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Mechanisms of cross and multiple herbicide resistance in *Alopecurus myosuroides* and *Lolium rigidum*

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Alopecurus myosuroides and *Lolium rigidum* have developed resistance to herbicides with several modes of action in many herbicide classes. *A. myosuroides* biotype Peldon A1 from England exhibits non-target site cross resistance to substituted urea and aryloxyphenoxypropionate herbicides (APP) due to enhanced metabolism. *L. rigidum* biotype SLR 31 from Australia has multiple resistance mechanisms, including both non-target site cross resistance and target site cross resistance. The majority of the SLR 31 population has enhanced metabolism of chlorsulfuron and diclofop-methyl and a mechanism correlated with altered plasma membrane response, which correlates with resistance to some APP and cyclohexanedione (CHD) herbicides. A small proportion of the population also has target site cross resistance to APP and CHD herbicides. While *A. myosuroides* and *L. rigidum* share common biological elements, they are not unique. Non-target site cross resistance and multiple herbicide resistance is predicted to develop in other weed species. The repercussions of cross and multiple resistance warrant proactive measures to prevent or delay onset.

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L'*Alopecurus myosuroides* et le *Lolium rigidum* ont développé des résistances croisées et multiples à des herbicides ayant différents modes d'action et provenant de diverses classes chimiques. Un biotype d'*A. myosuroides*, Peldon A1, possède une capacité élevée de dégradation métabolique des herbicides de types urée substituée et aryloxyphénoxypropionate (APP), lui conférant une résistance croisée non reliée à la cible d'action. Un biotype australien de *L. rigidum*, SLR 31, possède de multiples mécanismes de résistance, comprenant à la fois des mécanismes de résistance croisée non reliés à la cible et d'autres reliés à la cible. La majorité des individus de la population SLR 31 a une capacité élevée de dégradation métabolique du chlorsulfuron et du diclofop-méthyl, en plus d'un mécanisme associé à une altération de la membrane cellulaire, lequel est corrélé avec la résistance à plusieurs herbicides de types APP et cyclohexanediones

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(CHD). De plus, une faible proportion des individus de cette population possède une cible d'action modifiée conférant une grande résistance à tous les APP et CHD. Bien que la biologie d'*A. myosuroides* et de *L. rigidum* présente beaucoup de points communs, ces deux espèces ne sont pas uniques. Nous prédisons que les résistances aux herbicides de type croisée non reliée à la cible et les résistances multiples vont se développer chez d'autres espèces. Les implications potentielles de ces types de résistance justifient l'adoption de mesures préventives.

Nomenclature of chemical names cited in the text:

Chlorotoluron: *N'-(3-chloro-4-methylphenyl)-N,N-dimethylurea*; chlorsulfuron: *2-chloro-N-[[4-methoxy-6-methyl-1,3,5-triazin-2-yl]amino]carbonyl]benzenesulfonamide*; diclofop-methyl: *methyl(±)-2-[4-(2,4-dichlorophenoxy)phenoxy]propanoate*; fenoxaprop-ethyl: *ethyl(±)-2-[4-[(6-chloro-2-benzoxazolyl)oxy]phenoxy]propanoate*; fluazifop-butyl: *butyl(±)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoate*; isotroturon: *N-(4-isopropylphenyl)-N,N-dimethylurea*; quizalofop-ethyl: *ethyl(±)-2-[4-[(6-chloro-2-quinoxalinyloxy]phenoxy]propanoate*; sethoxydim: *2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one*; tetraclasis: *5-(4-chlorophenyl)-3,4,5,9,10-pentaazatetracyclo-5,4,1,0^{2,6},0^{8,11}-dodeca-3,9-diene*; tralkoxydim: *2-[1-(ethoxyimino)propyl]-3-hydroxy-5-(2,4,6-trimethylphenyl)cyclohex-2-one*; trifluralin: *2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)benzenamine*.

INTRODUCTION

Herbicides apply intense selective pressure on weed populations. They can select all available resistance mechanisms. Heritable changes conferring resistance are passed onto subsequent generations. It matters not if they affect herbicide metabolism, alter herbicide target sites or affect seed dormancy. All successful mechanisms can accumulate in treated populations (Powles and Matthews 1992).

Two weed species, *Alopecurus myosuroides* Huds. in England and *Lolium rigidum* Gaud. in Australia, have developed resistance to herbicides from many chemical classes having different modes of action. Some biotypes of both these species are resistant to most selective herbicides registered for their control. Where they occur, these resistant weeds are changing agronomic practice. They can negate successful use of selective herbicides in cereal crop rotations.

Weed populations may be resistant to more than one herbicide either by cross resistance or by multiple herbicide resistance. There are no universally accepted definitions of cross and multiple resistance (Moss and Rubin 1993; Rubin 1991). Definitions based on

the herbicide exposure history of biotypes have proven unsatisfactory because of difficulties inherent to obtaining reliable data. Our definitions, based on resistance mechanisms, are as follow. Cross resistance is defined as the expression of a mechanism that endows an ability to withstand herbicides from different chemical classes. It may be conferred either by a single gene or, in the case of quantitative inheritance, by two or more genes influencing a single mechanism. Two broad categories of mechanisms endowing cross resistance are recognised: target site cross resistance and non-target site cross resistance. Target site cross resistance occurs when a change at the site of action of one herbicide confers resistance to herbicides from a different chemical class that inhibit the same site of action. It does not necessarily result in resistance to all herbicide classes with a similar mode of action or indeed all herbicides within a given herbicide class. Non-target site cross resistance is conferred by a mechanism other than resistant target sites that is efficient over different chemical classes. Potential mechanisms include reduced herbicide uptake or translocation, enhanced herbicide detoxification, or changes in intra- or inter-cellular compartmentation.

Multiple resistance is the expression, within individuals or populations, of more than one resistance mechanism, endowing an ability to withstand herbicides from different chemical classes. The mechanisms in a population can include one or more mechanism which confers cross resistance.

This paper describes cross and multiple herbicide resistance in biotypes of two weed species: *A. myosuroides* (biotype Peldon A1), which illustrates non-target site cross resistance, and *L. rigidum* (biotype SLR 31), which typifies multiple herbicide resistance. Patterns of resistance exhibited by these weed biotypes are compared and the mechanisms of resistance identified are described.

RESISTANT *A. MYOSUROIDES*

Alopecurus myosuroides is an economically important annual grass weed of winter wheat crops in England and Europe. Herbicide-resistant populations have been detected on 60 farms in 10 counties of England. Many populations show resistance to a broad range of herbicides (Kemp *et al.* 1990; Moss 1992; Moss and Rubin 1993). The resistance pattern differs between biotypes, as does the level of resistance to various herbicides within a single biotype (Moss 1992). The best documented resistant biotype is Peldon A1. The mechanism conferring resistance in Peldon A1 is the subject of current research.

Biotype Peldon A1 was selected under a continuous wheat regime based on regular herbicide use (on average > 1.6 applications yr⁻¹) to control weeds. Herbicide selection occurred with substituted ureas, a dinitroaniline, sulfonyleureas and aryloxyphenoxypropionate (APP) herbicides. Peldon A1 is resistant to 23 different herbicides with several modes of action, including some which have not been applied to the population in the field (Moss 1992). Resistance is exhibited to some members of a herbicide class (*i.e.* tralkoxydim) but not others (*i.e.* sethoxydim) (Table 1). With the notable

exceptions of quizalofop-ethyl and fluzifop-butyl, all herbicides to which Peldon A1 is resistant are selective (and readily metabolised) in cereal crops.

Levels of resistance to substituted ureas vary from > 25 for chlorotoluron to > 5 for isoproturon. Despite continuous selection pressure, resistance to chlorotoluron has increased very slowly (Moss 1992). The site of action of chlorotoluron, photosystem II (PS II), has been examined in Peldon A1 and in the susceptible Rothamsted biotype. Electron transport in Peldon A1, measured by oxygen evolution of isolated thylakoids, is inhibited by chlorotoluron in a similar manner to the susceptible biotype, indicating no difference at the PS II active site (L.M. Hall *et al.*, unpublished data). Uptake and accumulation of ¹⁴C-chlorotoluron from aqueous media is similar in Peldon A1 and the susceptible biotype (Kemp *et al.* 1990). Therefore, neither changes at the PS II target site or in uptake and translocation of substituted ureas account for resistance in Peldon A1.

Peldon A1 shows enhanced metabolism of chlorotoluron and isoproturon. Peldon A1 had metabolised 3 times the amount of ¹⁴C-chlorotoluron of the susceptible biotype 96 h after treatment (HAT) (Kemp *et al.* 1990). Similarly, 24 HAT, Peldon A1 had metabolised 1.5 times more ¹⁴C-isoproturon than the susceptible biotype (Kemp *et al.* 1990).

Compared to the susceptible biotype, Peldon A1 is 10 and 4 times more resistant to diclofop-methyl and fenoxaprop-ethyl, respectively. The target site of diclofop-methyl and fenoxaprop-ethyl, the plastid enzyme acetyl CoA carboxylase (ACCase) extracted from resistant and susceptible biotypes, is equally inhibited by these herbicides (Hall *et al.*, unpublished data). Any contribution of differential absorption and translocation of diclofop-methyl and fenoxaprop-ethyl to resistance in Peldon A1 has yet to be examined. However, fenoxaprop-ethyl and diclofop-methyl are metabolised more rapidly in the resistant biotype Peldon A1 than in the susceptible one (Hall *et al.*, unpublished data). Peldon A1 had metabolised 2 times more ¹⁴C-fenox-

Table 1. A comparison of the resistance spectrum of *A. myosuroides* biotype Peldon A1 and *L. rigidum* biotype SLR31^a

Herbicide class	<i>A. myosuroides</i> Peldon A1			<i>L. rigidum</i> SLR 31		
	Resistant	Low level resistance	Susceptible	Resistance	Low level resistance	Susceptible
Substituted urea	chlorotoluron isoproturon diuron linuron					isoproturon
APP ^b	diclofop-methyl fenoxaprop-ethyl fluazifop-butyl quizalofop-ethyl			diclofop-methyl fenoxaprop-ethyl fluazifop-butyl quizalofop-ethyl haloxyfop-ethoxyethyl		
CHD ^c	tralkoxydim	sethoxydim		tralkoxydim	sethoxydim clethodim cycloxydim	
Sulfonylurea	chlorsulfuron			chlorsulfuron metsulfuron-methyl triasulfuron		sulfometuron-methyl
Imidazolinone	imazamethabenz			imazamethabenz imazethapyr		imazapyr
Dinitroaniline	pendimethalin		trifluralin ethalfluralin isopropalin	trifluralin ethalfluralin isopropalin pendimethalin		
Chloroacetamide				alachlor metolachlor propachlor		

^a The information in this table has been compiled from the following: Burnet *et al.* 1994a; Christopher *et al.* 1991; Heap and Knight 1990; Kemp *et al.* 1990; McAlister *et al.* 1994; Moss 1990,1992; S.R. Moss, personal communication; Tardif and Powles 1994.

^b Aryloxyphenoxypropionate.

^c Cyclohexanedione.

aprop than the susceptible biotype 24 HAT. Similarly, metabolism of ^{14}C -diclofop-methyl was more rapid in the resistant biotype.

As both substituted urea and APP herbicides are degraded more rapidly in Peldon A1, it is likely that enhanced herbicide metabolism imparts non-target site cross resistance in this biotype. Metabolism of chlorotoluron and diclofop in wheat depends upon the activity of cytochrome P₄₅₀ mono-oxygenases (Cyt P₄₅₀) (Gonneau *et al.* 1988; Zimmerlin and Durst 1992). There are indications that the enhanced ability of Peldon A1 to metabolise these herbicides is conferred by changes in the level or activity of Cyt P₄₅₀ enzymes. The Cyt P₄₅₀ inhibitor tetcyclasis has been shown to inhibit chlorotoluron metabolism in Peldon A1, and to increase phytotoxicity in whole plants growing in media containing chlorotoluron (Kemp *et al.* 1990).

While levels of resistance conferred by this non-target site resistance mechanism are not high, *A. myosuroides* is unique in the number of herbicides it is resistant to by a single detoxification mechanism.

RESISTANT *L. RIGIDUM*

Lolium rigidum, an annual grass weed abundant in Australia, exhibits the most extensive and complex herbicide resistance known (Heap and Knight 1990; Powles and Matthews 1992). It was recently estimated that > 2000 properties in Australia were infested with herbicide-resistant *L. rigidum*. The salient feature of *L. rigidum* has been its ability to develop multiple herbicide resistance (Holtum and Powles 1991; Powles and Matthews 1992). *Lolium rigidum* has exhibited resistance to at least 12 different herbicide classes affecting 8 different target sites (Burnet *et al.* 1994a, 1994b; McAlister *et al.* 1994; Powles and Matthews 1992). Some populations are resistant to a few herbicide classes whereas others are cross and multiple resistant and can be extremely difficult to control with selective herbicides (Powles and

Matthews 1992). *L. rigidum* biotype SLR 31 will be used to illustrate multiple herbicide resistance.

Biotype SLR 31 has developed resistance following selection with trifluralin and diclofop-methyl. This biotype now exhibits resistance to herbicides in 8 chemical groups including APPs, cyclohexanediones (CHD), sulfonyleureas, imidazolinones, dinitroanilines and chloroacetamides (Table 1). Biotype SLR 31 has accumulated a number of resistance mechanisms which confer both target site and non-target site cross resistance (Table 2).

SLR 31 metabolises both chlorsulfuron and diclofop-methyl at higher rates than a susceptible biotype. Chlorsulfuron half-life in SLR 31 was 6 h, compared to more than 12 h in the susceptible biotype (Christopher *et al.* 1991). The half life of diclofop in the resistant is 19 h, compared to 31 h in the susceptible (Holtum *et al.* 1991). While enhanced metabolism plays a significant role in resistance to chlorsulfuron, the contribution of diclofop metabolism to whole plant resistance remains uncertain. It is not yet established whether the enzymes which degrade chlorsulfuron are the same as those which degrade diclofop.

Resistance to APPs and CHDs in SLR 31 has been correlated with the capacity of membranes to recover from herbicide-induced membrane depolarisation (Häusler *et al.* 1991). The biochemical bases of this phenomenon are unclear as is the causal connection between membrane response and whole plant resistance. The recovery response and its relationship to herbicide compartmentation is continuing to be examined in our laboratory.

While the majority of the resistant SLR 31 population (88%) shows very low level of resistance to sethoxydim, 12% of the individuals are highly resistant to > 64 times the recommended field rate of this herbicide. These individuals are highly resistant to all the other CHD and APP herbicides tested (Tardif and Powles 1994). Thus, this subset of SLR 31 has a different mechanism of resistance to APP and

Table 2. Mechanisms of resistance documented in *L. rigidum* SLR 31

Herbicide	Site of action	Resistance mechanism	Proportion of population	Reference
APP and CHD ^a	ACCase ^b	Target site cross resistance: Resistant ACCase	12%	Tardif and Powles 1994
		Non target site cross resistance: Metabolism ^d	majority	Holtum <i>et al.</i> 1991
		Compartmentation (?)		Häusler <i>et al.</i> 1991
Chlorsulfuron	ALS ^c	Non target site cross resistance: Metabolism	majority	Christopher <i>et al.</i> 1991

^a APP: Aryloxyphenoxypropionate; CHD; Cyclohexanedione.

^b Acetyl-CoA carboxylase.

^c Acetolactate synthase.

^d Only tested with diclofop-methyl.

CHD herbicides than the balance of the population. ACCase extracted from the subset is resistant to inhibition by APP and CHD herbicides, whereas ACCase from the bulk SLR 31 biotype is susceptible (Matthews *et al.* 1990; Tardif and Powles 1994). This mechanism renders the subset of SLR 31 resistant to all APP and CHD herbicides.

The multiple mechanisms found in SLR 31 and their varying frequency can be explained by the herbicide exposure history and the biological characteristics of *L. rigidum*. Biotype SLR 31 was selected for many years with trifluralin, followed by several years of diclofop-methyl treatment. At the rate of diclofop-methyl used for *L. rigidum* control (375 to 500 g a.i. ha⁻¹), several mechanisms permit plant survival. Because *L. rigidum* is an obligate cross-pollinated species, herbicide survivors must cross with each other. Mechanisms of resistance thus accumulated in the progeny (Powles and Matthews 1992). Chlorsulfuron use followed, but its efficacy rapidly failed because the non-target site cross resistance mechanism of enhanced metabolism is effective against chlorsulfuron. When sethoxydim was first used on that population, it initially gave adequate control. The non-target site mechanism(s) effective against diclofop-methyl and chlorsulfuron did not protect against sethoxydim. However, individuals with a resistant form of ACCase survived sethoxydim selection, rapidly increasing

the frequency of resistant ACCase genes in the population.

CONCLUSION

A. myosuroides and *L. rigidum* share several biological characteristics. They are obligate cross-pollinated grass weeds, treated predominantly in cereal crops. They are highly fecund so individuals can contribute large number of seeds to a seed bank which has a rapid turnover. Many other weed species have the capacity to develop either non-target site cross resistance or multiple herbicide resistance and will do so in the future (Powles and Matthews 1992).

Weeds with broad spectrum resistance are difficult to control selectively. In both the United Kingdom and Australia, they have caused changes in cereal husbandry. Farmers are advised to prevent or delay the development of resistance by reducing their reliance on selective herbicides and integrating non-selective chemical and cultural weed control into their weed management strategies. In fields where resistant weeds occur, many farmers have discontinued continuous wheat cropping, introducing alternate crops and pastures into rotations. They rely heavily on non-selective and non-chemical control. If some selective herbicides are still effective, farmers are encouraged to use them infrequently, but at rates which give good control and under

optimum conditions. Integrated weed management has become a standard agronomic practice. While multiple resistance is rare in Canada, integrated weed management techniques, if adopted now, might prevent or delay its occurrence.

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