HERBICIDE RESISTANCE IN A WILD OAT BIOTYPE IS DUE TO MUTANT ACETYL COENZYME A CARBOXYLASE

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Summary. A biotype of wild oat (*Avena sterilis* ssp. *ludoviciana*) in Australia is resistant to a wide range of aryloxyphenoxypropionate (AOPP) herbicides. Possible mechanisms of herbicide resistance in this resistant (R) and a susceptible (S) biotype were investigated. Acetyl-CoA carboxylase (ACCase), the target site for herbicides and a key enzyme of fatty acid biosynthesis, from the R biotype was markedly less sensitive to ACCase inhibiting AOPP (diclofop) and cyclohexanedione (CHD) (tralkoxydim) herbicides than the S biotype. Uptake, translocation and metabolism of [^{14}C]diclofop-methyl were also investigated, but there were no differences between the two biotypes in any of these parameters. Hence, the most probable mechanism of herbicide resistance in this R wild oat biotype is due to a modified form of ACCase.

INTRODUCTION

Wild oats resistant to herbicides have been primarily reported from Canada, USA and Australia (1, 9, 10). In these cases, resistance has occurred following repeated selection with one herbicide or with herbicides from the same class or the same mode of action. Herbicide resistance may be due to the decreased sensitivity of the target site or alteration in uptake, translocation and metabolism of the herbicide. The target site of AOPP and CHD herbicides is acetyl coenzyme-A carboxylase (ACCase), a key enzyme in fatty acid biosynthesis, which catalyzes the conversion of acetyl-CoA to malonyl-CoA (6, 7, 12). Generally, ACCase from grasses is sensitive to both groups of herbicides while that of dicotyledons is insensitive (7, 8). In this paper the mechanism of herbicide resistance in a resistant wild oat biotype has been investigated.

METHODS

Resistant (R) and susceptible (S) wild oat biotypes collected from Bordertown, South Australia in 1989 were used. The R biotype was collected from a field which has been treated with diclofop-methyl and fluazifop-butyl for the previous six years and the S biotype was collected from unsprayed field near the infested area of R biotype (9). Seeds were germinated on 0.6% agar before being transplanted into sterilised potting soil and grown in a growth room. Growth conditions were 20°C, 14 h, 330 µmol photons/m/s light period/16°C, 10 h dark period. Plants at 2-3 leaf stage, the stage normally treated with dicofop-methyl in the field, were used for ACCase inhibition assays. ACCase was extracted and partially purified (13) from both biotypes and the response to herbicides was determined.

Plants at the 2-leaf stage were used for uptake, translocation and metabolism experiments. A solution of 5 mM [^{14}C]diclofop-methyl (a specific activity of 592 MBq/ml) made up in a commercial formulation blank (Hoegrass 36 EC, Hoechst Autralia) was applied as a 1 µL drop of the solution to the leaf axil of wild oat plants. In the translocation experiment, the tissue was divided into small fractions, i.e. meristem (1 cm above the root zone initiation), stem (from meristem up to 2 cm above leaf axil), first leaf, second leaf and third leaf. Each fraction was extracted separately with 80% methanol and the amount of radioactivity was determined by

liquid scintillation spectroscopy. Radiolabelled metabolites were separated by using reverse phase HPLC as previously described (4).

RESULTS AND DISCUSSION

Uptake, translocation and metabolism of [^{14}C]diclofop-methyl. Foliar absorption of diclofop occurred readily in both S and R biotypes with maximal uptake within the first 24 h. Very little label could be detected in the first or third leaves at any time. Most of the activity remained in the stem and leaf. Little label was found in the roots of either biotypes (Table 1).

Table 1. The distribution of radioactivity in wild oat seedlings growing in sand culture

Biotype	Time after treatment (h)	^{14}C activity (% of total uptake)		
		Stem	Leaf	Root
Susceptible	140	54	44	2
	180	40	56	4
Resistant	140	58	40	2
	180	43	54	3

In the translocation experiments, most of the label was present in the stem and second leaf of both biotypes. Slightly more radioactivity was found in the second leaf of the R biotype than in the S biotype at any time. At later time periods more of the radioactivity was observed in the upper parts of second leaf in both biotypes. However there was less translocation from the base of the second leaf in the S biotype (Figs 1A and 1B). This may be because the S plants were dying and therefore translocation was reduced.

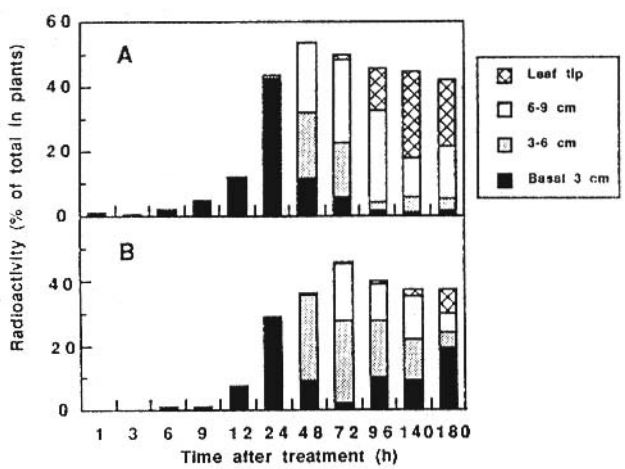

Figure 1. Distribution of [^{14}C]diclofop-methyl in the second leaf of resistant (A) and susceptible (B) wild oat biotypes.

The metabolism of [^{14}C]diclofop-methyl was similar in both biotypes. Conversion of diclofop-methyl to diclofop acid was rapid with less than 50% of applied label recovered as diclofop-methyl in either biotypes by 1 h after application (Table 2). Diclofop acid was slightly greater in the R biotype than in the S biotype at times greater than 12 h after treatment. However, the S biotype showed the herbicidal symptoms whereas the R showed little effect and recovered after 48 h. There were no differences in diclofop-methyl absorption, translocation and metabolism between the two biotypes of wild oat, which suggests that these processes do not account for the the mechanism of herbicide resistance. This is similar to the results found in diclofop resistant wild oats (1) or sethoxydim resistant annual ryegrass biotype SLR 3 (13).

Table 2. Diclofop metabolism in susceptible and resistant wild oats 1, 12, 24 and 48 h after treatment (HPLC data)

Biotype	Time after treatment (h)	^{14}C activity (% of total uptake)		
		Diclofop-methyl	Diclofop acid	Metabolites
Susceptible	1	47 ± 3	53 ± 1	-
	12	8 ± 1	58 ± 7	34 ± 6
	24	8 ± 3	42 ± 4	50 ± 4
	48	6 ± 1	25 ± 3	69 ± 1
Resistant	1	41 ± 1	59 ± 2	-
	12	11 ± 1	53 ± 2	36 ± 1
	24	6 ± 1	39 ± 3	55 ± 3
	48	5 ± 1	21 ± 2	74 ± 2

ACCase inhibition by herbicides. Partially purified ACCase from the S biotype, was inhibited by the AOPP and CHD herbicides. The enzyme purified from the R biotype was less sensitive to these herbicides. ACCase from R biotype was 52 times less sensitive to diclofop than ACCase from the S (Fig. 2A) and 3 times less sensitive to tralkoxydim (Fig. 2B). The result is similar to other observations that AOPP herbicides are more potent inhibitors of ACCase than CHD herbicides (3, 10).

At the whole plant level, the R biotype showed high levels of resistance to AOPP herbicides (diclofop, fluazifop, haloxyfop, fenoxaprop, quizalofop, propaquizafop and quinfurop) and a slight increase in resistance to the CHD herbicides (sethoxydim, tralkoxydim and cycloxydim) (9). This is reflected at the ACCase level with the level of resistance of the enzyme being greater for diclofop (52-fold) than tralkoxydim (3-fold). These results indicate that an altered form of ACCase in the R wild oat biotype is responsible for resistance to these herbicides. A similar mechanism of resistance to these herbicides has been reported for annual ryegrass resistant to sethoxydim in Australia (13) and Italian ryegrass resistant to diclofop in USA (3). In contrast, diclofop-resistant wild oats (*Avena fatua*) from Canada and Australia do not have a mutant ACCase (1, 5). In the case of wild oat from Canada, resistance is related to alteration in membrane properties (2). Currently there are over 20 biotypes of wild oats that have devloped

resistant to AOPP and CHD herbicides in Australia. The mechanism of resistance in the other biotypes has yet to be determined.

In conclusion, the most probable cause of resistance to a range of AOPP herbicides in the R biotype of wild oat is a mutant form of ACCase.

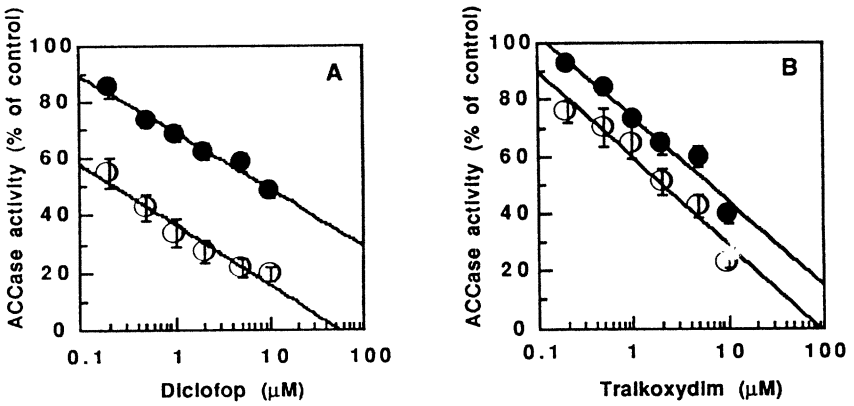

Figure 2. Inhibition of ACCase from susceptible and resistant wild oat biotypes by diclofop (A) and tralkoxydim (B). Open symbols, susceptible; closed symbols, resistant. Experiments were done in duplicate and repeated seven times.

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