ORIGINAL RESEARCH

Management of a herbicide-resistant ryegrass (*Lolium rigidum*) population in a crop rotation using alternative herbicides, row spacing, strategic nitrogen application and RR® canola (*Brassica napus*)

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Abstract

Rigid ryegrass (*Lolium rigidum* Gaud., henceforth, called ryegrass) is the most significant herbicide-resistant weed in Australian grain cropping. Failure to adequately control ryegrass causes grain yield losses of about 36%. Therefore, new approaches for the control of ryegrass are needed in diverse crop rotations. We studied the options for managing a high-density Acetyl CoA Carboxylase (ACCase)-resistant ryegrass population in a lupin (*Lupinus angustifolius* L.) - wheat (*Triticum aestivum* L.) - canola (*Brassica napus* L.) rotation, under dryland conditions, at Cunderdin (31.650908 º S, 117.238906 º E), Western Australia (WA). Field trials were conducted during 2012 to 2014.

In the 2012 lupin, and 2013 wheat crops, conventional herbicides (simazine in lupin, and trifluralin in wheat) and an alternative herbicide (dimethenamid-p in lupin, and pyroxasulfone in wheat) were tested. In 2014, Roundup Ready® (RR®) canola received two applications of glyphosate to control ryegrass. Three treatments of nitrogen (N) ((N_1) 25 kg N ha^{-1} as urea; (N_2) 50 kg N ha^{-1} as urea; and (N_3) 50 kg N ha^{-1} as urea ammonium nitrate (UAN)) were applied to the 2013 wheat, and the 2014 RR® canola. Each crop was grown at two row spacings (22 cm, or 44 cm). None of the management factors except the herbicides significantly decreased the ryegrass density. Indeed, N_3 (UAN) increased the emergence of ryegrass (more in 44 cm than 22 cm rows) compared to N_1 and N_2. Compared to urea N_1, N_3 reduced canola establishment by 28% and generally increased the grain yield of RR® canola by 11% but increased the density of ryegrass rather than controlling it. Dimethenamid-p, the alternative herbicide, decreased the ryegrass density in lupin and increased grain yield of lupin by 53%.

While pyroxasulfone, the alternative herbicide, had no significant effect on the ryegrass density compared, to trifluralin in wheat, it increased the wheat grain yield by 25%. However, the 99% reduction in ryegrass by two applications of glyphosate in RR® canola was by far the most effective weed control. The inclusion of RR® canola technology in the rotation was the most effective approach to control the ACCase-resistant ryegrass, under dryland conditions of Western Australia.

Keywords*:* Herbicides; crop rotation, *Lolium rigidum*, resistant rigid ryegrass, urea ammonium nitrate (Flexi N), Roundup Ready® (RR®) canola, trifluralin, simazine, dimethenamid-p, pyroxasulfone

Introduction

In Australia, the overall cost of weed management and grain yield losses due to weeds is estimated to be $3 billion, equivalent to $146 ha^{-1}. Rigid ryegrass (*Lolium rigidum* Gaud.; henceforth called ryegrass) accounts for 36% of the overall losses in revenue, and 28% of the losses in grain production in Australia (Llewellyn et al., 2016). Competition from ryegrass can reduce wheat (*Triticum aestivum* L.) grain yield by 42% under dryland cropping conditions (Hashem et al., 1998). Broadly, in North America, the annual losses in crop yields due to competition from weeds are estimated to be US $28 billion in corn (*Zea mays* L.) (Soltani et al., 2016), and US$16 billion in soybean (*Glycine max* L.) (Soltani et al., 2017).

Although the use of herbicides has greatly improved crop grain yields in Australia, increased reliance on herbicides for weed control has led to a significant increase in herbicide resistance in various weeds (Owen et al, 2007; Walsh et al., 2007; D'Emden et al., 2008). Ryegrass has evolved widespread resistance to various herbicide modes of action in Western Australia (WA) (Owen et al., 2014) and other parts of Australia (Boutsalis et al., 2012). In WA, 96% of the ryegrass populations were equally resistant to the Acetyl CoA Carboxylase (ACCase)-inhibiting herbicides, such as diclofop-methyl and Acetolactate Synthase (ALS)-inhibiting herbicides, such as sulfometuron, with cross-resistance in these two modes of action in 95% of the ryegrass populations tested (Owen et al., 2014).

However, resistance to other herbicides with different modes of action was significantly lower, with only 27% of the ryegrass populations showing resistance to other herbicides, including glyphosate (Owen et al., 2014). The adoption of integrated weed management (IWM) practices has increased in WA in response to the increase in herbicide resistant weeds (Llewellyn, 2016). Practices, such as increased competition by the crop (i.e., manipulation of row spacing, seed rate, competitive cultivars, etc.) (GRDC, 2014), windrow burning (Pannell et al., 2004), harvest weed seed control (Walsh et al., 2013) and the use of alternative herbicides, have become more common on WA farms.

Compared to wide row spacing, narrow row spacing is likely to facilitate the growth of crop plants with greater competitive ability than weeds (Minkey et al., 2000). Crops sown in wide rows are considered less competitive with weeds and are at an increased risk of seedling damage from close fertilizer placement. In addition, crops sown in wide rows reduced plant populations compared with those sown in narrower rows, even when fertilizer and seed were placed separately (Scott et al., 2013). However, the advantages of wide rows in Australian wheat, barley (*Hordeum vulgare* L.), canola (*Brassica napus* L.) and lupin (*Lupinus angustifolius* L.) may include improved stubble clearance, reduced fuel consumption, needs fewer ground-engaging components, increased speed of the sowing operation, and improved harvestability, seed size and grain quality but limited improvement of grain yield (Scott et al., 2013).

Some weed species are more efficient than crops in capturing nutrients from added fertilizers (Di Tomaso, 1995; Hashem et al., 2000; Blackshaw et al., 2003). Therefore, the addition of fertilizer can sometimes reduce crop grain yields by increasing weed growth. For example, Italian ryegrass *(Lolium multiflorum* L.) was two times more efficient than wheat plants at producing biomass and specific leaf area per unit of nitrogen (N) absorbed in a mixture of crop and weed (Hashem et al., 2000).

However, the placement and timing of applied fertilizers can increase access to nutrients by crops rather than weeds (Blackshaw et al., 2002; Dhima and Eleftherohorinos, 2001; Jørnsgard et al., 1996). For example, while weeds may have easy access to the N applied on the soil surface at sowing time, strategic N placement may maximise the access of crop plants to N compared to weeds. The widespread use of urea ammonium nitrate (UAN, henceforth, called N_3), applied as a liquid for in-season N application (Nelson, 2019), is a possible tool to direct N to the crop and decrease the access weeds may have to the N fertilizer.

Growers can improve production and monetary benefits from rotation with canola (GRDC, 2000). Despite known resistance to glyphosate in some weed species, glyphosate-resistant (GR) crops represent more than 80% of the 120 million ha of transgenic crops grown annually world-wide. This is attributed to the simple and superior weed control that GR crops deliver (Duke and Powles, 2009). In Australia, the genetically-modified (GM) canola was permitted for commercial production in Queensland (QLD) in 2003, New South Wales (NSW) and Victoria (VIC) in 2008, WA in 2010 (Office of the Gene Technology Regulator, OGTR, 2018. In South Australia, the State government lifted the moratorium on GM-canola in August 2019 (Heard, 2019).

The GM canola currently grown in Australia is resistant to glyphosate and can only be grown with the approval of the Office of the Gene Technology Regulator (OGTR), which carries out a science-based risk assessment before the crop is approved for release. In Australia, about 20% of the national canola crop is genetically modified (OGTR, 2018).

Since 2010, the area sown to glyphosate-resistant, Roundup Ready (RR®) canola in WA has grown to 34% of the total canola area, demonstrating an increasing growers' demand for this technology (DPIRD, 2019)**.** Already in the USA, about 93% of the canola crop is genetically-modified (Nestle, 2020) due to added benefits, such as ryegrass-free cropping for up to five years, control of nematodes, and disease break for cereals. In WA, a comparison of RR®, Clearfield® (CL) and Triazine-tolerant® (TT) canola by Zhang et al. (2014) found that RR® canola produced the highest grain yield at both the low (Cunderdin) and high (Kojonup) rainfall areas.

In a five-year-rotation study, Stanton et al. (2010) found that glyphosate-tolerant (i.e., RR®) and TT canola achieved high levels of ryegrass control and attained higher yields than the conventional system. They also found that glyphosate-tolerant canola provided extra control of broadleaf weeds and also achieved better seed oil levels when compared with the other canola systems. Based on the responses of 92 Australian farmers in a survey after 2008 growing season, Neilsen (2009) found that RR® technology increased canola yield by 20% and oil contents by 2% over CL and TT canola systems). Neilsen (2009) also noted that the level of weed control achieved using RR® canola was also superior to other herbicide-tolerant canola systems.

It, thus, appears that RR® canola technology can effectively be used to control herbicide-resistant ryegrass populations. However, diverse weed control methods are needed for ACCase-resistant ryegrass in crop rotations of legume, cereal, and canola.

Therefore, we conducted this study to assess the potential to manage a high density of a highly ACCase-resistant ryegrass population by: (a) application of alternative herbicides, (b) strategic management of N, and (c) the inclusion of RR® canola under normal and wide row spacing, in a lupin–wheat–RR® canola rotation.

Materials and Methods

Field site

Our rotation trial (lupin-wheat-RR® canola) was conducted within the dryland cropping systems of WA, on a sandy loam soil at Cunderdin, WA (31.5847843 S, 117.258432 E) during 2012 to 2014. The trial site had been cropped to wheat in 2011 and had a high density (1000 plants m^{-2}) of ACCase-resistant ryegrass in 2012. The resistance status of the site was confirmed in a glasshouse dose response experiment, reported below.

The site received an annual rainfall of 225 mm in 2012, 304 mm in 2013 and 360 mm in 2014 cropping years, while the long-term average annual rainfall at this site was 307 mm (Figure 1). During the study period, the daily mean minimum temperature was 14.4 ^{o} C in July and the daily mean maximum growing season temperature was 32.4 ^{o} C in October. The mean daily temperature did not vary markedly among years. A frost was recorded in the 4th week of July 2012, the first week of July 2013 and in the last week of June 2014. A mild frost was also noted in the middle of September 2014.

Field Study - Seed Bank Size and Density of Ryegrass

The trial site was 90 m wide in the east-west direction and 100 m long in the north-south direction and was fully fenced out for the duration of the trial, before the lupin crop was sown in 2012. The initial density of ryegrass at the field site was determined from five randomly selected locations within the untreated buffer zone of the trial site, using a 50 cm x 50 cm quadrat. The unit plot size was 20 m x 2 m.

All the unit plots were oriented in the in the north-south direction. Block 1 and 2 (a 'block' is the whole set of treatments of one replication, grouped together into one homogeneous block of land to minimise experimental error, this is also the replicate 1 and 2) were laid out next to each other in the east-west direction with a four (4) m gap in between the blocks. All plots of one replication were laid out within one block without any gap in between plots.

Blocks 3 and 4 were laid out on the north side of the trial area with a gap of 20 m from block 1 and 2. So, there was a buffer zone of 15 m between the south end of bock 1 and 2 and the fence on the south side of the trial area and a 20-m buffer between north end of block 3 and 4, and the fence on the north side of the trial area.

The buffer zone used for plant count in the untreated buffer zone was 5 m x 90 m along the south side of the block 1 and 2 (buffer zone 1) within the fenced area of the field site.

A strip of 2 m x 90 m on the north side of the buffer zone and the south side of block 1 and 2 (buffer zone 2) was sprayed with 1 L ha^{-1} of Roundup Ultra® Max (glyphosate 570 g L^{-1}) using a Ute-mounted boom sprayer. The buffer zone 1 (5 m x 90 m) was not sprayed with any grass herbicide, which allowed ryegrass to grow in this area.

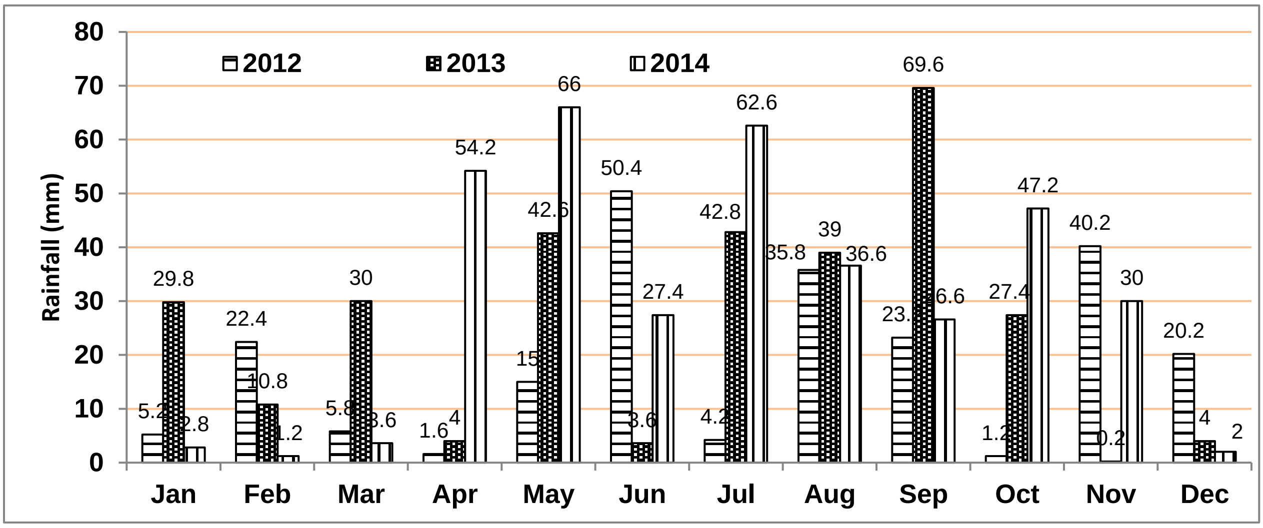

Figure 1. Monthly rainfall during 2012, 2013, and 2014 at Cunderdin Airfield (Station number: 10286), WA)

Ryegrass plants in the buffer zone were allowed to produce seed to mimic any failures of the herbicides in controlling the weeds. In each year, all treated plots were harvested using a 2-m wide plot harvester. The ryegrass (and canola crop in 2014) were harvested in the buffer zone using a 10-m wide header. The plant residues from the buffer were spread evenly within the harvested area and the ryegrass seed in the bin removed from the trial site.

To estimate the soil seed bank of the ryegrass population in 2012, soil samples were collected to a depth of 5 cm from 10 randomly selected locations within the trial area, using a 25 cm x 25 cm quadrat, before sowing of the crop. The existing emergence of ryegrass was recorded from each quadrat just before soil collection. Soil samples were transported to the glasshouse of DPIRD at Northam, WA and spread in a 3-cm thick layer on plastic trays (30 cm x 14 cm x 5 cm) and irrigated daily to keep the soil moist. The emergence of ryegrass was recorded at monthly interval for 15 months (from June 2012 to September 2013). After each counting, emerged seedlings were removed from the trays to prevent seed production. The seed bank size (viable seed number m^{-2}) was calculated by adding total field emergence before soil collection in 2012 to the total emergence of ryegrass in the glasshouse.

In the buffer zone 1, ryegrass plants were counted in a quadrat of 30 cm x 30 cm at five weeks after emergence (WAE) of the crop in 2012 and 2013. However, in 2014, ryegrass density was recorded 3 WAE in the untreated buffer zone of the study site sown to RR® canola. The density of ryegrass in the treated plots were also recorded at the same time as the buffer zone in each year.

Glasshouse Study to Confirm Resistance

To confirm and characterize the resistance in the ryegrass population at the study site (presumed herbicide-resistant, designated as 'R'), a dose response test was conducted under glasshouse conditions at Northam with diclofop-methyl, clethodim and glyphosate. In late June 2012, seedlings of ryegrass were collected from the trial site at 1- to 2-leaf stage. Roots and leaves were trimmed to 4 to 5 cm, and the seedlings then transplanted at 15 seedlings pot^{-1} to 5-L pots filled with a soil potting mix.

A susceptible population of commercially-available ryegrass (*cv.* Safeguard) (designated as 'S') was included in the test for comparison. Two weeks after transplanting, when seedlings had developed two to three fully expanded leaves, they were treated with 1/4x, 1/2x, 1x (label rate) and 2x rates of diclofop-methyl (Hoegrass®, 500 g diclofop methyl L^{-1}), clethodim (Select®, 240 g clethodim L^{-1}) and glyphosate (Roundup PowerMAX®, 540 g glyphosate L^{-1}) at 96 L of spray volume ha^{-1}.

The herbicides were applied using a laboratory closed-door belt-moving boom sprayer, equipped with three flat-fan nozzles at 200 kPa pressure moving at nine km hr^{-1}. The survival of the R and S biotypes of ryegrass seedlings was assessed at 24 days after herbicide application.

Field Study - Treatments in the Rotation Trial

Table 1 shows the herbicides, row spacing, and rates and sources of N used in the trials. Lupin (*cv.* Gunyidi) in 2012 was followed by wheat (*cv.* Mace) in 2013 and then by RR® canola (*cv.* 43Y23) in 2014. All herbicide treatments were applied in the plots using a Ute-mounted boom sprayer.

Lupin in 2012

The lupin crop was sown at 100 kg of seed ha^{-1} at two row spacings (22 or 44 cm) with fertilizer applied at 100 kg of Double Phos® ha^{-1} (17.7 P, 3.6 S, 16 Ca kg ha^{-1}) in mid-May.

To control ryegrass, the conventional herbicide simazine (H1) (simazine 500 g L^{-1}) at 1 kg ai ha^{-1} and an alternative herbicide Outlook® (dimethenamid-p 63.9% (H2)) at 720 g ai ha^{-1} were applied before sowing and were incorporated by the sowing operation. Subsequently, a commercial mixture of diflufenican (50 g ai ha^{-1}) and Metribuzin® 750 WG (metribuzin 750 g ai kg^{-1}) was applied at 112 g ai ha^{-1} at the seven-leaf stage of the lupin crop to control broadleaf weeds, such as wild radish (*Raphanus raphanistrum* L.) and capeweed (*Arctotheca calendula* L.) in each plot.

Photo 1 shows the lupin field, heavily infested with ryegrass and other weeds. The different degrees of weed control achieved by the herbicides are shown in Photo 2 (conventional herbicide - simazine) and Photo 3 (alternative herbicide - dimethenamid-p).

Photo 1. The lupin 2012 experimental site heavily infested with ryegrass, Cunderdin, WA

Table 1 Row spacing, conventional (H1) and alternative herbicides (H2), and nitrogen rates applied as treatments in each crop during 2012, 2013 and 2014 seasons at Cunderdin, Western Australia.

Year	2012	2013	2014
Crops	Lupin (cv. Gunyidi)	Wheat (cv. Mace)	RR® Canola (cv 43Y23)
Row spacing (cm)	22, 44	22, 44	22, 44
Herbicides	Simazine (H1) Dimethenamid-p (H2)	Trifluralin (H1) Pyroxasulfone (H2)	Glyphosate
Nitrogen (kg N ha-1)	Nil	N1 25 kg as urea N2 50 kg as urea N3 50 kg as UAN	N1 25 kg as urea N2 50 kg as urea N3 50 kg as UAN

	
Photo 2. The lupin plot treated with simazine that controlled ryegrass by 21% in the 2012 lupin crop at Cunderdin, WA	**Photo 3. The lupin plot treated with dimethenamid-p that controlled ryegrass by 61% in the 2012 lupin crop at Cunderdin, WA.**

Wheat in 2013

Wheat seeds (75 kg ha^{-1}) was sown at two row spacings (22 or 44 cm) with 100 kg of Double Phos® fertilizer ha^{-1} (17.7 P, 3.6 S, 16 Ca (%)) applied at sowing time. The conventional herbicide (H1) Triflur Xcel® 500 (500 g trifluralin L^{-1}) at 960 g ai ha^{-1} and an alternative herbicide (H2) Sakura® (850 g pyroxasulfone kg^{-1}) at 118 g ai ha^{-1} were applied to the soil surface four hours before sowing and incorporated by the sowing operation.

Herbicide 1 and 2 in the wheat crop were applied in the same plots as Herbicides 1 and 2 in the 2012 lupin crop plots. The objective here was to compare the cumulative effect of conventional herbicides (H1) against the cumulative effect of alternative herbicide (H2).

Three treatments of nitrogen (N) namely, (N_1) (25 kg N ha^{-1} as urea), (N_2) (50 kg N ha^{-1} as urea) and (N_3) (50 kg N ha^{-1} as urea ammonium nitrate, UAN) were applied to the wheat crop. Urea granules (N_1 and N_2) were drilled over the crop rows just in front of the tines while N_3 was injected 4 to 5 cm below the crop seed at the time of sowing.

A commercial mixture of bromoxynil (200 g bromoxynil L^{-1}) and MCPA (200 g MCPA L^{-1}) was applied at 400 g ai ha^{-1} to all plots when wheat was at Z14 stage to control broadleaf weeds.

RR® Canola Crop in 2014

RR® Canola was sown in 2014 across all the plots of the 2013 wheat crop at 3 kg of seed ha^{-1} with two row spacings (22 cm or 44 cm). A compound fertilizer, Agras® (16.1 N, 9.1 P, 14.1 S, 0.5 Ca, 0.06 Zn kg ha^{-1}) at 100 kg ha^{-1} mixed with an extra 40 kg K ha^{-1} and 16.5 kg S ha^{-1} (as potassium sulphate) was applied across all the plots of RR® canola.

The same three treatments of N were re-applied to RR® canola in the same plots as the wheat crop in 2013. As the compound fertilizer supplied some N, the amount of N applied in Agras® was deducted from each N treatment so the total N applied was the same as listed in the N treatments (Table 1). Roundup Attack® (570 g glyphosate L^{-1}) was applied at 900 g ai ha^{-1} in RR® canola at 2- and 5-leaf stages to control ryegrass.

Measurements

In the field trials, densities of lupin, wheat and ryegrass were recorded 5 WAE while in RR® canola, the density of ryegrass was recorded at 3 and 12 WAE. The density of ryegrass in the treated field plots was compared with the density of ryegrass in the buffer zone in each crop and expressed as a percentage of the density of the buffer zone 1 in each year. In the 2014 RR® canola crop, crop vigour was visually assessed in every plot at five-leaf stage, assuming the vigour as 100% in the buffer zone, where no N was applied.

Crop vigour of all the plots were assessed as per cent of the reference plot (buffer zone 1). Crop establishment of the 2014 RR® canola crop was also assessed visually considering the plot 4 with N_2 and 22 cm row spacing as a reference plot, where more than 90% canola plants emerged uniformly, and the crop establishment in all other plots was assessed as per cent relative to the reference plot.

Each crop (wheat, lupin, and RR® canola) was harvested by a plot harvester and the weight of clean grains per plot was recorded and then converted to grain yield per ha. The moisture content of grains was determined by moisture meter and grain yield obtained at 12% moisture content.

Design and Analyses

The glasshouse experiments were conducted in a completely randomised design with three replications. To determine the LD_{50} rate (lethal dose 50, a dose that would kill the 50% of the treated population), plant survival was analysed by probit analysis (GENSTAT 18th Edition) and then the LD_{50} ratio of the field-collected population (R) relative to the susceptible (S) biotype was determined to explain the degree of resistance.

The experimental design for the field study was a split-split-plot design with four blocks using a unit plot of 20 m by 2 m in each year. Row spacing was assigned to the main plots, herbicides to the sub-plots, and N treatments (in wheat and canola only) to the sub-sub-plots.

The data on lupin, wheat and ryegrass were separately subjected to two- or three-way analysis of variance by GENSTAT 18th Edition (VSN, 2015). The data on canola were analysed using the background herbicides (H1 and H2) applied in the previous lupin and wheat crops and, row spacing, and N rates applied in RR® canola. Means were separated by Fischer protected LSD at P = 0.05.

Results

Resistance, Seed Bank Size and Density of Ryegrass

In the glasshouse resistance experiment, 90% plants from the field (R) population survived at 1x (label rate) and 2x rates of diclofop-methyl and 80% survived at 1x and 2x rates of clethodim, while no plants survived at 1x or 2x rates of glyphosate (Figure 2). All the plants of the susceptible (S) population died at the 1x (label) rate of each of these herbicides.

The LD_{50} ratio of the R to the S populations was 36 for diclofop-methyl, 19 for clethodim and 1.0 for glyphosate, demonstrating that the R population was 36 times more resistant to diclofop-methyl and 19 times more resistant to clethodim but was highly susceptible to glyphosate. In the untreated buffer zone, the average density of ryegrass was 1000 ±64.9 plants m^{-2} in 2012, 525 ± 44.1 in 2013, and 901 ± 84.7 in the 2014 season. The soil seed bank size of ryegrass, determined before sowing the lupin crop in 2012, was 6518 ± 291 viable seed m^{-2} to a soil depth of 5 cm.

Ryegrass Control by Herbicides and RR® Canola Technology

Photo 4 shows RR® canola plots of blocks 3 and 4 in 2014. The levels of significance of each management factor and their interactions are presented in Table 2.

Photo 4. RR® canola plots of blocks 3 and 4 in 2014. On the left is the buffer plot, which was sprayed with Spray.Seed® (paraquat 125 g L^{-1} + diquat 125 g L^{-1}) at 1 L ha^{-1}) to ease in the harvest of RR® canola plots at the maturity.

In the 2012 lupin crop, simazine (Herbicide 1) reduced ryegrass density from 1000 plants m^{-2} to 794 plants m^{-2}, a 21% reduction in weed density (Table 3). The herbicide dimethenamid-p (Herbicide 2), applied in lupin, reduced ryegrass density from 1000 plants m^{-2} in the buffer zone 1 to 391 plants m^{-2} in the treated plots (Table 3), a 61% reduction.

In the 2012 lupin and 2013 wheat crops, there was a significant interaction effect of herbicides and row spacing on the density of ryegrass (Table 2).

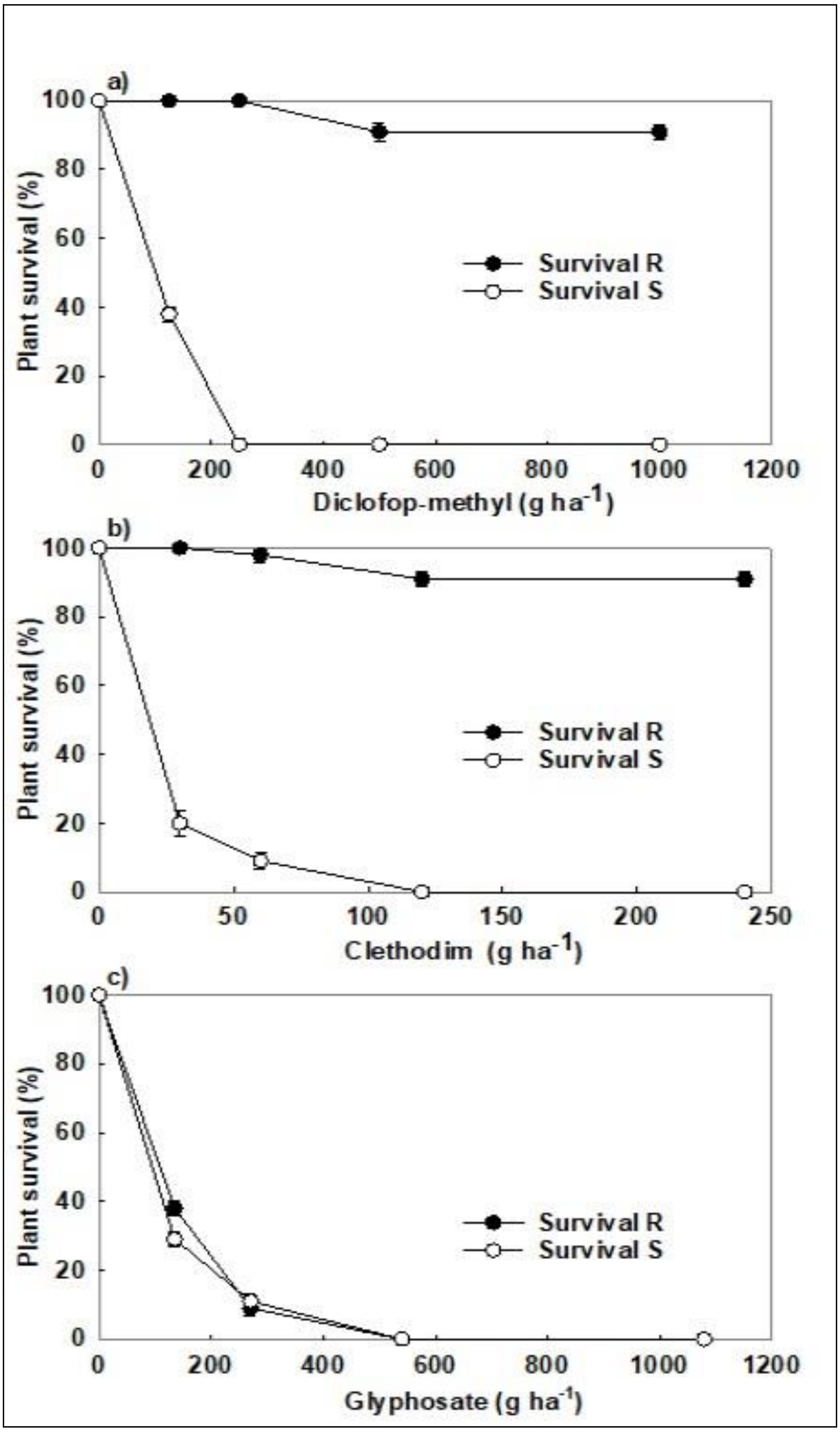

Figure 2 Plant survival (%) of the field-collected (R) population of ryegrass from the experimental site at Cunderdin, WA and the susceptible (S) population (*cv.* Safeguard) when treated with different rates of a) diclofop-methyl, b) clethodim or c) glyphosate at Northam in 2012. Where visible, vertical error bars in the graphs represent the standard errors (SE).

Table 2 Significance levels for the effect of row spacing (RS), herbicide (H) and nitrogen (N) and their interactions in the 2012 lupin, 2013 wheat and 2014 canola crops on ryegrass density, crop density and grain yield in a lupin-wheat-RR® canola rotation at Cunderdin, WA[1].

Treatments	Ryegrass density			Crop density			Crop grain yield		
	2012 lupin	2013 wheat	2014 canola	2012 lupin	2013 wheat	2014 canola	2012 lupin	2013 wheat	2014 canola
RS	ns	ns	ns	0.001	<0.001	<0.001	ns	<0.001	ns
H	<0.001	0.01	ns	ns	0.037	ns	<0.01	<0.001	ns
N	-	ns	ns	-	ns	<0.01	-	ns	0.061
RS*H	0.026	0.029	ns	ns	ns	ns	ns	ns	ns
RS*N	-	ns	ns	-	ns	ns	-	ns	ns
H*N	-	ns	ns	-	ns	ns	-	ns	ns
RS*H*N	-	0.05	0.001	-	ns	ns	-	ns	0.016

[1]ns = Not significant; "-"indicates N was not applied in the lupin crop.

Table 3 The effect of row spacing and herbicide treatments on the initial density of ryegrass in the 2012 lupin at Cunderdin, WA

Herbicide	Row spacing (cm)	Density of ryegrass (plants m^{-2})
Simazine	22	753
	44	835
Dimethenamid-p	22	445
	44	338
P-value		0.02

The overall density of ryegrass was halved with Herbicide 2 (dimethenamid-p) relative to Herbicide 1 (simazine) in the lupin crop (Table 3). However, under Herbicide 1 (simazine), the ryegrass density in the lupin crop was higher in 44 cm row spacing than 22 cm row spacing. In contrast, under the herbicide 2 (dimethenamid-p) ryegrass density was lower in 44 cm than 22 cm crop row spacing (Table 3).

In the 2013 wheat crop, there was a three way interaction between row spacing x herbicide x nitrogen. This interaction was due to the increase in ryegrass at the 44 cm row spacing with trifluralin when N_3 was applied (Table 4).

In the 2014 RR® canola, the average ryegrass density was 481 plants m^{-2} at 3 WAE (i.e. before the first application of glyphosate) and unaffected by row spacing, N fertilizer or prior herbicide treatments. Ryegrass density declined sharply to 7 plants m^{-2} (98.5% reduction) at 12 WAE, five weeks after second application of glyphosate in RR® canola (Table 4). The initial density of ryegrass in the buffer zone was 901 plants m^{-2} at 3 WAE in 2014.

Herbicide, Row Spacing and N Effects on Crop Density and Grain Yield

The herbicides did not affect the emergence (density) of lupin (Table 2, Table 5). On the other hand, wide row spacing decreased the lupin density from 63 to 49 plants m^{-2} (a reduction of 22%) but not its grain yield. Lupin grain yields were 53% greater with Herbicide 2 compared with Herbicide 1, an effect attributed to better ryegrass control with Herbicide 2 in the lupin crop.

Photo 4 (22 cm row spacing) and Photo 5 (44 cm row spacing) show the effect of row spacing on the growth of RR® canola. The row spacing of 44 cm reduced density of wheat from 136 to 106 plant m^{-2} (a reduction of 22%) (Table 5). The emergence of the wheat crop treated with trifluralin was 9% lower than with pyroxasulfone in 2013 (Table 5). At the 44 cm row spacing, wheat grain yield was reduced by 29% compared to the 22 cm row spacing (Table 5). Nitrogen source and rate did not affect the density of wheat (Table 2). The density of canola was reduced from 38 plants m^{-2} in 22 cm to 26 plants m^{-2} in 44 cm row spacing (a reduction of 28%) (Table 5).

In addition, the N3 (UAN) treatment reduced density (establishment) of canola by 15% compared to N_1 and 18% compared to N_2 but increased crop vigour of canola by 19% compared to N_1 and 12% compared to N_2 (Figure 3). The canola grain yield increased progressively with increases in N treatments, except at the 22 cm row spacing with the application of pyroxasulfone (alternative herbicide) (Table 6).

	
Photo 4. RR® canola plot sown at 22 cm row spacing and treated with N_1 in the 2014 experiment	**Photo 5. RR® canola plot sown at 44 cm row spacing and treated with N_3 in the 2014 experiment**

Table 4 The interaction of crop row spacing, herbicide type and applied nitrogen on the density of ryegrass plants in the 2013 wheat and the 2014 RR® canola at Cunderdin, WA[1].

Row spacing (cm)	Herbicides in wheat crop	Nitrogen (kg N ha^{-1})	Ryegrass in 2013 wheat crop (plants m^{-2})	Ryegrass in 2014 RR® canola crop (plants m^{-2})	
				3 WAE	12 WAE
22	Trifluralin (H1)	N_1	54	489	7
		N_2	51	380	7
		N_3	59	540	6
	Pyroxasulfone (H2)	N_1	44	508	6
		N_2	42	486	6
		N_3	76	296	6
44	Trifluralin (H1)	N_1	62	650	8
		N_2	67	510	9
		N_3	105	540	10
	Pyroxasulfone (H2)	N_1	35	448	5
		N_2	47	432	4
		N_3	51	488	5
P-value			0.05	ns	0.001
LSD.05			34.8	-	1.08

[1]N_1 = 25 kg N ha^{-1} as Urea, N_2 = 50 kg N ha^{-1} as Urea; N_3 = 50 kg N ha^{-1} as urea ammonium nitrate; WAE = week after emergence; RR = Roundup Ready.

Table 5 The effect of herbicides and row spacing on the crop emergence, and grain yield of crops from 2012 to 2014 in a lupin – wheat – RR® canola rotation at Cunderdin, WA[1].

Herbicides/Row spacing	Crop density (plants m^{-2}) 5WAE			Crop grain yield (kg ha^{-1})	
	2012 lupin	2013 wheat	2014 canola	2012 lupin	2013 wheat
Herbicide 1	57	115	31	394	1490
Herbicide 2	55	127	33	604	1870
LSD.05	ns	10.4	ns	85.7	192
22 cm	62.7	136	38	536	1970
44 cm	49.1	106	26	402	1390
LSD.05	3.72	18.0	3.5	ns	332.0

[1]Herbicide 1 = conventional herbicide: trifluralin for wheat and simazine for lupin; Herbicide 2 = alternative herbicides: pyroxasulfone for wheat and dimethenamid-p for lupin; WAE= weeks after emergence.

Table 6 The interaction of crop row spacing, herbicide type and applied nitrogen on canola grain yields in 2014, at Cunderdin, WA[1].

Row spacing (cm)	Herbicides in wheat crop	Nitrogen (kg N ha^{-1})	Grain yield (kg ha^{-1})
22	Trifluralin (H1)	N1	695
		N2	803
		N3	840
	Pyroxasulfone (H2)	N1	827
		N2	767
		N3	785
44	Trifluralin (H1)	N1	618
		N2	686
		N3	726
	Pyroxasulfone (H2)	N1	640
		N2	767
		N3	785
	P-value		0.016
	LSD.05		112.4

[1]N_1 = 25 kg N ha^{-1} as Urea, N_2 = 50 kg N ha^{-1} as Urea; N_3 = 50 kg N ha^{-1} as urea ammonium nitrate (Flexi N).

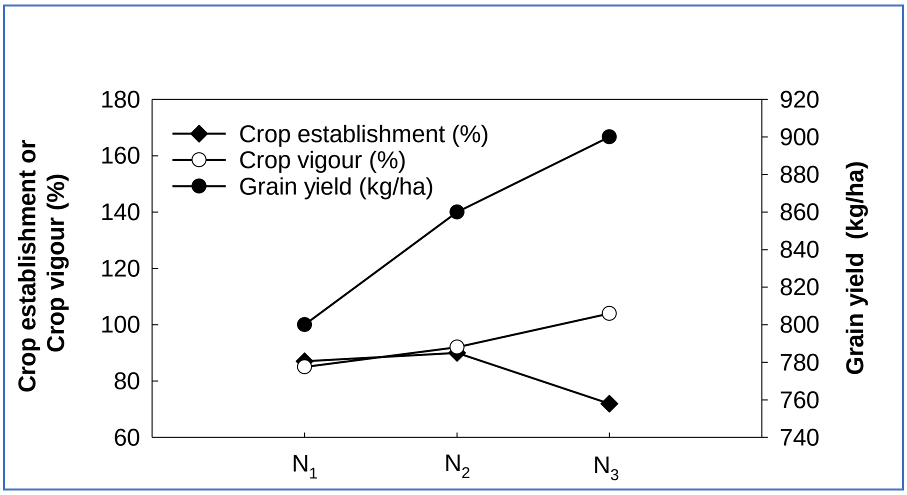

Figure 3 The effect of N on crop establishment (%), crop vigour (%) and grain yield of RR® canola in the 2014 season at Cunderdin, WA. N_1 = 25 kg N ha^1 (Urea), N_2 = 50 kg N ha^1 (Urea); N_3 = 50 kg N ha^{-1} (UAN). LSD (p=0.05) for crop establishment = 4.66%, crop vigour = 4.01%, and grain yield = 81.1 kg ha^{-1}.

Discussion

Relative Effectiveness of the Management Approaches on Ryegrass

RR® canola technology was effective in controlling the ACCase-resistant ryegrass population. At the end of this three-year crop rotation, the reduction in ryegrass was 99% compared to 1000 plants m^{-2} in the untreated buffer zone 1 in 2012. Most of the decrease can be attributed to the two applications of glyphosate in the RR® canola in 2014 (Photo 5).

Photo 5. A close-up photo showing dead ryegrass plants after second application of glyphosate in the RR® canola in the 2014 experiment Cunderdin, WA

By comparison, the other management approaches used had only modest or minor effects on ryegrass control. The rotation of herbicides, together with the rotation of crop species, reduced the ACCase-resistant ryegrass from 1000 plants m^{-2} to 586 (range 338 to 835) plants m^{-2} in the lupin crop in 2012, from 525 plants m^{-2} to 61 (range 44 to 79) plants m^{-2} in the 2013 wheat crop, and from 910 plants m^{-2} to only 7 (range 4 to 10) plants m^{-2} in the RR® canola crop in 2014.

Our results agree with published literature. For example, Zhang et al. (2014) compared RR®, Clearfield® (CL) and Triazine-Tolerant® (TT) canola and found that RR® canola produced the highest grain yield at both the low (Cunderdin) and high (Kojonup) rainfall areas of WA. In a five-year-rotation study, Stanton et al. (2010) found that glyphosate-tolerant (i.e. RR®) and TT canola achieved high levels of ryegrass control and attained higher yields than the conventional canola system.

Comparing the efficacy of weed control and yield advantages of herbicide tolerant crops with a standard herbicide treatment (sethoxydim plus ethametsulfuron) in a multi-site-year study in Canada, Harker et al. (2000) found that weed control in HT canola was highest with glyphosate, followed by imazethapyr/imazamox, and then glufosinate. In their study, the yield increases of glyphosate treatments over the standard treatment ranged from 13 to 39% but at some sites only. There is a general perception among some members of the public that the use of RR® canola could pose a risks to human health (when GM canola is consumed) and to the broader environment.

Row Spacing Effects on Crops and Ryegrass

Despite the effects of grain yields, there was little effect of narrow row spacing of lupin, wheat, or canola on the ryegrass density. In general, Fischer and Miles (1973) and Acciaresi and Chidichimo (2007) had earlier reported that seeding rate being constant, a reduction in the crop row spacing would increase the distance between plants within the row and is likely to result in an increased plant growth and grain yield due to lower intra-specific competition among the crop plants. An increase in grain yield at 22 cm row spacing was only found in wheat (42% higher in 22 cm than at 44 cm row spacing) in the present study. In wheat, the wider row spacing of 44 cm reduced the density of wheat by 22% which likely explained the decrease in grain yield.

In contrast, the decreased plant populations of lupin and canola did not affect the grain yields. Amjad and Anderson (2006) found a decline in wheat plant density with increased row spacing, even though the seed rate was constant. Unlike cereals, increased row spacings of canola do not usually result in grain yield reductions because canola plants are sufficiently plastic in producing similar biomass and grain yield in wide and narrow rows. This plasticity suggests that wide row spacing is an option for sowing canola (Harries et al., 2015).

Further, Patil and De (1978) reported that plants of *Brassica campestris* L. sown in wide rows utilized less water during the vegetative and flowering stages than the plants sown in close row spacing. In contrast, Kirkland (1993) and Weiner et al. (2001) have shown that very narrow row spacing (10 cm) or planting in a uniform grid can maximize the grain yield of cereal crops at higher seeding rates. Compared to wide row spacing, narrow row spacing is likely to facilitate crop plant with greater competitive ability than weeds (Minkey et al., 2000).

Herbicide Effects on Resistant Ryegrass in the Lupin and Wheat Crops

The present results suggest that there is scope for improved ryegrass control and crop grain yield by switching to alternative herbicides in lupin. Simazine was less effective than dimethenamid-p for ryegrass control in lupin. This may be due to the low rainfall (51% of long-term average) in May 2012, which resulted in the lupin crop being sown under dry conditions. Rainfall occurred about 16 days after sowing, and perhaps, simazine did not reach the root zone of ryegrass seedlings.

Previous studies had indicated that about 12.5 mm of rainfall in the USA (Peters, 2014) or 25 to 30 mm of rainfall in Australia (Nufarm, 2019) were needed after application on dry soil to disperse soil-applied herbicides, such as simazine into the soil, so that the herbicides can be absorbed by roots of weed seedlings. Gunasekara (2004) reported that the persistence of simazine is expected to be longer under dry conditions than wet conditions. Despite a half-life of 3-36 days of dimethenamid-p (APVMA, 2007), weed control in lupin by simazine was much lower than dimethenamid-p. The reason for lower efficacy of simazine in this study is unclear and needs further investigation.

Both herbicides applied in the wheat crop provided similar efficacy in controlling ryegrass in 2013. Although the vapour pressure of pyroxasulfone is lower than trifluralin, the similar efficacy of trifluralin was probably associated with the longer half-life of trifluralin than pyroxasulfone (Preston, 2017). However, the effects of herbicides applied in 2012 or 2013 were confined to those crops and had no significant influence on the density of ryegrass at 12 WAE in the canola crop of 2014.

The higher initial density of ryegrass with N_3 at 44 cm than N_1 or N_2 in the 2013 wheat crop, in the presence of soil-applied herbicide trifluralin (Table 4), suggests a possible stimulation of ryegrass emergence by the N_3 treatment.

These results demonstrate that greater herbicide incorporation by soils, thrown by the tines of the sowing machine, from the crop rows to the inter-row spaces, and increased competition from crop plants in narrow row spacing than wide row spacing, might have contributed to the greater reduction of ryegrass in this study at 22 cm than at 44 cm row spaces.

Although the effect of N fertilizers on the emergence of ryegrass was somewhat unclear in our study, Agenbag and De Villiers (1989) found that ammonium-containing fertilizers including UAN (N_3) were quite effective in stimulating germination and emergence of wild oat (*Avena fatua*) in sandy and loamy soil. However, the reason for a reduction of ryegrass density in the wider row spacing under pyroxasulfone is unclear from our study and needs further investigation.

In a related study, Yamaji et al (2016) reported that the low water solubility and the low vapour pressure of pyroxasulfone, applied to a sandy loam soil in a field that was free of clods, led to limited horizontal diffusion of this molecule on the soil surface, and also posed a low risk of volatilization.

As such, light incorporation in the wider row spacing might have maintained the availability of more pyroxasulfone molecules in the wheat crop to be accessed by ryegrass roots in the present study. Pyroxasulfone has the potential to provide weed control for an extended duration with low risk of runoff or volatilization (Yamaji et al. 2016).

In our study, no residual effects of the herbicides applied in previous wheat and lupin crops and their row spacing were evident on the initial density of ryegrass in the 2014 RR® canola crop. However, the grain yield of RR® canola in 2014 was influenced by the interaction of row spacing and herbicides (applied in 2013 wheat crop) and N.

Nitrogen Effects on Crops and Ryegrass

The hypothesis that placement of N fertilizer in the seeding row would favour crop N uptake relative to weeds was not supported by the results of our study. Indeed, the highest N rate, supplied as UAN (N_3), had higher initial weed density than N_1 or N_2 in the 2013 wheat crop, indicating a possible stimulation of ryegrass emergence by N_3.

The application of N_3 did not influence the density of the crop or ryegrass nor grain yield of the wheat crop. In contrast to our finding, Nelson (2019) reported greater grain yield and protein content in a wheat crop from applications of N_3.

The lack of response of grain yield to N_3 in the present study may be related to the soil N supply. Alternatively, the effective depth of N_3 placement in a wheat crop might need further investigation. However, in terms of the aims of our study, there was no support for the notion that N fertilizer placement close to the row of wheat could increase its competitiveness with ryegrass.

In our study, application of N_3 increased crop vigour and grain yield of RR® canola even though the ryegrass density at 3 WAE was not influenced by N treatments. Hence, with the placement of N fertilizer close to the RR® canola seeding row had no positive effect in suppressing weed competitiveness relative to the crop plants.

Herbicide Resistance and GM crops – Opportunities and Constraints

ACCase resistance in ryegrass was verified at the Cunderdin site, which is not a new occurrence within WA. Owen et al. (2014) reported that 96% of the ryegrass populations tested from WA were resistant to ACCase-inhibiting herbicide, diclofop-methyl, and the ALS-inhibiting herbicide, sulfometuron. Cross-resistance to these two modes of action (MOA) herbicides is also evident in in 95% of the ryegrass populations.

Ryegrass has also evolved resistance to ACCase in other regions of southern Australia. Boutsalis et al. (2012) reported up to 60% of the southeast Australian ryegrass populations had resistance to the ACCase herbicides such as diclofop-methyl, tralkoxydim, and pinoxaden.

Owen et al. (2014) also noted that resistance to other herbicide modes of action (MOAs) was significantly lower than for ACCase-inhibiting herbicides, with only 27% of the populations containing plants with resistance to other herbicides including glyphosate. The Cunderdin population of ryegrass was quite susceptible to glyphosate. Hence, this study at Cunderdin could be considered representative of the weed control challenges with herbicide-resistant ryegrass across the grain growing regions of WA, and possibly, elsewhere in Australia.

Despite the existence of resistance to glyphosate in some weed species, GR crops represent more than 80% of the 120 million ha of transgenic crops grown annually world-wide. The economic advantages of the technology, as well as the simple and superior weed control by glyphosate, are the reasons for its wide-scale adoption (Duke and Powles, 2009).

In the 1990s, researchers developed the canola crop with resistance to herbicides (CropLife Canada, 2020). This technology enables a farmer to use a herbicide without damaging the crop to control weeds that otherwise might compete with the canola for water and nutrients. This means that farmers can practice no-till or conservation tillage, which may reduce soil erosion, improve soil quality, reduce greenhouse gas emissions, and cut water use (CropLife Canada, 2020).

On the positive side, RR® canola (with GM herbicide resistance traits) has allowed producers to achieve superior weed control with the use of less total applied herbicide. Without the RR® canola technology, there will be ongoing selection pressure for weeds to develop resistance to the few other herbicide options available for use within canola crops. Despite the afore-mentioned, well-publicized advantages, the use of RR® technology for improved weed control and crop yields, needs to be considered in a broader context.

In our view, RR® canola (GM herbicide resistance traits) has allowed producers to achieve superior weed control with the use of less total applied herbicide. Without the RR® canola technology, there will be ongoing selection pressure for weeds to develop resistance to the few other herbicide options available for use in canola crops.

However, there are some genuine concerns among the communities about the RR technology that uses the glyphosate molecule. Hursh (2011) has rightly pointed out that glyphosate is such a widely used herbicide that the RR® canola varieties may trigger other weed control issues, particularly if canola volunteer plants are not controlled.

In a survey of soybean fields containing waterhemp (*Amaranthus rudis* J. D. Sauer) infestations across Missouri of the US, Rosenbaum and Bradley (2013) confirmed glyphosate resistance in 69% of the 144 populations of waterhemp, They noted that populations of glyphosate-resistant waterhemp were more likely to occur in fields where no other weed species were present at the end of the season. These were also the fields where continuous cropping of soybean was practised, which exclusively received glyphosate for several consecutive seasons, compared to fields with glyphosate-susceptible waterhemp. Evans et al. (2016) also reported that occurrence of glyphosate-resistant weed biotypes of *Amaranthus tuberculatus* (Moq.) J. D. Sauer was greatest in fields, which received the msot frequent glyphosate applications at high annual rates with only a few herbicides from other MOAs on an yearly basis.

They also noted that where other herbicide MOAs were mixed with glyphosate at the time of application, the likelihood of GR *A. tuberculatus* was reduced. Based on the meta-analysis, Chow (2019) reported that people who are highly exposed to glyphosate have up to 41% increased risks of developing non-Hodgkin Lymphoma (NHL) while the Environmental Protection Agency of the USA (EPA) declared that glyphosate is not likely to be carcinogenic to humans.

In Australia, based on the risk assessment by the OGTR (2012), it was concluded that the risks posed by the commercial release of RR® canola to human health, safety and the environment are no greater than those posed by conventional (non-GM) canola. Broadly, we agree that the continuous use of RR® canola may also increase the number of documented cases of glyphosate- resistance in annual ryegrass and other weeds in Australia. Glyphosate-resistant biotypes of ryegrass will survive in RR® canola unless other interventions, such as (a) alternative knockdown herbicides are used prior to sowing, cultivation at or prior to sowing and/or (b) in-crop herbicides from other mode of action (MOA) are used. Such practices are part of the best management package recommendations for minimising the risk of increased selection for the glyphosate-resistant biotypes (Preston, 2017).

RR® canola growers in Australia are encouraged to undertake a paddock risk assessment and develop a resistance management plan before growing RR® canola (Pritchard, 2014).

Glyphosate should not be used in the year following RR® canola (Pritchard, 2014). Fortunately, ryegrass plants with glyphosate resistance have a ‘fitness penalty’ (i.e. crops can compete better with glyphosate-resistant ryegrass than with glyphosate-susceptible ryegrass). This means some IWM tactics, such as growing a competitive crop, are likely to work better with glyphosate-resistant ryegrass than glyphosate-susceptible ryegrass (Pritchard, 2014). However, if RR® canola is grown frequently, not only will it increase the risks of diseases, but also the risks of evolving more glyphosate-resistant weed biotypes (GRDC, 2018).

Canada is now the biggest single producer of canola. More than 20 million metric tonnes of canola were produced in 2018, about half of it in Saskatchewan (CropLife Canada, 2020). Beckie et al. (2006) examined some agronomic, economic, and environmental impacts of herbicide-resistant (HR) canola, soybean, corn, and wheat in Canada after 10 years of growing herbicide-resistant (i.e., glyphosate-resistant, GR or Genetically-modified, GM) cultivars. They found that the rapid adoption of herbicide-resistant canola and soybean brought a net economic benefit to farmers.

Herbicide-resistant (HR) crops often have improved weed management, produced greater yields or economic returns, and have similar or reduced environmental impacts, compared with their non-HR crop counterparts.

In Canada, there has been no measured changes in volunteer weed problems associated with HR crops. However, in zero-tillage systems when glyphosate is used alone to control canola volunteers, there have been issues with weed biotypes with evolved resistance.

Weed shifts, as a consequence of HR canola, have also been documented, but a reduction in weed species diversity was not noted although gene flow from glyphosate-resistant canola to wild populations of bird’s rape (*Brassica rapa* L.) in eastern Canada occurred (Beckie et al., 2006).

The frequent use of HR crops in rotations and application of the same mode-of-action herbicide and/or multiple in-crop herbicide applications of the same mode of action over time can result in intense selection pressure for weed resistance. Therefore, diversifying the cropping systems and rotations are the key to sustainable agriculture. As such, the use of HR crops must adhere to this fundamental principles of farming and cropping systems diversity (Beckie et al., 2006).

Conclusions

The ryegrass population in our study was highly resistant to diclofop-methyl and clethodim but was highly susceptible to glyphosate. The initial soil seed bank of this herbicide-resistant ryegrass population was 6518±291 plants m^{-2}. None of the management factors, except herbicides, significantly decreased ryegrass density. Indeed, the fertilizer treatment N_3 (UAN) increased the emergence of ryegrass (more in 44 cm than 22 cm rows). This aspect needs to be investigated by further studies.

In the lupin crop of our study, dimethenamid-p reduced the ryegrass density by 61%, while simazine reduced the ryegrass density by 21% compared with the untreated buffer zone. The herbicides applied in the 2013 wheat crop had no significant effects on the resistant ryegrass density. However, the most striking weed control (99%) was in RR® canola, attributed to the double application of glyphosate.

This high level of weed control should reduce the soil seed bank of resistant ryegrass, over time. Once the ryegrass seed bank has been reduced to a low level, it is important for sustainable grain production to implement IWM practices and maintain low seed bank levels of herbicide-resistant ryegrass to minimize further development of herbicide resistant populations. Such IWM practices should include a range of physical, chemical, biological and mechanical approaches of weed control to deplete soil seed bank of weeds, kill resistant populations by effective and selective herbicides from different modes-of-action, and stop viable seed being set by resistant ryegrass. Additionally, IWM practices should also prevent viable weed seed being added to the soil seed bank, and the introduction of viable weed seed from external sources.

Finally, we emphasize that although the RR® canola technology appears to be a useful tool for effectively controlling resistant ryegrass and, perhaps, other weed species, this technology should be used carefully and judiciously. This requires strictly following the management guidelines of the OGTR (2018) to minimise the risks of further developments of glyphosate resistance in ryegrass and other weeds and potential health hazards.

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