Resistance to Aryloxyphenoxypropionate Herbicides in Wild Oat (*Avena ludoviciana*)

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Abstract

During 2002-2003 growing season, two field experiments in Fars and Khuzestan provinces and three greenhouse experiments at Plant Pest and Disease Research Institute, Tehran, were performed to evaluate the resistance of wild oat to aryloxyphenoxy propionate herbicides. Seed populations of wild oat were collected from nine locations in Fars and Khuzestan, in which seven populations were expected to be resistant to fenoxaprop-p-ethyl or clodinafop-propargyl and two of them were considered susceptible to these herbicides. In field experiments, fenoxaprop-p-ethyl at 75 g ai ha^{-1} was used in Fars and clodinafop-propargyl at 64 g ai ha^{-1} in Khuzestan to examine the response of wild oat populations treated at three-leaf stage to these herbicides. In greenhouse experiments, the response of 12 wild oat populations collected from three provinces (Fars, Khuzestan and Markazi) to fenoxaprop-p-ethyl, clodinafop-propargyl, and diclofop-methyl at 900 g ai ha^{-1} were examined in separate experiments. In each experiment, an untreated control of wild oat

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plants was maintained for each population (except Fars populations). Wild oat density, shoot dry weight, and visual rating of weed control were recorded. The results showed that wild oat populations in Khuzestan have developed resistance to aryloxyphenoxy propionate herbicides. But wild oat populations of Fars and Markazi are still controllable by this group of herbicides. However, there were some differences between the results of field experiments and those of the greenhouse experiments. For example, one of the populations (KR3) obtained from Khuzestan which was resistant to clodinafop-propargyl in field experiments only, showed resistance to all grass herbicides used in greenhouse experiments. In addition, two (KR1 and KR2) of them were resistant to all herbicides in greenhouse experiments only. Among populations collected from Fars, only one FR4 of them was resistant only to fenoxaprop-p-ethyl in greenhouse experiments. This indicated that the level of resistance of weeds is probably influenced by the environmental conditions.

Keywords: clodinafop-propargyl, diclofop-methyl, fenoxaprop-p-ethyl, resistance, wild oat, *Avena* spp.

چکیده

برای بررسی مقاومت علف‌هرز یولاف وحشی به علفکش‌های خانواده آریلوکسی فنوکسی پروپیونات‌ها، دو آزمایش صحرایی در سال زراعی ۸۲-۱۳۸۱ در استان‌های فارس و خوزستان و سه آزمایش گلخانه‌ای مؤسسه تحقیقات آفات و بیماریهای گیاهی به اجرا در آمد. در این بررسی، پنج توده یولاف وحشی از فارس و چهار توده از خوزستان که از هر استان یکی از توده‌ها حساس به علفکش و بقیه مشکوک به مقاومت بودند بررسی شدند. واکنش توده‌ها در فارس به علفکش فنوکساپروپ پی اتیل به میزان ۷۵ گرم ماده موثره در هکتار و در خوزستان به علفکش کلودینافوپ پروپارژیل به میزان ۶۴ گرم ماده موثره در هکتار ارزیابی شدند. در آزمایش‌های گلخانه‌ای، واکنش ۱۲ توده‌های یولاف وحشی نسبت به فنوکساپروپ پی اتیل و کلودینافوپ پروپارژیل و دیکلوفوپ متیل به ترتیب به میزان ۷۵، ۶۴، و ۹۰۰ گرم ماده موثره در هکتار بررسی شدند. نمره‌دهی مشاهده‌ای بر اساس رتبه‌بندی EWRC، تراکم و وزن خشک علف‌هرز یولاف وحشی چهار هفته پس از کاربرد علفکش‌ها معیار ارزیابی واکنش توده‌ها نسبت به علفکش‌ها بود. نتایج آزمایش‌ها نشان داد که

در آزمایش مزرعه‌ای در استان خوزستان توده KR3 مقاوم به کلودینافوپ پروپارژیل ارزیابی شد. در آزمایش‌های گلخانه‌ای نیز توده‌های KR1، KR2 و KR3 از خوزستان به علفکش‌های دیکلوفوپ متیل، کلودینافوپ پروپارژیل و فنوکساپروپ پی اتیل و همچنین توده FR4 از فارس به علفکش فنوکساپروپ پی اتیل مقاوم ارزیابی شدند. بر اساس این نتایج، برای جلوگیری از گسترش بیشتر مقاومت در خوزستان، کاهش کاربرد این دسته از علفکش‌ها توصیه می‌شود.

واژه‌های کلیدی: یولاف وحشی، مقاومت، فنوکساپروپ پی اتیل، کلودینافوپ پروپارژیل، دیکلوفوپ متیل.

Introduction

Herbicide resistance has become a major concern in weed management programs worldwide (Powels & Shaner, 2001; Burgos, 2004). In August 2005, International Survey of Herbicide-Resistant Weeds recorded 302 herbicide resistant weed biotypes (181 species) over 270,000 fields worldwide (Heap, 2005). Since the introduction of aryloxyphenoxy propionate herbicides in the 1970s, they have been widely used to control many grass weeds. This regular and widespread application has made several weed species resistant to these herbicides (Mousavi *et al.* 2005).

Wild oat grows as a weed in most wheat (*Triticum aestivum* L.) growing areas of the world (Thuston & Phillipson, 1976). The highest degree of herbicide resistance in *Avena* spp., has occurred to aryloxyphenoxy propionate (APP) and cyclohexanedione (CHD) herbicide groups. For example, several biotypes of wild oat (*A. fatua* L. and *A. sterilis* L.) that are resistant to these herbicides have been identified in North America, Europe and Australia (Mousavi *et al.*, 2005). Resistance of wild oat to acetyl-CoA carboxylase (ACCase) inhibitors was first reported in the Canadian prairies in 1990, 14 years after the registration of diclofop-methyl and 7 years after that in sethoxydim (Heap *et al.*, 1993).

Introduction of diclofop-methyl in 1980, clodinafop propargyl in 1994, and fenoxaprop-p-ethyl in 1993 caused significant improvements in herbicide efficacy and farmers became highly reliant on these herbicides in their cropping system. Many farmers in Iran have been using these herbicides for the last decade (Zand and Baghestani, 2002; Zand *et al.*, 2003). There are reports which indicate unexplained control failure of wild oat by diclofop-methyl, clodinafop propargyl, and fenoxaprop-p-ethyl, especially in Khuzestan. Such control failure of wild oat could not be attributed to improper herbicide application or other factors which may reduce herbicide performance. The objective of this study was to investigate the resistance to aryloxyphenoxy propionate herbicides in wild oat.

MATERIALS AND METHODS

Wild oat seeds collection

Thirteen wild oat populations were collected in spring 2001 from different fields (5 in Fars, and 4 in each of Khuzestan and Markazi provinces) which were under sequential wheat cropping for the previous seven years and were mostly treated by diclofop-methyl, clodinafop-propargyl, and fenoxaprop-p-ethyl. One of the populations at each location was susceptible and the others were suspicious to resistance (Table 1). Susceptible population seeds were collected from fields with no background of grass herbicide applications. As the seeds of Fars susceptible population did not germinate in the field due to the seed dormancy, this population was withdrawn from the experiments. In the field experiment, populations collected from Fars and Khuzestan provinces were studied only. However, in the greenhouse study all three provinces populations were studied.

Table1. Wild oat populations collected from each location in spring 2001.

Location	Population	
	Suspicious to resistance	**Susceptible population**
Fars	FR1, FR2, FR3, FR4	FS
Khuzestan	KR1, KR2, KR3	KS
Markazi	MR1, MR2, MR3	MS

Field studies

Two field experiments were conducted during 2002-2003 growing season in Fars (29° 46´ N, 52° 44´ E) and Khuzestan (32° 25´ N, 48° 23´ E) in fields that were fallow in the previous year. Both experiments were conducted in a factorial randomized complete block design with four replications. The treatments consisted of wild oat population (4 in Fars and 4 in Khuzestan), and herbicide dose in two levels (0 and recommended rate). Fenoxaprop-p-ethyl at 75 g ai ha^{-1} was used in Fars and clodinafop-propargyl at 64 g ai ha^{-1} was used in Khuzestan. Herbicide selection at each location was made based on the herbicide applied in the previous year.

The experiments were sown in October 2002 in Fars and Khuzestan to fields with no infestation to wild oat at sowing time. Fields were infested to wild oat manually. Wild oat seeds were broadcast by hand at 100 seeds m^{-2} on the soil surface of the plots at the initiation of the experiment immediately prior to planting winter wheat. All weeds emerged after this stage, were hand removed. Each plot consisted of 8 rows, 30cm apart and 5m long. The wheat cultivar used in each location was the common cultivar of that location, widely cultivated by farmers. Sowing density and amount of fertilizers applied were according to the recommendations of Iran Seed and Plant Research Institute, and Iran Water and Soil Research Institute, respectively. Herbicide treatments were applied at the 3-leaf stage of wild oat using a hand-held sprayer calibrated to deliver 300 L ha^{-1} of spray solution.

Visual percent weed control was estimated 3 weeks after herbicide application based on EWRC scale (Sandral *et al.*, 1997). Wild oat density and shoot dry weight were also measured at this stage. Weed density was determined by counting the number of wild oat plants from one Quadrat (1×1 m) per plot. All plants in the same quadrat were cut soil surface. Harvested plants were separated into wheat and wild oat, oven dried at 75 ^{o}C for 48 h and their dry weight were recorded.

Greenhouse study

Wild oat populations collected from provinces were used under separate experiments at greenhouse facilities of Plant Pest and Disease Research Institute, Tehran, to investigate their response to different herbicides. Each experiment was arranged in a randomized complete block design with factorial arrangement of treatments with 4 replications in fenoxaprop-p-ethyl and clodinafop-propargyl experiments and 5 replications in diclofop-methyl experiment. Similar doses as the field experiment were used for fenoxaprop-p-ethyl and clodinafop-propargyl. Clodinafop-propargyl was applied at 900 g ai ha^{-1}. The treatments of the greenhouse experiments were same as described under the field experiments.

Wild oat seeds were germinated on a moistend filter paper in Petri dishes for 48 h in the dark at 25 ^{o}C (Zand & Beckie, 2002). Among them, 10 seedlings with relatively uniform radical length (3-5 mm) were selected and planted 1 cm deep in 12cm diameter plastic pots containing a mixture of clay, sand, and manure (1:1:1 by volume). The pots were placed in a greenhouse with a 20/16 ^{o}C day/night temperature regime and a 16/8 h light/dark photoperiod supplemented with about 200 µmol m^{-2} s^{-1} photosynthetically active radiation illumination. Pots were irrigated daily to field capacity. Herbicides were applied using a cabinet sprayer equipped with an even flat fan nozzle calibrated to deliver 200 L ha^{-1} of spray solution at a pressure of 210 kPa, with a single pass over the foliage when wild oat plants reached to 3 to 4-leaf stage.

Visual percent weed control was estimated 3 weeks after herbicide application based on EWRC scale (Sandral *et al.*, 1997) and method of Beckie *et al.* (2000). Wild oat density and shoot biomass as a percentage of the untreated control (no herbicide application) were determined for all plants per pot, 4 weeks after herbicides application. Wild oat biomass dry weight was determined after oven drying at 75^{o}C for 48h.

Data analysis

All data were subjected to analysis of variance using SAS statistical software (SAS Institute, 1996). The assumptions of variance analysis were tested by insuring that the residuals were random, homogenous, with a normal distribution about a mean of zero. If the assumptions of variance were not adequately met, percent weed dry weight and population reductions were subjected to an arcsine square root transformation. Means were separated by Duncan multiple range test (DMRT) at the 0.01 level of significance. Field data were analyzed separately by location. Where the interaction between wild oat population and herbicide dose was significant, the discussion was made on interaction means. Because the reduction in wild oat density and shoot biomass in the greenhouse experiments were calculated as a percentage of untreated control (12 data sets), the data were analyzed as a randomized complete block design.

RESULTS AND DISCUSSION

Field studies

Fenoxaprop-p-ethyl significantly reduced wild oat density and shoot dry weight 3 weeks after herbicide application (WAHA) (Table 2). These results indicated that the susceptible and the suspect resistant populations of wild oat have been effectively controlled by fenoxaprop-p-ethyl, and hence wild oat populations in Fars are not resistant to this herbicide.

Table 2. Effect of fenoxaprop-p-ethyl on the mean density and shoot dry weight of wild oat plants under field conditions three weeks after herbicide application, in Fars 2002-2003.

Population	Treatments	Wild oat density (Plant m^{-2})	Shoot dry weight (g m^{-2})
FR1	Treated	2.00 b*	1.50 c
	Untreated	84.75 a	72.00 ab
FR2	Treated	1.00 a	0.00 c
	Untreated	82.25 a	71.50 ab
FR3	Treated	1.25 b	1.00 c
	Untreated	85.75 a	77.50 a
FR4	Treated	1.25 b	1.00 c
	Untreated	87.75 a	73.50 ab

*In each column, means followed by the same letter are not significantly different at α =1% according to DMRT test.

Statistical analysis for wild oat density and shoot dry weight in Khuzestan showed significant interaction between wild oat population and herbicide dose (Table 3). Clodinafop-propargyl significantly reduced wild oat density and shoot dry weight 3 weeks after herbicide application (WAHA) (Table 3). KS population which had the highest wild oat density at the untreated check was ranked the last when treated by this herbicide. KR3 showed the least reduction, and was grouped as resistant to clodinafop-propargyl. Similarly, KR1 which had the highest shoot dry weight at the untrea ted check was ranked the last when sprayed by clodinafop-propargyl (Table 3). Therefore, KR3 showed the strongest resistance to this herbicide. In fact, KR3 has accumulated 75% of its shoot dry weight 3 WAHA compared to the untreated control and could be considered as a resistant population. Thill and Lemerle (2001) have reported wild oat resistance to ACCase herbicides in many parts of the world. Owen (2001) has also stated that resistance of wild oat to ACCase herbicides in more than 1000 sites in Canada.

Greenhouse study

Response of populations to clodinafop-propargyl

Wild oat percent plant survival and shoot dry weight compared to untreated controls 4 WAHA, and visual rating 3 WAHA showed significant differences among populations (Table 4). KR3 showed complete plants survival and the least reduction in shoot dry weight followed by KR2 and KR1 under clodinafop-propargyl treatment. These results indicate that wild oat populations KR3, KR2, and KR1 were resistant to clodinafop-propargyl.

Table 3. Effect of clodinafop-propargyl on the mean density and shoot dry weight of wild oat plants under field conditions, three weeks after herbicide application, in Khuzestan 2002-2003.

Population	Treatments	Wild oat density (Plant m^{-2})	Shoot dry weight (g m^{-2})
KR1	Treated	13 c	100c
	untreated	78 ab	1870 a
KR2	Treated	12 c	100 c
	untreated	84 ab	925 b
KR3	Treated	60 b	2050 a
	untreated	77 ab	1100 ab
KS	Treated	9 c	80 c
	untreated	92 a	1362 ab

*In each column, means followed by the same letter are not significantly different at α =1% according to DMRT test.

Visual ratings also confirm these results. KR3, KR2 and KR1 showed the highest visual rating, respectively (Table 4). So, KR3, KR2 and KR1 could be considered as resistant populations to clodinafop-propargyl. Owen (2001) has stated that resistance of wild oat to ACCase herbicides has occurred in more than 1000 sites in USA. On the other hand percent plant survival in KS was 44.37, which indicates that this population has the potential to become resistant to clodinafop-propargyl (Table 4). Beckie *et al.* (2000) grouped weed accessions based on their survival and dry matter under herbicide application. Resistant populations are those with more than 50% weed survival and 80% dry matter accumulation compared with the untreated plants. These researchers also suggested that weed accessions could be rated as possibly resistant when weed survival and shoot dry weight accumulation in the treated plants were at least 50% of untreated plants.

Response of populations to diclofop-methyl

Wild oat plant survival and shoot dry weight compared to untreated control 4 WAHA, and visual rating 3 WAHA showed significant differences among populations (Table 5). KR2 showed the highest plant survival and the least reduction in shoot dry weight under diclofop-methyl treatment, but it did not significantly differ from KR3 and KR1 for the number of survived wild oat (Table 5). KR3, KR2 and KR1 also showed the highest visual rating (Table 5). Hence, KR3, KR2 and KR1 could be grouped as resistant populations to diclofop-methyl. Cavan *et al*. (2001) similarly reported wild oat resistance to diclofop-methyl and sethoxydim in Canada.

Response of population to fenoxaprop-p-ethyl

Percent plant survival and shoot dry weight of wild oat compared to untreated controls 4 WAHA, and visual rating 3 WAHA showed the same results as the other two herbicides (Table 6). KR3, KR2, KR1 and FR4 showed the highest plant survival and the least reduction in shoot dry weight when treated by fenoxaprop-p-ethyl, caused significant difference with all other populations (Table 6). Visual rating also confirms these results. KR3, KR2, KR1 and FR3 showed the highest visual rating (Table 6). According to the obtained results, KR3, KR2, KR1 and FR4 could be categorized as resistant population to fenoxaprop-p-ethyl.

Table 4. Mean plant survival, shoot dry weight and visual rating of wild oat populations treated by clodinafop-propargyl in the greenhouse study.

Wild oat population	4 WAHA		3 WAHA
	Survived plants (%)	Shoot dry weight (g m^{-2})	Visual rating
MS	0.0 e	28.66 d	1.0d
MR1	3.33 de	28.29 d	1.25 cd
MR2	12.67 d	36.30 cd	2.25 c
MR3	10.12 de	46.70 c	1.50 cd
FR1	0.0 e	42.04 cd	1.0 d
FR2	6.77 de	33.44 cd	2.0 c
FR3	0.0 e	45.32 c	1.0 d
FR4	11.20 d	36.99 cd	3.75 b
KS	44.37 c	41.08 cd	2.50 bc
KR1	83.75 b	78.09 b	8.25 a
KR2	87.85b	89.12ab	8.25 a
KR3	100a	98.39a	9.0 a

*In each column, means followed by the same letter are not significantly different at α =1% according to DMRT test.

WAHA = weeks After Herbicide Application.

The results of this study showed that wild oat populations in Khuzestan have developed resistance to aryloxyphenoxy propionate herbicides. Although the observed resistance level needs to be established by dose response test, application of these herbicides in Khuzestan should be avoided to prevent a higher degree of resistance. But wild oat populations of Fars and Markazi are still controllable by this group of herbicides. Of course it is noteworthy to mention that among the populations collected from Fars, only one of them was resistant to fenoxaprop-p-ethyl under greenhouse conditions. This indicates that resistance of weeds is influenced by environmental conditions. So inclusion of greenhouse experiments in weed resistance studies is necessary. Also, due to the initial resistance observed for some populations in Fars, application of these herbicides should be reduced.

Table 5. Mean plant survival, shoot dry weight and visual rating of wild oat populations treated by diclofop-methyl in the greenhouse study.

Wild oat population	4 WAHA		4 WAHA
	Survived plants (%)	Shoot dry weight ($g\ m^{-2}$)	Visual rating (3 WAHA)
MS	7.40 d	30.12 e	1.8 d
MR1	29.88 bc	35.36 de	3.0 cd
MR2	21.30 bcd	30.73 e	3.0 cd
MR3	15.01bcd	48.25 cd	3.8 bc
FR1	28.09 bc	43.76 cde	5.0 b
FR2	12.07cd	41.51 cde	3.6 b
FR3	16.81cd	41.98 cde	3.2 cd
FR4	36.59 b	50.35 c	5.0 b
KS	16.14 bcd	48.97 cd	4.0 b
KR1	95.48 a	94.31 b	9.0 a
KR2	96.68 a	97.54 a	9.0 a
KR3	93.54 a	91.79 a	9.0 a

*In each column, means followed by the same letter are not significantly different at α =1% according to DMRT test.

WAHA = weeks After Herbicide Application.

Table 6. Mean plant survival, shoot dry weight and visual rating of wild oat populations treated by fenoxaprop-p-ethyl in the greenhouse study.

Wild oat population	4 WAHA		3 WAHA
	Survived plants (%)	Shoot dry weight (g m^{-2})	Visual rating
MS	2.97c	27.64c	1.5c
MR1	5.39c	49.11cd	1.5c
MR2	3.48c	35.99ef	1.5c
MR3	7.21c	44.77cd	2.75b
FR1	5.49c	43.91cd	3.25b
FR2	2.91c	28.60ef	1.5c
FR3	3.86c	38.76cde	1.5c
FR4	64.76b	67.29b	8a
KS	16.66c	49.25c	3.25b
KR1	83.09a	92.94a	8.5a
KR2	92.86a	98.62a	8.75a
KR3	94.94a	98.54a	8.75a

*In each column, means followed by the same letter are not significantly different at α =1% according to DMRT test.

WAHA = weeks After Herbicide Application.

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