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Cross-resistance patterns of winter wild oat (*Avena ludoviciana*) populations to ACCase inhibitor herbicides

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Abstract The level of resistance and patterns of cross-resistance to clodinafop, sethoxydim, and pinoxaden were examined in 12 putative resistant and one susceptible populations of winter wild oat (*Avena ludoviciana*) collected from Fars Province, in the southwest of Iran. The responses of biomass and length of coleoptiles to the increasing dosages of the three herbicides were determined in both whole-plant and seed bioassays. In the whole-plant bioassay, all 12 putative resistant populations were found to be resistant to clodinafop with resistance ratios (R/S) ranging from 1.76 to >47.04. Most clodinafop-resistant populations exhibited low levels of cross-resistance to sethoxydim. Three highly sethoxydim-resistant

populations, F2, S2, and ES4, were slightly resistant to clodinafop. Six populations (M1, M2, F2, S2, S4, and ES4) showed high cross-resistance to pinoxaden with R/S values as large as 10.73 to 40.29. A highly clodinafop-resistant population, M2, was more sensitive to pinoxaden than the susceptible population. The results of the seed bioassay resembled those obtained from the whole-plant experiment suggesting seed bioassay as an inexpensive, rapid method for screening-resistant genotypes.

Keywords *Avena ludoviciana* Durieu · AED₅₀ values · Resistance ratio · Seed bioassay · Whole-plant assay

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Introduction

Various species of wild oat (*Avena* spp.) are among the most troublesome weeds worldwide in many crops (Holm et al. 1977). Winter wild oat (*Avena ludoviciana* Durieu.) is the prevailing *Avena* species in Iran, which is frequently found at high densities in winter crops such as winter wheat (*Triticum aestivum*) and barley (*Hordeum vulgare* L.) (Dezfoli 1997). It competes with winter crops and can cause severe yield losses of up to 44% in wheat (Montazeri 2007).

ACCase inhibitors constitute one of the major groups of commercially available herbicides for controlling grass weed species such as wild oat. They belong to three chemical families: aryloxyphenoxypropionates (APPs, fops), cyclohexanediones (CHDs, dims), and phenylpyrazolin (PPZ, dens) (Secor et al. 1989; Hofer et al. 2006). These herbicides block the first step in the

synthesis of fatty acids in the Gramineae by the inhibition of their chloroplastic acetyl-CoA carboxylase (ACCase) causing plant death (Burton et al. 1989). Since their introduction in the late 1970s (APPs) and 1980s (CHDs), the ACC-inhibiting herbicides have been widely used worldwide to control a number of grass weed species (Devine and Shimabukuro 1994). As a consequence, they rapidly selected, and are still selecting, resistant plants within grass weed species (Délye 2005) and rank third in terms of the frequency of resistance cases in the world (Heap 2015). The first case of ACCase inhibitor resistance in wild oat was reported in Western Australia in 1985 (Heap 2015) followed by many other reports thereafter (e.g., Morrison and Devine 1994, Seefeldt et al. 1994; Murray et al. 1996; Bourgeois and Morrison 1997; Beckie et al. 2002). Cross- or multiple-resistance patterns were also characterized in wild oat populations in some studies (Kern et al. 1996; Bourgeois et al. 1997; Friesen et al. 2000; Uludag et al. 2008).

In Iran, ACCase inhibitor herbicides were first introduced in 1994 for the control of grass weed species (Zand et al. 2007a) with a high efficacy in controlling wild oat (Montazeri et al. 2005). These herbicides have been extensively used by farmers for a decade in major cereal (mostly wheat) production regions of Iran such as Fars, Khuzestan, Khorasan, Golestan, and Markazi Provinces (Deihimfard and Zand 2005). However, field reports on the poor control of grass weeds raised the possibility of resistance evolution in regions with the history of using these herbicides (Zand et al. 2004). Results of through screenings confirmed the evolution of resistance in wild oat (Benakashani et al. 2006; Rastgoo et al. 2006; Zand et al. 2006) and little seed canary grass (*Phalaris minor* Retz.) populations (Elahifard et al. 2008; Gharekhloo et al. 2008), as the two major problematic grass weeds in winter crops in Iran. In a survey of 50 farmer's fields in Khuzestan Province, Zand et al. (2007b) characterized 52% of winter wild oat populations to be resistant to clodinafop.

Unsatisfactory control of winter wild oat from the application of clodinafop and sethoxydim has been criticized by local farmers in recent years in Fars Province. They blamed the quality of herbicides for the poor control, but a review of field history (repetitive use with no herbicide rotation), based on regular monitoring of fields by extension agents, raises the possibility of the evolution of resistance to these herbicides.

Phenylpyrazolin herbicide, pinoxaden (den group) (Hofer et al. 2006), has recently been also an ACCase inhibitor which was registered in Iran for the control of grass species in wheat and barley (Zand et al. 2007a). Even though the herbicide has not yet been used by farmers, it is likely that weeds suspected to be resistance to APPs or CHDs show resistance to pinoxaden as well. This has been demonstrated in a number of studies. For example, a tralkoxydim-resistant biotype of wild oat (*A. fatua*) (Uludag et al. 2008) and clodinafop-resistant population of little seed canary grass (*P. minor*) (Chhokar and Sharma 2008) have shown cross-resistance to pinoxaden. Cruz-Hipolito et al. (2011) also found that the two *A. fatua* biotypes from Chile and Mexico were resistant to APP, CHD, and PPZ herbicides.

It is necessary to detect resistance as early as possible to avoid the costly consequences of resistance spread. Seed bioassays, which are quick and inexpensive, have been found to be a useful and accurate tool for screening-resistant populations. Such assays have been used to discriminate between resistant and susceptible biotypes in green foxtail (*Setaria viridis*) (Beckie et al. 1990), Johnsongrass (*Sorghum halepense*) (Smeda et al. 1995), wild oat (*Avena fatua*) (Murray et al. 1996), and blackgrass (*Alopecurus myosuroides*) and ryegrass (*Lolium* spp.) (Letouze', A., and Gasquez, J. 1999).

The objective of this study was to quantify levels of resistance and investigate patterns of cross-resistance in wild oat (*A. ludoviciana*) populations to clodinafop, sethoxydim, and pinoxaden. We also examined the reliability of seed bioassays in determining herbicide resistance by comparing it with a whole-plant, standard experiment.

Material and methods

Plant material collection

Seed samples of 12 wild oat (*A. ludoviciana*) populations were collected from wheat fields of Fars Province in the southwest of Iran in 2006. The plant material collection was undertaken according to the protocols for screening weeds for resistance to herbicides as described in Beckie et al. (2000). All suspected seeds samples were collected from fields that were treated with, at least, one of

ACCase inhibitor herbicides for a minimum of 5 to 6 years. Seeds of a susceptible wild oat population (S) were collected from an area with no history of herbicide use. Both R-suspected and susceptible wild oat populations have never been treated with pinoxaden before. Crop and herbicide application history for fields where the suspected resistant biotypes were collected are summarized in Table 1. Both greenhouse and laboratory experiments were conducted at the Department of Weed Research, Plant Protection Institute, Tehran, Iran, in 2008 and 2009.

Whole-plant bioassay

To alleviate dormancy, the seeds of both putative resistant and susceptible wild oat populations were dehulled and then placed in petri dishes onto one sheet of filter paper (Whatman #1) saturated with 8 ml distilled water and 10 ppm gibberellic acid (GA3). The dishes were then incubated in a growth chamber set at 20 °C with a 16-h photoperiod for 3 days. Ten germinated seeds were randomly chosen and sown 1.5 cm deep into 500-ml plastic pots (12-cm-diameter) containing a mixture of clay, sand, and peat at equivalent volume ratio each. The emerged seedlings were then thinned to eight plants per pot. Plants were grown in a greenhouse with 25/20 °C day/night temperatures and 16-h supplemental lighting (PPFD of 400 $\mu\text{mol}/\text{m}^2/\text{s}$). Pots were watered as required, based on visual observation of the soil surface, and were fertilized with a dilute solution of water-soluble N:P:K fertilizer (9:4:7) at the rate of 5 g/L at the two-leaf stage. Four weeks after planting, the commercial formulations of clodinafop, sethoxydim, and pinoxaden were applied to 3–4 leaved wild oat plants using a moving-nozzle cabinet sprayer equipped with a flat-fan nozzle (8001E) calibrated to deliver 200 L ha^{-1} at 200 kPa in a single pass over the foliage. For a given herbicide, eight dosages of 0, 0.25X, 0.5X, 1X, 2X, 4X, 8X, and 16X of the recommended label rate (X) were applied. The recommended use rates for clodinafop, sethoxydim, and pinoxaden are 64, 375, and 45 g ai ha^{-1} , respectively. The aboveground biomass per pot was measured (oven-dried at 75 °C for 48 h) 4 weeks after application. The weed biomass data are expressed as a percentage of the nontreated control. The experiment was a completely randomized design with five replications. The effective dose of herbicide causing 50%

reduction in shoot dry weight (ED_{50}) was estimated from the log-logistic model (Seefeldt et al. 1995):

$$Y = c \frac{d-c}{1 + \exp(b(\log(x) - \log(Z)))}, \quad (1)$$

where Y is percent reduction in shoot biomass (as of nontreated control), x is the herbicide dose (g ai ha^{-1}), c and d denote the lower and upper limits, respectively, and b is the slope of the response curve and Z is the herbicide dose that gives a biomass reduction centered between the asymptotic values d and c . It should be noted that when $c \neq 0$, the Z parameter should not be interpreted as the dose that gives an absolute 50% reduction in the biomass. That is, the biomass reduction caused by an application rate equal to the Z is $\frac{d+c}{2}$ not $\frac{d}{2}$. As mentioned above, parameter d indicates the biomass production in the untreated control, and we need to estimate the dose that reduces this value by half. We will refer to this dose as “absolute ED_{50} ” and denote it by AED_{50} , which can be calculated from the parameters of Eq. 1 using the following formula:

$$\text{AED}_{50} = \left(\frac{Z^b d}{d-2c} \right)^{1/b} \quad (2)$$

The level of resistance (i.e., resistance ratio, R/S) was determined as the ratio of the AED_{50} of the resistant biotype to the AED_{50} of the susceptible biotype. All data were initially subjected to an ANOVA for testing the significance effect of herbicide doses using the PROC GLM procedure in SAS. Dose-response curves were fitted with the use of PROC NL MIXED of SAS, and the standard errors for both AED_{50} values and R/S ratios were estimated using the so-called delta method (Billingsley, 1986).

Seed bioassay

Ten dehulled seeds of *A. ludoviciana* were placed in 9-cm-diameter petri dishes onto one layer of filter paper (Whatman #1) moistened with 8 ml aqueous solution of potassium nitrate (KNO_3) at 0.1% (w/v). Dishes were then kept at 5 °C in darkness for 72 h to alleviate the residual seed dormancy to obtain uniform germination. Potassium nitrate was used to break seed dormancy as GA3 has been suggested to

Table 1 Herbicide use (graminicides) and cropping history from 2000 to 2006 at the locations where winter wild oat populations were collected from wheat fields in 2006

Population	Location	Crops planted	Herbicide applied
M1	Marv Dasht	Wheat-corn	Clodinafop (6 years)
M2	Marv Dasht	Wheat-corn-oilseed rape	Clodinafop (3 years), fenoxaprop (2 years), sethoxydim (1 year)
M3	Marv Dasht	Wheat-corn	Clodinafop (4 years), fenoxaprop (2 years)
M4	Marv Dasht	Wheat-corn	Not available
F2	Fasa	Wheat-corn	Clodinafop (4 years), fenoxaprop + clodinafop (1 year), fenoxaprop (1 year)
F3	Fasa	Wheat-corn	Clodinafop (4 years), clodinafop + fenoxaprop (1 year), fenoxaprop (1 yr)
S1	Sepidan	Wheat-corn	Not available
S2	Sepidan	Wheat-corn-barley-onion	Clodinafop (2 years), fenoxaprop (2 years), fenoxaprop + difenzoquat (1 year)
S3	Sepidan	Wheat-corn-rice	Clodinafop (4 years), diclofop (2 years)
S4	Sepidan	Wheat-corn	Not available
ES	Estahban	Wheat-corn	Clodinafop (2 years)
ES4	Estahban	Wheat-corn-oilseed rape	Clodinafop (4 years), sethoxydim (1 year)
S ^z	Sepidan	—	—

^zThe susceptible population has never been treated with any herbicide

interfere with seed assay results (Beckie et al. 2000). Following the chilling treatment, dishes were incubated at 20 °C with a 16-h photoperiod for 3 days. Seeds with 1-mm radical protrusion were then transferred to a second set of dishes, and 8 ml aqueous solution of the commercially formulated clodinafop, sethoxydim, and pinoxaden was applied to dishes at 0 (distilled water), 0.25X, 0.5X, 1X, 2X, 4X, 8X, and 16X of their discriminatory rates (X). A discriminatory concentration for a specific herbicide is the minimum herbicide concentration required to distinguish susceptible from resistant biotypes (Bourgeois et al. 1997) and was determined for a particular herbicide through some preliminary dose-response experiments. From these tests, the discriminatory concentrations for clodinafop, sethoxydim, and pinoxaden were estimated to be 0.32, 2, and 0.4 (mg ai ha⁻¹), respectively. The treated dishes were left in dark at 20 °C for 5 days followed by 2 days in light. After 7 days, the length of coleoptiles in ten seedlings was measured (Beckie et al. 2000) and expressed as a percentage of coleoptiles length of the nontreated control.

The experiment was conducted in a completely randomized design with four replications where a petri dish containing ten seeds constituted a replicate. ANOVA and dose-response curves fitting (Eq. 1) for coleoptiles length data were performed as described above.

Results and discussion

Whole-plant bioassay

The log-logistic model (Eq. 1) described the response of wild oat shoot biomass to increasing dosages of the three herbicides very well with R^2 values ranging from 0.95 to 0.99 (Figs. 1 and 2). The whole-plant dose-response experiment showed that the levels and pattern of cross-resistance to ACCase inhibitors were variables among the wild oat populations (Table 2). For the three tested herbicides, the susceptible biotype was completely controlled at rates much lower than the recommended rates suggesting that the susceptible biotype is highly sensitive to these herbicides. For example, the shoot biomass of the susceptible biotype was reduced by 50% with only 9.39 g ai ha⁻¹ of clodinafop (Table 2) compared with the recommended rate of 64 g ai ha⁻¹. Almost half the R-suspected populations could be controlled at the labeled use rate of clodinafop; however, their estimated AED₅₀ values were significantly higher than that of the susceptible population. The resistance ratio in the two most resistant populations (M2 and M3) was >47.04 (Table 2).

The level of resistance to sethoxydim was low: resistance ratio was smaller than five in nine populations. Resistance ratio for M2 did not exceed 1.49 indicating lack of resistance to sethoxydim in this population. Whereas F2 (R/S = 11.56), S2 (R/S = 7.72), and ES4

Fig. 1 Effect of clodinafop-propargyl dosages on the dry weight of some putative resistant and susceptible (*S*) populations of wild oat collected from counties of Marvdasht (a), Fasa (b), Sepidan (c), and Estahban (d) in the whole-plant bioassay

($R/S = 7.36$) populations showed the highest level of resistance to sethoxydim, they were among the least resistant populations to clodinafop. In contrast, the highly clodinafop-resistant populations (i.e., M1, M2, M3, M4, F3, and S3) exhibited low resistance level to sethoxydim (Table 2). According to the classification set out by Bourgeois et al. (1997), this pattern of cross-resistance can be categorized as type A, wherein a population is highly

resistant to APP herbicides and has no or low resistance to CHD herbicides. Populations that show low to moderate resistance to both herbicide groups are classified as cross-resistant type B. Type C is characterized by high levels of resistance to both APP and CHD herbicide groups; this group was not detected in our populations, while this type of cross-resistance was the most common, accounting for 44 of the 84 resistant wild oat lines, in the

Fig. 2 Effect of pinoxaden dosages on the dry weight of some putative resistant and susceptible (S) populations of wild oat collected from counties of Marvdasht (a), Fasa (b), Sepidan (c), and Estahban (d) in the whole-plant bioassay

study of Bourgeois et al. (1997). However, none of diclofop or clodinafop-resistant accessions of Italian ryegrass (*Lolium multiflorum* Lam.) was cross-resistance to the commercial dose of clethodim and sethoxydim in the study of Kuk et al. (2008). High resistance to APP herbicides and almost no resistance to CHD herbicides have been found in wild oat populations (Mansooji et al. 1992; Seefeldt et al. 1994; Uludag et al. 2008).

The resistance ratio for pinoxaden ranged from 0.35 to 40.29 (Table 2), indicating that some populations (e.g., M2 and ES) were more sensitive to the herbicide than the susceptible population, while others were highly resistant to pinoxaden (e.g., F2, S4, S2, and ES4). These two latter populations (i.e., S2 and ES4) also exhibited moderate cross-resistance to clodinafop and sethoxydim. The third most resistant populations to pinoxaden (i.e., F2) had the highest level of cross-

Table 2 The estimated herbicide rate required for 50% reduction (AED₅₀) in shoot dry matter of 12 putative resistant and one susceptible populations of winter wild oat and their corresponding resistance ratios (R/S) to three ACCase inhibitor herbicides

Population	Clodinafop		Sethoxydim		Pinoxaden	
	AED ₅₀ ^x (g ai l ⁻¹)	R/S	AED ₅₀ (g ai l ⁻¹)	R/S	AED ₅₀ (g ai l ⁻¹)	R/S
M1	139.67 (30.77)	<i>14.88</i> (3.90)	339.98 (30.40)	<i>3.13</i> (0.52)	46.24 (2.21)	<i>10.73</i> (3.35)
M2	>1024 ^y	–	157.63 (11.38)	<i>1.45</i> (0.23)	1.84 (0.98)	0.43 (0.26)
M3	>1024	–	249.10 (14.57)	<i>2.30</i> (0.35)	47.75 (7.38)	<i>11.08</i> (3.38)
M4	441.54 (108.49)	<i>47.04</i> (13.36)	388.28 (125.06)	<i>3.58</i> (1.26)	14.04 (3.39)	<i>3.26</i> (1.08)
F2	37.27 (5.36)	<i>3.97</i> (0.80)	1254.53 (73.20)	<i>11.56</i> (1.75)	130.69 (10.92)	<i>30.33</i> (9.71)
F3	120.12 (52.36)	<i>12.80</i> (5.86)	360.75 (36.75)	<i>3.32</i> (0.57)	26.63 (10.28)	<i>6.18</i> (2.06)
S1	29.56 (4.92)	<i>3.15</i> (0.69)	212.77 (25.43)	<i>1.96</i> (0.36)	14.31 (2.42)	<i>3.32</i> (1.17)
S2	49.46 (13.11)	<i>5.27</i> (1.59)	837.47 (320.01)	<i>7.72</i> (3.14)	172.73 (11.39)	<i>40.09</i> (12.66)
S3	127.59 (32.86)	<i>13.59</i> (4.00)	213.37 (9.07)	<i>1.97</i> (0.29)	14.27 (0.93)	<i>3.31</i> (1.05)
S4	39.36 (5.11)	<i>4.19</i> (0.81)	378.32 (53.33)	<i>3.49</i> (0.69)	173.57 (8.02)	<i>40.29</i> (12.58)
ES	16.48 (2.55)	<i>1.76</i> (0.37)	162.01 (30.85)	<i>1.49</i> (0.35)	1.49 (1.66)	0.35 (0.40)
ES4	34.54 (1.56)	<i>3.68</i> (0.55)	798.61 (88.08)	<i>7.36</i> (1.31)	103.99 (7.20)	<i>24.14</i> (7.64)
S ^z	9.39 (1.34)	1.00	108.54	1.00	4.31 (1.33)	1.00

Values shown in italics indicate herbicide-resistant populations as determined by a significantly R/S > 1

Values in parentheses indicate standard error

^x AED₅₀ values were estimated from the log-logistic model based on Eq. 2

^y Shoot dry weight reductions were less than 50% over the range of clodinafop application rates; thus, the AED₅₀ was not estimable

^z Susceptible population had never been treated with any of herbicides tested

resistance to sethoxydim but were only slightly resistant to clodinafop. Whereas M1 and M3 populations exhibited high cross-resistance to clodinafop and pinoxaden, they were only slightly resistance to sethoxydim. Pinoxaden could be a viable option for the control of clodinafop- and sethoxydim-resistant M2 as it showed high susceptibility to pinoxaden (Table 2).

Seed bioassay

The seed germination in all wild oat populations was higher than 90% suggesting the promoting effects of dormancy-breaking treatments in providing high and uniform germination (data not shown). The herbicide effect on coleoptiles length was detectable 7 days following incubation with large differences between populations (Table 3). Shoot length is more sensitive than root length to APP and CHD herbicides (Murray et al. 1996). The resistance ratio ranged from 0.68 (ES) to >26.21 (M2) (Table 3). M2 and ES were also found to be the most and least resistant populations in the whole-plant assay (Table 2). Like the pot experiment, all populations

showed low cross-resistance to sethoxydim except F3, which had an AED₅₀ value 12.56 times that of the susceptible population (0.41 mg ai ha⁻¹). Despite having the highest levels of resistance in the whole-plant assay, F2 and ES4 populations exhibited lower resistance ratios of 2.85 and 3.20 in the seed assay, respectively.

The seed assay was also highly efficient in quantifying the level of resistance to pinoxaden (Table 3). The results were congruent with those of the pot experiment. For example, M3, F2, S2, S4, and ES4 populations, which were previously found to be highly resistance to pinoxaden in the pot assay, were again ranked as the most resistant populations in the petri dish assay. On the contrary, the whole-plant assay suggested some degree of negative cross-resistance to pinoxaden in M2 population, but the data from the coleoptiles response of this population to pinoxaden showed a resistance ratio of 2.33. Similarly, the petri dish assay indicated a high level of resistance (R/S ratio: 10.98) in the S3 population (Table 3), but in the whole-plant assay, its R/S was only 3.31 (Table 2). With some narrow exceptions, the overall response of the wild oat populations in the seed

Table 3 The estimated herbicide rate required for 50% reduction (AED₅₀) in coleoptiles elongation of 12 resistant and one susceptible winter wild oat populations and their corresponding resistance ratios (R/S) to three ACCase inhibitor herbicides

Population	Clodinafop		Sethoxydim		Pinoxaden	
	AED ₅₀ ^x (mg ai l ⁻¹)	R/S	AED ₅₀ (mg ai l ⁻¹)	R/S	AED ₅₀ (mg ai l ⁻¹)	R/S
M1	0.95 (0.08)	<i>12.81</i> (1.61)	0.53 (0.04)	<i>1.29</i> (0.12)	0.63 (0.05)	<i>3.99</i> (0.35)
M2	> 5.12 ^y	—	0.49 (0.02)	<i>1.19</i> (0.08)	0.37 (0.04)	<i>2.33</i> (0.30)
M3	1.38 (0.33)	<i>18.54</i> (4.78)	0.42 (0.08)	1.01 (0.20)	1.83 (0.15)	<i>11.61</i> (1.06)
M4	0.55 (0.03)	<i>7.44</i> (0.82)	1.03 (0.26)	<i>2.52</i> (0.66)	0.52 (0.08)	<i>3.31</i> (0.52)
F2	0.74 (0.04)	<i>9.99</i> (1.07)	1.17 (0.15)	<i>2.85</i> (0.39)	1.77 (0.58)	<i>11.21</i> (3.72)
F3	1.20 (0.32)	<i>16.14</i> (4.54)	5.15 (1.16)	<i>12.56</i> (3.61)	1.09 (0.04)	<i>6.94</i> (0.36)
S1	1.95 (0.29)	<i>26.21</i> (4.64)	0.44 (0.01)	1.07 (0.06)	0.49 (0.07)	<i>3.14</i> (0.46)
S2	0.49 (0.04)	<i>6.57</i> (0.81)	1.06 (0.10)	<i>2.58</i> (0.28)	5.09 (0.48)	<i>32.29</i> (3.28)
S3	0.30 (0.02)	<i>3.98</i> (0.45)	0.41 (0.10)	0.99 (0.24)	1.73 (0.45)	<i>10.98</i> (2.92)
S4	0.36 (0.05)	<i>4.81</i> (0.77)	0.58 (0.03)	<i>1.41</i> (0.10)	2.50 (0.42)	<i>15.90</i> (2.75)
ES	0.05 (0.00)	0.68 (0.07)	0.26 (0.01)	0.65 (0.04)	0.29 (0.05)	<i>1.83</i> (0.33)
ES4	0.61 (0.07)	<i>8.27</i> (1.19)	1.31 (0.07)	<i>3.20</i> (0.24)	3.32 (0.71)	<i>21.08</i> (4.61)
S ^z	0.07 (0.01)	1.00	0.41 (0.02)	1.00	0.16 (0.01)	1.00

Values shown in italics indicate herbicide-resistant populations as determined by a significantly R/S > 1

Values in parentheses indicate standard error

^x ED₅₀ values were estimated from the log-logistic model based on Eq. 2

^y Plant survival was higher than 50% over the range of clodinafop application rates; thus, the AED₅₀ was not estimable

^z Susceptible population had never been treated with any of herbicides tested

bioassay was similar to that observed in the pot experiments (Table 2). Tal et al. (2000) also found a close correlation between the results from seed bioassay and those from the whole-plant assay suggesting that the petri dish bioassay could be a reliable, rapid, and inexpensive method for identifying populations of grass species resistant to ACCase-inhibiting herbicides.

According to the results of both the whole and seed assays, the resistance levels of most population to clodinafop were considerably higher than those to sethoxydim. This finding is in close agreement with many other reports (e.g., Mansooji et al. 1992; Seefeldt et al. 1994; Uludag et al. 2007; Kuk et al. 2008; Uludag et al. 2008). Although, pinoxaden was recently commercialized (2006) for use in Iran, and none of the wild oat populations has been exposed to this herbicide before, a high level of cross-resistance was detected in some populations.

The hexaploid wild oat (*Avena fatua*) has up to three unlinked ACCase gene loci assorting independently, and every homologous ACCase gene is able to carry

its own mutation. Therefore, each individual ACCase resistance mutation can confer relatively low-level herbicide resistance, in contrast to high-level resistance conferred by the same mutations in unrelated diploid weed species of the Poaceae family (Yu et al. 2013).

Our pattern of wild oat cross-resistance to pinoxaden, where the majority of populations (10 out of 12) evolved resistance to the herbicide, has rarely been detected in similar studies. For example, among the five wild oat populations tested for resistance to APP and CHD herbicides, only one biotype (R2) was highly cross-resistant to pinoxaden (R/S: 16.1) (Uludag et al. 2008). Similarly, of the 25 diclofop-resistant accessions of Italian ryegrass (*Lolium multiflorum*), five were resistant to pinoxaden (Kuk et al. 2008). Chhokar and Sharma (2008) also reported the same cross-resistance pattern in little seed canary grass (*P. minor*). However, the cross-resistance observed in our study along with other sparse instances may imply that the selection pressure from other ACCase inhibitors can result in cross-resistance to pinoxaden. Therefore, a pinoxaden's phenylpyrazolin chemistry and proposed different

ACCase-binding domains (Kuk et al. 2008) cannot necessarily dispose the risk of pinoxaden resistance.

Our study showed that some of the difficult-to-control populations of wild oat have indeed developed resistant to ACCase inhibitors. We found evidence of a negative cross-resistance pattern for clodinafop and sethoxydim where the highly clodinafop-resistant populations exhibited little or no resistance to sethoxydim and vice versa. Interestingly, some wild oat populations have already evolved resistance to pinoxaden while they have never been exposed to this recently registered herbicide. The results of seed bioassay resembled the whole-plant experiment suggesting seed bioassay as a reliable and yet inexpensive method for screening large number of samples for herbicide resistance.

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