Frequency, distribution and mechanisms of herbicide resistance in Western Australian wild radish (*Raphanus raphanistrum* L.) populations: a review

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Summary Wild radish is by far the most prevalent and economically damaging broad-leaf weed of cropping systems across the 10 million hectare WA wheatbelt. Wild radish populations are constantly treated with herbicides. Consequently, wild radish populations from the Western Australian wheatbelt have evolved resistance to many of the selective herbicides that are commonly used for their in-crop control. Recent survey results have identified alarmingly high numbers of wild radish populations with resistance to one or more of the sulfonylurea, auxin analog and phytoene desaturase inhibiting herbicides. Previously wild radish populations resistant to triazine herbicides have also been isolated from the WA wheatbelt. Our most recent survey determined that there is now a high frequency of populations with resistance to two or more of these distinct herbicide chemistries (multiple resistance). This paper reviews the current knowledge on herbicide resistance in wild radish populations covering the occurrence, distribution and describing the mechanisms involved.

Keywords Multiple resistance, cross resistance, chlorsulfuron, atrazine, 2,4-D amine, diflufenican.

INTRODUCTION

Wild radish (*Raphanus raphanistrum* L.) is the major dicotyledonous weed of cropping systems of the Western Australian (WA) wheatbelt (Alemseged *et al*. 2001). Herbicides have been used to control this weed since the introduction of the auxin analog herbicides in the 1940s. Further herbicide developments have facilitated the adoption of reduced tillage cropping practices that rely almost exclusively on herbicides for wild radish control in intensive cropping systems. This intensive use of herbicides has unfortunately facilitated the widespread evolution of herbicide resistance in wild radish populations across the WA wheatbelt.

ALS INHIBITING HERBICIDE RESISTANCE

Resistance to ALS inhibiting herbicides has previously been documented for wild radish populations from WA (Hashem *et al*. 2001, Walsh *et al*. 2001, Hashem and Dhammu 2002). Studies on a number of these wild radish populations determined that resistance is due to an altered target site. The most common change in these populations occurred at amino acid position 197 (relative to *Arabidopsis*) of the ALS enzyme, which in susceptible plants is proline but in resistant wild radish has been changed to any of serine, histidine, threonine, or alanine (Tan and Medd 2002, Yu *et al.* 2003, Friesen and Powles, unpublished results). Tan and Medd (2002) also reported a change at position 574 of tryptophan to leucine. These substitutions have been reported before for other weeds (reviewed in Tranel and Wright 2002) and so their presence in wild radish is not unexpected.

Recently we have identified a resistance mutation leading to a change of aspartate to glutamate at position 376 (Friesen and Powles unpublished results). This is a very interesting case of resistance because this mutation is very rare in higher plants. In fact only one report (Tardif *et al.* 2006) has suggested its occurrence in a naturally selected resistant weed population. Normally wild radish plants that are resistant to ALS inhibiting herbicides due to an altered target site have a robust level of resistance which allows them to survive, seemingly unaffected, very high (16 times the recommended rate) application rates of these herbicides (Yu *et al*. 2003, Walsh *et al*. 2004). This is not true of the substitution of aspartate for glutamate. This change appears to make the plants much less resistant to chlorsulfuron than substitutions of proline, for example (Friesen and Powles, unpublished results).

Resistance to chlorsulfuron is now a frequent and widespread phenomenon in wild radish populations from the WA wheatbelt. A recent survey found that 63% of wild radish populations were resistant to chlorsulfuron (Walsh *et al.* 2005). This is a three-fold increase on the 21% of populations identified in a survey conducted in 1999 (Walsh *et al.* 2001). This increase in chlorsulfuron resistance indicates the continued use and reliance on sulfonylurea herbicides for wild radish control in WA crop production systems.

AUXIN ANALOG HERBICIDE RESISTANCE

Despite the long term and extensive use of 2,4-D amine in WA cropping systems for the in-crop control of wild radish the first reported case of resistance

to herbicide was not documented until 2004 (Walsh *et al*. 2004). Until recently only a few examples of 2,4-D resistance in other weed species had been noted (reviewed by Coupland 1994, Sterling and Hall 1997). Currently there are now 24 weed species that have been documented as exhibiting resistance to auxin analog herbicides (Heap 2006).

Of those cases of auxinic resistance in weeds that have been studied worldwide, an altered target site has been clearly demonstrated in several unique weeds and weed populations (e.g. Peniuk *et al.* 1993, Goss and Dyer 2003, Abdallah *et al.* 2006). Non-target-site auxinic resistance in weeds has also been demonstrated (e.g., Barnwell and Cobb 1989, Coupland *et al.* 1990, Lutman and Heath 1990, Coupland *et al.* 1991, Weinberg and Hall 2004). The mechanisms of auxinic resistance in wild radish have not yet been identified.

Across WA there are now over 60% of wild radish populations exhibiting some level of resistance to 2,4-D amine (Walsh *et al*. 2005). Given the extensive and very high frequencies of resistance it is highly likely that resistance to this auxin analog herbicide has been present in WA wild radish populations for some time.

PHOTOSYSTEM II INHIBITING HERBICIDE RESISTANCE

Triazine resistance in a wild radish population from WA has previously been documented (Hashem *et al*. 2001a; Walsh *et al*. 2004). Resistance in this population was caused by an altered target site protein, i.e., the D1 protein of photosystem II, but the exact change was not identified. We have recently studied atrazine resistance in a different wild radish population to that examined by Hashem *et al.* (2001a) and have established that resistance is caused by a mutation leading to the substitution of serine at position 264 (relative to Arabidopsis) to glycine (Friesen and Powles unpublished). This mutation is very common and is almost always the mechanism for atrazine resistance in weed populations worldwide.

An interesting feature of this type of resistance is that it will not be readily transferred via pollen because it is encoded in the chloroplast genome and not the nucleus. This means that its main method of spread is via seed dispersal, as opposed to other types of resistance, which can be spread by seed and pollen flow. This may explain why triazine resistance is still relatively rare despite the many years of selection in lupin and canola crops with the photosystem II inhibiting herbicides simazine and atrazine respectively (Table 1).

Table 1. Number of wild radish populations with resistance (R) and developing resistance (DR) to chlorsulfuron, atrazine, 2,4-D amine and diflufenican, across the agronomic regions of the WA wheatbelt. (Modified from Walsh *et al.* 2005).

Region	Populations collected	Chlorsulfuron		2,4-D amine		Diflufenican		Atrazine	
		R	DR[2]	R	DR	R	DR	R	DR
		No. populations							
H1	12	9	1	1	7	0	9	0	0
M1	10	8	2	0	7	1	8	0	1
L1	10	5	2	1	8	0	3	0	1
H2	7	1	2	1	3	0	2	0	0
M2	5	2	2	1	3	0	1	0	0
L2	9	3	4	0	8	0	3	0	0
H3	7	1	1	0	4	0	1	0	0
M3	6	0	2	0	2	0	1	0	0
L3	7	0	3	0	5	0	6	0	1
H4	2	0	0	0	1	0	0	0	0
M4	3	0	1	0	1	0	2	0	0
L4	3	0	0	0	2	0	2	0	0
H5	0	0	0	0	0	0	1	0	0
M5	3	0	0	0	1	0	2	0	0
L5	1	0	0	0	0	0	0	0	0

[1] Resistance defined as ≥20% population survival.
[2] Developing resistance defined as between 1 and 19% population survival.

PDS INHIBITING HERBICIDE RESISTANCE

Resistance to the phytoene desaturase (PDS)-inhibiting herbicide, diflufenican has been documented in three wild radish populations from WA (Cheam and Lee 2004, Walsh *et al.* 2004). In both instances resistance to diflufenican developed following only four applications of this herbicide.

A PDS mutation leading to resistance in the aquatic weed hydrilla (*Hydrilla verticallata* (L.f.) Royle) has been identified (Michel *et al.* 2004), leading to the possibility that this or a similar mutation may have occurred in wild radish. Also, non-target-site resistance has been suggested for *Poa annua* (Hanson and Mallory-Smith 2000). However, no work to this effect has been reported for wild radish and at this stage the mechanism for resistance in wild radish is unknown.

DISTRIBUTION OF HERBICIDE RESISTANCE

Historically wild radish has been a problem weed principally of the northern region of the WA wheatbelt. In a recent survey approximately two thirds the of wild radish populations collected came from regions one and two (Table 1) (Walsh *et al*. 2005). Across this region wild radish populations have been under intense herbicide selection pressure in long-term continuous crop production systems based on no-till and stubble retention (D'Emden *et al*. 2005). It is no surprise then that this region has the highest proportion of resistant wild radish populations (Table 1). Across region one (H1, M1 and L1) 68% of wild radish populations were found to be resistant to chlorsulfuron. Additionally, 75% and 65% of these populations also contained plants that were resistant to 2,4-D amine and diflufenican respectively. Not only are there high frequencies of resistance to individual herbicides within wild radish populations in region one, but these populations have now accumulated multiple resistant mechanisms. Almost 90% of wild radish populations from region one now have two or more resistance mechanisms (Walsh *et al*. 2005).

There is a marked and continuous decline in the frequency of resistance in wild radish populations from the northern to the southern regions of the WA wheatbelt. Across the central region three (H3, M3 and L3) the frequencies of populations with resistance to chlorsulfuron, 2,4-D amine and diflufenican had decreased to 34, 50 and 35% respectively (Table 1). In the southern-most regions (4 and 5) there were only isolated populations with resistance to the four herbicides evaluated.

DISCUSSION

Wild radish populations from the WA wheatbelt currently have evolved at least four different mechanisms endowing resistance across four distinct herbicide chemistries. Many of these mechanisms are now occurring simultaneously within individual wild radish populations. At present across the WA wheatbelt there is a higher frequency of wild radish populations with multiple resistance mechanisms (48%) than populations that are herbicide susceptible (17%).

The challenge for WA farmers for now and the future is to preserve the availability of the herbicides they have remaining for the control of wild radish populations in their cropping systems.

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