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Seed and Seedlings Assays for Rapid Detection of Fenoxaprop Resistance in Sterile Wild Oat (*Avena Sterilis*)

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Abstract: Sterile wild oat (*Avena sterilis*) has developed resistance to fenoxaprop on a large area in Turkey. A Petri dish assay based on root and/or shoot elongation can be used as a fast and reliable method to evaluate the resistance level of a given population. The suggested discrimination rate for Petri dish rapid assay is 4 to 8 mg/l. In pot experiment, both, visual scoring or survival data was found as reliable as ED_{50} values which are calculated using shoot dry weight data. The discriminating rate for visual scoring and survival data are 180 g ai/ha (4 times the recommended rate) of fenoxaprop.

Key words: *Avena sterilis* (sterile wild oat), fenoxaprop, rapid test

INTRODUCTION

Herbicide-resistant grass weeds are a worldwide problem[1]. The first ACCase-resistant wild oat was detected at *Avena fatua* in Australia in 1985[2] followed by diclofop-resistant sterile wild oat recorded in 1989[3]. The first herbicide resistant weed case confirmed in Turkey was fenoxaprop resistant sterile wild oat[4].

The confirmation of resistance is a vital component for resistance management[5]. Studies on fast and reliable detection methods have been conducted. A good diagnostic test should be rapid, accurate, cheap, readily available and reliable. Field experimentation can provide some information for practical use but it is difficult to interpret results in the context of resistance[5] and is not reliable due to lack of a standard susceptible biotype at the same field[6]. Dose response experiments in pots under controlled conditions have been used to accurately characterize herbicide resistant weeds although results were not consistent in all cases[7]. Moreover, dose-response experiments are time and space consuming, and expensive, so they are not practical when a large number of samples is tested[8]. In vitro assays that measure photosynthetic competence, fluorescence, amount of pigment or protein, or activity of an herbicide's target enzyme can also identify herbicide resistance. These tests are generally need special facilities and equipments and are too complex and time consuming to be considered as rapid screening tests[8]. Petri dish assays with seeds are faster, inexpensive and require less space compared to pot assays[5]; but, they may not be as accurate as pot bioassays in determining the likely effect of resistance on herbicide activity in the field[8,9]. Moreover, dormancy in seeds can affect the performance of seed assays.

Several methods have been developed to detect resistance to ACCase inhibiting herbicides. Whole plant test, seed and seedling bioassay techniques, visual test, pollen test, GST activity test, and PCR markers have been exploited to detect ACCase resistance in grass weeds (10; 11; 12; 13; 14; 15). Some of the tests require more time and labor than others, while some require specialized laboratory procedures. Some of the tests are not compatible for detection of ACCase resistant wild oat.
The objective of this research was to develop a fast and accurate Petri dish method for detection of fenoxaprop resistance in wild oat, and to compare visual scoring and survival data with ED50 values to get faster decision in pot assays.

MATERIALS AND METHODS

Seedling assay with surviving plants: Sterile wild oat seedlings that survived commercial treatment of fenoxaprop or clodinafop in wheat fields were collected from 16 different fields and examined during the 1999-2000 cropping season (Table 1). Forty seedlings were uprooted with some soil from each field and transferred to a nethouse for 2 to 3 weeks of acclimation. Commercial formulations of fenoxaprop were used throughout the study, using a motorized backpack sprayer delivering a spray volume of 300 l/ha at 2 atm pressure. Herbicide was applied at 0, 1X (recommended dose), 2X, 4X and 8X recommended dose which is 45 g ai/ha. Three weeks after application (WAA), plants were visually

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Table 1: Sites where sterile wild oat plants and seeds were collected.

Populations	Location		
	Province	District	Village
AKR1	Adana	İmamoğlu	Ağzıkaraca
AKR2	Adana	İmamoğlu	Ağzıkaraca
BLG	Adana	Karaisalı	Topaktaş
BTP1	Adana	Kozan	Bağtepe
BTP2	Adana	Kozan	Bağtepe
CBY	Gaziantep	Oğuzeli	Çaybeyi
CYL	Adana	Yüreğir	Çaylı
DZC	Adana	Yüreğir	Düzce
GKY1	Adana	Karaisalı	Gökkuyu
GKY2	Adana	Karaisalı	Gökkuyu
GKY3	Adana	Karaisalı	Gökkuyu
GRD	Adana	Yüreğir	Gerdan
HZL	Adana	Yumurtalık	Hamzalı
KMH	Kahramanmaraş	Türkoğlu	Tarım İşletmesi
KMP	Kahramanmaraş	Türkoğlu	Tarım İşletmesi
KMT	Kahramanmaraş	Türkoğlu	Tarım İşletmesi
KRL1	Adana	Yüreğir	Karlık
KRL2	Adana	Yüreğir	Karlık
KRL3	Adana	Yüreğir	Karlık
KTA	Adana	Yüreğir	Köprüköy
MKU	Hatay	Reyhanlı	Universityfarm
SAL	Adana	Yüreğir	Sadıkali

evaluated using the Australian scale[16] with slight modifications. In this scale, 0 represents no effect as compared to untreated control and 5 represents fully necrotic dead plants without recovery. The experiment was arranged as a randomized complete block design (RCBD) with 4 replications. Data are presented as the mean evaluation score of all replicates with populations evaluated below 2 are considered susceptible.

Seedling assay grown from seeds: Before seed shed (May 2000), sterile wild oat seeds were collected from 20 fields from plants that survived fenoxaprop or clodinafop application during 1999-2000 cropping season (Table 1). Sterile wild oat seeds were also collected from two sites (KMH and KTA) that have never been exposed to herbicides to serve as sensitive (wild type, S) populations. Eight seeds from each population were dehulled, and soaked in 0.1% KNO_3 solution for two days, before planted in a pot containing potting medium pH 7.6, manure and sand (2:1:1 v/v). The pots were placed in a tarp covered greenhouse, which had natural light and temperatures. Plants were thinned at the 2 to 3 leaf stage to 5 plants/pot and fenoxaprop was applied as described above at 0, 1/4X, 1/2X, 1X, 2X, 4X and 8X recommended doses. Experiments were arranged in a RCBD with 4 replications for each herbicide and the experiments were repeated twice. Three WAA plants were visually rated, the surviving plants counted and shoot dry weight recorded in each pot.

Seed Assay: Seed assays were conducted in Petri dishes (90 mm in diameter) with selected populations identified from the previous experiments. Three populations identified as resistant (R), AKR2, GKY1 and KMT, two sensitive (S) populations, GKY2 and KRL1, and a wild type population KTA were tested. In preliminary experiments, four processes were examined: germinating intact seeds with DD water, germinating intact seeds in 0.1% KNO_3 solution, germinating dehulled seeds with DD water, and germinating dehulled seeds in 0.1% KNO_3 solution. This study have shown that the combination of intact seeds and KNO_3 is the most reproducible and efficient method, hence the whole experiment was repeated using this method. Sterile wild oat seeds (20/dish) were placed on two layers of filter paper in a Petri dish. Herbicide stock solutions were prepared with DD water or 0.1 % KNO_3 solution. Herbicide tested rates were 0, 0.04, 0.08, 0.16, 0.32, 0.64, 1.25, 2.50, 5.00, 10.00, 20.00, 40.00, 80.00 mg ai/L. Five ml of herbicide solution were added to each Petri dishe, which were placed in 20 ^{0}C in dark. DD water was added during the experiment as needed. Two weeks after seeding, root and shoot length were measured for each seedling. The experiment was arranged in RCBD with 3 replications.

Statistical Analysis: Shoot and root length data from the two experiments were pooled and subjected to nonlinear regression analyses. Dose response curves were obtained using a log logistic model[17], and the ED_{50} value (herbicide concentration causing 50% growth inhibition) was calculated for each population. The log logistic equation relating response Y to the herbicide rate x is:

$$Y = C + \{(D-C)/[1+\exp[b(\log(x)-\log(ED_{50}))]]\}$$

Where C = lower limit, D = upper limit, b = slope, and ED_{50} = dose giving 50% response[17]. Survival and visual rating evaluations were transformed using arcsine and subjected to ANOVA.

RESULTS AND DISCUSSIONS

Assay of surviving plants: Fenoxaprop applied to plants that survived the farmers' treatment with either fenoxaprop or clodinafop resulted in a variable response depending on the collection site. Some variation was also found among plants collected at the same field (Table 2). Some populations (BLG, KRL2, and KRL3) escaped the farmer's treatment, but were severely damaged by the recommended rate (1X) of fenoxaprop and were considered to be fenoxaprop sensitive. The GKY3, KMP and KMT populations did show only slight (if any) damage symptoms even when 8X rate of herbicide was applied and were considered resistant. AKR1, AKR2 and GRD populations showed resistance to 4X rate whereas two populations, GKY2 and SAL, tolerated only the recommended (1X) rate of fenoxaprop. The variation in response also indicates that some populations are still at

Table 2: Effect of fenoxaprop applied at different rates on sterile wild oat seedlings. Plants were collected from fields after treated with clodinafop or fenoxaprop by the farmer. The plants were exhumed from the soil, transferred to pots and two weeks later were treated with fenoxaprop. The plants were grown in a nethouse under the prevailing climatic condition.

Population	Herbicide rate (g ai/ha)			
	45	90	180	360
	Visual rating score*			
AKR1	1.5	1.5	2.0	2.5
AKR2	0.5	3.0	2.0	3.0
BLG	4.0	4.0	4.5	5.0
BTP1	3.5	0.5	3.0	4.5
BTP2	2.5	4.0	5.0	5.0
CYL	3.5	2.5	5.0	5.0
GKY1	2.5	4.0	3.0	-**
GKY2	0.0	4.0	5.0	-**
GKY3	0.5	1.0	2.0	1.5
GRD	2.5	2.0	2.0	3.5
KMP	0.0	0.5	1.0	0.5
KMT	2.0	0.5	1.5	0.0
KRL1	2.5	3.3	4.7	-**
KRL2	4.0	2.0	4.0	-**
KRL3	5.0	5.0	5.0	5.0
SAL	0.0	2.5	4.5	4.5

* Visual rating score: 0 = no damage; 5 = full shoot necrosis (no recovery)

** not tested

Table 3: Effect of fenoxaprop on sterile wild oat populations grown in pots from seeds collected from plants survived the treatments applied by the farmer. Visual rating was performed 3 weeks after treatment.

Populations	Herbicide rate (g ai/ha)					
	11	22	45	90	180	360
	Visual rating score*					
AKR1	0.0	0.0	0.0	0.5	2.0	3.0
AKR2	0.0	0.0	0.0	0.0	1.5	3.0
BLG	0.0	0.0	1.0	4.0	5.0	5.0
BTP1	0.0	0.0	0.0	4.0	5.0	5.0
BTP2	0.0	0.0	2.0	4.5	5.0	5.0
CBY	0.0	0.0	0.0	40.	5.0	5.0
CYL	0.0	0.0	0.5	3.5	5.0	5.0
DZC	0.0	0.0	0.0	0.0	1.0	3.0
GKY1	0.0	0.0	0.0	0.0	1.0	1.5
GKY2	0.0	0.0	1.0	4.0	3.0	4.0
GKY3	0.0	0.0	0.0	0.0	0.5	0.0
GRD	0.0	0.0	1.0	4.5	5.0	5.0
HZL	0.0	0.0	0.5	40.	5.0	5.0
KMP	0.0	0.0	0.0	0.5	2.0	3.0
KMT	0.0	0.0	0.0	0.5	2.5	3.0
KRL1	0.0	0.0	2.5	4.5	5.0	5.0
KRL2	0.0	0.5	2.0	4.0	5.0	5.0
KRL3	0.0	0.0	0.0	5.0	5.0	5.0
MKU	0.0	0.0	1.5	4.0	5.0	5.0
SAL	0.0	0.0	0.5	3.0	5.0	5.0
Susceptible	0.0	0.0	2.0	4.5	5.0	5.0

* Visual rating score: 0 = no damage; 5 = full shoot necrosis (without recovery)

an early stage of resistance evolution, containing certain proportion of sensitive individual plants. In spite of the variationin response to fenoxaprop, these results provided

Table 4: Effect of fenoxaprop on the survival of sterile wild oat plants grown from seeds in pots, as determined 3 wks after treatment.

Populations	Herbicide rate (g ai/ha)					
	11	22	45	90	180	360
	Plant survival (%)					
AKR1	100	100	100	97	75	40
AKR2	100	100	100	100	90	57
BLG	100	100	97	21	0	0
BTP1	100	100	82	0	0	0
BTP2	100	100	35	5	0	0
CBY	100	100	87	12	0	0
CYL	100	100	92	24	0	0
DZC	100	100	100	97	97	60
GKY1	100	100	100	97	97	100
GKY2	100	100	85	26	15	15
GKY3	100	100	100	100	95	100
GRD	100	100	87	5	0	0
HZL	100	100	92	0	0	0
KMP	100	100	100	85	78	56
KMT	100	100	100	90	47	55
KRL1	100	100	72	6	12	12
KRL2	100	100	77	12	0	0
KRL3	100	100	77	0	0	0
MKU	100	100	76	10	0	0
SAL	100	100	97	25	5	7
Susceptible	100	100	79	1	0	0

Table 5: Response of various sterile wild oat populations treated in Petri dish with fenoxaprop. The seed assay was performed using intact seeds germinated in 0.1% KNO_3 solution containing different concentration of fenoxaprop for 6 days. The results are given as a ratio of the ED_{50} (fenoxaprop rate (mg ai/L) causing 50% reduction in shoot or root elongation) value of the examined population (R) and the ED50 value of S population (KTA) serves as the susceptible (S) population..

Populations	R/S Ratio	
	Shoot	Root
GKY1	11.18	138.87
KMT	6.86	50.00
AKR2	6.31	45.81
GKY2	2.00	16.61
KRL1	0.61	4.69
KTA	1.00	1.00

the initial understanding of the resistant situation of sterile wild oat populations in the region. There were at least six populations, which have survived the high rates of 4X and/or 8X rates of the herbicide, which considered as resistant to fenoxaprop.

Assay with seedlings grown from seeds: Evaluation of the pot experiment was commenced in three different ways: visual scoring as described above; shoot dry weight, and rate of survival. According to the visual scoring (Table3) it seems that the best "discriminating rate" (12) was 4X, with six populations scored 2 or lower. However, when the 8X rate was used, only two populations (GKY1 and GKY3) were scored 2 or lower. Similarly, according to the 'survival' method (75% survival rate and above is considered R), the same six R

population were selected following treatment with the 4X rate of fenoxaprop, and the same two populations were rated as R after treatment with 8X (Table 4). These results suggest that visual scoring can be used when the herbicide is applied at 4X, as a fast and reliable method to estimate the resistance level of a given population.

Based on the dose response curves plotted from the shoot dry weight data and the ED50 values, we calculated the resistance/susceptible (R/S) ratio. Seven (AKR1; AKR2; DZC; GKY1; GKY3; KMP; KMT) of 20 populations examined, were regarded as resistant with a range of R/S ratio between 2.41 and >8.0 (4). Visual scoring and rate of survival methods resulted in a similar tendency and discriminated between the R and S populations in a manner similar to the shoot dry weight (R/S ratio) method. Survival rate was positively and visual rating was negatively correlated with dry weight data.

Seed Assay: Sterile wild oat root elongation was strongly inhibited by fenoxaprop as compared to the inhibition of shoot elongation (Table 5). Similar phenomena were observed in diclofop-resistant *Lolium rigidum*, fenoxaprop-resistant *Phalaris minor* and clodinafop-resistant *A. myosuroides* (12), whereas shoot length was found more sensitive than root length at fenoxaprop- and sethoxydim-resistant wild oat (18). When the ED_{50} values of certain populations were compared to that of a known sensitive population (KTA) the method differentiated between the very resistant populations (GKY1), highly resistant populations (AKR2 and KMT) and the moderately resistant populations that survived the farmer's treatment in the field (GKY2 and KRL1), but were considered as non-resistant in our other tests (Table3). Generally, the calculated R/S ratios were higher in the seed assay than in the seedling assay particularly when root length was compared. These relatively high R/S values may cause some overestimation of populations which are only slightly resistant such as GKY2. On the other hand the high 'sensitivity' of the method can be instrumental for identifying weed population at their early resistance evolution. The suggested discrimination rate for this rapid test is estimated to be 4 or 8 mg/l (data not shown). Those rates are in agreement with earlier data reported by Tal et al. (12) who suggested discriminating rates of 6 mg ai/L for diclofop-resistant *L. rigidum*, and 8 mg/l for fenoxaprop-resistant *P. minor*.

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