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Identification of glyphosate-resistant *Lolium rigidum* and *Raphanus raphanistrum* populations within the first Western Australian plantings of transgenic glyphosate-resistant canola

Michael B. Ashworth[A,B,C], *Michael J. Walsh*[A,B], *Ken C. Flower*[B], *and Stephen B. Powles*[A,B,D]

[A]Australian Herbicide Resistance Initiative, The University of Western Australia, Stirling Highway, Crawley, WA 6009, Australia.
[B]School of Plant Biology, The University of Western Australia, Crawley, Stirling Highway, WA 6009, Australia.
[C]Research Fellow, Department of Agriculture and Environment, School of Science, Curtin University, Bentley, WA, Australia.
[D]Corresponding author. Email: stephen.powles@uwa.edu.au

Abstract. Transgenic glyphosate-resistant canola was first commercially grown in Western Australia (WA) in 2010, providing an opportunity to obtain important baseline data regarding the level of glyphosate resistance in weeds following the exclusive use of glyphosate for in-crop weed control. In this study, two surveys (2010 and 2011) were conducted across the 14 Mha of the grainbelt of WA. The 2010 survey was carried out at the late-flowering stage of glyphosate-resistant canola, whereas the 2011 survey was conducted at an earlier growth stage (6–8 leaves), ~2–3 weeks after the second in-crop glyphosate application. During the surveys, 239 fields were visited, representing an estimated combined area of 24 000 ha. The 2011 survey alone represented a subsample of 23% of the total glyphosate-resistant canola planting in the WA grainbelt for that season. Glyphosate resistance was identified in one population of wild radish (*Raphanus raphanistrum* L.) and in eight annual ryegrass (*Lolium rigidum* L.) populations. None of the tested capeweed (*Arctotheca calendula* (L.) Levyns) populations were glyphosate-resistant. In this survey, no populations of barley grass (*Hordeum* spp.), brome grass (*Bromus* spp.), wild oat (*Avena* spp.) or small-flowered mallow (*Malva parviflora* L.) survived glyphosate application. Despite a long history of pre-seeding and fallow glyphosate use in WA, this survey found that glyphosate still provides excellent in-crop control of most species; however, some resistance is evident, requiring diverse weed control techniques to limit their spread.

Additional keywords: annual ryegrass, genetically modified crops, glyphosate resistance, herbicide resistance, Western Australia, wild radish.

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Introduction

Over the past 50 years, weed control in major crops has been achieved efficiently and economically with herbicides. Of all the herbicides available, glyphosate (*N*-(phosphonomethyl) glycine) is the world's largest selling and most important. Glyphosate inhibits the enzyme EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), preventing the biosynthesis of the aromatic amino acids required for the production of anthocyanins, lignin, growth regulators, phenolics and proteins (Amrhein *et al*. 1980; Steinrücken and Amrhein 1980). However, the efficacy of glyphosate is threatened by heavy reliance and the lack of weed-control diversity surrounding its use. In North and South America, the widespread adoption of glyphosate-resistant crops and the consequent ability to use glyphosate as an inexpensive, selective in-crop herbicide has applied a continuous and intense selection pressure on weed species. Glyphosate overuse has resulted in the evolution of glyphosate resistance in 14 weed species and in shifts towards a cohort of weed species that is only partially controlled by glyphosate (Heap 2014).

Australian dryland cropping systems contain some problematic, multiple herbicide-resistant weeds (Walsh *et al*. 2007; Owen and Powles 2009*a*; Michael *et al*. 2010; Owen *et al*. 2014). As a tool to control these multi-resistant species economically, glyphosate-resistant canola was first grown in Western Australia in 2010. However, in WA, glyphosate resistance has already been identified in annual ryegrass (*Lolium rigidum* L.) (Neve *et al*. 2004; Owen and Powles 2010), jungle rice (*Echinochloa colona* L.) (Gaines *et al*. 2012) and, most recently, wild radish (*Raphanus raphanistrum* L.) (Ashworth *et al*. 2014). Previous random surveys of the WA grainbelt at harvest have not identified glyphosate resistance in other problematic weed species including wild oat (*Avena* spp.) (Owen and Powles 2009*b*), barley grass (*Hordeum* spp.) (Owen 2014), brome grass (*Bromus* spp.)

(Owen 2014) or fleabane (*Conyza* spp.) (Owen *et al*. 2009). However, the identification of glyphosate resistance in these species may be concealed by the use of post-emergent herbicides.

The introduction of genetically modified glyphosate-resistant canola to the WA grainbelt, and the potential resultant exclusive use of glyphosate as an in-crop post-emergent herbicide provided a unique opportunity to establish important baseline data in 2010 concerning the prevalence of glyphosate resistance in weed populations in the WA grainbelt. In this study, we report the frequency and distribution of glyphosate-resistant weed species in the canola-growing regions of the WA grainbelt before the long-term adoption of transgenic glyphosate-resistant crops.

Materials and methods

Population collection

In 2010 and 2011, commercial plantings of glyphosate-resistant canola were identified by regional agronomists and consultants, with each field confirmed to have received two glyphosate applications (Fig. 1).

In 2010, 73 glyphosate-resistant canola fields were surveyed at the early flowering stage (GS 67; Lancashire *et al*. 1991), between 4 and 22 October (Fig. 1). Surviving wild radish plants were sampled by collecting all siliques (minimum 20) along three inverted-V transects, 100 m into each field. In total, 24 wild radish populations were collected. Wild radish siliques were stored in a glasshouse under ambient conditions over summer to break dormancy, before the seed was extracted with a modified 'grist mill'.

In 2011, 166 glyphosate-resistant canola fields were surveyed (Fig. 1) over a 4-week period between 17 May and 24 June, at the 6–8 true-leaf stage of canola (GS 18), ~2–3 weeks after the second recommended glyphosate application. Surviving weeds were carefully removed with soil and intact root systems. The shoots were trimmed to reduce transplant shock, wrapped in moistened paper towel, and express-posted to The University of Western Australia via an overnight courier service. On arrival, all samples were immediately planted into 180-mm-diameter plastic pots containing potting mixture (25% peat moss, 25% sand and 50% mulched pine bark) and allowed to re-grow. In total, 27 annual ryegrass, 19 wild radish and four capeweed populations (comprising a minimum of five plants per population) were collected.

Resistance testing

For all experiments, plants were grown outdoors during their normal growing season, May–November, at UWA. Plants were watered as required and fertilised weekly with 2 g Scotts

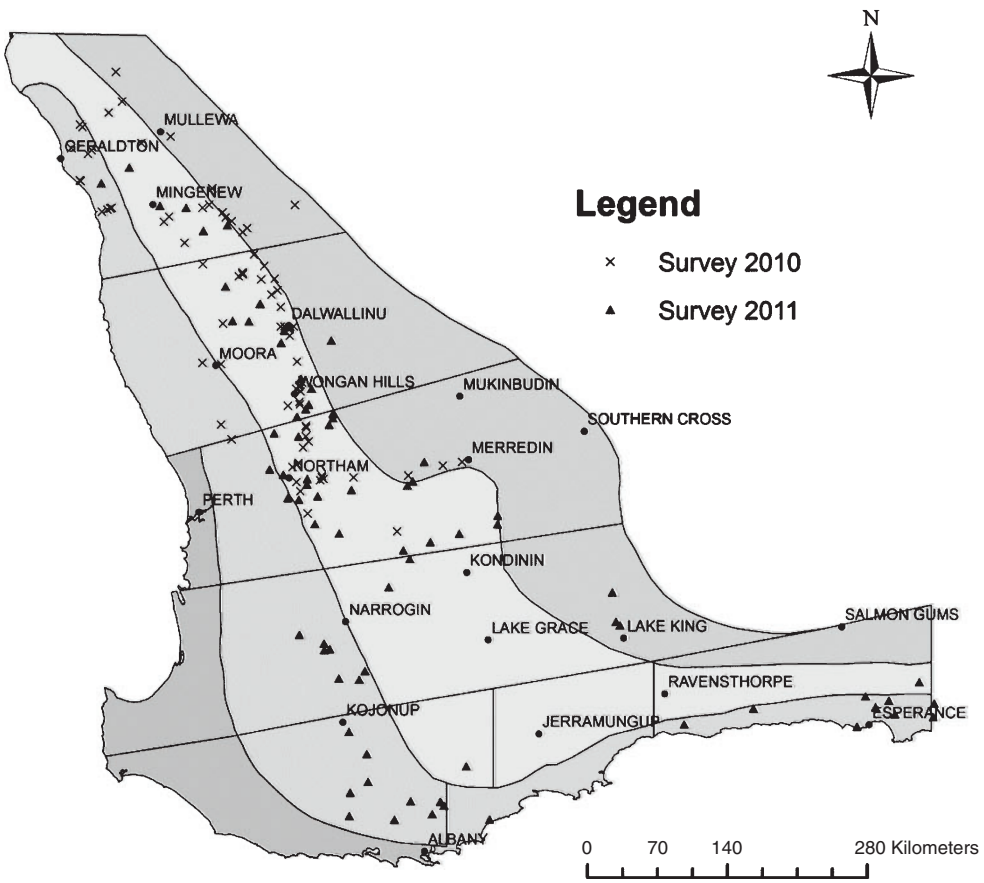

Fig. 1. Locations of surveyed glyphosate-resistant canola fields across the Western Australian grainbelt in 2010 and 2011. Average rainfall isohyets shown: high 450–750 mm, medium 324–450 mm, low <325 mm.

PolyFeed™ soluble fertiliser (Scotts Australia, Bella Vista, NSW) containing nitrogen 19% (urea 15%, ammonium 1.9%, nitrate 2.1%), phosphorus 8%, potassium 16%, magnesium 1.2%, sulfur 3.8%, iron 400 mg kg^{-1}, manganese 200 mg kg^{-1}, zinc 200 mg kg^{-1}, copper 100 mg kg^{-1}, boron 10 mg kg^{-1} and molybdenum 10 mg kg^{-1}. A known susceptible wild radish population, WARR7 (referred to hereafter as S1) (Walsh *et al*. 2004), and the transgenic glyphosate-resistant canola line (*Brassica napus* cv. Roundup Ready® Cobbler) were included as glyphosate-susceptible and -resistant controls, respectively. Herbicide treatments were applied by using a twin-nozzle laboratory sprayer fitted with 110° 01 flat-fan spray jets (TeeJet®, Glendale Heights, IL, USA) delivering herbicide in 100 L ha^{-1} of water at 210 kPa, travelling at a speed of 3.6 km h^{-1}.

Wild radish seed populations collected in 2010

In April 2011, ~250 seeds from each of the 24 wild radish populations collected in 2010 were germinated on solidified water agar (0.6% w/v), containing 1 μM of the germination stimulant karrikinolide (KAR1), in the dark for 2 days. Pre-germinated seeds were then planted into four separate replicate polystyrene foam trays (400 mm wide by 500 mm long by 150 mm deep) containing potting mixture (25% peat moss, 25% sand and 50% mulched pine bark). At the 2-true-leaf stage (GS 12), seedlings were treated with glyphosate (Roundup Ready Herbicide® 690 g kg^{-1}; Nufarm, Laverton North, Vic.) at 540 g ha^{-1}. Plant survival was assessed 42 days after treatment (DAT) by inspecting the rosette of each seedling. If new green growth was evident, the plant was deemed to have survived herbicide application. All surviving plants were cut back to the last newly emerging leaf to apply additional survival stress and allowed to re-grow for 14 days. Surviving populations were then re-treated with glyphosate (540 g ha^{-1}), with survival re-assessed at 42 DAT.

In 2012, the six wild radish populations identified in this experiment as being glyphosate-resistant (>20% plant survival) were tested in a full dose-response study to quantify their level of resistance. Population S1 was included as a susceptible control. Twenty seeds were planted in 180-mm pots containing potting mixture (25% peat moss, 25% sand and 50% mulched pine bark) and maintained outdoors. At the 2-true-leaf stage (GS 12), all populations were treated with glyphosate at 0, 270, 540, 810, 1080 and 2160 g ha^{-1}. Plant survival was assessed at 42 DAT. One population collected from Wongan Hills, WA (30°54′S, 116°43′E) (referred to hereafter as R), was deemed to be 2-fold glyphosate-resistant compared with the S1 population. Thirty surviving plants of this R population were re-potted into 305-mm plastic pots and isolated to prevent the ingress of foreign pollen. Once all individuals were flowering, plants were manually crossed using the 'beestick method' (Williams 1980) where flowers are crossed by using a bee carcass, ensuring a random pattern of cross-pollination. At maturity, all siliques were collected and milled as above. The extracted seed represented the glyphosate-selected progeny subset of the R population (referred hereafter as R1).

To confirm the heritability of glyphosate resistance in this wild radish population, a further dose-response study was conducted in 2013 with the R1 population and three known herbicide-susceptible wild radish populations: S1, WARR33 (referred to hereafter as S2) (Walsh *et al*. 2007) and WARR36 (referred to hereafter as S3) (Ashworth *et al*. 2014). Twenty seeds were planted into 180-mm plastic pots and maintained as above. At the 2-true-leaf stage (GS 12), plants were sprayed with glyphosate at 0, 150, 300, 450, 600, 750, 900 and 1800 g ha^{-1} for the S1–S3 populations and 0, 450, 750, 1050, 1500, 3000, 4500, 6000 and 7000 g ha^{-1} for the R1 population. All populations were assessed for survival at 42 DAT. Aboveground shoot biomass was harvested and dried at 65°C for 7 days before weighing.

Wild radish, annual ryegrass and capeweed plant populations collected in 2011

The surviving weed plants collected from the WA grainbelt in 2011 were transplanted into pots as previously described. Once annual ryegrass had ~25 mm of new leaf growth and the dicot species had two new leaves, all populations were tested with the Syngenta Resistance In-Season Quick-Test™ (RISQ) methodology (Walsh *et al*. 2001). The RISQ Test is an in-season method whereby field-sampled plants are herbicide-treated and monitored for injury symptoms. Glyphosate was applied at the recommended (for wild radish) field rate of 540 g ha^{-1} with plant survival assessed at 42 DAT by inspecting the new growth of each treated plant. If new growth was evident, the plant was deemed a survivor. Surviving plants were cut back and re-treated with glyphosate as done for the 2010 populations.

Of the 27 annual ryegrass populations collected as survivors in glyphosate-resistant canola crops, eight survived this repeated glyphosate–trimming treatment. They were re-potted into 250-mm plastic pots, isolated from each other to prevent the ingress of foreign pollen, and allowed to cross-pollinate randomly. At maturity, all seed heads were collected and threshed, with the extracted seed representing the glyphosate-selected progeny subset of each field-collected ryegrass population. These progeny were tested for resistance by planting 200 seeds into four replicate trays 200 by 250 by 50 mm containing potting mixture. The annual ryegrass populations VLR1 (Christopher *et al*. 1991) and NLR70 (Powles *et al*. 1998) were included as known susceptible and glyphosate-resistant controls, respectively. At the 2-leaf stage, seedlings were treated with single (450 g ha^{-1}) and double (900 g ha^{-1}) the recommended glyphosate rate for annual ryegrass control in Australia. At 42 DAT, plant survival was assessed, and survivors were severely trimmed and then re-treated with glyphosate (900 g ha^{-1}) once 20 mm of regrowth had occurred. Survival at 42 DAT was again assessed.

Weed prevalence in 2011 glyphosate-resistant canola fields

While inspecting glyphosate-resistant canola fields in 2011, visual estimates of the diversity and density of weeds species present were recorded by rating 10 replicate 0.0625 m^2 randomly placed quadrants per site. Species were identified as per Hussey *et al*. (2007). It was observed that plants surviving glyphosate had plant injury with fresh regrowth or were of advanced growth stage near plants showing glyphosate injury symptoms. Any weeds that emerged after glyphosate application were not considered survivors but were included in the assessment of weed density. These late-emerging weeds had no more than

2–3 leaves for grasses or two true leaves for dicots, with no herbicide injury symptoms. Glyphosate-affected barley grass, brome grass and wild oat could not be visually differentiated because of plant damage; therefore, they were grouped for initial plant density ratings. All late-emerging species could be differentiated and were reported individually.

Data analyses

Survival data were analysed by using non-linear regression analyses with the DRC package in R 3.0.0 (R Development Core Team 2011; www.R-project.org). Data were fitted to a 3-parameter log-logistic model (Eqn 1) where the upper limit was fixed at 1.0 (Streibig *et al*. 1993; Price *et al*. 2012):

$$Y = c + (1 - c/(1 + \exp(b(\log x - \log e))) \quad (1)$$

where Y denotes plant survival expressed as a percentage of the untreated control in response to herbicide dose x, c is the lower asymptotic value of Y, and b is the slope of the curve around e, which is the dose causing 50% mortality (LD_{50}) or biomass (GR_{50}). LD_{50} parameters were compared by using the selectivity indices (SI) function (R 3.0.0) to determine whether the ratios between these values were significantly different ($P < 0.05$). A lack-of-fit test was also applied to each fitted curve to ascertain the appropriateness of the model. The resistant and susceptible populations were compared by using a resistant to susceptible ratio (R/S) of the estimated LD_{50} values.

Results and discussion

Wild radish

In the 2010 glyphosate-resistant canola crop survey, wild radish populations were collected from 24 of the 73 fields surveyed in the northern region of the WA grainbelt. The subsequent screening of these populations identified one possible glyphosate-resistant population (designated as R), which exhibited 76% survival when screened at the recommended field rate for glyphosate (540 g ha^{-1}). At this rate, the S1 population was completely controlled, whereas the glyphosate-resistant canola control was unaffected. Dose-response screening quantified this R population to be 2-fold glyphosate-resistant (Table 1). A further dose-response study with the glyphosate-selected progeny of R (population R1) demonstrated that this resistance was heritable, increasing glyphosate resistance to 3.5-fold following selection compared with the pooled LD_{50} values of the three known susceptible populations (S1–S3) (Table 1). The mean survival of the R1 population was 84% at the recommended rate of glyphosate (540 g ha^{-1}) and 59% at double the recommended rate (1080 g ha^{-1}) (Fig. 2). Even though this population survived glyphosate treatment, its biomass was still dramatically reduced, exhibiting a GR_{50} of 350 g ha^{-1} compared with the pooled GR_{50} rate of 207 g ha^{-1} for the S1–S3 populations; therefore, the R biotype is not expected to have high fecundity within competitive crops.

A survey of 166 glyphosate-resistant canola fields across the WA grainbelt in 2011 did not identify glyphosate resistance in wild radish using the RISQ quick-test methodology. During this survey, wild radish survivors (whole plants) were collected from 23% of the glyphosate-resistant canola fields surveyed (Table 2). When glyphosate was applied at the recommended rate (540 g ha^{-1}), 62% of these populations had 100% mortality. Following trimming and glyphosate re-treatment, all populations died (Table 2).

In the 2011 weed-density survey, wild radish was the second most prevalent weed species, infesting 66% of glyphosate-resistant canola fields surveyed (Table 3). Of these fields, 86% contained economically damaging densities (>10 plants m^{-2}) (Streibig *et al*. 1989; Boz 2005) and 15% contained extremely high wild radish densities (>100 plants m^{-2}) (Table 3). Establishing the first commercial glyphosate-resistant canola plantings in WA on fields with such high wild radish densities is a dangerous practice, because it exposes populations to repeated applications of glyphosate, posing a significant risk of resistance evolution (Powles 2008). Exclusive reliance on glyphosate as an in-crop selective herbicide in glyphosate-resistant canola did not effectively control these high wild radish densities, because 33% of fields contained wild radish survivors following two glyphosate applications (Table 2). Of these sites, most (83%) contained >1 plant m^{-2}. Of concern were the 13 fields with surviving wild radish densities >10 plants m^{-2} following glyphosate treatment (Table 2). Resistance testing of these populations, however, did not identify any glyphosate-resistant populations.

Wild radish has a protracted emergence pattern during the growing season (Mekenian and Willemsen 1975; Cheam 1986).

Table 1. Parameter estimates from the three-parameter log-logistic model used to calculate LD_{50} and resistance to susceptible ratio (R/S) values for susceptible wild radish populations (S1–S3) and field-collected wild radish populations (R) collected in 2010 in Western Australia and its glyphosate-selected progeny (R1)

For LD_{50}, standard errors are in parentheses and *P*-value compares the difference between field-collected and susceptible control populations using the selectivity indices function in the DRC package in R v3.0.0. Lack-of-fit *P*-value for the appropriateness of the three-parameter model for field-collected dose-response 0.84, progeny dose-response 0.99

Designation	Population	Glyphosate resistance status	Location	Year	Coordinates	e (LD_{50}) (g ha^{-1})	*P*-value	R/S
S1	WARR7	Susceptible	Yuna	1999	–28.34S, 115.01E	384 (18)	–	–
R	–	Resistant	Wongan Hills	2010	–30.54S, 116.43E	752 (22)	<0.05	2.0
S1	WARR7	Susceptible	Yuna	1999	–28.34S, 115.01E	441 (28)		
S2	WARR33	Susceptible	Belmunging	2004	–29.11S, 116.26E	379 (45)		
S3	WARR36	Susceptible	Carnac Island	2013	–32.12S, 115.66E	421 (40)		
Pooled controls	WARR7, 33, 36	Susceptible	–	–	–	422 (21)	–	–
R1	–	Resistant	Wongan Hills	2010	–30.54S, 116.43E	1470 (102)	<0.05	3.5

Glyphosate must be applied in glyphosate-resistant canola before the 6-leaf stage and has no soil residual activity (Sprankle *et al*. 1975); therefore, late-emerging wild radish seedlings are not exposed to glyphosate in glyphosate-resistant canola crops, as demonstrated by 42% of surveyed fields containing late-emerging, non-glyphosate-affected wild radish populations (Table 2). Although late-emerging wild radish has been shown to have lesser effect on canola yield (Blackshaw *et al*. 2002), these individuals still significantly contribute to the weed seedbank. Historically, the use of non-transgenic, herbicide-tolerant canola varieties has allowed late-emerging cohorts to be treated with soil residual PSII or ALS herbicide chemistries (Kirkegaard *et al*. 2008); however, these chemistries cannot be used in glyphosate-resistant canola. With no residual control from glyphosate, crop competition is the only method available to suppress the growth of late-emerging wild radish populations in glyphosate-resistant canola. However, with 32% of the fields in 2010 containing wild radish at flowering, it is clear that crop competition alone was ineffective. In 2015, however, new glyphosate-resistant canola hybrid varieties with tolerance to PSII-inhibiting herbicides will be available to growers, providing an opportunity for longer season wild radish control in glyphosate-resistant canola crops (M. Tuffley, pers. comm.).

In summary, the study clearly demonstrates that glyphosate provided effective control of wild radish. However, in very large infestations, some survival was evident. In addition, late-emerging wild radish was not exposed to glyphosate, and therefore able to contribute to the weed seedbank. To ensure the sustainability of glyphosate-resistant cropping systems, additional weed-control diversity is required to ensure that surviving wild radish does not set seed. In general, the lack of

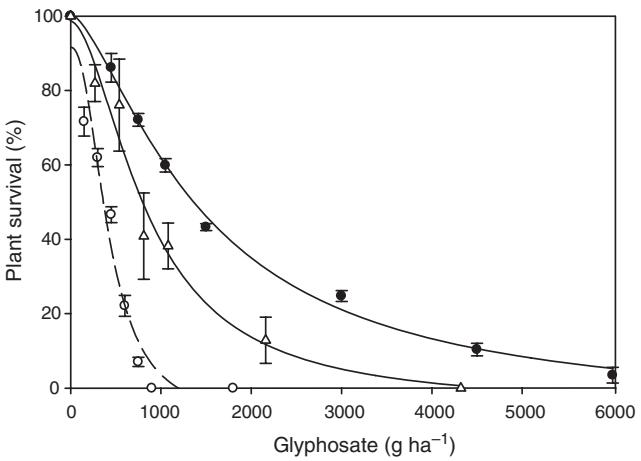

Fig. 2. Survival dose-response curve for the pooled susceptible wild radish populations S1, S2 and S3 (---○---) and the progeny subset of the glyphosate resistant R1 population (—Δ—) and R1 progeny subset population (—●—). Each symbol represents the mean of seven treatments for the susceptible and eight treatments for the resistant population. Plotted lineds are predicted using the Eqn 1. Vertical bars represent the mean ± s.e. ($n = 5$).

Table 2. Frequency, density and resistance of weed species present following glyphosate treatment in 166 surveyed glyphosate-resistant canola fields in 2011

Plants at an advanced growth stage (>2 leaves for grasses; >1 leaf for dicots) were considered to have survived glyphosate application; plants were considered to be late-emerging if they had <3 leaves (grasses) or <2 leaves (dicots). Resistance was confirmed in collected populations by spraying with glyphosate at once and twice the recommended rate. nd, Not determined

Species	No. of fields containing species following glyphosate treatment	No. of fields in each weed density category following glyphosate treatment				No. of fields with late-emerging populations	No. of surviving populations collected	No. of pops confirmed resistant
		<1 plant m^{-2}	1 plant m^{-2}	10 plants m^{-2}	20 plants m^{-2}			
Annual ryegrass	45	9	22	6	8	41	27	8
Wild radish	37	6	18	7	6	70	19	0
Barley grass	0	0	0	0	0	49	0	n.d.
Brome grass	0	0	0	0	0	18	0	n.d.
Wild oat	0	0	0	0	0	23	0	n.d.
Capeweed	4	3	0	1	0	0	3	0
Malva	0	0	0	0	0	0	0	n.d.

Table 3. Frequency and density of weed species present before glyphosate treatment in 166 glyphosate-resistant canola fields in 2011

Species	No. of fields containing species	No. of fields in each weed density category			
		Low (0–10 m^{-2})	Medium (10–50 m^{-2})	High (50–100 m^{-2})	Very high (>100 m^{-2})
Annual ryegrass	151	10	29	90	22
Wild radish	110	15	61	34	0
Barley grass, brome grass, wild oat	90	10	46	28	6
Capeweed	46	8	25	13	0
Malva	2	2	0	0	0

weed control in glyphosate-resistant crop phases has been shown to favour proliferation of uncontrolled species, leading to shifts in the weed flora (Reddy 2004; Culpepper 2006; Owen 2008). If sufficient weed control diversity is not incorporated, glyphosate-resistant canola crop phases may favour the further proliferation of uncontrolled species such as wild radish.

Annual ryegrass

In the 2011 glyphosate-resistant canola crop survey, annual ryegrass was the most common weed species, present in 91% (151 of 166) of glyphosate-resistant canola fields (Table 3). Plant densities were generally high, with 74% of fields containing >50 plants m^{-2} and 15% containing >100 plants m^{-2} (Table 3). Following glyphosate treatment, 30% of fields surveyed contained annual ryegrass survivors, with 18% of fields containing annual ryegrass densities >20 plants per m^{-2} (Table 2). From the 27 annual ryegrass populations collected in glyphosate-resistant canola, eight populations originating from the southern and eastern high-rainfall zone (450–700 mm $year^{-1}$) survived glyphosate treatment (540 g ha^{-1}) (Table 2, Fig. 1). Glyphosate resistance in these populations was heritable, with the progeny of all eight populations showing high levels of survival (>94%) following two successive treatments at double the recommended glyphosate rate for annual ryegrass control (900 g ha^{-1}). At this rate, the known glyphosate-resistant annual ryegrass population (NLR70) was unaffected, whereas the susceptible annual ryegrass population (VLR1) was killed. Therefore, 5% of glyphosate-resistant canola fields in this survey contained glyphosate-resistant annual ryegrass populations, reflecting a similar frequency of glyphosate resistance in random surveys (not containing glyphosate-resistant canola) conducted by Owen *et al.* (2014). Both studies demonstrate that glyphosate resistance in annual ryegrass is present in the southern and eastern, moderate–high rainfall zones (450–700 mm $year^{-1}$) of the WA grainbelt (Fig. 1). Despite the discovery of the first glyphosate-resistant annual ryegrass population (Neve *et al*. 2004) in the northern grainbelt of WA, the long history of glyphosate use in the central and northern grainbelt of WA has not resulted in a widespread increase in glyphosate-resistant populations, with no glyphosate-resistant annual ryegrass populations identified from these regions during this survey.

The introduction of glyphosate-resistant canola in WA and the continued prevalence of chemical fallowing in the northern and central agricultural areas (Oliver *et al*. 2010) will further increase glyphosate selection on the large populations of annual ryegrass found in this survey. This and previous surveys reveal that isolated glyphosate-resistant populations exist (Neve *et al*. 2004; Broster and Pratley 2006; Owen and Powles 2010); however, this and previous surveys by Owen *et al.* (2014) demonstrate that the majority of annual ryegrass populations are still susceptible to glyphosate. In order to preserve the efficacy of glyphosate, it is important that its use be combined with other weed-control strategies. Fortunately, no resistance has yet been detected in the WA grainbelt to the non-selective herbicide paraquat (Owen *et al.* 2014), which can be rotated with glyphosate. Pre-emergent herbicides are also effective at providing residual annual ryegrass control in canola and wheat (Gill and Holmes 1997). Annual ryegrass seed entering the seedbank can be virtually eliminated by using appropriately timed herbicide applications (i.e. crop topping) (Gill and Holmes 1997) or harvest weed-seed control techniques (Walsh *et al*. 2013).

Other weed species

No barley grass, brome grass or wild oat populations were found to have survived glyphosate applications in this study of glyphosate-resistant canola fields in WA, concurring with previous surveys by Owen *et al.* (2014) in which populations collected from cropping fields across the WA grainbelt were directly tested for resistance. However, glyphosate resistance has been recently documented in two populations of great brome (*Bromus diandrus* L.) in South Australia (Preston 2014). In the present survey, brome grass, barley grass and wild oats were found in 54% of fields, with 38% of sites containing high grass densities (>50 plants m^{-2}). Late-emerging populations of all three species were common, with 29% of fields contained late-emerging barley grass species, 11% brome grass species and 13% wild oat species. The widespread distribution and high weed densities of these species highlight that they should be carefully monitored for glyphosate resistance.

Capeweed is increasing in prevalence in WA cropping fields (Borger *et al*. 2012). In this study, capeweed was found in 28% of glyphosate-resistant canola fields, with four fields found to contain survivors following glyphosate treatment. However, testing of these populations revealed all were glyphosate-susceptible (Table 2). The 2011 survey also identified two fields containing populations of small-flowered mallow, which also did not survive glyphosate application, therefore were considered glyphosate-susceptible (Table 2).

Conclusion

Glyphosate-resistant crops offer an effective tool (i.e. glyphosate as an in-crop selective herbicide) for controlling the multiple herbicide-resistant annual ryegrass and wild radish populations prevalent in the WA grainbelt. This survey was conducted in the first year of commercial glyphosate-resistant canola plantings in WA, establishing that glyphosate controlled most wild radish and annual ryegrass populations. However, this survey identified one glyphosate-resistant wild radish population and eight glyphosate-resistant annual ryegrass populations collected from glyphosate-resistant canola fields. In light of the results from this and other surveys (Broster *et al*. 2011, 2012, 2014; Owen *et al.* 2014), it is important that weed control is diversified around the use of glyphosate to minimise the risks of glyphosate-resistance evolution (Sammons *et al*. 2007; Powles 2008). When weed-control diversity is incorporated, glyphosate-resistant cropping systems have been found to be sustainable, with only low levels of glyphosate resistance identified (Beckie *et al*. 2006; Beckie 2011). In Australia, incorporation of diversity may involve use of pre-seeding herbicide strategies such as ‘double knock’ (glyphosate followed by paraquat–diquat) (Borger and Hashem 2007), incorporation of residual herbicides (Beckie *et al*. 2011; Thornby *et al*. 2013), increased crop competition (Beckie *et al*. 2008), and harvest weed-seed control (Walsh *et al*. 2012, 2013), which intercepts and

controls herbicide-resistant weed seeds before they re-enter the soil seedbank.

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