Risk of glyphosate resistance in wide-row lupin cropping systems

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KEY MESSAGES

Using glyphosate as an inter-row annual ryegrass control results in faster development of ryegrass resistance than when glyphosate is applied annually as a pre-sow knockdown. The greatest risk occurs when glyphosate is used both pre-sowing and on the inter-row. However, there is a higher risk when glyphosate is sprayed on the inter-row but not used as a pre-sowing knockdown than when glyphosate is used every year as a knockdown but is not sprayed on the inter-row.

Farmers who use glyphosate for weed management in wide-row lupins may achieve good ryegrass control but they are risking glyphosate resistance and also using the herbicide off-label.

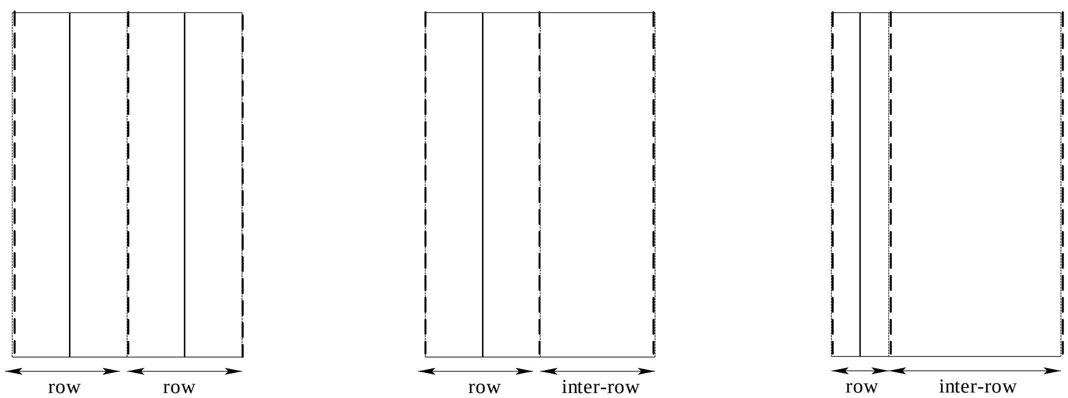
AIMS

The increasing availability of precision agriculture methods allows farmers to control weeds in wide-row systems by spraying non-selective herbicides between rows using a sprayshield. This practice is becoming increasingly popular in lupin crops in Western Australia (WA). However, intensive spraying between rows in crops may result in rapid development and spread of glyphosate resistance. This paper describes the application of a computer model to evaluate risk of glyphosate resistance in lupin wide-row systems in WA.

METHOD

We used a computer model that simulates the evolution and spread of glyphosate resistance in annual ryegrass to evaluate the risk of glyphosate resistance in wide-row lupin systems in WA under typical sequences of management choices.

The model assumes a wheat-lupin rotation, with the lupins planted in a wide-row scheme. Weeds are controlled in the wheat crop by application of a pre-plant knockdown, followed by application of an in-crop selective. Wide-row lupin crops are planted by removing every second row during seeding. Weeds in lupin crops are controlled by application of a pre-plant knockdown, followed by an in-crop selective on the row plus an application of a knockdown herbicide on the inter-row. The size of the row can be varied by varying the area shielded when the inter-row is sprayed. In this model, the row and inter-row widths sum to 52 cm, and the row width is set to be 4, 10, 20 or 30 cm.



(a) Wheat crop. **(b) Lupin crop with equal row and inter-row widths.** **(c) Lupin crop with wider inter-row width.**

Figure 7 Row spacings: solid lines show the position in which crop is sown and dashed lines show the boundaries between row and inter-row.

Two annual ryegrass populations are assumed; the first is composed of plants in the row and the second of plants in the inter-row. The two populations interact in the following ways:

1. After pollen is set, pollen is dispersed randomly across the two populations.
2. The number of seeds set is determined by a spatial competition model that allows plants in one population to have a competitive effect on plants in the other population.
3. After seed set and harvest, remaining seeds on plants are uniformly dispersed between the two populations.

Assumptions about development of herbicide resistance in annual ryegrass

1. Glyphosate resistance is conferred in full by a single dominant resistance gene and the gene mutation rate is 1 in 1000 million per year (Preston, personal communication, 2007).
2. Resistant plants have a reduced fitness effect of 0.33 relative to susceptible plants (Diggle et al. 2009).
3. The initial frequency of resistance genes is one in 1 000 000 (Neve et al. 2003).

We note that the third assumption is not necessarily true because glyphosate has been used in WA for weed control since around 1980. Thus, resistance may occur faster than this model suggests.

Herbicide survival rates for susceptible ryegrass

Spray conditions	Glyphosate/simazine pre-plant	Glyphosate/simazine inter-row	In-crop selective
70% germination before pre-sow spray	0.3*	0.01	0.05
60% germination before pre-sow spray	0.4	0.01	0.05
70% germination before pre-sow spray, poor in-crop control	0.3	0.01	0.2
60% germination before pre-sow spray, poor in-crop control	0.4	0.01	0.2

* Equivalent to an expected survival of 30% of susceptible ryegrass plants.

Plant life-cycle parameters

Seed pool parameters	Lupin	Ryegrass	Wheat
Initial no. of seed in seed pool (seeds/sqm)	0	500	0
Seeding rate (seeds/sqm)	60	0	100
Germination fraction	1	0.75	1
Seed pool survival fraction	0	0.7	0

Competition

The hyperbolic competition model used by Monjardino et al. (2003) was adjusted to allow for competitive effects from neighbouring plant populations, so that the yield/sqm for the *i*-th species is given by:

$$Y_i = \frac{n_i Y_i'}{\kappa_i + \sum_{k=1}^{M} w_k \sum_{j=1}^{N} n_j B_{ij}}$$

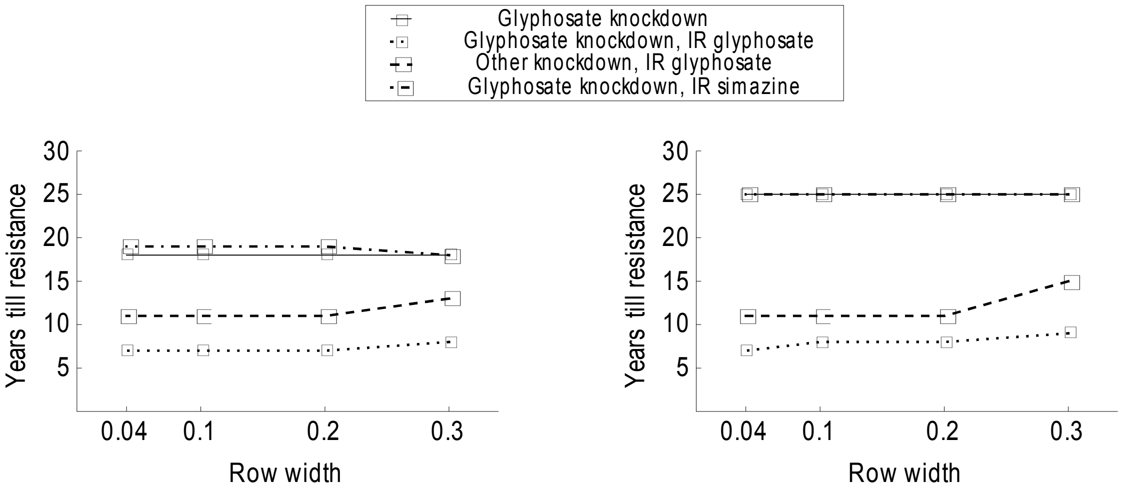
where N is the number of plant species present in the population, n_i is the number of plants in the population belonging to the *i*-th species, κ_i is the competition factor of the *i*-th species, B_{ij} is the inter-specific competition factor defining the antagonistic effect of the *j*-th species on the *i*-th species and w_k are weights defining the competitive effects of the M neighbouring populations on the population under consideration ($k = 1$ is the population on the row and $k = 2$ is the population on the inter-row). The weights w_k are calculated from the row and inter-row widths, by assuming that the distance across which plants compete follows a normal distribution, with standard deviation $\sigma = 0.1$ (so that 99% of competition occurs within 30 cm from the plant). That is, the weights correspond to a one-dimensional Gaussian filter with $\sigma = 0.1$ calculated for irregularly-sized cells.

Competition parameters	Lupin	Ryegrass	Wheat
Maximum seed set (seeds/m^2)	625	35 000	3 500
Competition factor	7	33	11

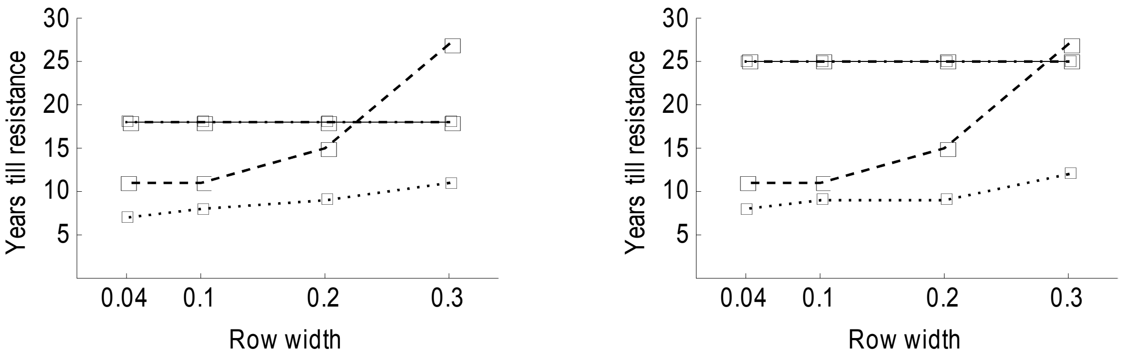
Inter-specific competition factors	Lupin	Ryegrass	Wheat
Lupin	1	0.25	0
Ryegrass	4	1	3
Wheat	0	0.33	1

RESULTS

The models were run for a period of 40 years. The number of years until 50% of ryegrass seeds have genes conferring glyphosate resistance under each of the simulated scenarios is shown in Figure 2.



(a) 70% germination before pre-sow spray. **(b) 60% germination before pre-sow spray.**



(c) 70% germination before pre-sow spray, with poor in-crop control. **(d) 60% germination before pre-sow spray, with poor in-crop control.**

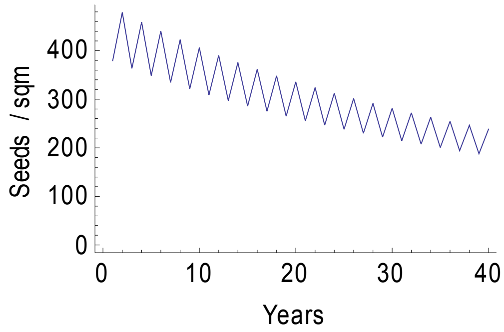
Figure 2 Time till development of resistance under different spray conditions, spray treatments and row widths (in metres).

When 70% of the ryegrass has germinated before applying the pre-sow herbicide, the number of years before resistance develops depends predominantly upon the selection pressure, with glyphosate remaining viable for slightly longer periods when larger row-sizes are used. Use of glyphosate as an inter-row treatment in lupin crops results in faster development of resistance than when glyphosate is used as an annual knockdown.

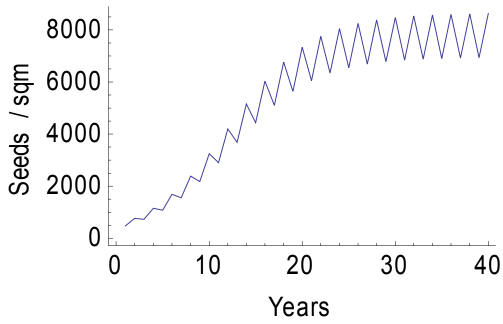
When the knockdown is applied after 60% of the ryegrass has germinated, the time to development of resistance increases when glyphosate is used as an annual knockdown, compared to when the knockdown is applied after 70% germination. This occurs because less ryegrass is exposed to selection pressure. However, development of resistance with the additional use of glyphosate for inter-row control is only slightly slowed compared to when the knockdown is applied after 70% germination. This is due to a corresponding increase in weed numbers caused by the less effective knockdown. This effect is shown in Figure 3, which shows the long-term impacts of different scenarios on the number of weed seeds when there is no resistance and the row and inter-row widths are equal.

In cases when glyphosate is used in the inter-row but ryegrass is poorly controlled in the crop, time till resistance increases with row size. This is again due to higher numbers of weeds in the system.

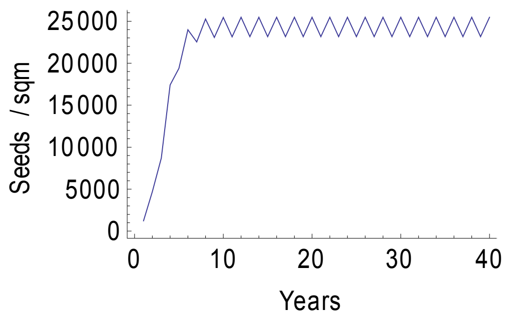
The use of glyphosate as an inter-row weed treatment results in faster development of resistance than when glyphosate is applied as an annual pre-sow knockdown in all cases, except when there is poor in-crop control and a very small inter-row size. The exception occurs when there are large numbers of susceptible weed plants in the row, i.e. ineffective overall weed management.



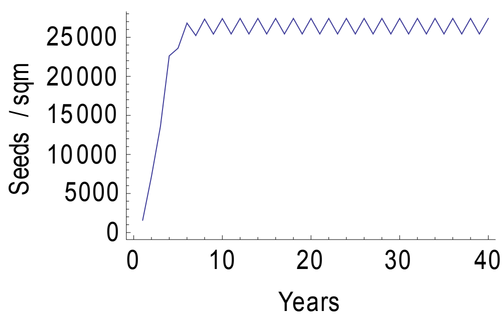
(a) 70% germination before pre-sow spray.



(b) 60% germination before pre-sow spray.



(c) 70% germination before pre-sow spray, with poor in-crop control.



(d) 60% germination before pre-sow spray, with poor in-crop control.

Figure 3 Ryegrass seed numbers through time, with no resistance.

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KEY WORDS

glyphosate resistance, wide-row, precision planting, simulation, model

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More glyphosate-resistant annual ryegrass populations within Western Australia

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KEY MESSAGES

- Three populations of annual ryegrass collected from the eastern wheat belt and Esperance area of Western Australia (WA) showed a 2.5–4 times resistance factor to glyphosate compared to a known susceptible population.
- One population of annual ryegrass collected from a vineyard in the Margaret river area showed a 10 times resistance factor to glyphosate compared to a known susceptible population.
- The total documented number of glyphosate-resistant annual ryegrass populations in WA is now nine.

AIMS

The aim of this study was to confirm resistance to glyphosate in several annual ryegrass populations collected from different regions of WA.

METHOD

Four suspect glyphosate-resistant populations (R biotype) of annual ryegrass were collected during the 2006 season from different regions of WA. Seed of R biotype GR1 was collected from a vineyard, in the Margaret river area in 2006 following application of glyphosate between rows of grape vines. Seed of R biotype BC1 was collected from a cropping paddock in the eastern wheat belt in 2006 following application of glyphosate as a knockdown and in-crop application of selective herbicides. Plants of R biotypes M23 and M24 that survived autumn application of glyphosate in 2006 were collected from two cropping paddocks in the Esperance region of WA in late autumn, grown outdoors at the Dryland Research Station, Merredin and seeds were collected from the surviving plants in spring 2006.

In 2007, plants of each biotype were grown under glasshouse conditions and treated with 1x and 2x label rates of glyphosate at the 3–leaf stage along with a known susceptible population, namely 'Safeguard' (S biotype), at the Cropping Systems Centre, Northam. Seeds from surviving plants (F1) were collected in spring 2007.

In 2008, dose response curve tests with glyphosate were conducted on the F1 plants of the R biotypes along with the S biotype under glasshouse conditions. Plants of each biotype were treated with different rates of glyphosate (0, 67.5, 135, 270, 540, 1080, 2160, 4320, and 8640 g a.i./ha). Plant survival of each population was recorded, LD_{50} (effective dose of glyphosate that killed 50 per cent of the treated population) determined by probit analysis (GENSTAT edition 10) and LD_{50} ratio of the R biotype to S biotypes calculated.

RESULTS

Dose response tests showed that plant survival in BC1, GR1, M23 and M25 biotypes of annual ryegrass was 25 per cent or greater at 540 g a.i./ha (label rate) of glyphosate while all the plants of S biotype (Safeguard) died at this rate (Figure 1). In the R biotypes, 17–32 per cent of plants survived at 1080 g a.i./ha, 8–31 per cent survived at 2160 g a.i./ha and 3–14 per cent survived at 4320 g a.i./ha of glyphosate. No plant survived in any of the R biotypes at 8640 g a.i./ha glyphosate. Among the R biotypes, clearly GR1 had the highest plant survival at the higher rates of glyphosate (Figure 1).

The LD_{50} ratio (an indication of the level of resistance in R biotype compared to S biotype) showed that the R biotype BC1, M23, M24 and GR1 were 2.5, 3, 4 and 10 times more resistant than the S biotype respectively (Table 1).

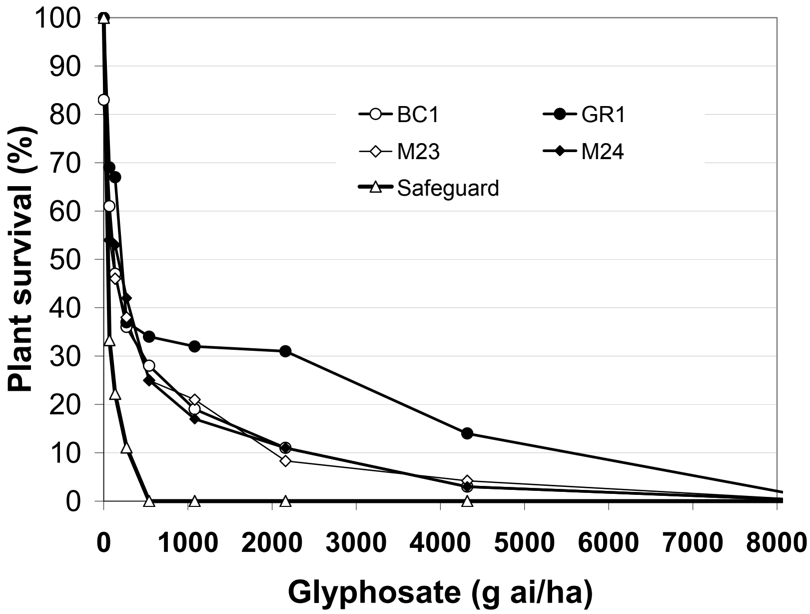

Figure 1 Response of F1 plants of resistant and susceptible biotypes of annual ryegrass to different rates of glyphosate under glasshouse conditions in 2008.

These results clearly established that the GR1 biotype that came from the vineyard near Margaret river was highly resistant to glyphosate. Continuous application of glyphosate between the rows of grape vines over many years might have resulted in the evolution of high resistance to glyphosate in this population of annual ryegrass.

The R biotypes such as BC1, M23 and M24 that came from cropping paddocks were 2.5–4 times more resistant than the S biotype. The growers reported partial control failure of these populations at > 500 g a.i./ha glyphosate prior to collection of seed or plants.

Table 1 **LD_{50} ratios, locations and situations of different glyphosate-resistant populations of annual ryegrass. The $LD_{50}$of the S biotype 'safeguard' was 157.5 g a.i./ha glyphosate**

Resistant biotypes of annual ryegrass	Location	Situation	LD_{50} ratio
BC1	Eastern wheat belt	Cropping area	2.5
GR1	Margaret river area	Vineyard	10.3
M23	Esperance area	Cropping area	3.1
M24	Esperance area	Cropping area	3.9

CONCLUSION

The total number of documented glyphosate-resistant populations in WA is now 9. Evolution of glyphosate resistance in annual ryegrass within the grain belt of WA is of great concern. An effective management plan is necessary to prevent the explosion and spread of these glyphosate-resistant populations within the WA wheat belt.

KEY WORDS

annual ryegrass, glyphosate resistance.

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Western Australian farmers are sowing herbicide-resistant weed seed into their cropping paddocks!

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KEY MESSAGES

- WA farmers have significant weed seed contamination in their crop seed.
- WA farmers are sowing herbicide-resistant weed seed into their cropping paddocks.

AIMS

The aims were to:

1. Determine the extent of weed seed contamination present in the crop seed sown in the WA grain belt farming systems.
2. Understand the effect of seed source (i.e. farmer retained, certified seed) and seed cleaning techniques on the degree of contamination.
3. Identify the herbicide resistance status of the infesting weed seeds.

BACKGROUND

Many Australian farmers store harvested seed for subsequent crop seeding (especially cereals) rather than purchase new seed. However, harvested crop seed can be contaminated with weed seed and if seed cleaning operations are only partially effective, weed seed can then be planted with the crop. Minimising the introduction of weeds into the farming system through sowing of clean crop seed is an important component of farm hygiene. As well as adding to the weed burden already present, foreign seed contamination may also introduce unwanted species and herbicide resistant biotypes into fields leading to long term yield losses and expensive management solutions. This has particular consequences for Western Australian cropping as herbicide resistant annual ryegrass (*Lolium rigidum* L.), wild oats (*Avena fatua* L.), and wild radish (*Raphanus raphanistrum* L.) populations are widespread throughout the WA grain belt (Owen et al. 2007, Walsh et al. 2007, Owen and Powles 2009).

METHOD

In 2007/8 a study was conducted to quantify the extent of weed seed contamination in grain seed used for sowing. Farmers were asked a series of questions about seed cleaning methods, whether crop seed was cleaned prior to sowing and the source of their crop seed. A total of 183 grain samples (~10 kg), of which half were wheat and half an alternative crop (i.e. barley, lupins, pea, canola and oats), were provided by 78 farmers from across the WA grainbelt (Figure 1).

Crop seed samples were cleaned by hand and the total weed seed contamination was determined. Weed seeds were then screened for herbicide resistance status using the most common herbicides used for the control of the particular weed species. Seedlings were sprayed at the 2–3 leaf stage and assessed for mortality 21 days after treatment.

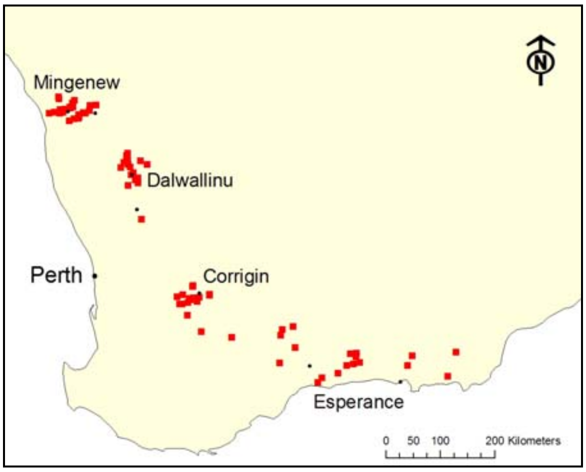

Figure 1 Map of the Western Australian grain belt, showing the regions where samples were collected in 2007 and 2008.

RESULTS

Grain Contamination

Of the 78 farms surveyed, the majority of crop seed samples (97%) were cleaned before crop sowing. Nearly all farmers surveyed (95%) grew their own grain for crop seed, with only 5 per cent purchasing external seed. Of farmers growing their own crop seed, the majority (70%), of crop seed was cleaned by external seed cleaners. Wheat accounted for over half of the samples, followed by barley and then lupins.

In total, 74 per cent of grain samples collected in this survey had some level of weed seed contamination even though 97 per cent of farmers stated that they had cleaned their grain. Contamination levels were highly variable between samples with an average of 14.1 ± 0.3 weed seeds per kg of crop seed (Table 1). The main contaminant weed was annual ryegrass (8.9 seeds/kg), which occurred in over half of all samples, followed by wild radish (2.7 seeds/kg), brome grass (*Bromus* sp) (1.3 seeds/kg) and wild oats (< 1 seeds/kg), which were all found in approximately one third of samples. A number of other weed seed contaminates were found in the grain sample but at low levels (Table 1). Figure 2 shows that annual ryegrass was also the most frequent contaminant followed by wild radish. Of the non-‘weedy’ species, volunteer cereal and legume grains accounted for 15–18 per cent of foreign material. There were at least 11 different weed species contaminating the grain samples surveyed (Table 1).

Table 1 **Average weed seed number infesting 1 kg of crop seed**

Species		Number of weed seeds per 1 kg crop-grain seed		
		All samples	Externally cleaned	Self cleaned (on farm)
Annual ryegrass	*Lolium rigidum*	8.9 ± 3.2	8.8 ± 4.2	9.8 ± 5.0
Wild radish	*Raphanus raphanistrum*	2.7 ± 0.7	1.3 ± 0.4	5.1 ± 2.0
Brome grass	*Bromus* spp.	1.3 ± 0.4	0.8 ± 0.3	2.6 ± 1.0
Wild oats	*Avena fatua*	< 1.0		
Barley grass	*Hordeum* spp	< 0.5		
Small-flowered mallow	*Malva parviflora*	< 0.5		
Doublegee	*Emex australis*	< 0.5		
Thistle	*Carthamus* spp.	< 0.5		
Silver grass	*Vulpia* spp	< 0.5		
Paddy melon	*Cucumis myriocarpus*	< 0.5		
Afghan melon	*Citrullus lanatus*	< 0.5		
Volunteer legume		< 0.5		
Volunteer cereal		< 0.5		

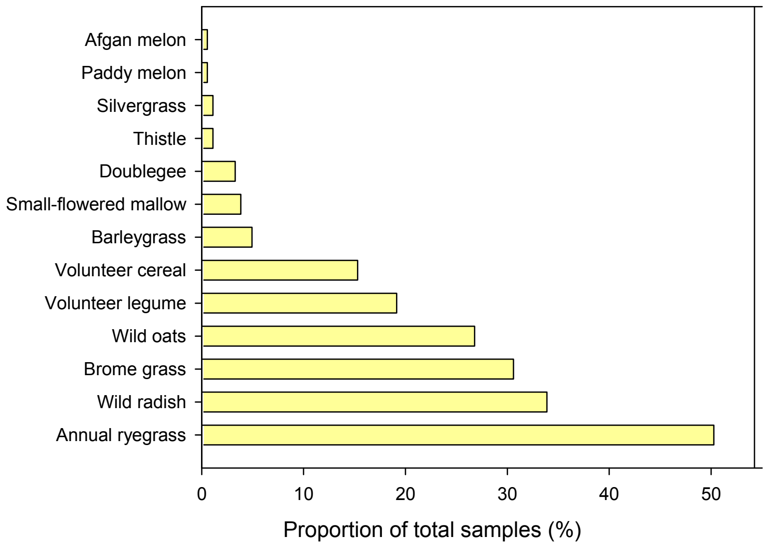

Figure 2 Seed contamination as a proportion of total samples (183).

Herbicide resistance status of weed seed contaminating crop seed

The annual ryegrass seeds collected from farmer grain samples were tested for herbicide resistance with Group A and B herbicides. The majority of these weed seed populations found in the crop seed were resistant to Group A—diclofop-methyl (84%) and Group B—sulfometuron (91%) (Table 2). Wild radish showed resistance to the group B herbicide chlorsulfuron (53% Table 2). Wild oat showed resistance to the Group A herbicide diclofop-methyl (40% Table 2), however, no resistance was found in brome grass to any Group A herbicide tested. Brome grass populations were not treated with Group B herbicides. The majority of wild radish resistant populations came from the more northern regions, while ryegrass resistance was spread across the whole state. This result is similar to that found in our recent field herbicide resistance surveys in WA (Owen et al. 2007; Walsh et al. 2007). All of the ryegrass populations resistant to the in-crop herbicides were controlled by the knockdown herbicide glyphosate (Table 2).

Table 2 **The percentage resistance of weed populations contaminating crop seed displaying resistance to each herbicide**

Weed	Herbicide	Group	Rate (g/ha)	Populations tested	% of Populations resistant
Annual ryegrass	diclofop-methyl	A	563	61	84%
	haloxyfop	A	52	4	50%
	clethodim	A	60	17	56%
	sethoxydim	A	186	6	33%
	sulfometuron	B	15	23	91%
	glyphosate	M	540	14	0%
Wild radish	chlorsulfuron	B	15	32	53%
	diflufenican	F	100	11	27%
Wild oat	diclofop	A	563	20	40%
Brome grass	fluazifop	A	79	31	0%
	clethodim	A	60	6	0%
	glyphosate	M	540	3	0%

CONCLUSION

The survey revealed that nearly all WA farmers use or employ crop seed cleaning techniques to remove contaminating weed seed prior to planting their crop. However, notwithstanding crop seed cleaning, significant weed seed remains in the crop seed sample, especially the two most important WA crop weeds; ryegrass and wild radish. It is therefore clear that many WA farmers are unknowingly introducing weed seed and herbicide resistance into their paddocks during the crop seeding process. This can be minimised by scrupulous seed cleaning, such as the use of a gravity table, to remove contaminating weed seed and reduce the risks of a weed burden on farm. With 'farmer saved' seed it is important to ensure a weed free paddock as seed grading is not always totally effective. Whilst there was a high proportion of crop samples with weed seed contamination it must be noted that 25 per cent of crop samples were weed free, indicating that it is possible to achieve clean crop seed. The seeding operation could be one way in which herbicide resistance occurs by movement of herbicide-resistant weed seed and attention needs to be given to crop hygiene to prevent such spread. Using only weed free crop seed may help to prevent the establishment and spread of new weed species, noxious weeds and herbicide resistance. Cost may be one factor preventing the improvement of grain-cleaning but this cost may be small compared to the cost incurred as a result of sowing weed seeds into cropping paddocks.

KEY WORDS

grain contamination, weed seed, herbicide resistance

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