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RESEARCH PAPER

Confirmed resistance to aryloxyphenoxypropionate herbicides in *Phalaris minor* populations in Iran

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Phalaris minor (littleseed canary grass) is a major weed in wheat fields in some parts of Iran. Diclofop-methyl, fenoxaprop-P-ethyl, and clodinafop-propargyl are three acetyl coenzyme A carboxylase (ACCase)-inhibiting herbicides that are commonly used to control this grass in wheat fields. Thirty-four *P. minor* populations with suspected resistance to ACCase-inhibiting herbicides were sampled from wheat fields in the provinces of Fars and Golestan in Iran. The dose–response assays that were conducted under controlled greenhouse conditions indicated that 14 populations were resistant to fenoxaprop-P-ethyl, seven populations were resistant to both fenoxaprop-P-ethyl and diclofop-methyl, and three populations were resistant to fenoxaprop-P-ethyl, diclofop-methyl, and clodinafop-propargyl. These populations showed different levels of resistance to the applied herbicides, compared to the susceptible population. These results suggest that different mechanisms of resistance could be involved. The enzyme assay revealed that the existence of modified ACCase in the three most-resistant populations (AR, MR4, and SR3) is responsible for the resistance of these populations.

Keywords: aryloxyphenoxy propionate, herbicide resistance, *Phalaris minor*.

Wheat (*Triticum aestivum* L.) is a major crop in Iran. In 2007, >6.5 million ha of wheat were planted in this country (Anonymous 2004). *Phalaris minor* is a common weed in some wheat-producing areas, especially in Fars, Khuzestan, and Golestan provinces (Minbashi Moeini *et al.* 2008). According to Singh (2007), littleseed canary grass became the major weed of wheat in northern India during the green revolution in the 1970s. This was due to revised agronomic practises, including the adoption of poorly competitive short-statured wheat varieties, leading to a significant yield reduction. As these practises also have been adopted in Iran, *P. minor* has become a major weed in some areas of Iran. This grass, under high fertility and moisture conditions, competes vigorously with wheat, reducing yields by ≤80% (Singh *et al.* 1999).

Diclofop-methyl (DIC), fenoxaprop-P-ethyl (FEN), and clodinafop-propargyl (CLD) are three aryloxyphenoxypropionate (APP) herbicides that are commonly used postemergence to control grass weeds, such as *Avena* spp. and *Phalaris* spp., in wheat and barley. Aryloxyphenoxypropionate herbicides inhibit the chloroplastic acetyl coenzyme A carboxylase (ACCase) action in the Poaceae family, preventing fatty acid synthesis and reducing the production of the phospholipids that are used in the membranes (Délye *et al.* 2002). Chlorosis, necrosis, and finally the death of plant tissue occur after applying these herbicides (Ball *et al.* 2007). Since their introduction in the 1970s and 1980s, ACCase-inhibitors have been used widely to selectively control grass weeds in crops. Their persistent use has resulted in the appearance of resistant grass weed biotypes. During recent decades, there has been an increasing number of reports of graminicide-resistant weeds. In 2010, Heap (2010) reported graminicide resistance in 38 grass weed species worldwide.

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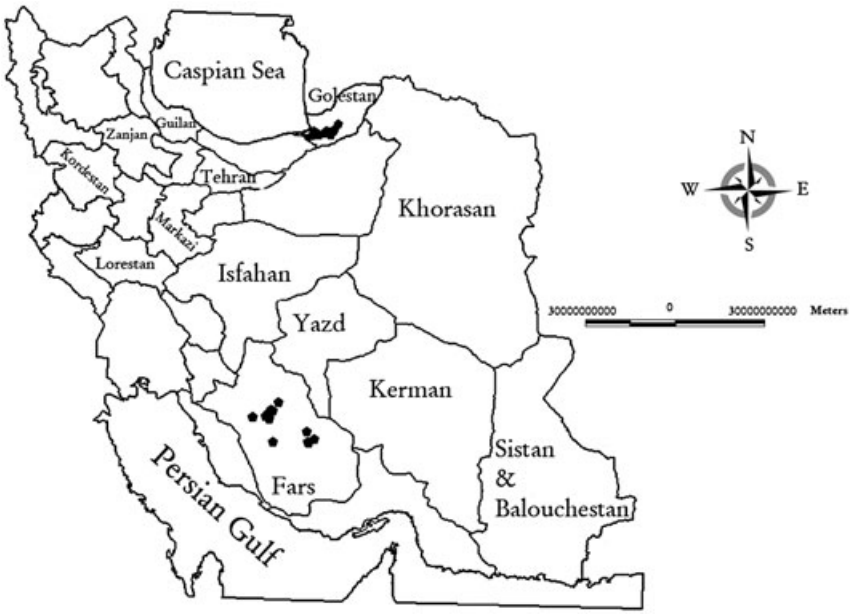

Fig. 1. Map of Iran, showing the approximate locations where *Phalaris minor* populations were sampled.

In recent years, several cases of poor *P. minor* control in wheat fields by APP herbicides have been noted, mainly in two provinces of Iran (Fars in the south and Golestan in the North), where these graminicides have been applied for several years (Fig. 1). The objectives of this current study were to: (i) confirm the resistance of *P. minor* to APP herbicides; (ii) quantify the level of resistance and cross-resistance patterns to DIC, FEN, and CLD; and (iii) determine the biochemical basis of resistance in the resistant populations, based on ACCase *in vitro* assays.

MATERIALS AND METHODS

Plant material

Seed samples of *P. minor* populations, selected on the basis of their suspected resistance to DIC, FEN, or CLD, were collected from locations throughout two provinces in Iran (Fars and Golestan) during 2005 and 2006 (Fig. 1).

As the susceptible standard, a population (ES) was collected from the region that never had been treated with ACCase-inhibiting herbicides.

Prescreening test

The seeds of both the resistant and susceptible populations were placed in Petri dishes on filter paper (Whatman International, Maidstone, England) that was moistened with distilled water and germinated in a regulated growth chamber at 25°C and constant darkness. After 48 h, four germinated seeds per plastic pot (9 cm diameter and 500 mL volume) were manually planted. The pots were filled with two-thirds loamy soil that was mixed with one-third sand. Then, the pots were distributed randomly in the greenhouse, where they were exposed to 16/8 h day/night photoperiods and 22/15°C day/night temperatures. They were watered every 2 days and fertilized twice per month with 2.4 g L^{-1} of N. Each pot was considered as one replication. The experiment was conducted in a completely randomized design, with three replications for each herbicide treatment. At the three-to-four-leaf stage, ~3 weeks after planting, the plants in each pot were sprayed with the recommended field dosage of the commercial formulation of the herbicides, DIC (900 g ai ha^{-1}; Bayer Cropscience, Frankfurt, Germany), FEN (67.5 g ai ha^{-1}; Bayer Cropscience), or CLD (60 g ai ha^{-1}; Syngenta, Madrid, Spain), using a Matabi automatic sprayer with a single 8001 flat-fan nozzle (Goizper, Guipuzkoa, Spain) that was calibrated to deliver 250 L ha^{-1} at 200 kPa.

Three weeks after spraying, the surviving plants were counted and were cut at the soil surface and the shoots were oven-dried for 48 h at 70°C. The dry-weight data then were expressed as a percentage of the respective unsprayed control.

In the prescreening test, the populations with a weight and surviving percentage of ≥80% and ≥50%, respectively, when compared to the control, were considered to be resistant to the applied herbicide. Due to a lack of

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space in the greenhouse, dose–response studies were conducted just for these populations.

Dose–response assay in the greenhouse

The seeds of the selected populations were germinated, manually planted in pots, and grown in the greenhouse, as explained above. At the three-to-four-leaf stage, ~3 weeks after planting, the plants were sprayed with the commercial formulation of DIC, FEN, or CLD at 0-, 0.25-, 0.5-, 1-, 2-, 4-, 8-, 16-, and 32-fold the recommended field dose of each herbicide by using an automatic sprayer with a single flat-fan nozzle that was calibrated to deliver 250 L ha^{-1} at 200 kPa. The recommended field dose for DIC, FEN, and CLD was 900, 67.5, and 60 g ai ha^{-1}, respectively. The experiment was repeated twice, once in Iran and once in Spain. The growing environment, spray application, and soil conditions were the same at both locations.

Three weeks after spraying, the surviving plants were counted and their aerial parts were harvested and oven-dried for 48 h at 70°C. The dry-weight data then were expressed as a percentage of the respective unsprayed control. A herbicide dose resulting in a 50% inhibition of weed growth (GR_{50}) was estimated for each herbicide by using the obtained dose–response curves.

Acetyl coenzyme A carboxylase extraction

Acetyl coenzyme A carboxylase isoforms were isolated by using the protocol of De Prado *et al.* (2000), with slight modifications, at the Agricultural Biochemistry Laboratory, Córdoba University, Cordoba, Spain. At the two-leaf stage, the plants were sprayed with a commercial formulation of the recommended field dose of DIC and FEN separately. The leaves (6 g on a fresh-weight basis) of all the populations were separated from the surviving plants at the three-to-four-leaf stage, ground in liquid N_2 in a mortar, and then added to 24 mL of extraction buffer (0.1 mol L^{-1} Hepes-KOH [pH = 7.5], 0.5 mol L^{-1} glycerol, 2 mmol L^{-1} ethylenediaminetetraacetic acid [EDTA], and 0.32 mmol L^{-1} phenylmethyl sulfonyl fluoride). The homogenate was mixed for 10 min with a magnetic stirrer and filtered sequentially through four layers of cheesecloth. The crude extract was centrifuged (24,000 *g* for 20 min). The supernatant that was obtained was fractionated with ammonium sulfate. The protein fraction, including the ACCase enzyme, was precipitated from the crude extract at 50% saturation of $(NH4)_2SO_4$. The supernatant was discarded, the pellet was resuspended in the extraction buffer (1 mL), and the resuspension was applied to a column (Sephadex G-25 PD-10; GE Healthcare, Chalfont St Giles, UK), which was equilibrated previously with S400 buffer. The desalted protein extract was used immediately for the ACCase enzyme activity assays.

Acetyl coenzyme A carboxylase assay (using diclofop acid and fenoxaprop acid)

The enzyme activity was assayed by measuring the adenosine triphosphate (ATP)-dependent incorporation of $NaH(^{14}C)O_3$ into an acid-stable ^{14}C product. The reaction product previously was shown to be (^{14}C)-malonyl-CoA. The assays were conducted in 7 mL scintillation vials that contained 0.1 mol L^{-1} Tricine-KOH (pH = 8.3), 0.5 mol L^{-1} glycerol, 0.05 mol L^{-1} KCl, 2 mmol L^{-1} EDTA, 0.5 mmol L^{-1} dithiothreitol, 1.5 mmol L^{-1} ATP, 5 mmol L^{-1} $MgCl_2$, 15 mmol L^{-1} $NaH(^{14}C)O_3$ (33 Ci mol^{-1}), 45 μL of enzyme fraction, and 5 mmol L^{-1} acetyl-CoA (lithium salt) in a final volume of 0.2 mL. The assay temperature was 34°C. The reaction was stopped after 5 min by adding 30 μL of 4N HCl. The acidified reaction medium was dried at 40°C under a stream of air. The dried samples were redissolved in 0.5 mL of 50% (v/v) ethanol, followed by a 3 mL liquid scintillation cocktail. Then, the acid-stable radioactivity was determined by a liquid scintillation counter (Beckman Instruments, Fullerton, CA, USA). The herbicide concentrations that resulted in a 50% inhibition of enzyme activity (I_{50}) were estimated for each herbicide by using the obtained concentration response curves.

Statistical analysis

Using a regression analysis and R software (drc add-on package) (Ritz & Streibig 2005; R Foundation for Statistical Computing 2006), the four-parameter log-logistic model (Eqn 1) was fitted to the data that were obtained from the greenhouse and the ACCase enzyme assay and the GR_{50} and I_{50} values were estimated for each population and each herbicide:

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

where e is the GR_{50} or I_{50}. The upper limit is d and the lower limit is c. The parameter b denotes the relative slope around e.

In cases where $c = 0$, the three-parameter model (Eqn 2), with the lower limit equaling zero, was refitted to the data (Ritz & Streibig 2005):

$$f(x,(b,d,e)) = \frac{d}{1+\exp\{b(\log(x)-\log(e))\}}. \qquad (2)$$

The resistance factor (RF), which is the ratio of the GR_{50} or I_{50} of the resistant population to the GR_{50} or I_{50} of the

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susceptible population, was considered as an index in order to compare the resistance level of the tested populations.

RESULTS

Prescreening

Among all the sampled populations, 14 populations were suspected of being resistant to FEN, seven out of 14 populations were suspected of being cross-resistant to FEN and DIC, and three populations were suspected of being cross-resistant to FEN, DIC, and CLD (Table 1). The ES population, which was controlled completely with the three herbicides, was considered to be the susceptible population.

Dose–response assay in the greenhouse

There was no significant difference between the results that were obtained from the whole-plant herbicide-screening assays that were conducted in Iran and Spain; thus, the data were pooled for the regression analyses (data not shown). The estimated GR_{50} values showed different levels of resistance to FEN for the different populations (Table 2). The ES population was 50% controlled with only 38 g ai ha^{-1} of FEN, while the amount of herbicide that controlled 50% of the population of GR2-1 and SR3 was 65.09 and 367.89 g ai ha^{-1}, respectively (Table 2). According to the estimated GR_{50}, the other populations ranged between these populations.

Although only 430 g ai ha^{-1} of DIC controlled the ES population by 50% (Table 3), GR2-1 and SR3 were 50% controlled with ~778 and ~5105 g ai ha^{-1}, respectively. Even in response to the maximum dose of DIC, the AR, MR4, and SR3 populations survived, confirming the high level of resistance to the applied herbicide of these populations in comparison with the other populations.

Using CLD, the estimated GR_{50} was 29.26 g ai ha^{-1} for ES and 260.45, 223.90, and 237.92 g ai ha^{-1} for SR3, AR, and MR4, respectively (Table 4). The RFs for all the populations were >1. These values were statistically significant and confirmed that the populations were resistant to the applied herbicides.

Acetyl coenzyme A carboxylase sensitivity assay

The results showed that the ACCase enzyme of the AR, SR3, and MR4 populations (the three most-resistant at the whole-plant level) were 37-, 72-, and 14-fold less sensitive to inhibition by FEN than the ACCase of the ES population. The I_{50} values of these four populations were 2268.8, 4449.7, 869.7, and 61.4 nmol L^{-1}, respectively (Table 5). A similar insensitivity of ACCase was observed in relation to DIC in the three most-resistant populations. The I_{50} values for DIC were 3999.3, 10379, 4448.15, and 127.75 nmol L^{-1} for the AR, SR3, MR4, and ES populations, respectively (Table 5).

DISCUSSION

Although the whole-plant assay clearly showed that the FR2, FR3, FR4, FR5, FR6, FR8, MR1, MR2, ER1, ER2, and GR2-1 populations were resistant to FEN and that the FR2, FR4, FR8, and GR2-1 populations also were resistant to DIC, the ACCase assay did not confirm these results at the enzyme level, resulting in a RF of ~1 (Table 5). It seems that these resistant populations have developed different mechanisms of resistance to the APP herbicides.

The dose–response assays confirmed that 14, seven, and three populations were resistant to FEN, DIC, and CLD, respectively. Resistance to APP herbicides has been reported in Italian ryegrass (*Lolium multiflorum*) (Stanger & Appleby 1989; De Prado *et al.* 2000), rigid ryegrass (*Lolium rigidum* Gaud.) (Llewellyn & Powles 2001), wild oat (*Avena fatua* L.) (Heap *et al.* 1993), rigid brome (*Bromus rigidus* Roth), *P. minor* (Tal *et al.* 2000), and *Setaria viridis* (De Prado *et al.* 2004). The calculated GR_{50} and associated RF of these populations indicated different levels of resistance to the applied herbicides. These populations indicated a range of a 2–10-fold, 2–12-fold, and 7–9-fold increase in resistance to FEN, DIC, and CLD, respectively, compared to the susceptible population (Tables 2–4). De Prado *et al.* (2000) reported that *L. multiflorum* had a RF of >10 to DIC. According to Tal *et al.* (2000), the RFs of *Alopecurus myosuroides* and *L. multiflorum* to FEN and CLD were 22.8 and 41.5, respectively. The resistance index of *Avena sterilis* to FEN and CLD was between 1.03 and >8 and 1.05 and >8, respectively (Uludag *et al.* 2007). Kotoula-Syka *et al.* (2000) found that the resistant/susceptible-values of *L. rigidum* for DIC and CLD were 97.5 and 33.9, respectively. They also detected resistant/susceptible-values of 6.9, 6, and 10.2 for this grass weed in relation to fluazifop, tralkoxydim, and sethoxydim, respectively.

This study also showed that three populations (AR, SR3, and MR4) had developed resistance to DIC, FEN, and CLD and that seven populations had developed resistance to both FEN and DIC. The cross-resistance of resistant grass weeds to APP herbicides is a common phenomenon and has been documented previously. Resistance to various APP herbicides has been reported in wild oat (Seefeldt *et al.* 1994). Heap and Knight (1986)

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Table 1. Results of the prescreening test (% of the control), applying the recommended dose of diclofop-methyl (900 g ai ha^{-1}), fenoxaprop-P-ethyl (67.5 g ai ha^{-1}), and clodinafop-propargyl (60 g ai ha^{-1})

Diclofop-methyl		Fenoxaprop-P-ethyl		Clodinafop-propargyl		Weed population	Province	Year
Survived	Dry weight	Survived	Dry weight	Survived	Dry weight			
25.00	33.11	41.67	35.11	0.00	0.00	GR1-1	Golestan	2005
75.00	77.03	75.00	76.82	16.67	26.31	GR2-1		
33.33	44.26	58.33	45.55	0.00	0.00	GR3-1		
25.00	34.45	33.33	27.31	0.00	0.00	GR4-1		
33.33	28.19	58.33	39.87	0.00	0.00	GR5-1		
33.33	40.07	58.33	32.33	0.00	0.00	GR6-1		
16.67	39.27	33.33	26.34	0.00	0.00	GR7-1		
8.33	14.70	8.33	17.28	0.00	0.00	GSH		
33.33	44.36	58.33	44.32	0.00	0.00	FR1	Fars	
83.33	80.65	91.67	76.75	33.33	29.93	FR2		
58.33	64.06	83.33	75.41	16.67	22.00	FR3		
75.00	80.72	91.67	81.89	41.67	35.25	FR4		
58.33	58.03	83.33	77.40	25.00	27.84	FR5		
58.33	57.12	83.33	78.84	25.00	25.36	FR6		
50.00	54.78	66.67	48.26	16.67	22.96	FR7		
83.33	81.51	100.00	81.61	50.00	44.95	FR8		
33.33	37.29	58.33	36.37	0.00	0.00	GR1	Golestan	2006
33.33	40.12	58.33	32.31	0.00	0.00	GR2		
41.67	48.40	58.33	41.20	0.00	0.00	GR3		
50.00	42.62	58.33	44.30	0.00	0.00	GR4		
16.67	52.34	41.67	44.11	0.00	0.00	GR5		
33.33	46.39	58.33	48.62	0.00	0.00	GR6		
41.67	36.99	66.67	44.75	0.00	0.00	GR7		
33.33	53.54	58.33	45.91	0.00	0.00	GR8		
41.67	33.86	50.00	46.53	0.00	0.00	GR9		
41.67	52.43	50.00	54.38	0.00	0.00	GR10		
50.00	43.79	66.67	55.63	0.00	0.00	GR11		
0.00	0.00	0.00	0.00	0.00	0.00	GS		
100.00	93.65	100.00	90.05	100.00	88.30	AR	Fars	
58.33	62.69	83.33	79.67	8.33	31.33	ER1		
41.67	63.95	91.67	79.80	16.67	35.47	ER2		
0.00	0.00	0.00	0.00	0.00	0.00	ES		
58.33	62.63	75.00	82.26	25.00	32.03	MR1		
58.33	58.66	83.33	80.33	33.33	35.38	MR2		
50.00	49.79	58.33	42.10	16.67	21.70	MR3		
100.00	92.72	100.00	90.77	100.00	88.21	MR4		
8.33	17.02	25.00	19.66	0.00	0.00	MS		
100.00	96.91	100.00	95.25	100.00	92.88	SR3		
8.33	12.71	16.67	13.94	0.00	0.00	SS		

reported that there was a level of cross-resistance to APP herbicides by *L. rigidum*. Mansooji *et al.* (1992) reported a biotype of *A. sterilis* that showed a high level of resistance to all APP herbicides and cross-resistance to cycloxydim, sethoxydim, and tralkoxydim. The cross-resistance of a resistant biotype of *A. sterilis* has been reported in relation to FEN and DIC (Greenwood *et al.* 1999). De Prado *et al.* (2004) affirmed that a resistant biotype of *S. viridis* had cross-resistance to clodinafop, diclofop, fluazifop-P, haloxyfop, clethodim, cycloxydim,

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Table 2. Estimated non-linear regression parameters for the *Phalaris minor* populations in response to fenoxaprop-P ethyl, based on the above-ground biomass

Resistance factor	R^2	*P*-value of the lack of fit	GR_{50} (SE)	Hill's slope (SE)	Upper limit (SE)	Lower limit (SE)	Weed population
9.40**	99.06	0.21	357.56 (18.08)	1.12 (0.06)	99.14 (1.39)	0.00	AR
9.66**	98.95	0.94	367.89 (60.91)	0.91 (0.09)	99.77 (1.29)	22.60 (4.89)	SR3
8.72**	98.19	0.16	332.01 (103.94)	0.71 (0.09)	98.19 (1.83)	0.00	MR4
2.23**	98.82	0.08	85.02 (4.95)	1.44 (0.09)	97.78 (2.07)	0.00	FR2
3.19**	99.15	0.14	121.45 (6.32)	1.35 (0.07)	97.17 (1.71)	0.00	FR3
3.18**	98.75	0.06	159.42 (8.29)	1.49 (0.09)	96.61 (1.69)	0.00	FR4
3.25**	98.91	0.07	123.83 (7.18)	1.27 (0.07)	98.64 (1.86)	0.00	FR5
3.83**	98.92	0.09	145.88 (8.23)	1.34 (0.08)	97.19 (1.79)	0.00	FR6
2.77**	97.94	0.06	105.64 (7.42)	1.44 (0.10)	97.68 (2.40)	0.00	FR8
3.08**	98.83	0.11	117.14 (7.18)	1.24 (0.07)	96.00 (1.90)	0.00	MR1
2.08**	98.85	0.10	79.23 (4.50)	1.42 (0.08)	96.83 (2.01)	0.00	MR2
2.98**	98.13	0.06	113.56 (8.91)	1.11 (0.07)	97.99 (2.33)	0.00	ER1
2.75**	98.93	0.07	104.73 (6.73)	1.11 (0.05)	98.38 (1.94)	0.00	ER2
1.71**	98.89	0.13	65.09 (3.58)	1.12 (0.05)	99.26 (1.78)	0.00	GR2-1
–	99.28	0.07	38.08 (1.71)	1.49 (0.07)	99.05 (1.83)	0.00	ES

**$P = 0.01$.

Table 3. Estimated non-linear regression parameters for the *Phalaris minor* populations in response to diclofop methyl, based on the above-ground biomass

Resistance factor	R^2	*P*-value of the lack of fit	GR_{50} (SE)	Hill's slope (SE)	Upper limit (SE)	Lower limit (SE)	Weed population
5.67*	98.71	0.57	2439.60 (638.36)	0.63 (0.06)	99.12 (1.36)	25.02 (5.07)	AR
11.87*	98.66	0.86	5105.00 (1860.00)	0.62 (0.07)	98.01 (1.12)	32.04 (6.65)	SR3
5.95**	98.55	0.36	2557.60 (448.21)	0.83 (0.08)	99.83 (1.40)	33.16 (3.66)	MR4
1.96**	98.51	0.06	819.62 (62.40)	1.21 (0.07)	99.27 (2.57)	0.00	FR2
1.93**	97.70	0.07	831.72 (71.59)	1.20 (0.09)	96.62 (2.84)	0.00	FR4
4.32**	97.90	0.06	1859.20 (158.25)	1.21 (0.09)	96.37 (2.54)	0.00	FR8
1.79**	99.12	0.08	778.36 (51.82)	1.20 (0.06)	100.01 (2.28)	0.00	GR2-1
–	98.53	0.07	430.01 (31.14)	1.22 (0.08)	96.62 (2.47)	0.00	ES

*$P = 0.05$; **$P = 0.01$.

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Table 4. Estimated non-linear regression parameters for the *Phalaris minor* populations in response to clodinafop-propargyl, based on the above-ground biomass

Resistance factor	R^2	*P*-value of the lack of fit	GR_{50} (SE)	Hill's slope (SE)	Upper limit (SE)	Lower limit (SE)	Weed population
7.65**	99.25	0.36	223.90 (15.60)	1.28 (0.11)	98.28 (1.45)	6.65 (2.84)	AR
8.90**	98.95	0.16	260.45 (39.94)	0.91 (0.08)	100.54 (1.28)	23.42 (4.22)	SR3
8.13**	99.03	0.19	237.92 (13.27)	1.22 (0.07)	96.17 (1.55)	0.00	MR4
–	99.75	0.63	29.26 (0.53)	2.70 (1.11)	99.84 (1.10)	0.00	ES

**$P = 0.01$.

sethoxydim, and tralkoxydim. The enzyme assay showed that the existence of a modified ACCase enzyme was responsible for the resistance in the three populations (AR, MR4, and SR3). A review of the other studies demonstrated that target-site mutation is a common mechanism of resistance to ACCase-inhibitors (Délye *et al.* 2003; Tal & Rubin 2004; Yu *et al.* 2007; Hochberg *et al.* 2009) and a biochemical basis of resistance to ACCase-inhibitors has been revealed in several resistant populations (Matthews *et al.* 1990; Holtum *et al.* 1991; Tardif *et al.* 1996). However, there were different levels of resistance among all the other resistant populations that were included in this study, suggesting that maybe other mechanisms, such as metabolism, are involved. Evidence for non-target site-based herbicide resistance is becoming increasingly prevalent. For example, it was indicated in a molecular survey for ACCase herbicide resistance in *A. myosuroides* in France that >75% of the resistant plants (in the 243 resistant populations that were analyzed) did not have ACCase gene mutations, indicating increased herbicide metabolism as the resistance mechanism (Délye *et al.* 2007; Yu *et al.* 2009). We shall be conducting a molecular study to reveal which mutation(s) has modified ACCase, endowing the resistant enzyme in the three most-resistant populations. We also are going to investigate the other mechanisms of resistance in further studies in order to find out the exact mechanisms that are responsible in the other resistant populations.

In general, the results demonstrate that resistance to APP-inhibiting herbicides has occurred in the *P. minor* populations in the wheat fields of Fars province, Iran. It is not surprising because Fars is one of the most important provinces for producing wheat in Iran and, each year, many hectares are planted very intensively with this crop. The use of herbicides has been the major approach to controlling weeds in the wheat fields and APP-inhibitors have been used for several years in this province. As we did not have any precise history of herbicide application in the sampled areas, it was difficult to give an acceptable explanation for the existence of large degrees of difference between the resistance levels of the populations. However, it would seem that, if the farmers do not change their weed management methods or do not use herbicides with different modes of action, resistance to ACCase herbicides will become a major problem in the sampled areas in the future.

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Table 5. Estimated I_{50} (nmol L^{-1}) and calculated resistance factor for the acetyl coenzyme A carboxylase enzyme of the *Phalaris minor* populations in response to fenoxaprop-P-ethyl and diclofop-methyl

Population	Herbicide			
	Fenoxaprop-P-ethyl		Diclofop methyl	
	I_{50} (SE)	Resistance factor	I_{50} (SE)	Resistance factor
AR	2268.80 (149.61)	37.19*	3999.30 (59.98)	31.30*
SR3	4449.70 (138.5)	72.47*	10379.10 (461.94)	81.25*
MR4	869.70 (127.52)	14.60*	4448.15 (141.48)	34.82*
FR2	73.85 (9.94)	1.20^{NS}	141.37 (20.38)	1.11^{NS}
FR3	64.29 (11.79)	1.05^{NS}	–	–
FR4	69.58 (10.64)	1.13^{NS}	138.94 (19.46)	1.09^{NS}
FR5	71.36 (14.71)	1.16^{NS}	–	–
FR6	66.82 (15.20)	1.09^{NS}	–	–
FR8	82.29 (31.27)	1.34^{NS}	167.62 (23.09)	1.31^{NS}
MR1	77.62 (13.29)	1.26^{NS}	–	–
MR2	80.12 (21.11)	1.30^{NS}	–	–
ER1	68.90 (17.32)	1.12^{NS}	–	–
ER2	72.13 (14.03)	1.17^{NS}	–	–
GR2-1	60.78 (12.65)	0.99^{NS}	129.16 (28.51)	1.01^{NS}
ES	61.40 (15.78)	–	127.75 (31.29)	–

*$P = 0.05$. NS, not significant.

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