Frequency and distribution of herbicide resistance in *Raphanus raphanistrum* populations randomly collected across the Western Australian wheatbelt

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Summary

In 2003, a random survey was conducted across the Western Australian wheatbelt to establish the extent and frequency of herbicide resistance in *Raphanus raphanistrum* populations infesting crop fields. Five hundred cropping fields were visited, with 90 *R. raphanistrum* populations collected, representative of populations present in crop fields throughout the Western Australian wheatbelt. Collected populations were screened with four herbicides of various modes of action that are commonly used for the control of this weed. The majority of Western Australian *R. raphanistrum* populations were found to contain plants resistant to the acetolactate synthase (ALS)-inhibiting herbicide chlorsulfuron (54%) and auxin analogue herbicide, 2,4-D amine (60%). This survey also determined that over half (58%) of these populations were multiple resistant across at least two of the four herbicide modes of action used in the screening. Only 17% of *R. raphanistrum* populations have retained their initial status of susceptibility to all four herbicides. The distribution patterns of the herbicide-resistant populations identified that there were higher frequencies of resistant and developing resistance populations occurring in the intensively cropped northern regions of the wheatbelt. These results clearly indicate that the reliance on herbicidal weed control in cropping systems based on reduced tillage and stubble retention will lead to higher frequencies of herbicide-resistant weed populations. Therefore, within intensive crop production systems, there is a need to diversify weed management strategies and not rely entirely on too few herbicide control options.

Keywords: herbicide resistance survey, wild radish, chlorsulfuron, atrazine, 2,4-D amine, diflufenican.

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Introduction

Raphanus raphanistrum L. (wild radish) is an important weed of agricultural regions worldwide and is the major dicotyledonous weed of cropping systems of the Western Australian (WA) wheatbelt (Alemseged *et al*., 2001). This problematic annual weed species is highly competitive with annual crops, frequently leading to large yield reductions (Panetta *et al*., 1988; Cousens *et al*., 2001; Blackshaw *et al*., 2002; Eslami *et al*., 2006). The difficulty in controlling *R. raphanistrum* arises from its flexible germination requirements, high reproductive capacity and longevity of seeds in the soil (Reeves *et al*., 1981; Panetta *et al*., 1988; Cheam & Code, 1995; Blackshaw *et al*., 2002).

The introduction of the auxin analogue herbicides in the 1950s heralded the start of herbicidal control of *R. raphanistrum* populations. Further herbicide developments have facilitated the adoption of reduced tillage and stubble retention practices that rely almost exclusively on herbicides for *R. raphanistrum* control in intensive cropping systems. However, *R. raphanistrum* has considerable genetic diversity (Cheam & Code, 1995) and this intensive use of herbicides has facilitated the

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evolution of herbicide resistance in populations within these cropping systems. Over the last decade, resistance to chlorsulfuron (Hashem *et al*., 2001a; Walsh *et al*., 2001), atrazine (Hashem *et al*., 2001b), diflufenican (Walsh *et al*., 2004) and 2,4-D amine (Walsh *et al*., 2004) have been documented for *R. raphanistrum* populations collected from the WA wheatbelt. An earlier survey determined high frequencies of chlorsulfuron resistance in randomly collected crop-field *R. raphanistrum* populations (Walsh *et al*., 2001). However, this limited survey was not across the entire WA wheatbelt and focused on only one herbicide. Therefore, this study aimed to determine the frequency of resistance to four commonly used selective herbicides within randomly collected *R. raphanistrum* populations across the whole WA wheatbelt.

Materials and methods

Seed collection

Crop fields were surveyed over a 4-week period (November and December) just prior to the 2003 grain harvest. In total, 500 cropping fields were visited across the WA wheatbelt (Fig. 1), encompassing an 8 million hectare cropped area. Crop fields were visited at random with 5 km intervals (geo-referenced) in each of 15 agronomic zones of the WA wheatbelt (Fig. 1) (pasture fields were not sampled). Fields were surveyed by two people walking in an inverted 'V' 100 m into the crop, collecting pods from any *R. raphanistrum* plants encountered. Over 500 fields were surveyed in this manner, with *R. raphanistrum* populations collected from 90 fields. Populations were present in a further 47 fields but were not collected, due to either very low densities (< 5 plants found) or immature plants had not produced pods at the time of collection. No *R. raphanistrum* plants were found in the remaining 366 surveyed fields. Collected populations were stored in a non-air-conditioned glasshouse at UWA, Nedlands campus. The following April, *R. raphanistrum* populations were individually processed using a modified gristing mill to remove intact seed from the pods. *R. raphanistrum* seed collections were subsequently stored in the laboratory.

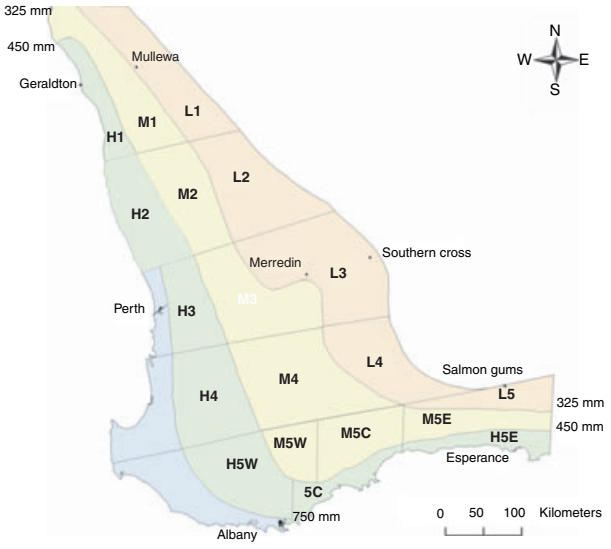

Fig. 1 Agronomic zones of the Western Australian wheatbelt based on growing season temperatures (decreasing from north to south) and rainfall (decreasing from west to east).

Resistance testing

During the 2004, 2005 and 2006 growing seasons (May–September), 100 seeds of each of the 90 *R. raphanistrum* populations were planted in foam boxes (50 cm × 40 cm × 15 cm) containing potting mix (50% composted pine bark, 25% peat and 25% river sand). The plants were grown outdoors at the University of Western Australia (UWA) where they were watered and fertilised as required. Germination and seedling emergence was high (> 80%), ensuring that *c*. 80 individual seedlings were treated for each population. When the seedlings had reached the 2–3 leaf stage, they were treated with herbicide (upper recommended field rates) using a custom-built, dual nozzle cabinet sprayer fitted with 11001 Teejeet® flat fan nozzles delivering herbicide in 100 L ha^{-1} water at 210 kPa and a speed of 3.6 km h^{-1}. Known resistant and susceptible *R. raphanistrum* populations were used as controls for each herbicide treatment. In all experiments, 100% mortality occurred in the known susceptible population (WARR 7), whereas there was always very high proportions (> 90%) of plant survival in the known resistant populations with all herbicides used (data not shown). Herbicide effects were assessed by determining seedling survival 21 days after the final herbicide treatment. Plant count data were converted to percentage population survival for presentation.

Classification of resistant and developing resistant populations

Resistant

R. raphanistrum populations were classified as resistant if 20% or more of the individuals in the population survived the herbicide. The definition of a resistant population used here is: 'Population survival of 20% or greater would result in observed commercial failure in the field, resulting in this herbicide being no longer effective on this weed population'.

Developing resistance

Where there was 1–19% of *R. raphanistrum* plants in a population survived the population was classed as developing resistance. The definition of developing resistance as used here is: 'A population survival level of 1–19% indicates a significant number of resistant individuals in the population that is likely to result in commercial failure the next time this herbicide is applied'.

Susceptible

When all plants were killed by the herbicide treatment.

ALS-inhibiting herbicide

R. raphanistrum populations were screened for resistance to chlorsulfuron (Glean, 750 g a.i. kg^{-1}, WDG; Dupont Australia, North Sydney, NSW, Australia) in 2004 and 2006. When *R. raphanistrum* seedlings were at the 2–3 leaf stage chlorsulfuron was applied at 10 g ha^{-1} with adjuvant [0.25% *V/V* BS1000 (1000 g L^{-1} alcohol alkoxylate) Cropcare Australasia Pty, Murarrie, Qld, Australia]. Seedlings were assessed 21 days after treatment. Seedlings that survived chlorsulfuron were counted and then cut back to the youngest emerging leaf. To confirm seedling resistance, these seedlings were allowed to regrow for 7 days before retreating with chlorsulfuron applied at the same rate. Final plant survival was then recorded 21 days after this second treatment application. There were only minor differences ($< 5\%$) in seedling survival between the first and second herbicide treatments. Results for the 2004 and 2006 chlorsulfuron screens and results were combined for presentation.

Photosystem II inhibiting herbicide

R. raphanistrum seedlings at the 2–3 leaf stage were treated with atrazine (Gesaprim® 500 SC, 500 g a.i. L^{-1}; Syngenta Crop Protection, North Ryde, NSW, Australia) applied at 1 kg ha^{-1} with adjuvant [2.0% *V/V* Hasten, 704 g a.i. L^{-1} esterfied (ethyl based) oilseed rape oil and non-ionic surfactants; Victorian Chemicals, Coolaroo, Vic, Australia]. Seedlings were assessed 21 days after treatment. Survivors were counted and then cut back to the youngest emerging leaf. To confirm seedling resistance, these survivors were allowed to regrow for 7 days before retreating with atrazine applied at the same rate. Plant survival was then recorded 21 days after this second treatment application with only small differences ($< 10\%$) in seedling survival observed between the first and second herbicide treatments. To confirm resistance status, *R. raphanistrum* populations were screened in this manner with atrazine in 2004 and 2005 with the results combined for presentation.

Auxin analogue herbicide

When *R. raphanistrum* seedlings had reached the 2–3 leaf stage they were treated with a commercial 2,4-D amine (Amicide 625®, 625 g a.i. L^{-1}; Nufarm Limited, Laverton North, Vic, Australia) applied at 625 g ha^{-1}. Plant survival was assessed when the *R. raphanistrum* plants had reached the reproductive stage and began to flower. Because of the slow kill rate of 2,4-D amine, survival assessments were delayed until it could be confirmed that plants had progressed to the reproductive stage. To confirm resistance status, *R. raphanistrum* populations were screened in this manner with 2,4-D amine in 2004 and 2005 with the results combined for presentation.

Phytoene desaturase inhibiting herbicide

R. raphanistrum seedlings at the 1–2 leaf stage were treated with 100 g ha^{-1} of diflufenican (Brodal®, 500 g a.i. L^{-1}; Bayer Crop Science Pty, East Hawthorn, Vic, Australia) formulation. Plant survival was assessed 21 days after treatment. Survivors were counted and cut back to the youngest emerging leaf. Seedlings were allowed to regrow for 7 days before retreating with the same herbicide treatment to confirm seedling resistance. Plant survival was then recorded 21 days after this second treatment and only minor differences ($< 5\%$) in seedling survival were observed between the first and second herbicide treatments. To confirm resistance status, *R. raphanistrum* populations were screened in this manner with diflufenican in 2004 and 2005 with the results combined for presentation.

Results and discussion

Resistance to ALS-inhibiting herbicides

Screening of *R. raphanistrum* populations randomly collected from WA cropping fields determined that there were very high frequencies of populations that are resistant to the ALS-inhibiting herbicide chlorsulfuron. Approximately one-third (32%) of collected populations were found to be resistant ($> 19\%$ survival) (Table 1 and Fig. 2) and a further 22% were classified as developing resistance (1–19% survival). Given that the randomly collected populations are representative of WA wheatbelt *R. raphanistrum* populations, a total of 54% of crop-field *R. raphanistrum* populations have plants that are resistant to the ALS-inhibiting herbicide chlorsulfuron. This is a substantial increase in just 4 years on the 21% of populations identified in the initial survey conducted in 1999 (Walsh *et al.*, 2001). Additionally, there were, on average, high proportions of resistant individuals (53%) in resistant populations

Table 1 Percentage of resistant *R. raphanistrum* populations and resistant individuals in these populations in each category for each herbicide

Resistance categories	Chlorsulfuron (%)	Atrazine (%)	2,4-D amine (%)	Diflufenican (%)
Susceptible (0)	46	85	40	54
DR (1–19)	22	12	57	39
R (>20)	32	3	3	1

Zero indicates fully susceptible populations, 1–19% (survival) indicates the percentage of populations developing resistance (DR), and >20% (survival) indicates a resistant population (R).

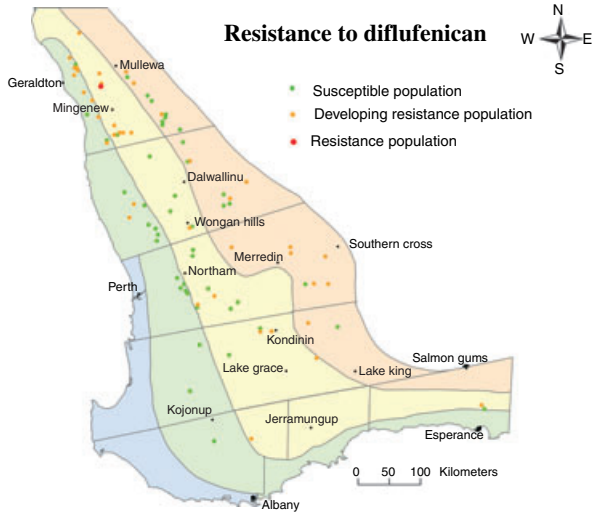

Fig. 2 Map of south-western Western Australia showing the *R. raphanistrum* wild radish populations that are resistant, developing resistance or susceptible to the ALS-inhibiting herbicide chlorsulfuron.

(data not presented). This level of survival in resistant populations, along with the observed increase in chlorsulfuron resistance indicates the continued use and reliance on chlorsulfuron and other ALS-inhibiting herbicides for weed control in WA crop production systems.

High frequencies of chlorsulfuron resistant and developing resistant *R. raphanistrum* populations were identified in the northernmost regions of the WA wheatbelt, with reduced frequencies of resistance to this herbicide identified elsewhere in the wheatbelt (Fig. 2). The highest frequencies of resistant populations were evident in the H1 (75%) and M1 (80%) regions, followed by L1 region where half of the populations collected were identified as chlorsulfuron resistant (Table 2 and Fig. 2). Frequencies of resistant populations in region two (H2, M2 and L2) were around half those of region one (H1, M1 and L1). However, frequencies of developing resistance *R. raphanistrum* populations were, on average, much higher in region 2 (36%) than in region one (16%). Therefore, when combined, there were similar frequencies of populations with some level of chlorsulfuron resistance across these two regions. Across regions one and two, *R. raphanistrum* populations have been under intense herbicide selection pressure in long-term intensive crop production systems based on reduced tillage and stubble retention (D'emden *et al.*, 2006). Much lower frequencies of chlorsulfuron resistance (<40%) were identified in *R. raphanistrum* populations collected from the southern regions. This is most likely due to both reduced occurrence (Table 2) and lower densities of *R. raphanistrum* populations in these regions, combined with lower cropping intensities. Thus, there is a reduced selection pressure for the evolution of chlorsulfuron resistance in the southern regions.

Table 2 Per cent of *R. raphanistrum* populations that are resistant (R) or developing resistance (DR) to herbicides in each agronomic zone (refer to Fig. 1 for agronomic zones)

Region	Populations collected	Chlorsulfuron		Atrazine		2,4-D amine		Diflufenican	
		R	DR	R	DR	R	DR	R	DR
H1	12	75	8	8	33	8	58	0	75
M1	10	80	10	10	20	0	70	10	80
L1	10	50	30	0	20	10	80	0	30
H2	8	13	25	0	13	0	38	0	25
M2	5	40	40	0	0	20	60	0	20
L2	9	33	44	0	11	0	89	0	33
H3	8	13	13	0	13	0	50	0	13
M3	7	0	29	0	0	0	29	0	14
L3	8	0	38	13	0	0	63	0	75
H4	2	0	0	0	0	0	0	0	0
M4	4	0	25	0	0	0	25	0	50
L4	3	0	0	0	0	0	67	0	67
H5	1	0	0	0	0	0	0	0	0
M5	3	0	0	0	0	0	33	0	67
L5	0								

The mechanism(s) responsible for chlorsulfuron resistance in *R. raphanistrum* populations very likely includes an altered target site. Resistance to ALS-inhibiting herbicides is the most common form of resistance in weed populations worldwide, indicating the relative ease with which plants can evolve resistance to these herbicides (Saari *et al.*, 1994; Heap, 2007). In the majority of documented cases, resistance to ALS-inhibiting herbicides is due to an altered target (Saari *et al.*, 1994; Tranel & Wright, 2002). Specifically in *R. raphanistrum*, ALS-inhibiting herbicide resistance has previously been reported in a number of *R. raphanistrum* populations from WA (Hashem *et al.*, 2001a; Walsh *et al.*, 2001; Hashem & Dhammu, 2002), as well as in populations from New South Wales (Tan & Medd, 2002) and South Africa (Smit & Cairns, 2001). Studies on a number of these *R. raphanistrum* populations determined that resistance is conferred by 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 *R. raphanistrum* has been substituted with any of serine, histidine, threonine or alanine (Tan & Medd, 2002; Yu *et al.*, 2003; Friesen and SB Powles, unpubl. obs.). However, substitutions at position 574 of tryptophan to leucine (Tan & Medd, 2002) and aspartate to glutamate at position 376 (Friesen and SB Powles, unpubl. obs.) have also been observed in *R. raphanistrum*. Therefore, the potential of *R. raphanistrum* populations to evolve resistance to ALS-inhibiting herbicides is evident in the numerous amino acid substitutions occurring on the ALS enzyme.

Photosystem II inhibiting herbicide resistance

The survey revealed that, resistance to the photosystem II inhibiting herbicide, atrazine, remains relatively rare in *R. raphanistrum* populations collected from the WA wheatbelt. Only three populations were found to be resistant, with a further 12% classified as developing resistance to atrazine (Table 1 and Fig. 3). Additionally, on average, 21% and 12% of plants survived atrazine in resistant and developing resistance populations respectively. Similarly low frequencies of resistance to triazine herbicides have also been observed in annual ryegrass populations collected from the same areas, with a recent survey by Owen *et al.* (2007) finding very little atrazine resistance. These low frequencies of atrazine resistance are surprising, given the history of use of triazine herbicides in WA crop production systems. Simazine, a closely related herbicide to atrazine, has been used extensively for weed control in lupin (*Lupinus angustifolius* L.) cropping systems in WA for over 25 years. However, it is interesting to note that developing

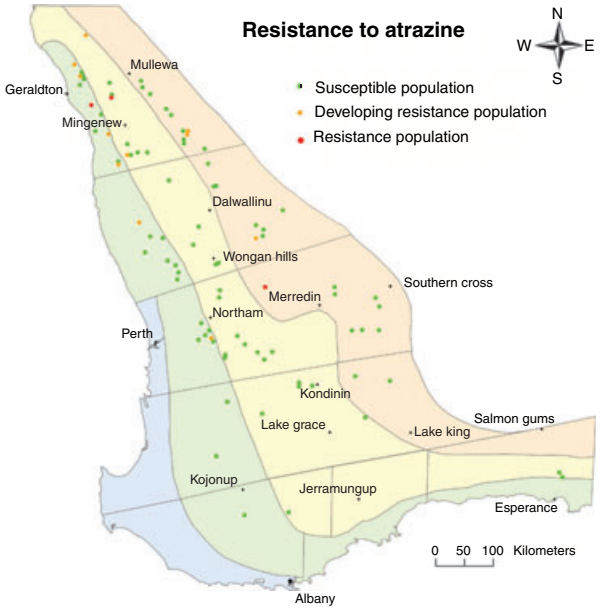

Fig. 3 Map of south-western Western Australia showing the *R. raphanistrum* populations that are resistant, developing resistance or susceptible to the photosystem II inhibiting herbicide atrazine.

resistant *R. raphanistrum* populations were primarily collected from region one, which is also the same area of the WA wheatbelt with the highest intensity of lupin crop production (Fig. 3). The low levels of triazine resistance in WA are in contrast with the high frequency of resistance to triazine herbicides that has occurred globally. Worldwide, triazine herbicide resistance is second only to ALS-inhibiting herbicide resistance in documented cases of herbicide-resistant weed populations (Heap, 2007). Triazine resistance has previously been documented in *R. raphanistrum* populations from WA (Hashem *et al.*, 2001b; Walsh *et al.*, 2004). Resistance in the population documented by Hashem *et al.* (2001b) was caused by an altered target site protein, i.e. the D1 protein of photosystem II, but the exact change was not identified. Recent studies on atrazine resistance in another *R. raphanistrum* population from WA established that resistance is caused by the well-known target site mutation leading to the substitution of serine at position 264 (relative to *Arabidopsis*) to glycine (Friesen and SB Powles, unpubl. obs.). This mutation is very common and is almost always the mechanism responsible for atrazine resistance in weed populations worldwide (Tian & Darmency, 2006). 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 oilseed rape (*Brassica napus*) crops with the photosystem II inhibiting herbicides simazine and atrazine respectively (Table 1).

Auxin analogue herbicide resistance

The survey revealed that across the WA wheatbelt, 60% of the *R. raphanistrum* populations exhibited some level of resistance to the auxin analogue herbicide 2,4-D amine. The majority (57%) of *R. raphanistrum* populations were classified as developing resistance, with a further 3% classed as resistant populations (Table 1). Unlike chlorsulfuron resistance, the distribution of these populations was comparatively uniform across the wheatbelt (Table 2 and Fig. 4). Populations with developing resistance were found throughout the WA wheatbelt, with higher concentrations in regions one and two. Given the extensive distribution and very high frequencies of resistance to 2,4-D amine, it is likely that resistance to this herbicide has been present in WA *R. raphanistrum* populations for some time. Despite the long term and extensive use of 2,4-D amine in WA cropping systems for the in-crop control of *R. raphanistrum,* the first reported case of resistance to this 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 reported (Coupland, 1994; Sterling & Hall, 1997). Currently, there are 24 weed species that have been documented as exhibiting resistance to auxin analogue herbicides (Heap, 2007). Of the cases that have been studied, an altered target site has been clearly demonstrated in several species as the mechanism responsible for resistance to these herbicides (Peniuk *et al.*, 1993; Goss & Dyer, 2003; Abdallah *et al.*, 2006). However, non-target-site resistance to auxin analogue herbicides has also been demonstrated in a number of weed biotypes (Barnwell & Cobb, 1989; Lutman & Heath, 1989; Coupland *et al.*, 1990, 1991; Weinberg *et al.*, 2006). The mechanism/s of auxin analogue resistance evident in *R. raphanistrum* populations identified here have not yet been identified.

Phytoene desaturase inhibiting herbicide resistance

High levels of developing resistance to the phytoene desaturase (PDS) inhibiting herbicide diflufenican were identified in *R. raphanistrum* populations randomly collected from across the WA wheatbelt. Although only one population was found to be resistant to diflufenican, 39% of the *R. raphanistrum* populations were classified as developing resistance to this herbicide (Table 1). Similar to 2,4-D amine resistance, *R. raphanistrum* populations with developing resistance to diflufenican were found uniformly throughout the wheatbelt (Fig. 5). There was no apparent pattern of resistance to this herbicide with high frequencies of developing resistance populations present in several regions (Table 2).

Resistance to the PDS-inhibiting herbicide, diflufenican has previously been documented in *R. raphanistrum* populations from WA (Cheam & Lee, 2004; Walsh

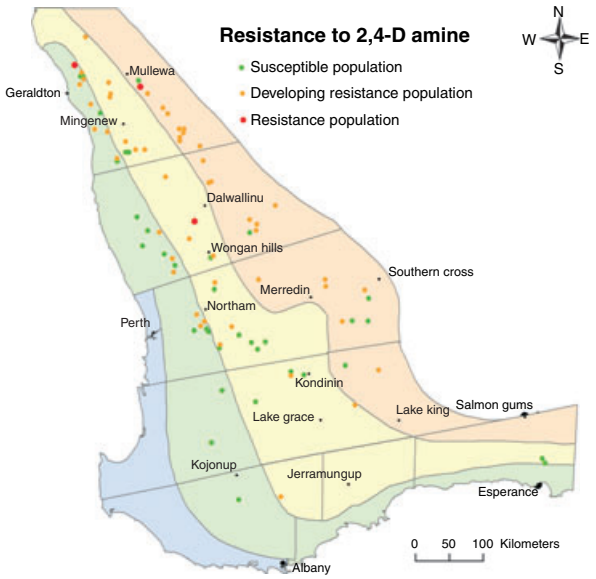

Fig. 4 Map of south-western Western Australia showing the *R. raphanistrum* populations that are resistant, developing resistance or susceptible to the auxin analogue herbicide 2,4-D amine.

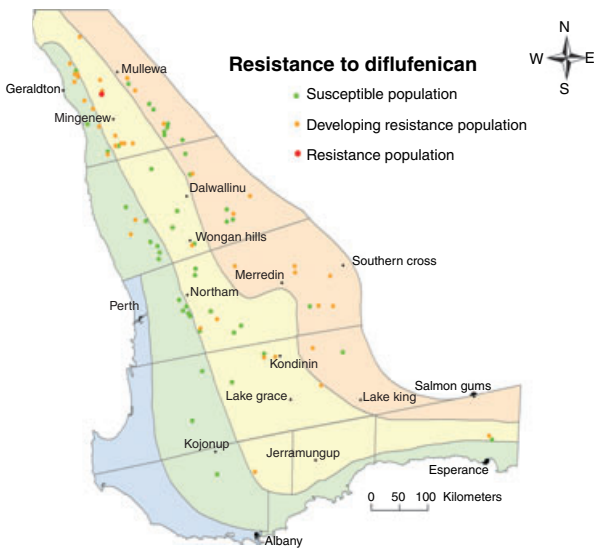

Fig. 5 Map of south-western Western Australia showing the *R. raphanistrum* populations that are resistant, developing resistance or susceptible to the phytoene desaturase inhibiting herbicide diflufenican.

et al., 2004). In two instances, resistance to diflufenican developed following only four applications of this herbicide (Walsh *et al*., 2004). A mutation of the PDS enzyme leading to resistance to the PDS inhibiting herbicide flouridone 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 *R. raphanistrum*. Moreover, non-target-site resistance has been suggested as the mechanism for resistance to norflurazon in *Poa annua* L. (Hanson & Mallory-Smith, 2000). However, no work to this effect has been reported for *R. raphanistrum* and at this stage, the mechanism for resistance in *R. raphanistrum* is unknown.

Multiple herbicide resistance

As all populations examined in this survey were independently treated with the four above-mentioned herbicide classes, the extent of multiple herbicide resistance was determined. More than half of the *R. raphanistrum* populations randomly collected from across the WA wheatbelt displayed multiple herbicide resistance across the different herbicide modes of action (Table 3). The majority (60%) of *R. raphanistrum* populations contained plants multiple resistant across two (35%), three (18%) or four (7%) herbicide modes of action. The most common patterns (20% of populations) of multiple resistance involved combined resistance to chlorsulfuron and 2,4-D (Table 4). A further 17% of populations had multiple resistance to three modes of action, 2,4-D amine, chlorsulfuron and diflufenican. Resistance to chlorsulfuron in *R. raphanistrum* populations is most likely due to an altered ALS enzyme. Although the mechanism/s conferring resistance to 2,4-D amine and diflufenican are unknown, an altered ALS enzyme has no effect on the activity of these herbicides. Therefore, at least two different mechanisms are present in individual *R. raphanistrum* plants.

Table 3 Regional and wheatbelt proportions of populations containing plants with resistance to between 0 and 4 herbicides

	Populations with resistance to following herbicide no. (%)				
Region	4	3	2	1	0
H1	25	25	42	8	0
M1	20	60	0	20	0
L1	0	40	40	20	0
H2	0	0	38	38	25
M2	0	0	80	20	0
L2	0	33	44	22	0
H3	0	0	38	25	38
M3	0	0	14	43	43
L3	13	13	38	25	13
H4	0	0	0	0	100
M4	0	0	50	0	50
L4	0	0	33	67	0
H5	0	0	0	0	100
M5	0	0	33	33	33
Wheatbelt average	7	18	35	23	17

Table 4 Frequency of resistance patterns among *R. raphanistrum* populations with resistance

Herbicide resistance patterns	Population frequency (%)
Susceptible to all herbicides	17
Chlorsulfuron	2
Atrazine	2
2,4-D amine	10
Diflufenican	9
Chlorsulfuron + atrazine	1
Chlorsulfuron + 2,4-D amine	20
Chlorsulfuron + diflufenican	6
Atrazine + 2,4-D amine	0
Atrazine + diflufenican	1
2,4-D amine + diflufenican	7
Chlorsulfuron + atrazine + 2,4-D amine	2
Chlorsulfuron + atrazine + diflufenican	2
Chlorsulfuron + 2,4-D amine + diflufenican	14
Atrazine + 2,4-D amine + diflufenican	0
Chlorsulfuron + atrazine + 2,4-D amine + diflufenican	7

Multiple resistance across three modes of action has previously been identified in populations collected from the WA wheatbelt. Walsh *et al*. (2004) examined two populations (WARR 5 and WARR 6) collected from fields exposed to herbicide selection in continuous crop production systems for 20+ years. WARR 5 was found to be multiple resistant to atrazine, diflufenican and 2,4-D amine, with WARR 6 being multiple resistant to chlorsulfuron, diflufenican and 2,4-D amine. This survey identified the simultaneous resistance to all four of these herbicides in 7% of *R. raphanistrum* populations. This result indicates the stacking of multiple resistance mechanisms in *R. raphanistrum* individuals and populations, posing major control challenges in WA crop production systems.

Multiple resistance was found to be most prevalent in *R. raphanistrum* populations from the northernmost region of the WA wheatbelt. The highest incidence of multiple resistance in *R. raphanistrum* populations was found in populations collected from region one, where *c*. 80% of these populations contained individuals with multiple resistance to between two to four herbicides (Table 3 and Fig. 6). Further evidence of the high incidence of multi-resistance in *R. raphanistrum* populations from this region is seen in the fact that most of these populations exhibited resistance to three or four herbicide modes of action. None of the *R. raphanistrum*

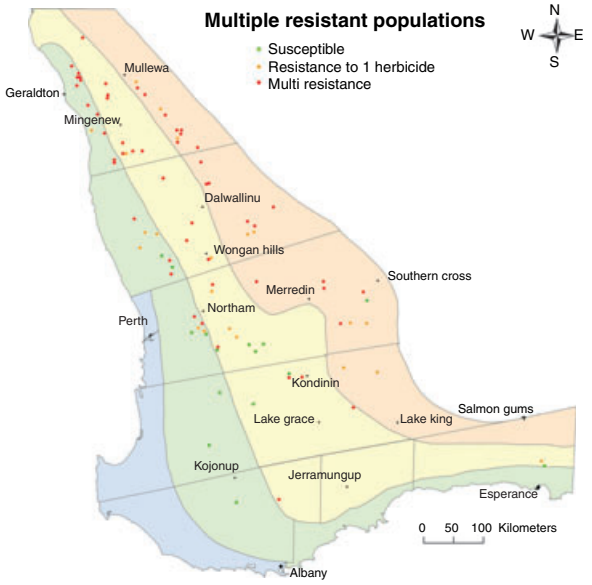

Fig. 6 Map of south-western Western Australia showing the *R. raphanistrum* populations that are multi-resistant to two to four herbicides, resistant to 1 herbicide or susceptible to all four herbicides.

populations collected from this region were found to have retained their original susceptible status to all four herbicides. High frequencies of multiple resistance were also found in region two, where high proportions of populations were resistant to two herbicide modes of action. Resistance to two or more herbicides was less frequent in *R. raphanistrum* populations collected from regions three, four and five.

Conclusions

This survey conducted over an *c.* 8 million hectare region devoted to grain production revealed very high frequencies of resistance to the herbicides relied upon for *R. raphanistrum* control. The most prevalent resistance occurred to the highly effective ALS-inhibiting herbicide chlorsulfuron. High frequencies of developing resistance to 2,4-D amine and diflufenican were also identified. The most dramatic and biggest challenge to crop producers are the very high frequencies of populations/individuals with multiple resistance across two, three or four of the herbicides commonly used for their control. The results from this study indicate the current and future difficulties that producers face in controlling *R. raphanistrum* populations within cropping systems where weed management programmes are heavily reliant on herbicides. Therefore, the future control of *R. raphanistrum* needs to incorporate alternative control measures, either in the management of herbicide-resistant populations or in an effort to avoid the evolution of herbicide resistance.

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