DICLOFOP-METHYL RESISTANCE IN A BIOTYPE OF WILD OATS (*AVENA FATUA* L.)

P. Boutsalis, J.A.M. Holtum and S.B. Powles
Dept. of Agronomy, Waite Agricultural Research Institute,
University of Adelaide, PMB 1 Glen Osmond S.A. 5064

Summary. A biotype of *Avena fatua* L. from York, Western Australia, was examined for resistance to diclofop-methyl and cross-resistance to other wild oat herbicides. The biotype was confirmed as resistant to diclofop-methyl but not resistant to a range of other wild oat herbicides. Acetyl-CoA carboxylase, an enzyme considered by many to be the target site of diclofop-methyl, from the susceptible and resistant biotypes did not exhibit differences in extractable activity, substrate affinity or herbicide sensitivity. It is suggested that the mechanism of diclofop-methyl resistance in the wild oat biotype does not involve the possession of an herbicide-insensitive acetyl-CoA carboxylase.

INTRODUCTION

In 1985 a farmer noted that diclofop-methyl failed to control wild oats (*A. fatua* L.) infesting a field near York, 60 km east of Perth. The field was cultivated under a continuous wheat/lupin rotation and had been exposed to diclofop-methyl annually since 1979. Field trials conducted by Piper (6) confirmed this population was not controlled by diclofop-methyl.

We report here on the response, under controlled conditions, of the biotype to nine graminicides used for wild oat control. The herbicides tested included five aryloxyphenoxypropionate graminicides, three cyclohexanedione graminicides and the phenylurea herbicide, chlortoluron. Diclofop-methyl, tralkoxydim and chlortoluron are used to control wild oats in cereal crops, whereas the others can only be used in dicotyledonous crops because of their toxicity to monocotyledonous species. Chlortoluron is not registered for use in Australia but is widely used for selective weed control in cereal crops in Europe.

Diclofop-methyl is an inhibitor of the chloroplastic enzyme acetyl-CoA carboxylase (1; ACCase). We examined the herbicide-inhibition and extractable activities of ACCase from susceptible and resistant *A. fatua* in order to determine whether resistance to diclofop-methyl was attributable to differences in the expression or herbicide-sensitivity of the enzyme from the resistant biotype.

MATERIALS AND METHODS

Plant material. Seed from wild oats that survived 375 g a. i. ha^{-1} diclofop-methyl was collected from a trial near York in 1987. Susceptible wild oat seed was collected from Saddleworth, 120 km north-east of Adelaide.

Seedling growth. Dehusked, punctured seeds were placed on filter paper moistened with 5 ml 0.05 g a. i. l^{-1} thiram in 9 cm petri dishes. Germination took place under a 12 h, 20°C day/12 h, 16°C night regime. Photon flux density during the photoperiod was 19 $\mu E\ m^{-2}\ s^{-1}$. Seedlings were transplanted into 15 cm pots (5 plants/pot) containing recycled soil. Pots were grown outdoors during May to September, the normal growing season for this species.

Herbicide treatment. Herbicides tested included diclofop-methyl, fluazifop-butyl, haloxyfop-methyl, fenoxaprop-ethyl, quizalofop-ethyl, tralkoxydim, sethoxydim, alloxydim and chlortoluron. Surfactant (0.2% v/v Agral 600) and spray oil (1% v/v Ampol D-C-tron Spray Oil) were added with each herbicide. Plants were sprayed at the 1.5-2 leaf stage, the stage which they would be exposed to herbicides in typical farming systems. Plants were treated with a twin nozzle laboratory sprayer delivering 113 l ha^{-1} at a pressure of 250 kPa and a speed of 1 m sec^{-1}. After treatment the plants were kept indoors overnight and then returned outdoors the following morning.

Except for quizalofop-ethyl and diclofop-methyl, five rates were sprayed per herbicide with five pots per rate. For diclofop-methyl nine rates were sprayed. Only two rates of quizalofop-ethyl were sprayed. The pot experiments were repeated three times during the growing season.

Measurements. Forty one days after spraying, the plant survival and dry weights were recorded. Plants were designated as dead if no new green plant material was evident. Green plant material was dried for three days at 85°C before dry weights were measured.

Statistical analyses. The experiment was conducted as a completely randomized factorial experiment. Dry weights and the number of plants surviving per pot were analysed by an analysis of variance using the statistical package GENSTAT 5. The results were tested at the 5% significance level.

Extraction and assay of acetyl CoA carboxylase. The methods of Matthews *et al.* (5) were employed.

RESULTS AND DISCUSSION

Diclofop-methyl resistance. In comparison to the susceptible control the biotype from York, Western Australia, is resistant to diclofop-methyl (Figure 1). The LD_{50} (the herbicide rate required to kill 50% of the population) for the resistant biotype was about 3-fold greater than for the susceptible biotype.

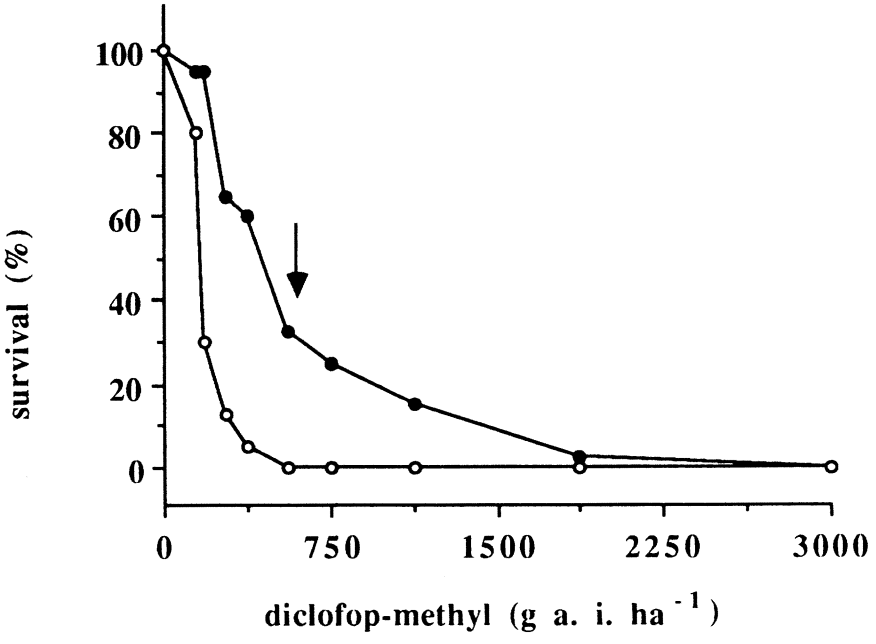

Figure 1. Effect of diclofop-methyl on percentage survival of the resistant (●) and susceptible (○) *A. fatua* biotypes. Each point is the mean value for survival of 75 plants. Analysis of variance demonstrated that the response of both wild oat biotypes was statistically significant for the diclofop-methyl treatment ($P < .001$). The arrow indicates the recommended rate for wild oat control.

At 563 g a.i. ha^{-1}, the rate recommended for control of wild oats, 33% of plants from the resistant biotype survived whereas there were no survivors from the susceptible biotype (Figure 1, Table 1). This survival will be a minimum estimate because in the field, even under optimum environmental conditions that favour herbicide uptake, the highest control of susceptible wild

oats normally obtained is about 95%. In our experiments, 95 % control was obtained at about 375 g a.i. ha^{-1} a rate at which 60 % of the resistant plants survived (Figure 1). Our observations are consistent with those of Piper (6).

Above 1875 g a.i. ha^{-1}, greater than three times the recommended rate, there were no survivors of either biotype. Application of similar high rates by farmers to control such a resistant biotype is uneconomical.

Effect of alternative wild oat herbicides. The diclofop-methyl resistant biotype did not show any resistance to other aryloxyphenoxypropionate herbicides tested, nor did it show cross-resistance to cyclohexanedione herbicides or to chlortoluron (Table 1). These observations are in direct contrast to those of Mansooji *et al.* (4) for a diclofop-resistant biotype of *Avena sterilis* from the Bordertown region of South Australia. The *A. sterilis* biotype exhibits resistance to a range of aryloxyphenoxypropionate compounds but not to any cyclohexanediones tested.

Table 1. Rates of application of herbicides required to kill 50% of resistant and susceptible wild oats and survival of both biotypes at the recommended rates.

herbicide*	LD_{50}		survival	
	resistant	susceptible	resistant	susceptible
	g a.i.ha^{-1}		%	
Aryloxyphenoxypropionates				
diclofop-methyl	442	165	33	0
fluazifop-butyl	16.8	23.7	0	0
haloxyfop-methyl	12.9	12.9	0	0
fenoxaprop-ethyl	7.8	7.8	5	0
quizalofop-ethyl	12.7	11.9	0	0
Cyclohexanediones				
tralkoxydim	10.8	15.6	0	0
sethoxydim	28	28.5	0	3
alloxydim	49	79	0	0
Phenylurea				
chlortoluron	750	750	0	0

* Recommended rates in g. a.i. ha^{-1} are :- diclofop-methyl 563; fluazifop-butyl 125; haloxyfop-methyl 52; fenoxaprop-ethyl 90; quizalofop-ethyl 48; tralkoxydim 150; sethoxydim 93; alloxydim 350; chlortoluron 1500.

Acetyl CoA carboxylase from resistant and susceptible *Avena fatua*. The aryloxyphenoxypropionate and cyclohexanedione graminicides are inhibitors of the enzyme acetyl-CoA carboxylase (ACCase). We examined ACCase from wild oats to determine whether the mechanism of resistance to diclofop-methyl might be invested in either an herbicide-insensitive ACCase or in the increased expression of ACCase.
The extractable activities of ACCase from resistant and susceptible biotypes were similar. The activity from susceptible plants was about 1347 n moles (g. fr. wt.)$^{-1}$ h^{-1} whereas that from the resistant plants was about 1142 n moles (g. fr. wt.)$^{-1}$ h^{-1}. It is therefore unlikely that differences in the expression of ACCase contribute to diclofop-methyl resistance. A similar observation was made for diclofop-methyl resistant annual ryegrass, *L. rigidum* biotype SR31 (3, 5).

In crude extracts the affinities of ACCase for the substrate acetyl-CoA were similar. Fifty percent of maximum activity was measured in the presence of 0.16 mM and 0.15 mM acetyl-CoA in extracts from resistant and susceptible plants, respectively. The similarity of these values suggests that differences in the affinity of ACCase for acetyl-CoA are unlikely to contribute to diclofop-methyl resistance in the resistant biotype.

Despite the observation that, at the whole-plant level, the York biotype was three-fold less sensitive to the herbicide diclofop than the susceptible biotype, the concentrations of diclofop-acid required to inhibit ACCase from both biotypes by 50% (I_{50} values) were similar. ACCase from the susceptible plants exhibited an I_{50} value of 0.4 μM whereas ACCase from the resistant plants exhibited an I_{50} value of 0.3 μM. In diclofop-resistant weeds for which it has been suggested that resistance is endowed by an insensitive enzyme, the I_{50} values for the enzyme from resistant tissue are about 100-fold those for the enzyme from susceptible tissues (2). We therefore consider it unlikely that the resistance to diclofop-methyl in the York *A. fatua* biotype is due the presence of a diclofop-insensitive ACCase. In addition to inhibition by diclofop-acid, ACCase from both biotypes was inhibited by fluazifop-acid, haloxyfop-acid, sethoxydim and tralkoxydim.

Other, not necessarily mutually exclusive, possible mechanisms for resistance include reduced changes to membrane potentials, reduced diclofop-methyl uptake, reduced translocation to the site of action or detoxification of the herbicide to non-phytotoxic products. These possibilities are under investigation.

REFERENCES

1. Burton, J.D., Gronwald, J.W., Somers, D.A., Connelly, B.C., Gengenbach, B.G., and Wyse, D.L. 1987. Biochem. Biophys. Res. Commun. 148, 1039-1044.
2. Gronwald, J.W., Eberlein, C.V., Betts, K.J., Rosow, K.M., Ehlke, N.J., and Wyse, D.L. 1989. Plant Physiol. 89s, 115.
3. Holtum, J.A.M., Matthews, J.M., Liljegren, D.R., and Powles, S.B. 1990. Proc. 9th Aust. Weeds Conf.
4. Mansooji, A.M., Holtum, J.A.M., Matthews, J.M., and Powles, S.B. 1990. Proc 9th Aust. Weeds Conf.
5. Matthews, J.M., Holtum, J.A.M., Liljegren, D.R., Furness, B., and Powles, S.B. 1990. Plant Physiol. in press.
6. Piper, T. 1990. Proc. 9th Aust. Weeds Conf.