Weed Technology 2016 30:423–430

Alternative Herbicides for the Control of Clethodim-Resistant Rigid Ryegrass (*Lolium rigidum*) in Clearfield Canola in Southern Australia

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Rigid ryegrass is the most-troublesome, herbicide-resistant weed in cropping systems of southern Australia. Field experiments were undertaken at Roseworthy, South Australia, in 2013 and 2014, to identify effective herbicide options for the control of clethodim-resistant rigid ryegrass in Clearfield canola. PPI trifluralin + triallate followed by (fb) POST imazamox + imazapyr + clethodim + butroxydim had the lowest plant density of rigid ryegrass in 2014 and provided superior control compared with the standard grower practice of PPI trifluralin + triallate fb POST imazamox + imazapyr + clethodim in 1 of 2 yr. Propyzamide either alone or as a split application (PPI fb POST) or in combination with clethodim provided similar rigid ryegrass control to that of the standard grower practice (38 to 553 plants m^{-2}). Rigid ryegrass treated with PPI dimethenamid-P, pethoxamid, pethoxamid + triallate, and PPI trifluralin fb carbetamide POST produced significantly more seeds than the standard grower practice, which would lead to reinfestation of subsequent crops. Canola yield responded positively to effective herbicide treatments, especially in 2014, when rigid ryegrass density was greater. PPI dimethenamid-P and pethoxamid alone or in combination with triallate and propyzamide were ineffective in reducing rigid ryegrass density and seed production to levels acceptable for continuous cropping systems.

Nomenclature: Butroxydim; clethodim; dimethenamid-P; imazamox + imazapyr; pethoxamid; propyzamide; triallate; trifluralin; rigid ryegrass; rigid ryegrass, *Lolium rigidum* Gaudin; Clearfield canola; *Brassica napus* L.

Key words: Herbicide strategies, preplant incorporated herbicides, resistance management, weed control.

Rigid ryegrass is widely distributed in many countries (Heap 2015), including the cropping region of southern Australia (Boutsalis et al. 2012; Gallagher et al. 2004; Gill 1996). The widespread distribution of this species in southern Australia has been attributed to its adaptation to the local climate, its extensive use as a pasture species in the past, and its high level of genetic variability (Charmet et al. 1996; Gill 1996). During the past two decades, herbicides have become the most common method of rigid ryegrass control in Australian cropping systems. This heavy reliance on herbicides for rigid ryegrass control in Australia has led to the widespread evolution of herbicide resistance (Boutsalis et al. 2012) to 11 herbicides with different modes of action (Heap 2015).

Rigid ryegrass is a prolific seed producer (Rerkasem et al. 1980) that enables any plants surviving a herbicide application to produce a large number of seeds, even in extremely competitive surroundings (McGowan 1967). High fecundity in rigid ryegrass enables this species to readily replenish its seedbank and ensure weed infestation in the following crops. Rigid ryegrass is highly competitive against broadleaf crops and can significantly reduce productivity (Hashem et al. 2011; Lemerle et al. 1995). In southeastern Australia, rigid ryegrass is considered the most important weed of canola crops (Lemerle et al. 2001).

Clethodim, an acetyl-coenzyme A carboxylase (ACCase)-inhibiting cyclohexanedione herbicide, has been widely used for the selective control of annual and perennial graminaceous weeds in many broadleaf crops (Burke et al. 2002, 2004; Vidrine et

DOI: 10.1614/WT-D-15-00078.1

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Table 1. 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					
2013	417	341	Lentil[c]	October 30	May 15	July 2
2014	470	311	Lentil	November 3	May 22	July 20

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

[b] POST herbicide treatments were applied to rigid ryegrass at 3 to 4-leaf stage of growth.

[c] Lentil, *Lens culinaris* Medik.

al. 1995). Clethodim is considered to be at a lower resistance risk than other ACCase-inhibiting herbicides, with only 2 of 11 target-site mutations (amino acid substitutions) in weed populations conferring resistance to this herbicide (Beckie and Tardif 2012). Asp-2078→Gly and Cys-2088→Arg substitutions in the plastidic ACCase enzyme have been identified as the most-predominant mutations endowing clethodim resistance at field rates (Yu et al. 2007). In previous studies, Délye et al. (2008) reported that Leu-1781, Gly-2078, and Ala-2096 mutations in the ACCase gene may also confer resistance to clethodim in the field if conditions are not optimal for herbicide efficacy or where reduced herbicide rates are used. Although clethodim has been effective in controlling rigid ryegrass for many years (Boutsalis et al. 2012), heavy reliance on this herbicide has resulted in the evolution of clethodim resistance in rigid ryegrass populations in Australia (Saini et al. 2014, 2015a,c; Yu et al. 2007). In a recent survey, Boutsalis et al. (2012) reported that nearly 60% of the field populations of rigid ryegrass in southeastern Australia had evolved resistance to clethodim. Previously, local growers were able to control clethodim-resistant populations by simply increasing the herbicide dose. However, this is no longer possible because several rigid ryegrass populations can no longer be controlled with even doses greater than the recommended field rates (Boutsalis et al. 2012; Saini et al. 2014, 2015b). Moreover, high rates of clethodim (> 120 g ha^{-1}) in canola can have phytotoxic effects, such as delayed flowering, distorted flower buds, and possible grain-yield suppression (Zerner 2013).

PPI herbicides can improve the performance of grass-selective herbicides, such as clethodim by reducing the density of rigid ryegrass requiring in-crop control. Traditionally, trifluralin was used to provide PRE weed control. However, many rigid ryegrass populations in southern Australia have now evolved resistance to trifluralin (Boutsalis et al. 2012). Increasingly, multiple resistance across many herbicide chemistries is severely reducing herbicide options available for the control of rigid ryegrass in crop production systems (Walsh and Powles 2007). To date, there are few options available for the selective control of clethodim-resistant rigid ryegrass in broadleaf crops. Therefore, research was conducted to identify effective herbicide options for the control of clethodim-resistant rigid ryegrass in Clearfield canola.

Materials and Methods

Field Experiment Description. Two field experiments were conducted at the Roseworthy campus of the University of Adelaide, located in the lower north region of South Australia (34.51°S, 138.68°E at 68 m above sea level), in different fields over the growing season of 2013 and 2014 to identify alternative herbicides for the control of clethodim-resistant rigid ryegrass in Clearfield canola. Both experimental sites were infested with a high density of clethodim-resistant rigid ryegrass. Resistance status of both field populations was previously confirmed by whole-plant dose–response studies (data not presented). 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. Rainfall received at the site during the study period and the long-term average are shown in Table 1 (Anonymous 2015a). The amount of rainfall received for one month after the application of PPI herbicides is presented in Figure 1. A pre-seeding application of glyphosate at

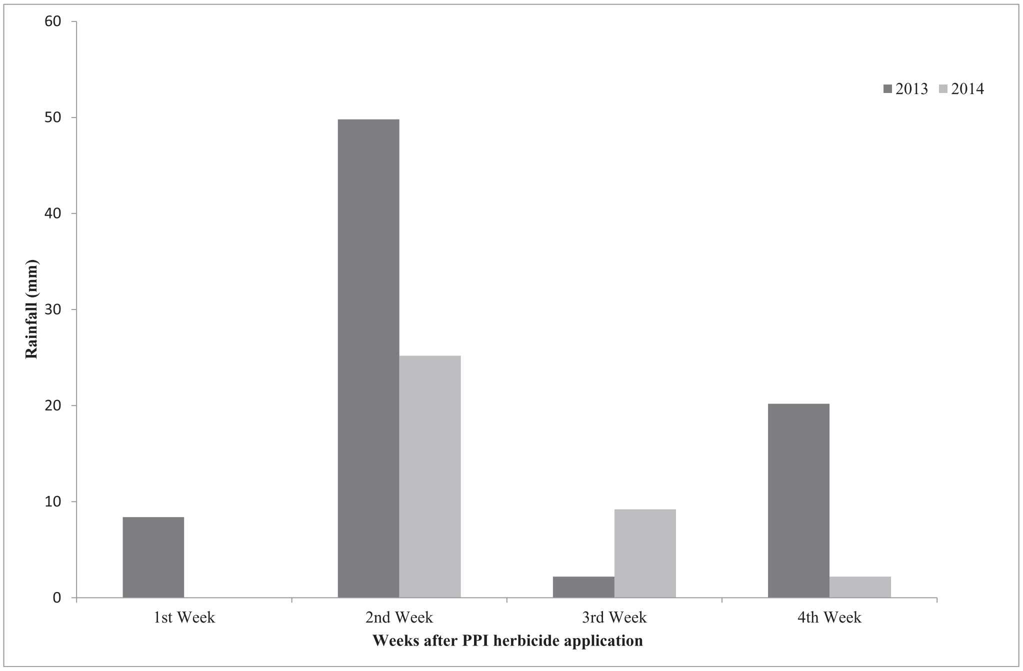

Figure 1. Amount of rainfall received after application of PPI herbicides in 2013 and 2014.

900 g ha^{-1} and oxyfluorfen at 22 g ha^{-1} was made to control existing weeds in the field.

Clearfield canola (cv. 'Pioneer 45Y82') was sown at a depth of 2 cm at a seed rate of 5 kg ha^{-1} (for a target of 50 plants m^{-2}). The trials were sown on May 17, 2013, and May 23, 2014, using a no-till plot seeder fitted with knife-point tines 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 80 kg ha^{-1} of diammonium phosphate (18 kg N ha^{-1} and 20 kg P ha^{-1}) banded below the seed at sowing. Additional N was broadcast applied on the August 2, 2013, and August 8, 2014, in the form of 40 kg ha^{-1} of urea (20 kg N). The experiments were laid out in a randomized complete-block design with four replications. All 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 trifluralin + triallate followed by (fb) POST imazamox + imazapyr + clethodim is considered the standard grower practice for the district and was used as the control in this study. The dose and timing of other herbicide treatments are presented in Table 2. As per label recommendations, 1% (v/v) paraffinic oil mixed with nonionic surfactant Supercharge (Crop Care Australasia Pty. Ltd., Queensland, Australia) was added to clethodim and butroxydim tank mix when rigid ryegrass had reached three- to four-leaf growth stage.

Rigid ryegrass plant and spike density were assessed with a 0.25-m^2 quadrat placed at four random locations in each plot. Assessments of rigid ryegrass plant density were taken 5 and 12 wk after planting (WAP) before and after POST herbicide application. Spike density was assessed 14 to 16 WAP when all the spikes had emerged. Rigid ryegrass seed production was assessed (16 to 18 WAP) in October before seed shed.

Seed production of rigid ryegrass was determined by measuring the spike length of 20 rigid ryegrass plants selected randomly from each plot, and average spike length per plot was calculated. There was no significant effect of herbicide treatments on spike length (data not shown). Separately, 50 plants from the experiment were randomly collected and total spike length per plant and numbers of seeds per plant were counted. The data obtained from 50 plants were fitted to a functional linear model (Equation 1) to derive a relationship between total spike length per plant and total number of seeds per

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Table 2. Effect of herbicide treatments on rigid ryegrass plant and spike density in Clearfield canola in 2013 and 2014.[a,b]

Treatments			Rigid ryegrass			
			Weed density		Spike density	
PPI	Followed by POST	Rate	2013	2014	2013	2014
		g ai ha^{-1}	plant m^{-2}		spikes m^{-2}	
Trifluralin + triallate	Imazamox + imazapyr + clethodim	960 + 1,000 fb 25 + 11 + 120	47 d	632 cd	44 e	79 e
Trifluralin + triallate	Imazamox + imazapyr + clethodim + butroxydim	960 + 1,000 fb 25 + 11 + 120 + 20	31 d	128 e	31 e	104 e
Dimethenamid		540	186 a	1,697 a	142 ab	660 a
Propyzamide		500	63 cd	553 d	92 cd	245 bcde
Propyzamide	Clethodim	500 fb 120	65 cd	385 de	15 e	48 e
Propyzamide		1,000	40 d	364 de	56 de	195 cde
Propyzamide	Propyzamide	500 fb 500	38 d	318 de	62 de	52 e
Pethoxamid		1,880	198 a	1,643 a	156 a	358 bc
Pethoxamid + triallate		1,880 + 1,000	101 bc	1,088 abc	140 ab	334 bcd
Pethoxamid	Propyzamide	1,880 + 500	156 ab	787 bcd	100 bcd	143 de
Trifluralin	Carbetamide	960 fb 1,200	60 cd	1,206 ab	130 abc	422 b

[a] Abbreviation: fb, followed by.

[b] Means within the same column followed by the same letters are not significantly different according to Fisher's protected LSD test at P = 0.05. Data for rigid ryegrass plant density per square meter were square-root transformed before mean comparisons. Data presented are the nontransformed mean values.

plant using SigmaPlot version 12.5 (Systat Software Inc., Melbourne, VIC, Australia). The linear model fitted was

$$y = bx$$

where y is the number of seeds per plant, x is the spike length per plant, and b is the slope of the line.

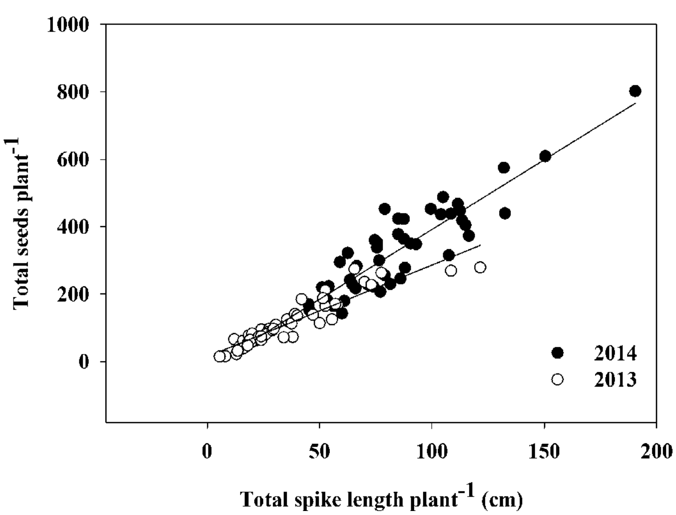

Figure 2. The linear relationship between total spike length of rigid ryegrass per plant and total seeds produced per plant over 2 yr. The linear regression analysis was performed using Equation 1, in 2013, $y = 3.0x$; $R^2 = 0.85$; and in 2014, $y = 3.9x$; $R^2 = 0.83$.

The linear equation thus obtained (2013, R^2 = 0.85; 2014, R^2 = 0.83) (Figure 2) was used to estimate the seed production per square meter. The number of seed produced per unit area was based on the average spike length and the spike density per square meter of rigid ryegrass and shown as the number of seeds per square meter.

Canola seed yield was determined by harvesting plots with a small-plot harvester. Seeds were then dried to uniform moisture and cleaned, and yield was determined. Harvest dates in each experiment are shown in Table 1. A seed subsample of 500 g was used to determine the 1,000-seed weight.

Statistical Analyses. Weed control (plant and spike density, and rigid ryegrass seed production) and crop data (seed yield and seed weight) were analyzed with ANOVA (GenStat version 15.3, VSN International, Hemel Hempstead, U.K.). A square-root variance-stabilizing transformation was used for rigid ryegrass plant density and spike density before analysis to normalize the distribution of residuals. Means of the transformed data were separated using Fishers protected LSD test at P = 0.05, but the original means have been reported in the tables. SigmaPlot was used to derive linear relationships between spike density of

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Table 3. Effect of herbicide treatments on seed yield of canola and seed production of rigid ryegrass in 2013 and 2014.[a,b]

Treatments			Seed yield		Rigid ryegrass seeds	
PPI	Followed by POST	Rate	2013	2014	2013	2014
		g ai ha^{-1}	kg ha^{-1}		seed m^{-2}	
Trifluralin + triallate	Imazamox + imazapyr + clethodim	960 + 1,000 fb 25 + 11 + 120	1,732 a	1,706 abc	1,404 e	5,404 d
Trifluralin + triallate	Imazamox + imazapyr + clethodim + butroxydim	960 + 1,000 fb 25 + 11 + 120 + 20	1,643 ab	1,787 ab	838 e	7,915 d
Dimethenamid		540	1,475 b	1,407 c	5,798 bc	45,347 a
Propyzamide		500	1,617 ab	1,651 abc	4,577 cd	17,270 cd
Propyzamide	Clethodim	500 fb 120	1,571 ab	1,842 a	540 e	3,662 d
Propyzamide		1000	1,660 ab	1,598 abc	2,262 de	14,572 cd
Propyzamide	Propyzamide	500 fb 500	1,683 ab	1,934 a	2,366 de	3,633 d
Pethoxamid		1,880	1,599 ab	1,355 c	8,855 a	27,107 bc
Pethoxamid + triallate		1,880 + 1,000	1,590 ab	1,615 abc	7,369 ab	26,451 bc
Pethoxamid	Propyzamide	1,880 + 500	1,636 ab	1,762 ab	5,610 bc	9,640 d
Trifluralin	Carbetamide	960 fb 1,200	1,442 b	1,444 bc	7,247 ab	33,299 ab

[a] Abbreviation: fb, followed by.

[b] Means within the same column followed by the same letters are not significantly different according to Fisher's protected LSD test at P = 0.05.

rigid ryegrass and its seed production, and between spike density of rigid ryegrass and seed yield of canola.

Results and Discussion

Weed Control. Because there were differences in the efficacy of herbicide treatments between the 2 yr, data for each year were analyzed and are presented separately (Table 2). PPI trifluralin + triallate fb POST imazamox + imazapyr + clethodim + butroxydim was more effective ($P < 0.05$ in 2014) in reducing rigid ryegrass density (2013, 34%; 2014, 80%) than the standard grower practice of PPI trifluralin + triallate fb POST imazamox + imazapyr + clethodim (Table 2). Greater improvement in weed control in 2014 because of the addition of butroxydim may be associated with the higher density of rigid ryegrass present at the site. Either single (PPI) or split application (PPI fb POST) of propyzamide and its mixture with clethodim provided similar ($P > 0.05$) weed control as the standard grower practice did. A single application of PPI propyzamide at the lower rate (500 g ha^{-1}) was less effective in reducing rigid ryegrass plant density as compared with the PPI trifluralin + triallate fb POST imazamox + imazapyr + clethodim + butroxydim treatment ($P < 0.05$ in 2014). During both growing seasons, the rainfall received was above the long-term average, which may have favored the activity of propyzamide on rigid ryegrass. Propyzamide can be an effective option for rigid ryegrass control provided sufficient rainfall is received after planting to keep the seedbed moist (Walker and Roberts 1975). Kleemann and Gill (2012) also reported effective control of rigid ryegrass with propyzamide (> 85%) in fava bean (*Vicia faba* L.). The PPI application of trifluralin fb carbetamide POST (60 plants m^{-2} [2013]; 1,206 plants m^{-2} [2014]) provided inferior weed control compared with farmers' standard practice in 2014 but not in 2013. PPI dimethenamid-P and pethoxamid remained less effective in reducing rigid ryegrass density (198 to 1,697 plants m^{-2}) compared with the farmers' standard practice. Lower weed control in these treatments could be related to their inherently poor activity on rigid ryegrass or the absence of any POST herbicide in these treatments.

In both years of the study, spike density and seed production of rigid ryegrass was significantly influenced by the herbicide treatments (Tables 2 and 3). In general, rigid ryegrass spike density data closely followed the trends observed for rigid ryegrass plant density (Table 2). Dimethenamid-P and pethoxamid PPI were less effective in reducing rigid ryegrass spike density relative to the growers' standard practice ($P < 0.05$). A significant reduction in rigid ryegrass spike density was observed with PPI trifluralin + triallate fb POST imazamox +

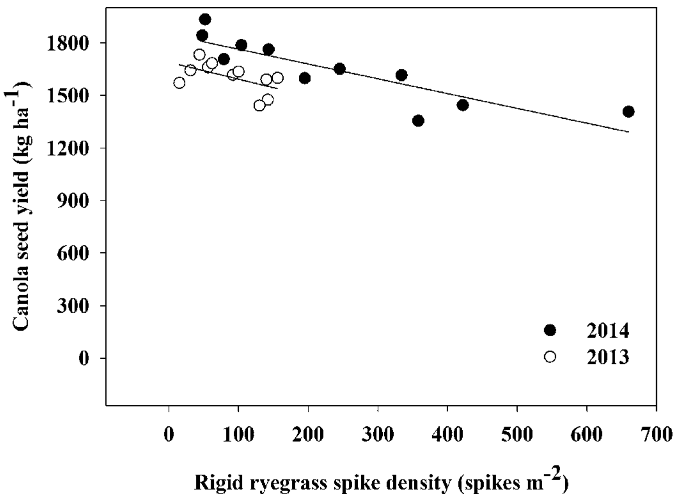

Figure 3. Effect of rigid ryegrass spike density on seed yield of canola over 2 yr. The linear regression analysis was performed using Equation 1, in 2013, $y = 1{,}689.88 - 0.97x$; $R^2 = 0.32$ and in 2014, $y = 1{,}848.06 - 0.84x$; $R^2 = 0.75$.

imazapyr + clethodim + butroxydim (31 to 104 spikes m^{-2}) and propyzamide treatments (PPI, split application or in combination with clethodim) (15 to 245 spikes m^{-2}) compared with dimethenamid-P and pethoxamid PPI treatments. In 2014, spike density levels were numerically higher than those observed in 2013, which appears to be related to higher rigid ryegrass infestation.

None of the herbicide treatments completely prevented seed production of rigid ryegrass. During both years, PPI trifluralin + triallate fb POST imazamox + imazapyr + clethodim + butroxydim, PPI propyzamide fb clethodim POST, PPI propyzamide (1,000 g ha^{-1}), and split application of propyzamide (PPI fb POST) were more effective ($P < 0.05$) in reducing seed production of rigid ryegrass (540 to 14,572 seeds m^{-2}) in comparison with the less-effective dimethenamid-P, but rigid ryegrass seed set in these treatments was similar to that of the growers' standard practice (Table 3).

Similar to the weed-control trend, pethoxamid PPI fb propyzamide POST in 2014 resulted in a significantly less rigid ryegrass seed production than did pethoxamid itself or its combination with triallate, trifluralin fb carbetamide, and dimethenamid. Propyzamide at the lower rate (500 g ha^{-1}) proved less effective in reducing rigid ryegrass seed production (> 4,500 seeds m^{-2}; $P < 0.05$ in 2013) relative to the growers' standard practice. PPI herbicides dimethenamid-P, pethoxamid alone or in combination with triallate, and trifluralin PPI fb carbetamide POST resulted in greater rigid ryegrass seed production (5,610 to 45,347 seeds m^{-2}) ($P < 0.05$) than that of the growers' standard practice and PPI trifluralin + triallate fb POST imazamox + imazapyr + clethodim + butroxydim. The high levels of weed seed production in some of these treatments will undoubtedly cause production problems in succeeding crops. The results from this study indicate that PPI herbicides are inadequate to effectively control clethodim-resistant rigid ryegrass in Clearfield canola in southern Australia. Furthermore, none of the herbicide treatments was effective in reducing rigid ryegrass seed production to low levels (~100 seeds m^{-2}). PPI propyzamide fb clethodim was most effective in reducing rigid ryegrass seed set (540 seeds m^{-2}), but it will not prevent future weed infestations.

Crop Response. There were significant differences between herbicide treatments in seed yield of canola (Table 3) in both years of this study. Because of ineffective weed control, rigid ryegrass in PPI dimethenamid-P in 2013, pethoxamid in 2014, and PPI trifluralin fb POST carbetamide in 2013 caused 8 to 20% reduction in seed yield relative to the growers' standard practice of PPI trifluralin + triallate fb POST imazamox + imazapyr + clethodim (Table 3). Significant differences in seed yield of canola, from 60 to 100%, because of rigid ryegrass interference have been previously reported (Lemerle et al. 1995, 2011). Propyzamide as a split application (PPI fb POST) and as a mixture with clethodim, PPI trifluralin + triallate fb POST imazamox + imazapyr + clethodim + butroxydim, and PPI pethoxamid fb propyzamide POST produced greater seed yield of canola in 2014 than did dimethenamid-P and pethoxamid PPI treatments. This difference in canola seed yield among herbicide treatments was related to the level of weed control provided by these treatments.

Rigid ryegrass spike density showed a negative linear relationship with seed yield of canola (2013, $R^2 = 0.32$; 2014, $R^2 = 0.75$) (Figure 3). The greater impact of rigid ryegrass spike density on canola yield in 2014 appears to be related simply to the much greater weed density at the experimental site (Table 2). Canola 1,000 seed weight was unaffected by the herbicide treatments in 2013 (3.9 to 4.0 g [1,000 seed^{-1}]) and 2014 (2.8 to 3.0 g [1,000

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seed^{-1}]) (data not presented). The lower seed weight in 2014 appears to be associated with a much higher rigid ryegrass density and lower spring rainfall in 2014 than in 2013.

In this study, no herbicide treatment was able to completely prevent seed production of rigid ryegrass. The use of soil-residual herbicides in conjunction with clethodim and butroxydim provided the most-effective control of clethodim-resistant rigid ryegrass, but no herbicide treatment was able to completely prevent seed production. Among the PPI herbicides, propyzamide was the most promising option. However, rigid ryegrass was able to produce a large amount of seed, even with the propyzamide treatment, which is likely to cause reinfestation of subsequent crops. Use of glyphosate near crop maturity to reduce weed seed production may have an important role in managing rigid ryegrass in Australian cropping systems, especially if growers are unable to use POST herbicides because of resistance (Anonymous 2015b). Growers also need to consider use of nonchemical tactics as a part of integrated management program to minimize crop-yield loss and to slow down the evolution of clethodim resistance.

Acknowledgments

A Ph.D scholarship by the Australian Centre for International Agricultural Research (ACIAR) supported R.K.S. during this research program at The University of Adelaide, South Australia. We also wish to thank Lovreet Singh Shergill, Malinee Thongmee Burk, and Benjamin Fleet for providing technical assistance.

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Received May 30, 2015, and approved December 10, 2015.

Associate editor for this paper: Robert Nurse, Agriculture and Agri-Food Canada.