Canola is widely grown in Australia to provide diversity within winter crop rotations. Its production is heavily reliant on triazine tolerant varieties but Clearfield® and conventional varieties are also significant components of the industry. Glyphosate tolerant varieties have been introduced, thereby providing an alternative technology to be incorporated into the farming systems. To evaluate the potential impact of the relative herbicide technologies, a comparison of herbicide tolerant canola weed management systems was undertaken at Wagga Wagga, Australia over a 5-year rotation. Near isogenic lines of conventional, glyphosate tolerant and triazine tolerant varieties were evaluated for their abilities to control annual ryegrass (Lolium rigidum), the most challenging weed of temperate crops in Australia. Glyphosate tolerant and triazine tolerant canola achieved high levels of ryegrass control and attained higher yields than the conventional system. Glyphosate tolerant canola provided extra control of broadleaf weeds and also achieved better seed oil levels when compared with the other canola systems. There were positive flow on weed management benefits for the remainder of the crop sequence from the weed control achieved in the initial crop, particularly following glyphosate tolerant canola. Subsequent control of volunteer canola in all treatments was readily achieved by using paraquat/diquat. The glyphosate tolerant weed management system was more profitable than the triazine tolerant system, although no allowance was made for technology costs as they were not known at the time of study. These outcomes demonstrate the potential value of herbicide tolerant canola to Australian farmers.


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Herbicide tolerant canola systems and their impact on winter crop rotations

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Abstract

Canola is widely grown in Australia to provide diversity within winter crop rotations. Its production is heavily reliant on triazine tolerant varieties but Clearfield® and conventional varieties are also significant components of the industry. Glyphosate tolerant varieties have been introduced, thereby providing an alternative technology to be incorporated into the farming systems. To evaluate the potential impact of the relative herbicide technologies, a comparison of herbicide tolerant canola weed management systems was undertaken at Wagga Wagga, Australia over a 5 year rotation. Near isogenic lines of conventional, glyphosate tolerant and triazine tolerant varieties were evaluated for their abilities to control annual ryegrass (Lolium rigidum), the most challenging weed of temperate crops in Australia. Glyphosate tolerant and triazine tolerant canola achieved high levels of ryegrass control and attained higher yields than the conventional system. Glyphosate tolerant canola provided extra control of broadleaf weeds and also achieved better seed oil levels when compared with the other canola systems. There were positive flow-on weed management benefits for the remainder of the crop sequence from the weed control achieved in the initial crop, particularly following glyphosate tolerant canola. Subsequent control of volunteer canola in all treatments was readily achieved by using paraquat/diquat. The glyphosate tolerant weed management system was more profitable than the triazine tolerant system, although no allowance was made for technology costs as they were not known at the time of study. These outcomes demonstrate the potential value of herbicide tolerant canola to Australian farmers.

Keywords: transgenic; glyphosate; crop rotation; herbicide resistance
Introduction

Transgenic (or genetically modified, GM) crops are planted on more than 100m hectares worldwide and canola (Brassica napus) comprises around 5% of that area (Graef et al. 2007). The introduction of transgenic varieties in the mid-1990s has resulted in their widespread adoption by farmers in those countries which allow GM crops to be grown commercially. In canola this technology is exclusively herbicide tolerance and its adoption by farmers is reported to be due to simpler, more effective weed control (Buth 2007, Stringam et al. 2003). In Canada, more than 90% of canola production is from herbicide tolerant varieties covering an area of nearly 6 m ha (Buth 2007, Dill 2005, Gianessi 2005). Gilroy (2007) reported that there was a 40% reduction in herbicide costs and a saving of around 6000t of herbicides in 2007 due to GM adoption in Canada.

In Australia, canola has been grown since the 1970s in rotation with winter cereals, providing a disease break crop as well as a commercial return in its own right (Norton et al., 1999). The lack of selective broadleaf herbicides to control cruciferous weeds such as wild radish (Raphanus raphanistrum) in that crop (Senior and Bavage 2003) was an impediment to wider adoption until 1993 when triazine tolerant (TT) canola varieties, derived from conventional breeding methods, provided control of such weeds. TT canola was rapidly included in crop rotations, particularly in Western Australia, despite the fitness penalty resulting in a reduction in yield (Beversdorf et al. 1988, Devine 2005) and oil (Salisbury et al. 1999, Radcliffe 2002). The high frequency of TT canola raises the prospects of both herbicide resistance in weeds such as annual ryegrass (Lolium rigidum) and wild radish and crop rotation limitations arising from the high level of triazine use, particularly if springs and summers are dry (Norton et al., 1999).

In Australian temperate cropping systems, glyphosate herbicide is applied as a pre-plant non-selective herbicide to remove emerged weeds prior to sowing a crop and for fallow weed control. The introduction of glyphosate tolerant crops provides the opportunity for this herbicide to be used differently as an in-crop herbicide for broad spectrum selective post-emergence weed control. As such, it provides an alternate mode of action post-emergent herbicide to complement the use of pre and/or post emergent herbicides, particularly where herbicide resistance in weeds has evolved to current herbicides. The use of glyphosate in this manner is likely to control a wide
range of both grass and broadleaved weeds and reduce the range and volume of herbicide active ingredients currently used for weed control in canola.

An issue of concern for Australian farmers, however, is the threat of herbicide resistance in weeds of crops. Although resistance to glyphosate is not common (Broster and Pratley 2006), it has been reported in annual ryegrass (Pratley et al. 1996, 1999, Powles et al. 1998), a major weed of winter crops. Most reports of resistance, however, have been where resistant plants have been selected under repeated glyphosate applications in non-crop situations (Preston 2009). The introduction of glyphosate tolerant canola varieties changes the pattern of use but intensifies the selection pressure for resistance to this herbicide and thus management of the risk to avert the evolution of weed resistance to glyphosate is paramount. However there has been limited commercial experience of the production of transgenic canola in Australia, due to moratoria imposed by governments preventing field research on, or commercial production of GM crops. These moratoria were lifted in some states from 2008, thereby allowing transgenic canola to be grown commercially.

The applicability of GM canola to Australian conditions is thus unknown. An evaluation of the glyphosate technology is required with respect to its ability to effectively complement existing canola weed management system options, its relative yield and economic returns, and carryover impacts in a rotation in relation to weed and herbicide management.

**Methodology**

We evaluated the effect of the inclusion of glyphosate tolerant canola in a typical winter crop sequence relative to other common canola weed management options. The weed focus was annual ryegrass because this is the most challenging weed of winter crop production systems in Australia due to its propensity to evolve herbicide resistance to a range of herbicides currently applied for its control (Broster and Pratley 2006). The project was conducted in compliance with the strict protocols and conditions applied by the Office of the Gene Technology Regulator, Australia. The introduction of the government moratoria on field research on GM crops placed limitations on extending the research beyond that presented in this paper including different sites and years.
The project was conducted through five seasons from 1999 to 2003 at Charles Sturt University, Wagga Wagga, Australia in a degraded long term pasture field consisting of subterranean clover (*Trifolium subterraneum*) and annual ryegrass. Paterson’s curse (*Echium plantagineum*) and silver grass (*Vulpia spp*) were known to be present in the field. The soil is a eutrophic brown dermosol (Chen and McKane, 1997) and an electromagnetic survey was used to locate plot area for maximum site uniformity.

Prior to the autumn break of year 1, 15 kg/ha (around 750 seeds/m²) annual ryegrass were broadcast across the site to provide a uniform, dense seed bank. Individual plots were 10m x 1.8m with 1.2m buffers to minimise spray drift effects. Annual ryegrass control was compared using near-isogenic lines of conventional (CC), glyphosate tolerant (GT) and triazine tolerant (TT) canola sown at 4 kg/ha. Near isogenic lines were used to remove varietal differences.

Treatments chosen reflected the range of practices used on Australian farms in the winter cropping regions. They were replicated three times in a randomised split-split plot design, with main plots pre-sowing cultivation (+/- an “autumn tickle” to stimulate early weed emergence), sub-plots the degree of soil disturbance at sowing (minimal or full soil disturbance) and sub-sub plots the selective herbicide regime (untreated control, pre-emergent only, post-emergent only, pre- and post-emergent) using herbicides appropriate to each canola system (Table 1). Sub-plot treatments also included a knockdown application of either glyphosate applied at minimum tillage (495 g a.i./ha) and full soil disturbance (360 g a.i./ha) label rates, or cultivation used instead of a glyphosate application for weed control prior to sowing with full soil disturbance. Fertilisers were applied in line with regional recommendations being 170kg/ha of Pivot Stimulus™ (30-00-00-15). Flutriafol fungicide was applied at 100g a.i./ha to minimise blackleg infestation and 80 g a.i./ha methidathion insecticide was applied post-sowing, pre-emergence for red legged earth mite control.

Carryover effects of treatments on weed densities and crop yields were monitored for a further three years under a crop rotation of wheat (cv Diamondbird, 77kg/ha), lupins (cv Wonga, 80kg/ha) and wheat (cv Diamondbird, 80kg/ha). Herbicide management practices are outlined in Table 1. All crops were direct drilled with narrow points and fertilisers applied according to regional recommendations with 100 kg/ha grain legume fertiliser (13.9-00-6.5-17.2).
Five 0.1 m² random quadrats per plot were used to monitor weed density and composition three times each season, i.e. prior to application of pre-emergent herbicides, prior to application of post-emergent herbicides, and six weeks after application of post emergent herbicides. Plots were harvested individually each season using a plot harvester, with grain subsamples retained for quality testing.

Gross margins were calculated based on New South Wales Department of Primary Industries farm enterprise budgets (www.dpi.nsw.gov.au). Pesticide and fertiliser costs per hectare were calculated using average retail prices and actual application rates. Machinery costs were based on the New South Wales Department of Primary Industries guide to tractor and implement costs (www.dpi.nsw.gov.au). Other typical costs that could be incurred for a commercial crop (e.g. windrowing, harvesting, cartage, insurance and industry levies) were included in all calculations.

A base price of $300 per tonne for canola was assumed. An oil premium/discount of 1.5% per 1% variation from 42% oil content was calculated based on oil tests conducted by AgSeeds, Horsham, Victoria for each plot. A technology fee for the glyphosate tolerant canola was not included in the calculations as this had not been determined for the Australian market at that time.

In year 5, the three isolines used in Year 1 were superimposed on each of the original canola systems. As previous herbicide treatments were still yielding differences in annual ryegrass populations, 108 plots were used comprising equal number of plots per original canola system, providing 12 replicates per treatment combination. Each canola system was managed as per year 1 following a pre-planting application of glyphosate at 612 g a.i./ha one week prior to sowing. Although, the GT treatment allowed for two post-emergent applications of glyphosate, only one application was required. Weed populations were monitored in early August after post-emergent herbicide application and grain yields were recorded as before at the end of the season.

**Statistical analysis**

Mean weed counts for each plot were transformed using ln(count + 1) to normalise variances prior to the analyses of variance. Initial annual ryegrass density was included as a covariate in the analysis of subsequent annual ryegrass data to address some variations in initial densities. *Post hoc* Fisher’s protected LSD tests
were used to separate significantly different means. Data were back-transformed into counts for presentation.

**Results**

Main plot effects of light pre-season cultivation (‘autumn tickle”) to stimulate weed germination were not significant (P>0.05), presumably due to the time and rate at which annual ryegrass seed was broadcast over the site. As main plot effects were not significant, and sub-plot level results were inconclusive, data are discussed here only in terms of the herbicide regimes imposed.

Overall, the GT system was the most productive system over the first four years in terms of crop yield and weed control when selective herbicides were used post-emergent, either alone or in conjunction with a pre-emergent herbicide. Weed burdens are recorded in Table 2 and the ability of the GT system to provide broad spectrum selective post-emergent weed control is demonstrated. Herbsicide regimes within the CC and GT systems provided similar levels of annual ryegrass control, although control of other weeds was less effective in the CC system. Annual ryegrass control in the TT system was largely reliant upon pre-emergent herbicide application, as post-emergent use of triazine herbicides only provided limited control of annual ryegrass, although it provided some control of other weeds. The better performance of the triazines when used pre-emergent is likely to be due to the triazines being more efficacious through root absorption in seedlings compared to foliar absorption by established plants.

The different herbicide regimes in each canola system resulted in different yield outcomes (Table 2). Highest yields were obtained where both pre-emergent and post-emergent herbicides were used and lowest yields where no selective herbicides were used due to the associated weed burden. Average oil content of harvested grain was 44.0%, 45.5% and 42.9% for CC, GT and TT lines respectively.

The initial herbicide management regimes had significant effects on annual ryegrass density in year 1 (P<0.05) which in turn had a significant impact on ryegrass densities(P<0.05) and crop production (P<0.05) in the second year (Table 3). Thus, in year 2, annual ryegrass was more abundant in the former post emergent CC system treatments and least abundant in the pre + post-emergent plots (P<0.05). Annual ryegrass was less dense where triazines had been used pre-emergent in the
TT system (P<0.05), whilst there was no consistent trend over time within the GT system except that the overall weed population was lower than for the other canola systems.

Similar to the initial year, the differences in the crop yield in the second year are a reflection of the weed burden (Table 3). The level of annual ryegrass control achieved by using the recommended practice for both pre- and post-emergent herbicides resulted in wheat yield being highest where this practice had been used in the CC, GT and TT systems (6.93, 7.38 and 6.91t/ha, respectively).

In the third year, there was evidence (P<0.01) of higher lupin yield where post-emergent herbicides had been used in the initial CC and GT systems (0.84 and 0.90 t/ha, respectively) compared with when post-emergent herbicides had not been used (0.69 and 0.76 t/ha, respectively). Differences within the initial TT system were not significant with respect to herbicide regime.

Poor seasonal conditions limited wheat yields in the fourth year, with just over 2 t/ha of wheat harvested across all systems and herbicide regimes. Differences between initial plots were evident in the CC system (P<0.05), with the lowest yield (1.95 t/ha) observed in the post emergent only treatment. This appears to be related to the poor annual ryegrass control in the second year (Table 3), rather than to the initial herbicide regime. Subsequent testing of annual ryegrass seed samples through the Charles Sturt University herbicide resistance program indicated that resistance to diclofop-methyl was present albeit at a low level, which may explain the poor control observed.

Gross margins indicated that the near-isogenic lines of canola used in the CC and GT systems provided similar economic returns when the same pre-emergent herbicide only was used (Table 4). Use of glyphosate for post emergent weed control in the GT system led to higher returns than when diclofop-methyl was used in the CC system, presumably through a combination of developing diclofop-methyl resistance in the annual ryegrass and the broad spectrum weed control achieved with glyphosate. The TT system was most profitable when triazines were used pre-emergent. Use of glyphosate as a post-emergent selective herbicide within the GT system provided consistently higher gross margins compared with the other systems.
In Year 5 when the canola isolines were superimposed on each of the isolate plots from Year 1, outcomes were consistent with those in the first year. Weed and crop yield data are shown in Table 5. Annual ryegrass control was again superior in the 2003 GT treatment and this was reflected in canola yields being highest in that treatment. The CC treatment was consistently lowest in canola grain yield due to a consistently higher broadleaf weed population as well as annual ryegrass. The carryover effect from the GT treatment in Year 1 was still evident in year 5 in grain yields and initial ryegrass densities.

Discussion

The use of glyphosate as a post-emergent herbicide was superior to triazine pre-emergent or to grass herbicides pre-emergent and post-emergent in the conventional line. This impact carried forward to the next crop, in this case wheat, in both weed burden and crop yield. Glyphosate provided broadleaved weed control in addition to grass control. These outcomes were achieved using isolines differing only in herbicide technology whereas previous comparisons, where benefits of glyphosate have been shown, were confounded by other varietal differences (eg Harker et al. 2006). Experience in other countries has shown rapid adoption of transgenic herbicide tolerant varieties, particularly glyphosate tolerant (ie Roundup Ready®) varieties. In Canada, where triazine tolerant varieties have not been adopted, transgenic canola provides increased profits, more flexible rotations and more effective weed control, considered a weakness in conventional varieties (O'Donovan et al. 2006). Moreover, these GM varieties are suited to no-till systems and farmers have indicated that adoption is more related to management benefits than specifically to yield or profit (Stringam et al. 2003).

Although the glyphosate tolerance technology provides for two post-emergent applications, our experience was that the second application was not required. Similar experience was reported by O'Donovan et al. (2006) that there was no advantage in two glyphosate applications.

Herbicide resistance was measured at a low level of incidence to the post-emergent grass herbicide used in the CC system but there was no evidence of resistance evolving to glyphosate. In Australia, herbicide resistance is a substantial problem, particularly in annual ryegrass (Broster and Pratley 2006), hence the focus on this species in this research. Conventional canola production is also under challenge.
from annual ryegrass because of its ability to evolve resistance to herbicides used for its control. The ACCase-inhibiting chemicals in particular are now extensively compromised by resistance and this has a significant impact on canola production which depends heavily on these chemicals. The availability of glyphosate as a post-emergent herbicide provides new options for resistant weed control. In this context, the introduction of herbicide tolerant canola varieties provides new weed control options for resistant ryegrass, as described by Shaner (2000). However the risk of resistance to glyphosate needs to be carefully managed to minimise the risk of widespread glyphosate resistance. This herbicide is fundamental to the conservation farming practices adopted by the great majority of Australian farmers. Manjardino et al. (2005) have modelled the introduction of glyphosate tolerant canola into Australian farming systems over a 20 year time frame. They showed the advantages of broad spectrum weed control by glyphosate tolerant varieties and the better economics compared to TT varieties, results consistent with our work. They cautioned on the need for good herbicide management to minimise the risks of glyphosate resistance in the weed population. Similar outcomes were reported by Neve et al. (2003). Experience also shows a weed shift to those species that are naturally more tolerant to glyphosate, with Owen (2008) reporting the increased incidence of waterhemp (Amaranthus tuberculatus), horseweed (Conyza canadensis), and giant ragweed (Ambrosia trifida) as a result of post-emergent selection pressure by glyphosate in US crops.

The use of paraquat/diquat as the knockdown herbicide in the year following canola readily controlled volunteers, including glyphosate tolerant volunteers, in the next crop. Pageau and Lejeunesse (2008) showed that, in Canada, there were adequate herbicide options for control of volunteers in a following barley crop. Rainbolt et al. (2004) showed in US crops that paraquat and diuron provided control of glyphosate tolerant volunteers whereas glyphosate provided adequate control of other herbicide tolerant variety volunteers. Harker et al. (2006), also in Canada, showed that in low disturbance direct seeding situations suited to GM crops, there was enhanced canola seed mortality and reduced secondary seed dormancy and persistence in the soil seed bank. A comprehensive study of minimum tilled and direct drilled fields in South Australia found that the canola seedbank and volunteers rapidly declined, thereby minimising the risk of herbicide tolerant canola becoming a weed problem (Baker and Preston 2008).
TT canola performed second to the GT isoline in the experiment reported here through better weed control than the CC isoline. The availability of triazine tolerant varieties has enabled the development of the canola industry in Western Australia because it facilitated the control of wild radish in that crop, although resistance is evolving to triazines in that state (Walsh et al. 2007). Whereas yields were often inferior to conventional varieties (up to 26%, Potter and Salisbury, 1993; 15-20%, Salisbury and Wratten, 1999), due to the genetic deficiency of the TT varieties, the overall benefit gained from the superior weed control both within the canola crop and the following crop rotation provided a significant benefit to the farmer. The long term downside is the potential environmental impact due to the residual nature of the triazine herbicides (Parsons, 1995) applied for weed control in TT canola. Glyphosate tolerant canola provides another agronomic option for farmers and will likely replace TT canola because of its better weed control, improved varieties and the overall improvement in the gross margin it is likely to deliver. In addition, due to the environmentally friendly profile of glyphosate, its application will not attract the same long term environmental issues that face TT canola.

This project compared glyphosate tolerant canola with existing herbicide tolerant options in canola, using annual ryegrass as the target weed. The experiment demonstrated that the glyphosate tolerant system was at least as productive as, and generally more productive than, the current canola systems. The control of ryegrass was more effective with glyphosate when applied in a GT canola system and the effects, both agronomic (i.e. improved weed control) and economic, carried over into later seasons of the crop rotation. Using actual costs and product prices for the years in question, the glyphosate tolerant canola had higher gross margins due to cost savings, better weed control and improved product quality. These findings are consistent with those of O’Donovan et al. (2006) who reported superior weed control, higher canola yields and net revenues in Roundup Ready® canola as well as reduced levels of active ingredient entering the environment.

The research undertaken demonstrates that, in southern Australia, a glyphosate tolerant canola system, when compared with conventional and triazine tolerant systems: delivered better control of both annual grass and broadleaved weeds; produced positive carryover effects into following years of the rotation; provided a better financial outcome; and delivered improved product quality.

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