse experiments were conducted to confirm resistance to a number of group 1 herbicides. The suspected resistant downy brome accession was tested for resistance to fluazifop-P and tested for cross-resistance to other aryloxyphenoxy propionate (APP) and cyclohexanedione (CHD) herbicides, including quizalofop-P, sethoxydim, and clethodim. Data recorded included plant-mortality counts and biomass. Tests revealed that the La Grande downy brome accession was highly resistant to fluazifop-P and sethoxydim at all tested rates. The La Grande accession suffered no mortality from fluazifop-P or sethoxydim treatments up to the maximum tested rate of eight times (8×) the labeled recommendation. The La Grande accession was resistant to quizalofop-P and clethodim at the labeled rate or less but was susceptible to application rates higher than the labeled rate. The control downy brome accession was completely susceptible to fluazifop-P, quizalofop-P, and clethodim at all rates and exhibited increasing susceptibility with increasing sethoxydim rate. This pattern of cross-resistance differs from that of a previously reported case of ACCase resistance in downy brome.

Nomenclature: Clethodim; fluazifop-P; quizalofop-P; sethoxydim; downy brome, *Bromus tectorum* L. BROTE; creeping red fescue, *Festuca rubra* L.

Key words: Acetyl-coenzyme A carboxylase, herbicide resistance, lipid biosynthesis inhibitor, graminicide.

In spring 2005, a downy brome population with possible resistance to acetyl-coenzyme A carboxylase (ACCase) inhibitor (group 1) herbicides was found in a commercial creeping red fescue seed production field, in Union County, OR. Group 1 herbicides encompass two chemical families, aryloxyphenoxy propionates (APP) and cyclohexanediones (CHD). Herbicides in both chemical families inhibit chloroplastic ACCase action in Gramineae and prevent fatty acid synthesis, thus reducing production of phospholipids used in membranes (Delye et al. 2002). Necrosis of plant tissue and death follow herbicide application. The APP herbicides, fluazifop-P or quizalofop-P, and CHD herbicides, sethoxydim or clethodim, control annual and perennial grass weeds in broadleaf crops, fallow, and noncrop areas (WSSA 2002). Resistance to APP and CHD herbicides has been attributed to an altered target site resulting in high-level resistance or to low-level metabolism-based resistance (Volenberg and Stoltenberg 2002). Recent research demonstrated a nuclear source for sethoxydim resistance. Resistance to sethoxydim has been bred into foxtail millet [*Setaria italica* (L.) Beauv.] by transferring a nuclear dominant gene (Wang and Darmency 1997). One point mutation (isoleucine to leucine) in the chloroplastic ACCase allele of green foxtail [*Setaria viridis* (L.) P.Beauv.] (Delye et al. 2002), of Italian ryegrass (*Lolium multiflorum* Lam.) (White et al. 2005), and of slender foxtail (*Alopecurus myosuroides* Huds.) (Brown et al. 2002), conferred resistance to sethoxydim. As of February 2006, 35 gramineous weed species have been reported to display resistance to group 1 herbicides (Heap 2006).

Prolonged, repeated use of group 1 herbicides has led to documented APP- and CHD-resistant grass weeds. Resistance to APP herbicides has been reported in Italian ryegrass (Stanger and Appleby 1989), rigid ryegrass (*Lolium rigidum* Gaud.) (Llewellyn et al. 2001), wild oat (*Avena fatua* L.) (Heap et al. 1993), and johnsongrass [*Sorghum halepense* (L.) Pers.] (Barrentine et al. 1992). Three reports of APP-resistant annual bromes include: quizalofop-P–resistant rigid brome (*Bromus rigidus* Roth) reported in Western Australia in 2005 and two instances of haloxyfop-R-methyl resistant ripgut brome (*Bromus diandrus* Roth) reported in Victoria, Australia, in 1999 and 2002 (Heap 2006). Resistance to multiple APP herbicides has been reported in wild oat (Seefeldt et al. 1994).

Cross-resistance between APP and CHD herbicides has been reported in giant foxtail (*Setaria faberi* Herm.) (Volenberg and Stoltenberg 2002), johnsongrass (Bradley et al. 2001; Smeda et al. 1997), Chinese sprangletop [*Leptochloa chinensis* (L.) Nees. (Maneechote et al. 2005), green foxtail [*Setaria viridis* (L.) Beauv.] (de Prado et al. 2004), and large crabgrass [*Digitaria sanguinalis* (L.) Scop.] (Wiederholt and Stoltenberg 1995). A downy brome population with reported resistance to acetolactate synthase (ALS) inhibitors from repeated ALS inhibitor applications to research plots also exhibited multiple resistances to several herbicide classes including APP and CHD herbicides (Park and Mallory-Smith 2005). This previously reported downy brome population exhibited multiple herbicide resistance due, partly, to an enhanced metabolism mechanism. This previously reported downy brome population is different from the downy brome population evaluated in the present study. The population evaluated in this study was directly selected by repeated applications of ACCase-inhibitor herbicides under commercial field-application conditions.

Resistant grass-weed populations develop through repeated applications of APP and CHD herbicides over variable numbers of years. Resistance to APP and CHD herbicides in giant foxtail developed after 6 out of 9 yr of APP and CHD herbicide use (Stoltenberg and Wiederholt 1995), or in wild oat, after 8 yr of APP and CHD herbicide use (Seefeldt et al. 1994). Examples of rapid development of resistant populations include resistant annual ryegrasses after four exposures to diclofop (Heap and Knight 1990) or three exposures to sethoxydim (Tardif et al. 1993).

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Table 1. Downy brome mortality response to fluazifop-P, quizalofop-P, sethoxydim and clethodim.[a]

Treatment[b]	Fluazifop-P		Quizalofop-P		Sethoxydim		Clethodim	
	R	S	R	S	R	S	R	S
	% mortality							
Untreated	0	0	0	0	0	0	0	0
0.5×	0	100	0	100	0	5	0	100
1×	0	100	0	100	0	86	0	100
2×	0	100	13	100	0	86	53	100
4×	0	100	100	100	0	98	98	100
8×	0	100	98	100	0	100	100	100
LSD (0.05)	NS	NS	23	NS	NS	23	32	NS

[a] Abbreviations: R, La Grande downy brome accession; S, Columbia Basin Agricultural Research Center, Pendleton, OR, (CBARC98) downy brome accession; NS, nonsignificant.
[b] Treatment is the amount times the label rate.

The objectives of these experiments were (1) to confirm group 1 herbicide resistance in a 2005 downy brome population, collected from a commercial field site in eastern Oregon that had been subjected to repeated field use rates of fluazifop-P, and (2) to quantify cross-resistance of this downy brome population to quizalofop-P, sethoxydim, and clethodim applied at field-application rates.

Materials and Methods

Suspected resistant downy brome plants were collected from a commercial seed production field of creeping red fescue var. 'Fenway' in Union County, OR, on June 10, 2005. The 5.3-ha field had been planted to creeping red fescue in spring 1998 and received one or more annual applications of fluazifop-P and other group 1 herbicides for approximately 7 yr. After a number of years, downy brome plants in the field survived repeated fluazifop-P herbicide treatments in 2.4 to 2.8 ha of the field. Collected downy brome plants were placed in a greenhouse to mature. Greenhouse-grown seed (designated 'La Grande') was collected July 1, 2005, dry-stored in paper bags for 92 d, and used to test for resistance to fluazifop-P,[1] quizalofop-P,[2] sethoxydim,[3] and clethodim.[4]

Greenhouse experiments were conducted as a factorial in a randomized complete-block design with two brome accessions, susceptible and suspected resistant; six herbicide treatment rates, including an untreated control; with five replications; and repeated two times for a total of three runs. Control downy brome seed was collected in 1998 at the Columbia Basin Agricultural Research Center, Pendleton, OR (designated 'CBARC98') from a population not previously exposed to group 1 herbicides.

Seeds were planted in commercial potting mix[5] in 1-L pots and thinned to one plant per pot before being treated. Greenhouse day/night temperatures for all experiments were 21/17 C, and all plants were watered daily through a drip system. Plants were fertilized at 35 and 42 d after treatment (DAT) with 80 ml of fertilizer[6] at a concentration of 4 g in 1 L water. Plants received supplemental lighting for 12 to 14 h d^{-1}. Herbicides were applied to La Grande and CBARC98 downy brome accessions at dosages proportional to 0.5, 1.0, 2.0, 4.0, or 8.0 times the recommended dosages on labels. Fluazifop-P treatments were 0, 0.07, 0.14, 0.28, 0.56, or 1.12 kg ai ha^{-1}. Quizalofop-P treatments were 0, 0.09, 0.19, 0.37, 0.74, or 1.48 kg ai ha^{-1}. Sethoxydim treatments were 0, 0.28, 0.56, 1.13, 2.25, or 4.5 kg ai ha^{-1}. Clethodim treatments were 0, 0.07, 0.14, 0.28, 0.56, or 1.12 kg ai ha^{-1}. Fluazifop-P treatments included a nonionic surfactant at 0.25% v/v, as recommended on the product label. Quizalofop-P, sethoxydim, and clethodim treatments included a crop oil concentrate at 1% v/v, as recommended on the product label. All herbicide treatments were sprayed once when downy brome was in the two to three-leaf stage with an overhead moving, flat-fan nozzle sprayer calibrated to deliver 157 L ha^{-1} at 207 kPa. A mortality count was made, and aboveground biomass was harvested at approximately 47 DAT, dried for 48 h at 60 C, and weighed.

Statistical analysis of data included ANOVA of biomass data expressed as a percentage of the control from the three runs for fluazifop-P, quizalofop-P, sethoxydim, and clethodim herbicides using PROC MIXED to calculate standard errors (SAS Institute 2005). Four-parameter logistic analysis on boxcox-transformed, biomass data were used to calculate GR_{50} values for the control downy brome treated with sethoxydim and for the La Grande accession treated with quizalofop-P and clethodim (R Development Core Team 2006). The four-parameter logic function is given by the formula in Equation 1.

$$f[x, (b, c, d, e)] = c + \frac{d - c}{1 + \exp\{b[\log(x) - \log(e)]\}} \quad [1]$$

With four parameters b, c, d, and e. The parameter e is also denoted GR_{50}, and it is the dose producing a 50% biomass reduction, which is half-way between a maximum biomass, d, and a minimum biomass, c. The parameter b denotes the relative slope around e (R Development Core Team 2006). ANOVA on arcsine-transformed, percentage-mortality data separated means with the LSD test (SAS Institute 2005).

Results and Discussion

The susceptible, control downy brome accession (CBARC98) was completely susceptible to fluazifop-P, quizalofop-P, and clethodim, resulting in complete mortality at all applied herbicide rates (Table 1). In addition, the CBARC98 population exhibited increasing mortality and decreasing biomass with increasing sethoxydim dose (Tables 1 and 2). The La Grande downy brome suffered no mortality at any tested rate of fluazifop-P or sethoxydim (Table 1) and exhibited very little to no biomass reduction with increasing herbicide rates (Table 2). At the labeled rate (1×) or less of quizalofop-P or clethodim, La Grande downy brome, exhibited no mortality, but mortality increased at rates higher than the labeled rate (Table 1). Similarly, La Grande downy

Table 2. Biomass response of resistant (R) and susceptible (S) downy brome to group 1 herbicides applied at five rates as a percentage of untreated plant biomass within an accession.[a]

Herbicide	Label rate	Rate	% of Control[b] R	% of Control[b] S
		kg ai ha^{-1}	%	
Fluazifop-P	0.5×	0.07	107.8	0.3
SE ± 9.6	1×	0.14	102.3	0.6
	2×	0.28	99.2	0.6
	4×	0.56	101.7	0.4
	8×	1.12	88.4	0.5
Quizalofop-P	0.5×	0.09	99.9	2.3
SE ± 4.4	1×	0.19	90.1	2.1
	2×	0.37	26.4	2.0
	4×	0.74	2.4	2.1
	8×	1.48	2.2	2.8
Sethoxydim	0.5×	0.28	94.6	43.7
SE ± 8.6	1×	0.56	96.2	15.5
	2×	1.13	93.5	5.2
	4×	2.25	107.6	3.5
	8×	4.5	104.5	3.6
Clethodim	0.5×	0.07	101.0	2.4
SE ± 2.9	1×	0.14	79.8	2.2
	2×	0.28	6.9	2.4
	4×	0.56	2.4	2.3
	8×	1.12	2.2	2.5

[a] Abbreviations: R, La Grande downy brome accession; S, Columbia Basin Agricultural Research Center, Pendleton, OR, (CBARC98) downy brome accession.

[b] Values are percentages of the nontreated control ± SE as estimated by PROC MIXED.

brome biomass decreased in response to increasing quizalofop-P or clethodim rate (Table 2).

Calculated GR_{50} values and associated resistance factors were used to compare the CBARC98 control and La Grande downy brome populations for all of the tested herbicides (Table 3). Because of complete mortality of CBARC98 to three of the selected herbicides used in this study, only approximate GR_{50} values could be established (Table 3). Where GR_{50} values could be calculated, a four-parameter logistic analysis on boxcox-transformed biomass data calculated GR_{50} values for the control downy brome treated with sethoxydim and the La Grande accession treated with quizalofop-P or clethodim (R Development Core Team 2006) (Table 3).

These results indicate that a high level of resistance exists in the La Grande population to fluazifop-P and sethoxydim with resistant : susceptible (R/S) resistance ratios of > 16 and > 23, respectively. A somewhat intermediate resistance exists to quizalofop-P and clethodim in this downy brome population, with R/S resistance ratios of > 3 and > 2, respectively. The resistance ratios calculated for the La Grande downy brome indicated a much higher level of resistance to fluazifop (> 16 vs. 1.9) in comparison to the downy brome resistance reported earlier (Park and Mallory-Smith 2005). This much higher level of fluazifop resistance in the La Grande downy brome suggests that a different resistance mechanism is likely present than the enhanced metabolism from the earlier report.

Results of this study are of interest because they illustrate varying downy brome resistance patterns to group 1 herbicides. It is possible that the La Grande downy brome exhibit an altered site of action in the ACCase enzyme, compared with the metabolism-based resistance in downy brome reported earlier. Any one of four mutations of the ACCase gene may confer resistance to different APP and CHD herbicides (Maneechote et al. 2005). However, further testing is needed to characterize the mechanism of resistance in the La Grande downy brome population. Whole-plant assays at field rates, such as this one, cannot determine the physiological cause of resistance in the La Grande downy brome population but can call attention to a weed-control problem resulting from repeated use of a single herbicide group. La Grande downy brome population developed resistance to selected group 1 herbicides under commercial field-application pressure.

These results support research results in Australia on distribution of resistance attributes across geographical areas. Studies of wild oat (Heap et al. 1993) and large crabgrass (Volenberg and Stoltenberg 2002) resistance to APP and CHD herbicides have shown that biotypes from different locations can evolve resistance independently and have different patterns of cross-resistance. In random surveys of fields in Western Australia, resistant populations of rigid ryegrass had differing resistance to APP and CHD herbicides (Llewellyn and Powles 2001). Field history and geography circumscribe any recommendations that may be made. Herbicide use recommendations developed for Union County, OR, for APP and CHD herbicide treatment of downy brome may not apply to downy brome populations in other geographic areas.

Table 3. Estimate of GR_{50} and a resistance ratio of resistant and susceptible downy brome biotypes.[a]

Herbicide	GR_{50}[b] R	GR_{50}[b] S	Ratio
	kg ai ha^{-1}		
Fluazifop-P-butyl	> 1.12	< 0.07	> 16
Sethoxydim	> 4.5	0.20 (0.03)	> 23
Quizalofop-P	0.23 (0.03)	< 0.09	> 3
Clethodim	0.17 (0.01)	< 0.07	> 2

[a] Abbreviations: R, La Grande resistant accession; S, Columbia Basin Agricultural Research Center, Pendleton, OR, (CBARC98) downy brome susceptible accession.

[b] Asymptotic standard error for estimated GR_{50} values are in parentheses.

Sources of Materials

[1] Fusilade DX, Syngenta Crop Protection, Inc., P.O. Box 18300, Greensboro, NC 27409.

[2] Assure II, E. I. DuPont de Nemours & Co., Inc., Agricultural Products, 1007 Market Street, Wilmington, DE 19898.

[3] Poast Plus, BASF Corporation, 100 Campus Drive, Florham Park, NJ 07932.

[4] Select, Valent U.S.A. Corporation, P.O. Box 8025, Walnut Creek, CA 94596.

[5] Sunshine Mix # 3 potting mix, Sun Gro Horticulture, Inc., 110 110th Avenue NE, Suite 490, Bellevue, WA 98004.

[6] Miracle-Gro All Purpose Plant Food, Scotts Miracle-Gro, 14111 Scottslawn Road, Marysville, OH 43041.

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