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Alternative Herbicides for the Management of Clethodim-Resistant Rigid Ryegrass (*Lolium rigidum*) in Faba Bean (*Vicia faba* L.) in Southern Australia

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Two field experiments were conducted during 2012 and 2013 at Roseworthy, South Australia to identify effective herbicide options for the management of clethodim-resistant rigid ryegrass in faba bean. Dose–response experiments confirmed resistance in both field populations (B3, 2012 and E2, 2013) to clethodim and butroxydim. Sequencing of the target site of acetyl coenzyme A carboxylase gene in both populations identified an aspartate-2078-glycine mutation. Although resistance of B3 and E2 populations to clethodim was similar (16.5- and 21.4-fold more resistant than the susceptible control SLR4), the B3 population was much more resistant to butroxydim (7.13-fold) than E2 (2.24-fold). Addition of butroxydim to clethodim reduced rigid ryegrass plant density 60 to 80% and seed production 71 to 88% compared with the standard grower practice of simazine PPI plus clethodim POST. Clethodim + butroxydim combination had the highest grain yield of faba bean (980 to 2,400 kg ha^{-1}). Although propyzamide and pyroxasulfone plus triallate PPI provided the next highest levels of rigid ryegrass control (< 60%), these treatments were more variable and unable to reduce seed production (6,354 to 13,570 seeds m^{-2}) to levels acceptable for continuous cropping systems.
Nomenclature: Clethodim; rigid ryegrass, *Lolium rigidum* Gaudin; faba bean, *Vicia faba* L.
Key words: Herbicide strategies, PPI herbicides, target site mutation, weed control.

En 2012 y 2013, se realizaron dos experimentos de campo en Roseworthy, en el sur de Australia, para identificar opciones de herbicidas efectivos para el manejo de *Lolium rigidum* resistente a clethodim en campos de haba. Experimentos de respuesta a dosis confirmaron la presencia de resistencia a clethodim y butroxydim en ambas poblaciones de campo (B3, 2012 y E2, 2013). La secuenciación del sitio activo del gen de acetyl coenzyme A carboxylase identificó la mutación aspartate-2078-glycine en ambas poblaciones. Aunque la resistencia a clethodim de B3 y E2 fue similar (16.5 y 21.4 veces más resistentes que el control susceptible SLR4), la población B3 fue mucho más resistente a butroxydim (7.13 veces) que E2 (2.24 veces). La adición de butroxydim a clethodim redujo la densidad de *L. rigidum* 60 a 80% y la producción de semilla 71 a 88%, al compararse con la práctica estándar de los productores de aplicar simazine PPI más clethodim POST. La combinación de clethodim + butroxydim tuvo el mayor rendimiento de grano de haba (980 a 2,400 kg ha^{-1}). Aunque propyzamide y pyroxasulfone más triallate PPI brindaron los segundos niveles de control de *L. rigidum* más altos (< 60%), estos tratamientos fueron más variables e incapaces de reducir la producción de semillas (6,354 a 13,570 semillas m^{-2}) a niveles aceptables para sistemas de cultivo continuo.

Rigid ryegrass is one of the most troublesome herbicide-resistant weeds in Australia (Jones et al. 2005), and it has evolved resistance to at least 11 major herbicide mode-of-action groups (Heap 2015). As a result of its suitability to the climate, its extensive use as a pasture species, and its prolific seed production (Rerkasem et al. 1980), rigid ryegrass is present in high numbers across the southern Australian grain belt (Pannell et al. 2004). It is highly competitive against pulse crops and can significantly reduce grain yield (Hashem et al. 2011; Lemerle et al. 1995). Rigid ryegrass can produce more than 1,000 seeds per plant even in extremely competitive surroundings (McGowan 1967), which enables the plants surviving weed control to readily replenish the seed bank and reinfest subsequent crops. In early to late 1980s rigid ryegrass was effectively controlled in crops with grass-selective herbicides. However, overreliance on the acetyl coenzyme A carboxylase (ACCase)-inhibiting and acetolactate synthase-inhibiting herbicides has led to the evolution of widespread resistance to these important herbicide groups (Boutsalis et al. 2012).

As many populations of rigid ryegrass have evolved resistance to the grass-selective herbicides, control options for rigid ryegrass in broadleaf crops have become far more limited. Clethodim, an

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ACCase-inhibiting cyclohexanedione herbicide, has been widely used to provide selective control of grass weeds in many broadleaf crops (Burke et al. 2004; Burke and Wilcut 2003; Vidrine et al. 1995). Clethodim is deemed to be the lowest-resistance-risk herbicide, with only 2 of 11 target-site mutations found in weed populations endowing resistance to this herbicide (Beckie and Tardif 2012). Aspartate-2078-glycine and cystine-2088-arginine mutations in the plastidic ACCase enzyme have been identified as the main mutations endowing clethodim resistance at field rates (Yu et al. 2007). Délye et al. (2008) reported that leucine-1781, glycine-2078, and alanine-2096 mutations in the ACCase gene may also confer resistance to clethodim in the field if conditions are not optimal for herbicide efficacy, or with reduced application rates. Repeated use of clethodim over the last 2 decades has resulted in the evolution of clethodim resistance in rigid ryegrass populations (Yu et al. 2007), with as many as 60% of fields across southeastern Australia having some level of clethodim resistance (Boutsalis et al. 2012). In the past, clethodim resistance was managed by increasing its dose; however, this is no longer a viable option for growers as several populations of rigid ryegrass are now resistant to clethodim doses greater than the recommended field rate (120 g ai ha^{-1}).

For growers with clethodim-resistant rigid ryegrass, there are few herbicide options available for its selective control in broadleaf crops. Consequently, the use of PPI herbicides has increased to reduce reliance on grass-selective herbicides like clethodim. Traditionally trifluralin PPI was used to control rigid ryegrass in southern Australia. However, many rigid ryegrass populations across southern Australia have now evolved resistance to trifluralin (Boutsalis et al. 2012). Given the increasing prevalence of clethodim-resistant rigid ryegrass in southern Australia, there is an urgent need to identify alternative herbicides for its control. Therefore, the objective of this study was to identify effective herbicide options for the control of clethodim-resistant rigid ryegrass in faba bean, which is an important pulse crop in South Australia.

Materials and Methods

Dose–Response Experiments. Dose–response experiments were conducted to determine the resistance status of two field populations (B3 and E2) to clethodim and butroxydim during the growing seasons of 2012 and 2013. Seedlings at one-leaf growth stage were randomly collected from two different experimental sites at Roseworthy located in the Lower North region of South Australia (34.51°S, 138.68°E at 68 m above sea level) and transplanted into 9.5 cm by 8.5 cm by 9.5 cm pots containing cocoa peat potting mix (Boutsalis et al. 2012). There were nine seedlings per pot and the pots were arranged in a randomized complete block design with three replications. The plants were maintained outdoors under natural conditions and watered and fertilized as needed. A highly characterized herbicide-susceptible (S) rigid ryegrass biotype (SLR4) served as the control population (Wakelin and Preston 2006). At the two- to three-leaf growth stage, rigid ryegrass seedlings were treated with clethodim and butroxydim. Clethodim was applied at 0, 7.5, 15, 30, 60, and 120 g ai ha^{-1} for S plants, and 0, 30, 60, 120, 240, and 480 g ha^{-1} for R plants. Butroxydim was applied to both resistant and susceptible populations at 0, 5.6, 11.3, 22.5, 45, and 90 g ai ha^{-1}. The recommended dose of clethodim in Australia is 120 g ha^{-1} and for butroxydim 45 g ha^{-1}. As per label recommendations, esterified canola oil mixed with nonionic surfactant Hasten™ (Victorian Chemical Co. Pty. Ltd., Victoria, Australia) at 1% (v/v) was added to clethodim and 1% (v/v) paraffinic oil mixed with nonionic surfactant Supercharge® (Crop Care Australasia Pty. Ltd., Queensland, Australia) to butroxydim. The herbicides were applied by using a laboratory moving boom sprayer (Tee-Jet 110° flat fan, Spraying Systems, Wheaton, IL) equipped with a single nozzle (Hardi ISO F-110-01, Hardi, Adelaide). The output volume of the sprayer was 103 L ha^{-1} at a pressure of 250 kPa and a speed of 1 m s^{-1}. Control plants were not treated with herbicide. Plants were returned and maintained outdoors after herbicide treatment. Three weeks after spraying, surviving plants were counted and recorded as alive if they had strongly tillered since herbicide application and plants showing chlorosis, stunting, and mortality were considered as susceptible (Powles et al. 1998). The surviving plants were harvested and oven dried at 80 C for 2 d. The dry weight data were expressed as a percentage of the respective unsprayed control.

Sequencing of *ACCase* gene. Fresh leaf material (~ 1 cm^2) was harvested from young leaves of at least 12 resistant plants of both field populations

Table 1. Primer sequences used for amplification and sequencing of the carboxyl transferase (CT) domain of the *acetyl coenzyme A carboxylase* (*ACCase*) gene in *Lolium rigidum* from genomic DNA.

Primer	Sequence 5′ 3′
Acclr9	ATGGTAGCCTGGATCTTGGACATG
Acclr6	GGAAGTGTCATGCAATTCAGCAA
AccCT 2F	CCACTCCTGAATTTCCCAGTGG
AccCT 2R	CGCGATTTGAGTGTACAAAGGCTG
AccCT MidF	CCTGAGAATACATGTGATCCTCGTG
AccCT MidR	CCATTTCCCTTGGCTGTCATCAATGCC

(B3 and E2), snap frozen in liquid nitrogen, and stored at −20 C until its use. DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Australia) in accordance with the manufacturer's instructions. Primers were designed to amplify regions in the carboxyl tranferase (CT) domain known to be involved in sensitivity to ACCase herbicides. Two sets of primers covering all seven known mutation sites (1781, 1999, 2027, 2041, 2078, 2088, and 2096) were designed against the blackgrass (*Alopercurus myosuroides* Huds.) (accession number AJ310767) *ACCase* gene sequence (Table 1) and were used to amplify a 1.5-kb fragment covering nearly the entire CT domain without any intron. The range of amino acids covered by the fragment was equivalent to codons 1658–2157 in blackgrass. A nested polymerase chain reaction (PCR) approach was used with oligo set Acclr9 and Acclr6 (Zhang and Powles 2006) followed by oligo set AccCT 2F and AccCT 2R (Malone et al. 2014). MyFi DNA polymerase kit (Bioline, Australia Pty Ltd, Alexandria, NSW, 1435) was used to run PCR reactions of 25 μl that contained 80 to 100 ng of DNA template, 1× MyFi reaction buffer, 0.8 μM of each specific primer, and 2 units of MyFi DNA polymerase. Amplification was carried out in an automated DNA thermal cycler (Eppendorf Mastercycler® Gradient, Germany) with PCR conditions as described by Malone et al. (2014).

The PCR products were examined on 1.5% agarose gels stained with 1× SYBR® Safe DNA gel stain. Samples were electrophoresed in 1× TAE buffer (40 mM Trizma base, 1 mM Na_2EDTA, pH to 8 with glacial acetic acid) at 90 V and photographed under UV light (λ302 nm). DNA fragment sizes were estimated by comparing their mobility with bands of known sizes in a low-mass molecular weight marker (Invitrogen, Australia). PCR products were sequenced by Australian Genome Research Facility Ltd., Australia using primers CT Mid F and CT Mid R (Malone et al. 2014) to obtain sequence data covering the full CT domain fragment. DNA sequence data were assembled, compared, and analyzed using ContigExpress from the Vector-NTi Advance 11.5 programs (Invitrogen) and all sequences visually rechecked using the chromatogram files.

Field Experiments. Two field experiments were conducted in different fields (B3 and E2) over the growing seasons of 2012 and 2013 at Roseworthy located in the Lower North region of South Australia (34.51°S, 138.68°E at 68 m above sea level). The soil at the field sites was a calcarosol (McKenzie et al. 2001) with organic matter content of 2 to 2.5% and a pH (water) of 7 to 7.5 in 0- to 20-cm layer. The long-term average annual rainfall at Roseworthy is 434 mm and average growing season rainfall (April to October) is 321 mm. Rainfall received at the site in 2012 and 2013 is shown in Table 2 (Australian Bureau of Meteorology 2014). Before the start of the experiment, the field sites were treated with glyphosate (900 g ai ha^{-1}) and oxyfluorfen (22 g ha^{-1}) for preplant weed control. Faba beans (cv. Nura) were sown at a depth of 5 cm at a seed rate of 150 kg ha^{-1} (for a target of

Table 2. Summary of rainfall, cropping history, harvest, and herbicide application dates for the two study sites.

Year	Rainfall		Previous crop	Harvest date	Spray application date	
	Annual	GSR[a]			PPI	POST[b]
	mm					
2012	337	243	Lentil	November 27	May 21	July 20
2013	417	341	Barley	November 18	May 16	July 2

[a] Abbreviation: GSR, growing-season (April to October) rainfall.

[b] POST herbicide treatments were applied to rigid ryegrass at three- to four-leaf stage of growth.

Table 3. Dose of clethodim and butroxydim required for 50% mortality (LD_{50}) and for 50% biomass reduction (GR_{50}) of resistant (R) and susceptible (S) rigid ryegrass populations with confidence intervals (CI) in parentheses. R/S is the ratio of LD_{50} and GR_{50} of resistant and susceptible populations.

Population	LD_{50} (g ha^{-1})	R/S	GR_{50} (g ha^{-1})	R/S
Experiment 1 (May 2012)				
Clethodim				
B3	68.2 (54.5, 85.3)	16.5	38.1 (30.4, 47.7)	9.7
SLR4	4.13 (2.5, 6.8)	-	3.9 (2.4, 6.4)	-
Butroxydim				
B3	26.9 (24.2, 29.9)	7.1	6.3 (3.6, 11.1)	1.6
SLR4	3.8 (3.0, 4.8)	-	4.0 (3.4, 4.7)	-
Experiment 2 (May 2013)				
Clethodim				
E2	58.1 (49.9, 67.7)	21.4	32.7 (26.4, 40.4)	12.3
SLR4	2.7 (2.7, 2.8)	-	2.7 (2.6, 2.7)	-
Butroxydim				
E2	7.7 (6.9, 8.7)	2.2	5.0 (4.3, 5.8)	1.6
SLR4	3.5 (2.6, 4.7)	-	3.0 (2.1, 4.5)	-

30 plants m^{-2}). The crop was sown on May 22, 2012 and June 5, 2013 using a no-till plot seeder fitted with knife-point openers (16 mm) and press wheels. Plots were 10-m long and contained six crop rows spaced 25 cm apart. Fertilizer rate was consistent with the local grower practice of 100 kg ha^{-1} of diammonium phosphate (18 kg N and 20 kg P ha^{-1}) banded below the seed at sowing. The experiments were established in a randomized complete block design with four replicates. Herbicides (PPI and POST) were applied using an all-terrain vehicle fitted with a spray boom delivering 100 L ha^{-1} water volume at a pressure of 200 kPa. Herbicide treatment of PPI simazine (1,350 g ha^{-1}) followed by POST clethodim (120 g ha^{-1}) is considered the standard grower practice for the district. The dose and timing of other herbicide treatments are presented in Table 4. As per label recommendations, clethodim was applied with spray adjuvant Hasten at 1% (v/v) (vegetable oil, Victorian Chemicals) and butroxydim with non-ionic surfactant Supercharge when rigid ryegrass had reached the three- to four-leaf growth stage.

Effect of Herbicides on Rigid Ryegrass Density and Seed Production and Grain Yield of Faba Bean. Rigid ryegrass plant and spike density were assessed in a 0.25-m^2 quadrat placed at four random locations in each plot. Assessments on rigid ryegrass plant density were taken 12 weeks after sowing (WAS) before and after POST herbicide application. Spike density was assessed 16 to 18 WAS in October when all spikes had emerged. Seed production of rigid ryegrass was determined by

Table 4. Effect of herbicide treatments on rigid ryegrass plant and spike density, and seed production in faba beans grown at Roseworthy in 2012 and 2013.[a]

		Rigid ryegrass[b]					
		Weed density		Spike density		Seed production	
Treatment	Rate	2012	2013	2012	2013	2012	2013
	g ai ha^{-1}	Plant m^{-2}		Spikes m^{-2}		Seed m^{-2}	
Simazine PPI + clethodim POST	1,350, 120	181 d	112 bc	350 c	8 a	15,873 cd	73 a
Simazine PPI + clethodim + butroxydim POST	1,350, 120 + 45	73 ab	22 a	105 a	2 a	4,539 a	9 a
Dimethenamid PPI	720	300 e	185 bcd	748 d	281 bc	3,5169 e	15,074 b
Pyroxasulfone PPI	100	113 bc	208 bcd	271 bc	219 b	1,2498 bc	10,067 ab
Pyroxasulfone + triallate PPI	100 + 800	83 ab	199 bcd	149 ab	252 bc	6,354 a	13,171 b
Prosulfocarb + *S*-metolachlor PPI	2,000 + 300	173 d	325 d	393 c	449 c	18,704 d	20,775 b
Prosulfocarb + *S*-metolachlor + triallate PPI	2,000 + 300 + 800	147 cd	221 cd	335 c	287 bc	16,134 cd	15,032 b
Propyzamide PPI	500	64 a	101 b	172 ab	256 bc	7,742 ab	13,570 b

[a] Means within the same column followed by the same letters are not significantly different according to P = 0.05.

[b] Rigid ryegrass plant density m^{-2} data were square root transformed before mean comparisons. Data presented are the nontransformed mean values.

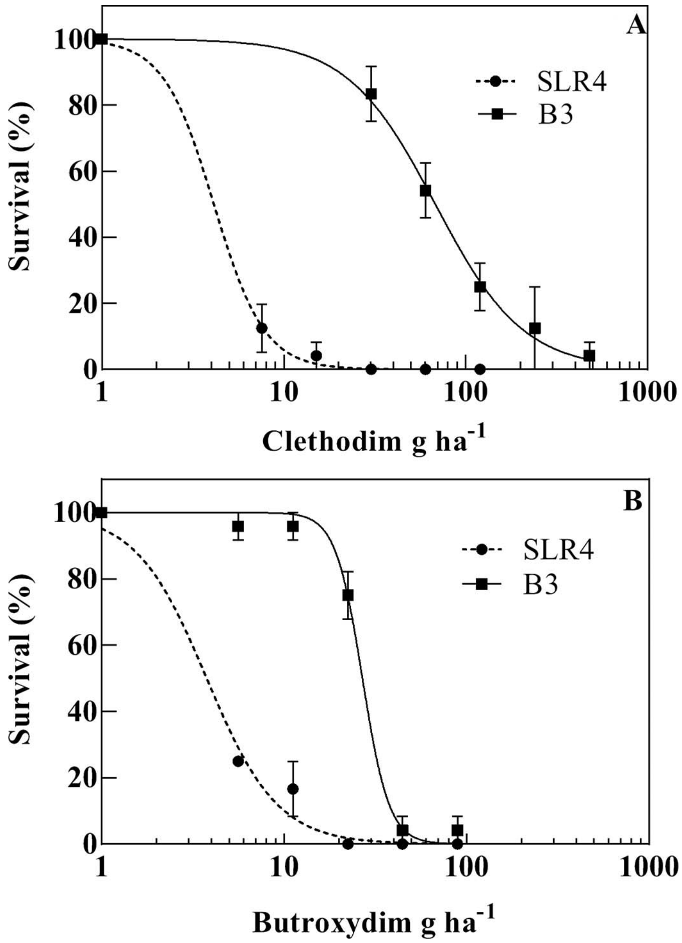

Figure 1. Dose–response experiments undertaken in May 2012 (1A and 1B) showing the response of field populations of ryegrass B3 (■) and E2 (▼) and a known susceptible population (●) to clethodim and butroxydim. Recommended rate of clethodim is 120 g ha^{-1} and butroxydim is 45 g ha^{-1}. Each data point is a mean of three replicates and bars represent standard error of the mean. Nonlinear regression analysis was performed using the equations (1A): $y = 100/1 + 10^{(1.833 - x) \times -1.795}$, (1B): $y = 100/1 + 10^{(1.430 - x) \times -5.902}$, where y is the plant survival (%) or biomass reduction (%) and x is the log dose of the herbicide used.

counting the number of spikes of 20 rigid ryegrass plants randomly sampled from each plot. The number of seeds produced per plant was determined by multiplying the number of spikes by the average number of seed in each spike. The number of seed produced per unit area was based on the weed density and shown as number of seeds per square meter. Faba bean grain yield was determined with a small plot harvester when the crop had reached physiological maturity and 12% grain moisture content. Harvest dates in each experiment are shown in Table 2. A grain subsample of 500 g was used to determine the average grain weight (g [100 seeds]$^{-1}$).

Statistical Analyses. Weed control (plant and spike density, and rigid ryegrass seed production) and crop data were analyzed separately with ANOVA (Genstat 5 Committee 2003). A square-root variance-stabilizing transformation was used for rigid ryegrass plant density data before analysis. Original means are reported with mean separation of the transformed data. Means were separated using LSD at $P = 0.05$. Data (plant survival and dry weight) from dose–response experiments were analyzed using log-logistic equation (Graphpad Prism v.6.0; GraphPad Software, San Diego, CAia) to calculate the dose of herbicide required to produce 50% reduction in plant survival (LD_{50}) and dry weight (GR_{50}). The model fitted was

$$y = 100/\left[1 + 10^{(\log IC_{50} - x) \times b}\right] \qquad [1]$$

where y is the plant survival (%) or biomass reduction (%), x is the log dose of the herbicide used, IC_{50} is the dose of herbicide required to produce 50% reduction in plant survival or biomass, and b is the slope of the curve.

Results and Discussion

Dose–Response Experiments. Dose–response experiments confirmed resistance in both field populations (B3 and E2) to clethodim, whereas the susceptible population (SLR4) was controlled with less than the recommended field rate (< 120 g ha^{-1}) of clethodim (Figures 1A and 2A). The LD_{50} values for clethodim were 68.2 and 58.1 g ha^{-1} for R populations B3 and E2, respectively. In contrast, the S population showed 50% mortality at 4.13 and 2.71 g ha^{-1}, respectively (Table 3). On the basis of the LD_{50} values, the R populations B3 and E2 were approximately 16 and 21 times more resistant to clethodim than the S population SLR4 (Table 3). Similar levels of resistance (20-fold) were reported for several populations of rigid ryegrass from the Western Australian wheat belt (Yu et al. 2007). According to Yu et al. (2007), the ACCase mutation present, the homo-/heterozygous status of a plant for a specific mutation, combinations of different resistance alleles, and herbicide rate used in the field contribute to the overall level of resistance

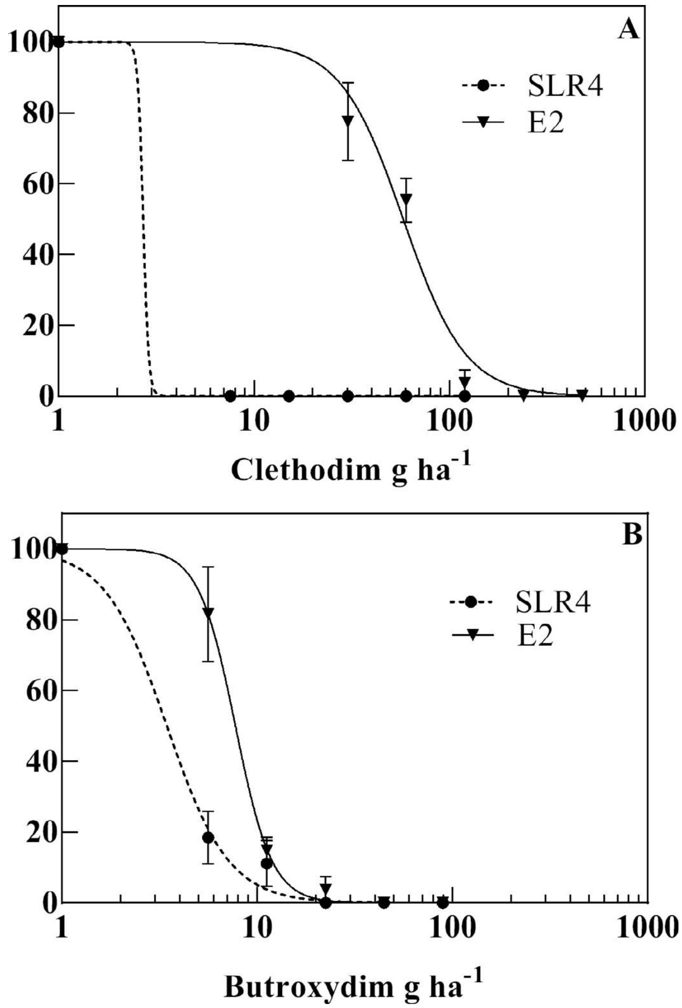

Figure 2. Dose–response experiments undertaken in May 2013 (2A and 2B) showing the response of field populations of ryegrass B3 (■) and E2 (▼) and a known susceptible population (●) to clethodim and butroxydim. Recommended rate of clethodim is 120 g ha^{-1} and butroxydim is 45 g ha^{-1}. Each data point is a mean of three replicates and bars represent standard error of the mean. Nonlinear regression analysis was performed using the equations (2A): $y = 100/1 + 10^{(1.766 - x) \times -2.743}$, (2B): $y = 100/1 + 10^{(0.8882 - x) \times -4.629}$, where y is the plant survival (%) or biomass reduction (%) and x is the log dose of the herbicide used.

to clethodim. The clethodim rate causing 50% reduction of shoot growth (GR_{50}) for the S population was 3.93 and 3.97 g ha^{-1} as compared with 38.1 and 32.7 g ha^{-1} for R populations B3 and E2, respectively.

Dose responses of the R populations (B3 and E2) and the S (SLR4) to butroxydim (Figures 1B and 2B) were different. The susceptible population (SLR4) was easily controlled at the recommended butroxydim field rate of 45 g ha^{-1}, with LD_{50} values of 3.77 and 3.45 g ha^{-1}, whereas R populations B3 and E2 required 26.9 and 7.73 g ha^{-1} of butroxydim, respectively (Table 3). Population B3 was more resistant to butroxydim when compared with the E2 population in terms of plant survival (LD_{50}), but plants that survived herbicide treatment were stunted in growth as indicated by the modest GR_{50} values (1.60) of both field populations. Cross-resistance to butroxydim in clethodim-resistant field populations is not surprising given that many of the ACCase mutations can endow resistance to other ACCase-inhibitor herbicides including clodinafop, diclofop, fluazifop, haloxyfop, sethoxydim, tralkoxydim, and pinoxaden (Yu et al. 2007).

Sequencing of *ACCase* gene. A target-site mutation for clethodim resistance was identified in both field populations of rigid ryegrass. The nucleotide sequences of the field populations differed from that of the susceptible population by a single nucleotide. Sequencing results revealed an aspartate-2078-glycine substitution in the CT domain of plastidic ACCase in both field populations of rigid ryegrass. Amino acid modification at position 2078 is known to provide strong resistance to clethodim (Délye et al. 2008) and has been already confirmed in rigid ryegrass (Yu et al. 2007), black-grass (Délye 2005), and wild oats (*Avena fatua* L.) (Cruz-Hipolito et al. 2011). The continuous use of clethodim over an extended period on rigid ryegrass populations that already had resistance to other ACCase-inhibiting herbicides might be selecting for the accumulation of amino acid modifications within the *ACCase* gene that contribute to clethodim resistance.

Effect of Herbicides on Rigid Ryegrass Density and Seed Production. Rigid ryegrass density was affected ($P < 0.05$) by herbicide treatments in both years of the study (Table 3). PPI simazine followed by POST clethodim plus butroxydim had the lowest density of rigid ryegrass in 2012 (73 plants m^{-2}) and 2013 (22 plants m^{-2}). Even though rigid ryegrass was confirmed resistant to both clethodim and butroxydim (Table 3), the mixture of the two reduced rigid ryegrass density relative to the standard grower practice of simazine PPI followed by clethodim POST by 60% in 2012 and 80% in 2013. As mentioned earlier, plants surviving butroxydim in the dose–response study were stunted ($GR_{50} = 1.6$) and indicated modest levels of resistance in both of these populations. Conse-

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quently, application of clethodim–butroxydim mixture significantly reduced rigid ryegrass plant and spike density in the field (Table 4). Among PPI herbicide treatments, propyzamide was the most effective option against rigid ryegrass; however, additional weed control relative to the grower practice was much higher in 2012 (65%; $P < 0.05$) than in 2013 (10%). In 2012, there was 42 mm of rainfall in the week after herbicide application as compared with 23 mm of rainfall within this period in 2013. In previous research, Walker and Roberts (1975) showed that weed control with propyzamide was positively correlated with the amount of rainfall received in 7 d after application. Therefore, greater rainfall after herbicide application in 2012 may be responsible for better weed control with propyzamide in 2012 than in 2013. Kleemann and Gill (2012) reported much higher levels of rigid ryegrass control with propyzamide (> 85%) in faba bean; however, the herbicide was applied early postsowing PRE rather than PPI as in this study. Failure of propyzamide to control rigid ryegrass in 2013 because of dry conditions reflected in high spike density (256 spikes m^{-2}) and seed set (> 13,000 seeds m^{-2}; Table 4). PPI applications of dimethenamid-P, prosulfocarb plus *S*-metolachlor, and pyroxasulfone alone or in combination with triallate provided modest (~ 54%) and in some instances no reduction in rigid ryegrass density relative to the standard grower practice in 2012 and 2013. Pyroxasulfone and prosulfocarb plus *S*-metolachlor in wheat were shown to provide 64 to 94% control of rigid ryegrass (Boutsalis et al. 2014; Kleemann et al. 2014). The combination of limited competitive ability of faba bean with weeds (Felton et al. 2004) and severe weed infestation at the experimental sites could have been responsible for the ineffectiveness of PPI herbicides used alone.

Rigid ryegrass spike number and seed production were significantly ($P < 0.05$) influenced by herbicide treatment in both years of the study (Table 4). Similar to the trend for weed control, PPI simazine followed by POST clethodim plus butroxydim had the lowest spike density (2 to 105 spikes m^{-2}). High rigid ryegrass plant density was recorded early in the growing season during 2013. However, greater rainfall received in late winter (July through August) in 2013 (111 mm) than in 2012 (59 mm) appeared to favor faba bean growth and its ability to suppress weed growth in the wetter season. Consequently, rigid ryegrass produced much greater spike density in simazine followed by POST clethodim plus butroxydim in 2012 than in 2013. This herbicide treatment also proved more effective in reducing seed production of rigid ryegrass in 2013 (two spikes m^{-2}; < 50 seeds m^{-2}) than in 2012 (105 spikes m^{-2}; 4,539 seeds m^{-2}). The lower level of resistance to butroxydim in the E2 population (2013) as compared with the B3 population (2012) may have contributed to this difference in rigid ryegrass density. Furthermore, faba beans are known for their high sensitivity to water stress (Mwanamwenge et al. 1999), which may have reduced their competitive ability against rigid ryegrass under drier conditions experienced in 2012. PPI herbicides resulted in a large buildup in the seed bank of rigid ryegrass, which could have serious effects on the productivity of subsequent crops in the rotation. These results suggest that PPI herbicides alone are inadequate for rigid ryegrass management in faba bean.

Effects of Herbicides on Grain Yield and Grain Weight of Faba Bean. In the absence of effective control, rigid ryegrass was extremely competitive against faba bean and caused large reductions ($P < 0.05$) in grain yield (Table 5). Reduction in grain yield due to rigid ryegrass competition has also been previously reported in other pulse crops (Hashem et al. 2011; Lemerle et al. 1995; McDonald 2003). In 2012, faba bean produced up to 50% less grain yield in treatments with low herbicide efficacy as compared with simazine PPI followed by clethodim plus butroxydim POST. However, the yield penalty in treatments with low weed control was < 15% in 2013. Greater sensitivity of faba bean to rigid ryegrass in 2012 could be the result of greater weed density and lower growing-season rainfall (Table 2), which could have exacerbated competition for soil water. In both years, the highest grain yields were recorded in simazine followed by clethodim plus butroxydim (980 to 2,400 kg ha^{-1}), which was most effective against rigid ryegrass. Faba bean grain yield in propyzamide (890 to 2,350 kg ha^{-1}) was similar to the simazine PPI followed by clethodim plus butroxydim POST treatment. Even though pyroxasulfone plus triallate PPI controlled rigid ryegrass 54% in 2012, faba bean yield at 550 kg ha^{-1} was lower than the standard grower practice simazine

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Table 5. Effect of herbicide treatments on grain yield (kg ha^{-1}) and grain weight (g [100 seeds]$^{-1}$) of faba beans grown at Roseworthy in 2012 and 2013.[a]

Treatment	Rate	Grain yield		Grain weight	
		2012	2013	2012	2013
	g ai ha^{-1}	kg ha^{-1}		g (100 seeds)$^{-1}$	
Simazine PPI/clethodim POST	1,350 , 120	900 bc	2,110 a	576 bc	57.9 a
Simazine PPI/clethodim + butroxydim POST	1,350, 120 + 45	980 c	2,400 c	582 c	55.8 a
Dimethenamid PPI	720	490 a	2,170 ab	534 a	56.8 a
Pyroxasulfone PPI	100	680 ab	2,190 abc	552 abc	57.3 a
Pyroxasulfone + triallate PPI	100 + 800	550 a	2,190 abc	547 ab	55.1 a
Prosulfocarb + *S*-metolachlor PPI	2,000 + 300	860 bc	2,160 ab	559 abc	54.9 a
Prosulfocarb + *S*-metolachlor + triallate PPI	2,000 + 300 + 800	860 bc	2,060 a	572 bc	55.2 a
Propyzamide PPI	500	890 bc	2,350 bc	553 abc	57.6 a

[a] Means within the same column followed by the same letters are not significantly different according to LSD at the P = 0.05 level.

PPI plus clethodim and butroxydim POST at 900 kg ha^{-1}. Failure of faba bean to benefit from weed control as a result of treatment with pyroxasulfone could be associated with crop injury (data not shown). Similarly in previous research, Walsh et al. (2011) observed biomass reduction in faba bean with pyroxasulfone application.

Faba bean grain weight was not affected by the herbicide treatments in 2013 (54.9 to 57.9 g [100 seeds]$^{-1}$). However, in 2012, poor weed control with dimethenamid-P (53.4 g [100 seeds]$^{-1}$) significantly reduced faba bean grain weight as compared with simazine followed by clethodim plus butroxydim (58.2 g [100 seeds]$^{-1}$) (Table 5). Variation in crop response over the 2 yr is likely due to more competition between rigid ryegrass and the crop for water during the reproductive phase.

At present, there are no new grass-selective herbicides registered for use in broadleaf crops for the control of clethodim-resistant rigid ryegrass in Australia. The field studies reported here examined the efficacy of some currently registered herbicides for controlling clethodim-resistant rigid ryegrass in faba bean. From the herbicides examined, simazine PPI followed by clethodim plus butroxydim POST was the most effective treatment for control of clethodim-resistant rigid ryegrass. Of the herbicides applied PPI, propyzamide appeared to be the most effective; however, rigid ryegrass was still able to set a large amount of seed, which could have serious implications for weed management and productivity of subsequent crops in the rotation. Although the combination of simazine PPI with clethodim plus butroxydim POST was effective for the control of clethodim-resistant rigid ryegrass in faba bean, resistance to both of these herbicides is steadily increasing in field populations of rigid ryegrass. There is an urgent need for the adoption of nonchemical weed control tactics to reduce the current heavy dependence on herbicides for weed control in Australian cropping systems. However, herbicides are likely to remain an important component of Australian cropping systems. Therefore, a serious effort is needed to identify effective alternatives to clethodim and butroxydim for POST use in broadleaf crops in southern Australia.

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