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Diverse systems and strategies to cost-effectively manage herbicide-resistant annual ryegrass (*Lolium rigidum*) in no-till wheat (*Triticum aestivum*)-based cropping sequences in south-eastern Australia

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ABSTRACT

Context. Herbicide-resistant annual ryegrass (*Lolium rigidum*; ARG) is a major weed of commercial significance globally, including no-till wheat-based production systems in south-eastern Australia. **Aims.** To compare the cost-effectiveness of different crop sequences and intensities of weed management to control ARG in rainfed grain production. **Methods.** Two on-farm studies in southern New South Wales, Australia, compared the effect of combinations of 3-year crop-sequence options (continuous wheat, 1- or 2-years of break crops), conservative or aggressive weed-control measures, hay cuts, legume brown manure (BM), and/or weed-free winter fallow on in-crop ARG infestations and soil seedbanks. Gross margins were calculated for each combination of treatments to compare system economic performance. **Key results.** Double-breaks consisting of two consecutive broadleaf crops, or canola–cereal hay, were frequently the most profitable and effective ARG control strategies. Single canola or lupin crops, BM, cereal hay, and fallow all significantly reduced subsequent in-crop ARG and seedbank numbers compared with continuous wheat. Aggressive in-crop control measures in wheat were more expensive than those applied to break crops. Gains in ARG control could be lost by a single year of poor weed control. **Conclusions.** High levels of control (>95%) over three consecutive seasons are required to reduce ARG seedbanks, and this is most cost-effectively achieved with diverse crop sequences. **Implications.** Farmers with high populations of ARG can reduce seedbanks by growing three crops sequentially that achieve complete weed seed control. This must be followed with ongoing high levels of control in subsequent years to keep ARG seedbanks low.

Keywords: break crop, cover crop, crop competition, crop rotation, fallow, profitability, soil nitrogen availability, systems agronomy, weed control, weed seedbank.

Introduction

Herbicides have facilitated global adoption of conservation agriculture (CA) by enabling its first principal, namely, the direct placement of seed and fertiliser without the use of tillage to control weeds (FAO 2022). Elimination of tillage also assists with application of the second principle of CA, namely, retention of crop residues to provide soil cover. However, the strong evolutionary pressure imposed by herbicides, especially in no-till, residue-retained systems, has led to a world-wide development of herbicide resistance in many crop weeds (Powles and Yu 2010; Matzrafi *et al.* 2021; Heap 2022).

Research studies and surveys of farmers' fields have demonstated that herbicide resistance in weeds can develop rapidly, and both the frequency and spectrum of herbicide resistance has increased globally over recent decades (Powles and Yu 2010; Norsworthy *et al.* 2012; Owen *et al.* 2014; Broster *et al.* 2019; Busi *et al.* 2021; Broster *et al.* 2022; Heap 2022). This has had large economic consequences and represents a significant threat to crop yields and food security if it is not addressed (Varah *et al.* 2020; Storkey *et al.* 2021).

Annual ryegrass (rigid ryegrass, ARG; *Lolium rigidum* Gaud.) is an important weed of many wheat (*Triticum aestivum*)-based cropping systems throughout the world and has evolved resistance to multiple herbicide modes of action, aided by high genetic variability, obligate outcrossing and prolific seed production (Bajwa *et al.* 2021; Beckie and Jasieniuk 2021; Matzrafi *et al.* 2021). This is certainly the case in the wheat-producing regions of Australia, where two-thirds of respondents in a national survey of grain producers reported the presence of herbicide-resistant weeds on 40–50% of their cropping land and ranked ARG as both the most frequent herbicide-resistant weed and the most expensive to manage (Llewellyn *et al.* 2016). High prevalence of resistance in this species has been confirmed in ARG collected during on-farm surveys in all the major grain-production zones of Australia, with >90% of ARG populations frequently displaying resistance to one or more herbicide modes of action (Harries *et al.* 2020; Busi *et al.* 2021; Broster *et al.* 2022). Resistance to various modes of action have also been reported for ARG in cereal cropping systems from France, Spain, Greece, Saudi Arabia, Tunisia, Israel, Iran, South Africa and Chile (Beckie and Jasieniuk 2021; Heap 2022).

A wide range of strategies has been proposed to manage herbicide-resistant weeds (e.g. Gill and Holmes 1997; Lemerle *et al.* 2004; Walsh and Powles 2007; Flower *et al.* 2012; Norsworthy *et al.* 2012; Lemerle *et al.* 2014; French *et al.* 2015; Borger *et al.* 2016; Kleemann *et al.* 2016; Saini *et al.* 2016; Walsh *et al.* 2018, 2019; Harries *et al.* 2020; Kumar *et al.* 2020; Bajwa *et al.* 2021; Beckie and Jasieniuk 2021; Brunharo *et al.* 2022; Walsh and Powles 2022). The strategies generally comprise one or more of the following approaches:

1. Diverse and tactical use (mixing and rotating) of herbicide modes of action to slow the development of herbicide resistance and to kill individuals that survived previous chemical-control measures.
2. Enhanced crop competition to suppress weed growth and reduce weed seed production.
3. Reducing the density of weed seedlings reinfesting subsequent crops by minimising the replenishment of the soil weed seedbank and encouraging seedbank decline either by preventing seed-set by weeds that escape early control measures, or collecting, removing and/or destroying weed seeds at the end of the growing season.

Diverse and tactical use of different herbicide modes of action is facilitated by the third principle of CA, namely, species diversification through varied crop sequences (FAO 2022). Diverse crop sequences are underused in many regions of Australia due to farmer perception that broadleaf break crops are more risky and less profitable than are wheat and barley (*Hordeum vulgare*), which typically represent ~75% of the total 20–25 million ha of land grown to grain each year (Robertson *et al.* 2010; Collins and Norton 2019; ABARES 2022).

In their review of break-crop effects on wheat, Angus *et al.* (2015) highlighted a gulf that exists between break-crop research and commercial practice. Grain growers often identify grass weed control as the main benefit of break crops (Moodie 2012); yet, most published break-crop experiments generally report results in weed-free conditions and do not consider weeds as a treatment effect. Because past break-crop experiments have controlled for the factor that is of most interest to farmers and advisors, Angus *et al.* (2015) proposed that future research focus on the prospective role of break crops to manage weeds in wheat-based cropping sequences. This could include the deployment of herbicides with alternative modes of action as well as competition and weed suppression by break crops, and different uses for break crops, including cover crops being either terminated early (brown manuring) or harvested for hay prior to weed seed maturation and/or dispersal.

A series of group discussions and farmer interviews held as part of a participatory action research process with members of the FarmLink grower group (https://farmlink.com.au) in southern New South Wales (NSW), Australia, during 2011 and 2012, showed several key knowledge gaps in the management of herbicide-resistant weeds. All participants acknowledged that herbicide-resistant weeds were increasingly becoming a problem on their farm. However, in common to other similar exercises undertaken elsewhere in the world (Schroeder *et al.* 2018), the FarmLink grain-growers had limited knowledge of the efficacy of individual or combined weed-control strategies for ARG management in crop sequences and were uncertain about the economic viability of the different options available. Furthermore, although most farmers were aware of previous Australian research that demonstrated that competition from in-crop weeds reduces the yield of grain crops (e.g. Lemerle *et al.* 1995; French *et al.* 2015), many were unable to estimate the scale of yield losses that might be occurring on their own farm. The most frequent questions posed by participants experiencing difficulties managing ARG in their cereal-based cropping operations were as follows:

1. How much grain yield am I losing if there is ARG in my wheat fields?
2. Can ARG be managed more cost-effectively with break crops in a diverse crop sequence than in wheat crops?
3. Is it possible to adequately control herbicide-resistant ARG in wheat crops with increased inputs and newer, more costly, herbicides with different modes of action to those used in the past?

To address these questions, the effectiveness of different strategies to manage ARG was assessed on two farms in southern NSW with high existing ARG seedbanks. The experimental treatments investigated were developed in participatory consultation with FarmLink growers and advisors. The strategies included contrasting intensities of weed-control measures applied to continuous wheat sequences (conservative or aggressive; different herbicide choice, increased plant density and higher nutrient supply; Lemerle *et al.* 2004; Walsh *et al.* 2018; Bajwa *et al.* 2021), as well as the inclusion of 1-year or 2-year (double) breaks of the broadleaf grain crops canola (rapeseed; *Brassica napus*) grown under different management intensities (conservative or aggressive; different herbicide choice, hybrid vigour, increased plant density and higher nutrient supply; Lemerle *et al.* 2014; Bajwa *et al.* 2021), and/or lupin (narrow-leaf lupin; *Lupinus angustifolius*). Broadleaf break crops were specifically used to provide opportunities to diversify herbicide modes of action (Walsh and Powles 2007; Broster *et al.* 2019; Harries *et al.* 2020) and to provide opportunities to reduce ARG seed viability and seed-set through pre-harvest spray-topping (crop-topping) of lupin with non-selective herbicide following crop maturity to kill immature ARG seeds (Walsh and Powles 2007; French *et al.* 2015; Kleemann *et al.* 2016; Bajwa *et al.* 2021). Brown manuring of field pea (*Pisum sativum*) cover crops and wheat cereal hay cutting to either kill or remove ARG to prevent ARG soil seedbank recruitment and imposing a weed-free winter fallow to deplete soil ARG seedbanks were also evaluated (Gill and Holmes 1997; French *et al.* 2015; Kleemann *et al.* 2016; Walsh *et al.* 2019; Bajwa *et al.* 2021). Our aim was to compare the cost-effectiveness of different combinations of crop sequences and intensities of weed management for control of in-crop ARG infestations and soil seedbanks in rainfed grain production.

Materials and methods

Site and soil descriptions

The research was undertaken on grain-cropping farms located near Eurongilly, in southern NSW, Australia. Experiment 1 was located on Eurongilly Road (−34.93, 147.76) approximately 6 km north-west from Experiment 2 on Dollarvale Road (−34.92, 147.81). Sites were chosen on the basis of the land-owner identification of paddocks with severe ARG infestations that had become difficult to control with the standard suite of registered pre- and post-emergent herbicides and a history of two consecutive wheat crops in the preceding growing seasons.

The susceptibility/resistance of the existing ARG populations at both sites was evaluated by a commercial provider (Plant Science Consulting, South Australia) on live plants sampled from the field in March 2012 and 2013 for Experiments 1 and 2 respectively (Table 1). The endemic ARG population was resistant to haloxyfop, clethodim and pinoxaden (Group 1) herbicides and iodosulfuron-methyl-sodium, imazamox and imazapyr (Group 2) herbicides to varying degrees (30–95%), but was still susceptible to glyphosate (Group 9), atrazine (Group 5) and butroxydim (Group 1) herbicides (Table 1; HRAC 2020). Because live plants rather than seeds were used for resistance testing, pre-emergent herbicides were not included in the assay, and resistance status of ARG to them at the sites is unknown. However, a 2014 survey of resistance from the same region where the experiments were conducted found that none of the 65 ARG populations sampled was resistant to the commonly used pre-emergent herbicide trifluralin, nor to newer and more expensive products containing pyroxasulfone or prosulfurocarb + S-metolachlor, despite a widespread resistance to Group 1 and 2 herbicides (Broster *et al.* 2022).

Table 1. Herbicide-resistance assessment of annual ryegrass (ARG) populations at the chosen farm cropping paddocks for Experiment 1 and Experiment 2 on adjacent farms at Eurongilly, NSW (determined in March 2012 and March 2013 respectively).

Herbicide	Herbicide group[A]			Survival (%) rating	
	HRAC and WSSA number code	Legacy HRAC letter code		Expt 1	Expt 2
39 g/ha haloxyfop	1	A	Fop	70 RR	
52 g/ha haloxyfop	1	A	Fop		90 RR
72 g/ha clethodim	1	A	Dim	55 R	
120 g/ha clethodim	1	A	Dim		5 R
30 g/ha pinoxaden	1	A	Dim	65 RR	50 RR
45 g/ha butroxydim	1	A	Dim	0 S	5 R
20 g/ha Iodosulfuron-methyl-sodium	2	B	Sulfonylurea	95 RRR	60 RRR
20 g/ha imazamox + 9 g/ha imazapyr	2	B	Imidazolinone		30 R
1800 g/ha atrazine + 1% Hasten	5	C1	Triazine		0 S
675 g/ha glyphosate	9	G	Glycine		0 S

[A]Source: HRAC (2020) https://hracglobal.com/tools/hrac-moa-2020-revision-description-and-master-herbicide-list.
Resistance-ratings: RRR, plants tested have strong resistance; RR, medium-level resistance; R, low-level but detectable resistance; S, no detection of resistance.

This information guided the choice of herbicides (especially for pre-emergent herbicides that were still effective options) used for the different strategies in the experiments.

The soils at the sites were classified as a Kurosol at Experiment 1 and Chromosol at Experiment 2 (Isbell 2021; Table 2). Due to the acidity of the top soil at Experiment 1, 2.5 t of lime/ha was surface-applied in April 2012 without incorporation, about 3 weeks before sowing. No soil amelioration was deemed necessary for Experiment 2 (Table 2). Details of monthly, growing-season (April–October) and annual rainfall at the two sites during the experiments are provided in Table 3.

Experimental design and treatments

The studies were established in autumn 2012 (Experiment 1) and 2013 (Experiment 2) and each ran for 3 years. The experiments compared the economic outcomes and efficacy of ARG control by multiple weed-management strategies that involved combinations of the following:

1. Contrasting sequences of crops
   (a) *Continuous wheat (identified by treatment codes 0.1–0.4; Table 4)*. Wheat grown for grain in each of the 3 years of the experiments. Given that the farm sites had a previous history of two consecutive wheat crops prior to commencement of experimentation, the final wheat in this system represented 5 years of continuous wheat.
   (b) *Single-break sequences (identified by treatment codes 1.1–1.12; Table 4)*. An alternative to a wheat grain crop was grown in either Year 1 or Year 2 of the 3-year cropping sequence. Year 1 break options included canola and lupin grown for grain, field pea cover crop used as brown manure (BM; pea crop killed by a non-selective herbicide during early pod fill to kill ARG prior to seed maturation, with crop residues left as a mulch), and a weed-free winter fallow (i.e. replacing a winter crop with bare soil fallow kept free of all weeds for 18 months). Consequently, first year single-breaks consisted of either break crop–wheat–wheat, or fallow–wheat–wheat sequences. The only single-break option evaluated in Year 2 was a canola grain crop (i.e. wheat–canola–wheat sequence).
   (c) *Double-break sequences (identified by treatment codes 2.1–2.5; Table 4)*. Two consecutive alternatives to wheat grain crops grown in Years 1 and 2. These double-breaks consisted of lupin, field pea BM or fallow in Year 1, followed by canola in Year 2, or canola in Year 1, followed by wheat cut for cereal hay in spring prior to ARG seed dispersal, with any regrowth terminated with non-selective herbicides. Double-break sequences represented either legume–canola–wheat, fallow–canola–wheat, or canola–cereal hay–wheat sequences.
2. Contrasting intensities (conservative or aggressive) of weed-control measures
   (a) Low-input, conservative weed management (identified by the acronym C), representative of local farmer practice applied to Year 1 and/or Year 2 wheat grain crops and Year 1 canola.
   (b) A more aggressive higher-cost and higher-input weed-control regime (acronym A) applied to Year 1 and/or, Year 2 and Year 3 wheat grain crops and Year 1 and/ or Year 2 canola that used higher plant densities and nutrient supply, and, in the case of canola, a vigorous hybrid, to enhance the crop's capacity to compete with ARG and newer, higher-cost pre- and/or post-emergent herbicides with modes of action different from those more commonly used by farmers to improve the prospects of killing ARG populations that had developed resistance to herbicides previously used.

Table 2. Soil characteristics of on-farm cropping paddocks chosen for Experiment 1 and Experiment 2 at Eurongilly, NSW, prior to establishing research treatments in 2012 and 2013 respectively.

Analysis	Soil depth (m)	Site	
		Experiment 1	Experiment 2
pH ($CaCl_2$)	0–0.1	4.5	5.3
	0.1–0.2	4.5	4.6
Olsen available P (mg/kg)	0–0.1	29	44
Available mineral N (kg N/ha)	0–1.6	87	90

Table 3. Monthly, growing-season, and annual rainfall (mm) during on-farm experimentation between 2012 and 2015 at Eurongilly, NSW.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	GSR[A]	Annual
2012	38	81	153	11	40	23	45	15	21	25	74	31	179	557
2013	19	83	25	7	40	88	53	42	37	14	7	16	281	432
2014	16	20	64	52	32	67	20	18	33	24	30	79	247	456
2015	63	18	0	99	23	76	75	84	11	22	141	24	392	638
LTA[B]	39	43	36	26	31	49	46	41	49	44	47	50	286	501

[A]GSR, growing-season rainfall (April–October).
[B]LTA, long-term average rainfall (1991–2020).

An outline of the different crop sequences and weed-management treatments are provided in Table 4 and described in more detail below.

Experimental details

All crops were sown with a three-point linkage plot seeder equipped with six Flexi-Coil 250 kg break-out tines (Flexi-Coil Australia, St Marys, NSW, Australia) at 0.3 m row spacing and fitted with Agmaster® boots, 12 mm knife-points and 420 mm × 70 mm 'V' tyre press wheels (Agmaster, Welshpool, WA, Australia). The initial size of individual wheat, break crop or fallow main plots was 40 m in length × 1.8 m. Each treatment was replicated four times in a randomised complete block design.

Insect pests and fungal diseases were controlled with registered insecticides and fungicides where appropriate, such that they did not affect yield or crop competitive ability. All crop and fallow treatment plots were maintained weed-free over the first and subsequent summer periods prior to sowing the next series of crop treatments, by using various applications of glyphosate and 2,4-D amine.

Table 4. Overview of the combination of crop sequence and conservative (C) or aggressive (A) weed-control measures that underpin the different experimental treatments evaluated at the two on-farm study sites at Eurongilly, NSW.

Treatment code	Year 1	Year 2	Year 3
Continuous wheat over 3 years			
0.1	Wheat(C)	Wheat(C)	Wheat(A)
0.2	Wheat(C)	Wheat(A)	Wheat(A)
0.3	Wheat(A)	Wheat(C)	Wheat(A)
0.4	Wheat(A)	Wheat(A)	Wheat(A)
Single-break in 3 years			
1.1	Wheat(C)	Canola(A)	Wheat(A)
1.2	Wheat(A)	Canola(A)	Wheat(A)
1.3	Canola(C)	Wheat(C)	Wheat(A)
1.4	Canola(C)	Wheat(A)	Wheat(A)
1.5	Canola(A)	Wheat(C)	Wheat(A)
1.6	Canola(A)	Wheat(A)	Wheat(A)
1.7	Lupin	Wheat(C)	Wheat(A)
1.8	Lupin	Wheat(A)	Wheat(A)
1.9	Field pea BM	Wheat(C)	Wheat(A)
1.10	Field pea BM	Wheat(A)	Wheat(A)
1.11	Fallow	Wheat(C)	Wheat(A)
1.12	Fallow	Wheat(A)	Whea (A)
Double-break over 3 years			
2.1	Canola(C)	Wheat hay	Wheat(A)
2.2	Canola(A)	Wheat hay	Wheat(A)
2.3	Lupin	Canola(A)	Wheat(A)
2.4	Field pea BM	Canola(A)	Wheat(A)
2.5	Fallow	Canola(A)	Wheat(A)

Year 1: the experimental areas at both sites received an initial knockdown spray in early March (720 g/ha glyphosate, 36 g/ha oxyfluorfen and 300 g/ha 2,4-D low volatile ester) followed by a second knockdown application of glyphosate (1350 g/ha) prior to establishment of treatments.

The Year 1 wheat (cv. Spitfire) grain crop under C management was sown with target populations of 75 plants/m^2, low nitrogen (N) and phosphorus (P) fertiliser application rates (42.5 kg N/ha, 5.5 kg P/ha), using standard pre-emergent (trifluralin and diuron) and in-crop herbicides (prosulfocarb and *s*-metalochlor at 2–3-leaf stage of wheat and 1–2-leaf stage of ARG) for weed control (Supplementary material Table S1). The wheat(A) weed-management strategy consisted of newer and higher-cost registered pre- and post-emergent herbicides that were recently available at the time of experimentation and which had modes of action different from those that had been standard farmer practice, increased crop plant density (to 150 plants/m^2) with higher N and phosphorus P fertiliser inputs (87.5–151.5 kg N/ha, 16.5 kg P/ha; Table S1).

The Year 1 canola(C) crop was an open-pollinated triazine-tolerant (TT) cultivar (cv. Crusher in Experiment 1 and cv. Stingray in Experiment 2) sown at target populations of 40 plants/m^2, with standard farmer N and P fertiliser applications (63.5–87.5 kg N/ha, 5.5 kg P/ha), pre-emergent (trifluralin and atrazine) and in-crop (butroxydim and atrazine) herbicides. The canola(A) treatment received more N and P fertiliser (108.5–172.5 kg N/ha, 15.5 kg P/ha) and used a glyphosate-tolerant (Roundup Ready®; RR) hybrid (cv. Hyola 505RR in both experiments) because of its greater vigour than that of TT-canola (Lemerle *et al.* 2014), and to provide different herbicide options for weed control (Table S1).

The lupin (cv. Mandelup) grain crop was sown at target populations of 40 plants/m^2 with either 75 kg/ha (7.5 kg N/ha, 16.5 kg P/ha; Experiment 1) or 25 kg/ha (2.5 kg N/ha, 5.5 kg P/ha; Experiment 2) of mono-ammonium phosphate (MAP), with 960 g/ha trifluralin and 1980 g/ha simazine, as pre-emergent incorporated by sowing (IBS) herbicides, and 45 g/ha butroxydim applied in-crop (lupin at 5–6-leaf and ARG at 1–4-leaf stage). In Experiment 1, the lupin crop was also spray-topped at mid-pod ripening stage (French *et al.* 2015; Bajwa *et al.* 2021) with 100 g/ha paraquat in mid-November to kill immature seeds of in-crop ARG, but problems with site access prevented spray-topping in Experiment 2.

The field pea (cv. Morgan) grown for BM was sown at target populations of 40 plants/m^2, with 25 kg/ha MAP at sowing, 960 g/ha trifluralin and 900 g/ha simazine as pre-emergent IBS. A tank mix of 900 g/ha glyphosate, 90 g/ha clopyralid and 10 g/ha carfentrazone-ethyl was used to terminate both the pea crop and ARG in early September which was followed up with 1125 g/ha glyphosate in mid-October.

In both experiments, canola and lupin were sown in late April, whereas the field pea and wheat were sown in mid-May, reflecting optimal recommended sowing dates for each species in southern NSW.

The weed-free chemical winter fallow commenced in late August–early September with an application of 900 g/ha glyphosate (both Experiments 1 and 2) and 3 g/ha metsulfuron-methyl (Experiment 1 only). Plots were re-sprayed with 900 g/ha glyphosate in mid-October followed by 720 g/ha paraquat 14 days later.

Year 2: in the second cropping year, 1083 g/ha glyphosate and 18 g/ha oxyfluorfen were applied to kill emerged ARG seedlings at both sites prior to sowing. Each Year 1 main plot was split into three 13.3 m subplots, in a split-plot design. Wheat was sown into designated subplots in all Year 1 treatments, whereas canola was sown into subplots after wheat, lupin, field pea BM, and fallow main plots. Wheat was grown for cereal hay only following the Year 1 canola to provide an additional double-break option (Table 4). Growing two consecutive canola crops was not considered for inclusion in the study because this carries a high risk of reduced productivity and yield losses due to blackleg (*Leptosphearia maculans*) disease (Hegewald *et al.* 2018).

Both conservative and aggressive weed-control measures were again imposed on the wheat grain crops (cv. Gauntlet in Experiment 1 and cv. Suntop in Experiment 2; Table S2). All Year 2 canola crops received aggressive weed management, using either an imidazolinone-tolerant hybrid (Hyola575CL; Experiment 1), or a glyphosate-tolerant hybrid (44Y26RR; Experiment 2). The imidazolinone-tolerant canola was grown in Experiment 1 rather than a glyphosate-tolerant hybrid because the residual herbicide metsulfuron applied in Year 1 fallow treatment precluded the use of hybrids that were susceptible to the Group 2 herbicide residues.

All Year 2 treatments received 100 kg/ha of ammonium sulfate (21 kg N/ha, 24 kg S/ha) at sowing, and, in Experiment 1, the wheat designated to be cut for hay (sown at target populations of 150 plants/m^2) was also supplied with an additional 16 kg N/ha as urea. The rates of MAP applied, along with all herbicides (and fungicides) and intensity of weed-control categories applied for the respective Year 2 wheat and canola crops are presented in Table S2. Details of the seed-dressings used in both Year 1 and 2 are shown in Table S3.

The wheat and canola grain crops were subsequently top-dressed with different rates of urea at the stem-elongation growth stage on the basis of pre-sowing measurements of soil mineral N to ensure an equivalent supply of plant-available soil N regardless of the preceding Year 1 treatment to achieve target wheat yields of 4 t/ha (soil mineral N + fertiliser N = ~160 kg N/ha) for wheat(C), or 6 t/ha (soil mineral N + fertiliser N = ~240 kg N/ha) for wheat(A), and 3.5 t/ha (soil mineral N + fertiliser N = ~280 kg N/ha) for canola. Details of pre-sowing concentrations of soil mineral N detected in Experiment 1 and 2 subplots and the amounts of N supplied to individual treatments are provided in Tables S4–S8 (note: presented values do not include the small amounts of N accompanying the MAP applied at sowing).

Year 3: in the final year in both experiments, all plots were sown to wheat (cv. Suntop in Experiment 1, cv. Lancer in Experiment 2), and were managed with aggressive (A) weed-control measures. Seed was sown at 150 plants/m^2 with 70 kg/ha MAP (7 kg N/ha, 15 kg P/ha), and in Experiment 2 an additional 21 kg N/ha as ammonium sulfate was also applied.

As with the Year 2 grain crops, urea was top-dressed at different rates to individual treatments on the basis of pre-sowing autumn measurements of soil mineral N in Experiments 1 and 2 to balance the plant-available soil N, to ensure all wheat crops were supplied with similar amounts of available N (Tables S4–S8) that were sufficient to achieve target grain yields of 5 t/ha (soil mineral N + fertiliser N = ~200 kg/ha N).

Any emerged ARG seedlings were killed prior to sowing with glyphosate and either carfentrazone-ethyl or oxyfluorfen at label rates. The pre-emergent herbicides pyroxasulfone at 100 g/ha and tri-allate at 1000 g/ha were used in both experiments, and no in-crop herbicides were applied.

Determining the impact of ARG competition on wheat yield

To examine the effect of ARG weed populations on wheat yield, in-crop ARG shoot dry matter (DM) at crop maturity was used as a surrogate measure of competition. To this end, the wide range of ARG shoot DM data recorded in the average growing-season rainfall year of 2013 under Year 1 wheat conservative weed-control measures in Experiment 2, and in the Year 2 wheat(C) crops in Experiment 1 were used to quantify the impact of ARG competition on wheat grain yields. Although pre-sowing concentrations of soil mineral N differed across Year 2 Experiment 1 wheat(C) subplots following the various Year 1 treatments (144–250 kg N/ha; Table S4), measurable N supply (soil mineral N + fertiliser N) to each treatment was balanced as described above, to control for initial differences in soil mineral N. The resulting grain protein of all wheat(C) crops exceeded 11.5% (data not shown), confirming that grain yield was not limited by soil N availability (Russell 1963).

Measurements

Soil sampling and analysis

In total, 30 soil core samples (0.03 m in diameter) were collected at random across the proposed experimental sites to a depth of 0.2 m for site characterisation in early April, prior to establishment of each experiment (2012 Experiment 1 and 2013 Experiment 2). Soils were analysed according to the methods described by Rayment and Lyons (2011) for surface and subsurface soil pH ($CaCl_2$; 0–0.1 and 0.1–0.2 m) and surface-soil Olsen available P (0–0.1 m). A further 12 soil cores (three 0.042-m-diameter cores in each of the four replicate block areas) were sampled to the anticipated maximum rooting depth of 1.6–1.7 m of wheat (Kirkegaard and Lilley 2007). Each of the deep soil cores was separated into the following segments: 0–0.1, 0.1–0.2, 0.2–0.4, 0.4–0.6, 0.6–0.8, 0.8–1.0, 1.0–1.3, 1.3–1.6 m. The soils from individual depth intervals from the three cores in each block were combined, mixed and subsampled for soil mineral N analyses (nitrate and ammonium; Rayment and Lyons 2011).

In late March or early April of Years 2 and 3, soils were again sampled to a depth of 1.6 m for pre-sowing soil mineral N determinations. In Year 2, this represented three 0.042-m-diameter soil cores collected in the central four sowing rows from the middle and both ends of each of the individual Year 1 main plots. In Year 3, two 0.042-m-diameter soil cores were collected approximately 1–1.5 m from both ends of each subplot. Each core was separated into the various depth intervals as outlined above. A further six 0.03-m-diameter cores collected to 0–0.1 m and 0.1–0.2 m depth at random across each plot or subplot were added to the equivalent 0.042-m-diameter core samples. The bulked soil was then mixed and subsampled. The resulting soil samples from each depth were stored separately in coolers in the field and later frozen on return to the laboratory, until analysed for soil mineral N.

Plant sampling and analysis

Wheat above-ground biomass, grain yield, ARG DM and spike density at crop maturity were quantified by removing all above-ground shoot material (wheat and ARG) from four equally distributed 0.458 m^2 quadrat sample areas in each main plot collected across the four central rows (total 1.83 m^2) in Year 1 and from a single 0.458 m^2 sample area from each subplot in Years 2 and 3. Wheat and ARG plants were separated and all ARG spikes counted. The wheat and ARG samples were dried at 70°C for at least 48 h before weighing for dry-matter (DM) determinations. Wheat grain yields were determined by mechanically harvesting 39–40 m of the middle four rows in each wheat(C) and wheat(A) main plot in Year 1 and from 9–12 m of each wheat subplot in Years 2 and 3. Wheat grain protein contents and grain moisture were measured using a near-infrared (NIR) transmission analyser (CropScan 3000B; Next Instruments International, Sydney, NSW, Australia) calibrated using a 20–20 stable-isotope mass spectrometer (Europa Scientific, Crewe, UK). Screenings were determined using a grain shaker with 2 mm sieve according to Australian Grains Industry standards.

Canola above-ground DM, ARG DM and spike density at crop maturity were determined at 50–70% seed colour change, as described above for wheat in Year 1 and as two 0.82 m sections (total 2.0 m^2) in Year 2. The separated canola samples were retained in aerated bags for 3 weeks to dry and mature (mimicking drying in a windrow). The canola was then threshed to separate the grain from the shoot residue. All canola samples were then dried at 70°C for at least 48 h before DM was recorded. Grain moisture, and oil and protein concentrations were determined by NIR calibrated with nuclear magnetic resonance (NMR) spectroscopy (Oxford Instruments, Oxon, UK) and mass spectrometry analysis. The remainder of the plot was harvested with a header to a height of 0.2 m.

The lupin grain crop was sampled twice in Year 1. Above-ground DM was sampled once during mid-pod fill (peak biomass prior to senescence and the commencement of leaf fall), and again at maturity for grain yield. Protocols to determine lupin DM and grain yield, ARG DM and spike density were the same as described for wheat. The remainder of the plot was harvested at maturity, with a header to a height of 0.1–0.15 m.

For all grain crops, a heavy ground-length tarpaulin shroud was attached to the rear of the plot harvester in each of 3 years of experimentation, to ensure that all ARG seeds and crop stubble remained on the harvested subplot regardless of weather conditions.

Field pea above-ground DM, ARG DM and spike density were determined as described for wheat immediately before application of non-selective herbicide in early September. The remaining field pea residues were retained as standing stubble.

Estimates of wheat cereal hay DM yields in Year 2 of both experiments were determined by removing all shoot material (wheat and ARG) 0.1 m above the ground from the middle four rows in one 0.5 m section (total 0.61 m^2 area) from each hay treatment subplot in late September, prior to ARG seed dispersal. The wheat and ARG components were separated. Hay biomass DM was measured after drying samples at 70°C for at least 48 h. The remainder of the hay treatment areas were slashed to a similar height, and all cut material was collected and removed from the site.

Assessment of ARG soil seedbank populations

In late March 2012 (Experiment 1) and 2013 (Experiment 2), prior to the establishment of the Year 1 treatments, the initial ARG seedbanks were determined by removing 40 surface-soil cores (0.05 m deep × 0.058 m in diameter) from across each of the trial sites. The soil was stored at 4°C for 2 weeks before being placed into free-draining seedling trays in a glasshouse and watered daily for 3 months. All ARG seedlings that germinated and emerged over that period were recorded and the cumulative number was taken to represent the relative size of the ARG seedbank. To ascertain the effect of the imposed experimental treatments on ARG seedbank numbers, 10 surface cores (0.05 m deep × 0.058 m in diameter) were removed in the autumn of Year 2 (early April), and eight cores were removed from the 13.3 m subplots in early April of Year 3, and again prior to commencement of the following growing season, 4 years after the initial trial establishment.

The glasshouse process outlined above was undertaken for each plot and subplot sample.

Economic analyses

The economic analyses used actual spot prices and costs from the period of experimentation. Total input costs for seed, fertiliser, herbicides and foliar fungicides, and operational costs for sowing, spraying, fertiliser top-dressing, harvesting and grain transport were calculated for each cropping year over the 3-year duration of the on-farm studies from farm gross margin and enterprise planning guides from the South Australian Grains Industry Trust (SAGIT; https://grdc.com.au/resources-and-publications/all-publications/publications/2019/farm-gross-margin-and-enterprise-planning-guide) and NSW Department of Primary Industries (NSW DPI; https://www.dpi.nsw.gov.au/agriculture/broadacre-crops/guides/publications/weed-control-winter-crops). Summaries of the calculated costs of N fertiliser are shown in Tables S5 and S8, and herbicide and total production costs are provided in Tables S6 and S9.

Gross margins were determined from the difference between total production costs for each treatment (i.e. inputs and operational costs) and estimated income was derived from crop grain yields or cereal hay on the basis of commodity prices on the day of harvest from cash prices offered at the nearby GrainCorp terminal at Junee, NSW (−34.51, 147.34; https://www.awb.com.au/daily-grain-prices), except for the glyphosate-tolerant canola, which was required to be delivered to Stockinbingal, NSW (−34.30, 147.53), at an assumed additional freight cost of A$5 per tonne (A$1 = ~US$0.69). The wheat and canola grain prices during experimentation both tended to be 10–15% and 8–20% lower respectively, than was the 15-year long-term average (2007–2021). In the case of the lupin grain and cereal hay, the commodity prices were similar to the long-term average in 1 year, but 26% or 22% higher respectively in the other year they were included as experimental treatments. Despite these fluctuations in value, it was assumed that the relative rankings of treatments and the observed trends in profit (gross margins) calculated using the commodity prices that were current at the time of experimentation would also be representative of longer-term outcomes.

Statistical analyses

The experiments were analysed using linear mixed models fitted using REML directive in GENSTAT (16th edn; https://vsni.co.uk/software/genstat). The blocking structure was a row–column layout nested within blocks (Block/(Row × Col). The Year 1 treatments were assigned in a randomised-block design with four blocks. The Year 2 treatments were randomly assigned to the subplots within each Year 1 main plot. Outputs of the statistical analyses of the experimental data are presented either as a standard error (s.e.) of the mean, or least significant difference (l.s.d.), and the significant level of propability (*P*-value). The Year 1 means for grain yield, GM, ARG spike numbers and DM, and Year 2 mean ARG seedbank counts were calculated at the main-plot level. It was assumed sampling was sufficient so that the means and s.e. would be equivalent for all subplots within each main plot.

The responses analysed in the ARG spike and soil seedbank data sets required log-transformation or square-root transformation to normalise the residual distribution. Statistical comparisions were performed on the transformed scale and an estimated l.s.d. was derived by multiplying the maximum standard error of difference (s.e.d.) by two. The transformed data were then back-transformed on the original scale to provide logical values for the reader to understand. For both experiments, *P*-values are indicated for ARG spike number and seedbank counts for Year 1 main plot treatment, Year 2 subplot treatment and main plot × subplot interactions.

Full details of the statistically significant treatment differences for Year 1 main plot and Year 2 subplot interactions are provided for Experiment 1 spike numbers in Table S10. In Experiment 1, no significant interaction between main plot × subplot treatment effects were observed in seedbank counts for Year 3 and the final Year 4 deteminations (i.e. measured in the autumn following 3 years of experimental treatments); however, the main significant main plot and subplot effects can be found in Table S11.

Details of statistically significant treatment differences for Year 1 main plot and Year 2 subplot interactions for spike numbers and soil seedbanks for Experiment 2 are presented in Table S12. Because there were significant main plot × subplot interactions in the final ARG weed seedbank counts for Experiment 2, these have been identified with letters to show significant differences between sequences on the basis of the statistical analyses of the transformed data.

For analysis of ARG seedbanks in Year 4, fixed effects were crop years (Crop year 1 + Crop year 2 + Crop year 1 × Crop year 2), with the random effects being block, row and column (Block.Row + Block.Column + Block.Row.Column).

Results

Impact of cropping sequence and treatments on crop production and profit

Experiment 1

Annual rainfall in the first year of the study (2012) was ~50 mm higher than the 501 mm long-term average, owing to heavy rain in March (153 mm) prior to the commencement of the study; however, rainfall over the crop growing-season (April–October) was more than 100 mm less than the long-term average (286 mm; Table 3). Growing-season rainfall resembled the long-term average during Years 2 (2013) and 3 (2014), although total annual rainfall in those years was lower than the long-term average (Table 3).

Year 1: grain yields of 2.0–3.2 t/ha for wheat were unusually low; especially compared with the yields measured for canola (3.0–3.5 t/ha), which normally would be expected to be ~60% of wheat (Holland *et al.* 1999). The low tiller numbers measured in both wheat(C) and wheat(A) at maturity (average of 1.98 tillers/plant; data not shown), despite establishment of reasonable plant populations (~90 and 140 plants/m^2; data not shown), suggested an underlying, unidentified constraint to early wheat root and/or shoot growth at the site (Ryan *et al.* 2003). PREDICTA B® DNA-based soil tests (South Australian Research and Development Institute, Urrbrae, SA, Australia; Ophel-Keller *et al.* 2008) taken at the site prior to commencement of the experiment in 2012 showed low levels of all major soil-borne cereal pathogens (data not shown); consequently, disease was considered to have not played a major role. However, a measurable level of *Pratylenchus neglectus* (1 per g of soil) did increase to 3 per g of soil in wheat treatments when measured prior to sowing in 2013; so, nematode damage of wheat roots cannot be dismissed as a contributing factor. The lupin grain crop yielded 3.1 t/ha and the field pea BM represented 4.5 t/ha of legume residues (Table 5).

Higher profits were achieved for canola (A$1166–A$1259/ha) and lupin (A$683/ha) than wheat, which in turn showed little difference in gross margin among the weed-control intensities (A$250–$257/ha), despite the wheat(A) achieving >1 t/ha higher grain yield than wheat(C) (Table 5). This arose from the higher cost of the herbicides applied to wheat(A) than wheat(C) (A$142/ha cf. A$56/ha) and higher overall production costs (A$586/ha cf. A$283/ha; Table S6).

Year 2: the pre-sowing autumn determination of soil mineral N was higher following fallow (250 kg N/ha), field pea BM (231 kg N/ha) and lupin (204 kg N/ha), than after wheat (169–172 kg N/ha), or canola (144–155 kg N/ha; Table S4). This resulted in applications of a lower rate of top-dressed fertiliser N (46 kg N/ha) to achieve Year 2 target grain yields for wheat(A) (6 t/ha) and canola(A) (3.5 t/ha) crops, than following any of the Year 1 wheat or canola treatments (92–119 kg N/ha; Table S5).

The lowest Year 2 wheat yield (1.5 t/ha) was recorded for the wheat(C)–wheat(C) sequence and was significantly smaller than the yield for wheat(C) grown after any other Year 1 treatment (2.8–5.2 t/ha; Table 5). By comparison the biomass accumulated by the wheat cereal hay represented 7.4–8.1 t DM/ha, and grain yields across all Year 2 wheat(A) crops fell within a narrow range (4.6–5.2 t/ha) regardless of the Year 1 treatment (Table 5). Unlike Year 1, the yield ratio of Year 2 canola(A) (3.2–3.6 t/ha) to wheat(A) was much closer to the anticipated 0.60 value (Holland *et al.* 1999).

Despite canola grain yields being about two-thirds of wheat(A), the gross margins of all canola crops (A$964–$1159/ha), except where canola followed wheat(C) (A$820/ha), were considerably greater than those of wheat grain crops grown after fallow or any of the break-crop treatments (A$480–$799/ha; Table 5). Profit for the continuous wheat grain crop systems ranged from A$170 to $642/ha across the various combinations of wheat(C) and wheat(A) weed-control measures, and the gross margin calculated for cereal hay represented A$533–$644/ha.

Year 3: the pre-sowing autumn measurements of soil mineral N in Year 3 ranged from 101–168 kg N/ha across all treatment subplots (Table S4). The final wheat(A) crops were top-dressed at 40 kg N/ha in all systems except where wheat(C) had been grown in Year 2, which required higher rates of N application (53–86 kg N/ha; Table S5) to achieve the Year 3 prospective target grain yields (5 t/ha). The resultant grain yields in Year 3 ranged from 3.1 to 4.5 t/ha, resulting in individual Year 3 gross margins of A$677–$911/ha (Table 5).

The five systems with the highest cumulative profit over the full 3 years of the study (A$2501–$2650/ha) included canola–cereal hay double-break and single-break systems that had canola grain crops in Year 1. The five systems with the lowest cumulative profits (A$1164–$1616/ha) either had field pea BM in Year 1 or were continuous wheat systems where wheat(C) had been grown in both Year 1 and Year 2 (Table 5).

Experiment 2

The growing-season rainfall was similar to the long-term average in both of the first 2 years of Experiment 2, although the 2013 growing season was characterised by a particularly dry start, and total annual rainfall was lower than the long-term average by 70 mm in 2013 and 45 mm in 2014 (Table 3). By contrast, both growing-season and annual rainfall were ~106 and 137 mm higher respectively, than was the long-term average in the final year of cropping (2015; Table 3).

Year 1: grain yields ranged from 2.2 to 4.0 t/ha for wheat, from 1.6 to 1.9 t/ha for canola, and 2.6 t/ha for lupin (Table 6). The wheat and canola grain yields were lower than achieved by the equivalent crops sown nearby in Experiment 1 with the same growing-season rainfall (refer to Year 2 data in Table 5).

Lupin was the most profitable crop (A$724/ha; Table 6) owing to its low costs of production and high grain value in 2013 (Tables S9, S13), but it is worth noting that the ranking of treatment profit would not change if lupin gross margins were recalculated using the lower long-term average grain price (Table S13). The low canola grain yields, high fertiliser-N and production costs of canola(A) (Tables S8, S9) resulted in canola profits of only 15–30% of that achieved by the same Year 1 treatments in Experiment 1 (compare Tables 5 and 6). Again, the higher herbicide and production costs for wheat(A) than wheat(C) (Table S9) resulted in similar gross margins (A$318 and $359) despite the higher wheat(A) grain yields (Table 6).

Table 5. Crop grain yields and gross margins (GM) for the different weed-control strategies in Experiment 1 at Eurongilly, NSW (2012–2014), and the cumulative 3-year GM.

Year 1			Year 2			Year 3			Years 1–3
Treatment code	Yield (t/ha)	GM (A$/ha)	Crop	Yield (t/ha)	GM (A$/ha)	Crop	Yield (t/ha)	GM (A$/ha)	Cumulative GM (A$/ha)
0.1-Wheat(C)	2.0 ± 0.10	250	Wheat(C)	1.5 ± 0.18	170	Wheat(A)	3.3 ± 0.20	745	1164
0.2-Wheat(C)	2.0 ± 0.10	250	Wheat(A)	4.6 ± 0.11	536	Wheat(A)	4.0 ± 0.29	824	1610
0.3-Wheat(A)	3.2 ± 0.03	257	Wheat(C)	2.9 ± 0.23	510	Wheat(A)	3.7 ± 0.26	772	1539
0.4-Wheat(A)	3.2 ± 0.03	257	Wheat(A)	5.0 ± 0.26	642	Wheat(A)	4.2 ± 0.24	855	1755
1.1-Wheat(C)	2.0 ± 0.10	250	Canola(A)	3.0 ± 0.19	820	Wheat(A)	4.0 ± 0.13	677	1747
1.2-Wheat(A)	3.2 ± 0.03	257	Canola(A)	3.3 ± 0.26	964	Wheat(A)	3.1 ± 0.13	686	1908
1.3-Canola(C)	3.0 ± 0.15	1166	Wheat(C)	2.8 ± 0.14	480	Wheat(A)	3.8 ± 0.37	753	2399
1.4-Canola(C)	**3.0 ± 0.15**	**1166**	**Wheat(A)**	**4.7 ± 0.11**	**537**	**Wheat(A)**	**3.8 ± 0.28**	**828**	**2532**
1.5-Canola(A)	**3.5 ± 0.11**	**1259**	**Wheat(C)**	**2.8 ± 0.09**	**489**	**Wheat(A)**	**4.1 ± 0.26**	**788**	**2536**
1.6-Canola(A)	**3.5 ± 0.11**	**1259**	**Wheat(A)**	**4.7 ± 0.11**	**533**	**Wheat(A)**	**4.5 ± 0.26**	**858**	**2650**
1.7-Lupin	3.1 ± 0.25	683	Wheat(C)	3.5 ± 0.21	651	Wheat(A)	3.9 ± 0.33	811	2145
1.8-Lupin	3.1 ± 0.25	683	Wheat(A)	5.1 ± 0.06	726	Wheat(A)	3.9 ± 0.04	863	2272
1.9-Pea BM	(4.5 ± 0.16)[A]	−160[B]	Wheat(C)	3.0 ± 0.13	525	Wheat(A)	3.8 ± 0.30	826	1191
1.10-Pea BM	(4.5 ± 0.16)[A]	−160[B]	Wheat(A)	5.0 ± 0.09	707	Wheat(A)	4.3 ± 0.18	911	1457
1.11-Fallow	Nil	−45[B]	Wheat(C)	4.2 ± 0.49	799	Wheat(A)	3.5 ± 0.20	835	1589
1.12-Fallow	Nil	−45[B]	Wheat(A)	5.2 ± 0.07	761	Wheat(A)	4.5 ± 0.25	900	1616
2.1-Canola(C)	**3.0 ± 0.15**	**1166**	**Wheat hay**	**(8.1 ± 0.48)[A]**	**644**	**Wheat(A)**	**3.8 ± 0.16**	**720**	**2531**
2.2-Canola(A)	**3.5 ± 0.11**	**1259**	**Wheat hay**	**(7.4 ± 0.72)[A]**	**533**	**Wheat(A)**	**3.7 ± 0.11**	**709**	**2501**
2.3-Lupin	3.1 ± 0.25	683	Canola(A)	3.2 ± 0.35	967	Wheat(A)	4.1 ± 0.27	721	2371
2.4-Pea BM	(4.5 ± 0.16)[A]	−160[B]	Canola(A)	3.3 ± 0.21	1019	Wheat(A)	3.4 ± 0.20	679	1538
2.5-Fallow	Nil	−45[B]	Canola(A)	3.6 ± 0.37	1159	Wheat(A)	3.7 ± 0.15	696	1810

Grain yield data represent the mean ± s.e. The bold treatment codes indicate the five most profitable systems.
Treatment identify code numbers commencing with '0' indicate 3 years of continous wheat grain crops. Codes commencing with '1' or '2' represent sequences with either a single- or double-break from wheat grain production over 3 years respectively. See Table 4 for a full description of treatment strategies and sequences.
[A]Values in parentheses represent measures of above-ground dry matter for field pea BM in Year 1 and wheat cereal hay in Year 2.
[B]Because there was no income, the negative GM represents the full value of the production costs.

Year 2: unlike Experiment 1 where the autumn concentrations of soil mineral N were similar following wheat and canola, the pre-sowing soil mineral N measured after wheat(C) in Experiment 2 (82 kg N/ha) was substantially lower than where either wheat(A) or canola was the preceding crop (134–162 kg N/ha; Table S7). Whether this arose from a carry-over of fertiliser N from Year 1 wheat(A) and canola, or greater scavenging of soil mineral N by the higher ARG burden in wheat(C) (see below) is open to speculation, but the net result was that the Year 1 legume (141–166 kg N/ha) and winter fallow treatments (179 kg N/ha; Table S7) did not provide the same degree of savings in fertiliser N or production costs as observed in Experiment 1. The rates of fertiliser N supplied ranged from the basal ammonium sulfate application of only 21 kg N/ha to cereal hay and some wheat(C) treatments, to 41–153 kg N/ha across the other crop and intensity of weed-control combinations (Table S8).

Grain yields of <3.0 t/ha for wheat and <2.0 t/ha for canola were lower than expected (Table 6). Especially given the close to average growing-season rainfall (Table 3) and that the Experiment 2 wheat cereal hay biomass (7.9 t DM/ha; Table 6) was similar to measures of hay DM observed in Experiment 1 when wheat(A) grain yields of 4.2–5.2 t/ha and 3.0–3.6 t/ha for canola were recorded (Table 5). Visual assessment of wheat tillers and head numbers at maturity indicated that 20–30% of tillers developed no spikes and ~40% of emerged spikes displayed frost damage (data not shown); so, frost was likely to have been the major contributing factor for the reduced yield.

Year 2 gross margins were highly variable, ranging from a loss of –A$18/ha to A$442/ha across wheat grain crops and from A$82 to $285/ha for canola (Table 6) and were heavily influence by Year 1 treatments and total production costs (Table S8). Wheat cereal hay proved to be the most profitable option (A$933–$937/ha; Table 6).

Table 6. Crop grain yields and gross margins (GM) for the different weed-control strategies in Experiment 2 at Eurongilly, NSW (2013–2015), and the cumulative 3-year GM.

Year 1			Year 2			Year 3			Years 1–3
Treatment code	Yield (t/ha)	GM (A$/ha)	Crop	Yield (t/ha)	GM (A$/ha)	Treatment	Yield (t/ha)	GM (A$/ha)	Cumulative GM (A$/ha)
0.1-Wheat(C)	2.2 ± 0.08	318	Wheat(C)	2.1 ± 0.06	129	Wheat(A)	4.1 ± 0.05	547	994
0.2-Wheat(C)	2.2 ± 0.08	318	Wheat(A)	2.7 ± 0.05	−18	Wheat(A)	3.6 ± 0.10	586	886
0.3-Wheat(A)	4.0 ± 0.07	359	Wheat(C)	2.7 ± 0.13	369	Wheat(A)	3.9 ± 0.13	631	1359
0.4-Wheat(A)	4.0 ± 0.07	359	Wheat(A)	2.8 ± 0.06	118	Wheat(A)	4.3 ± 0.08	612	1089
1.1-Wheat(C)	2.2 ± 0.08	318	Canola(A)	1.7 ± 0.10	82	Wheat(A)	4.4 ± 0.05	550	950
1.2-Wheat(A)	4.0 ± 0.07	359	Canola(A)	1.7 ± 0.07	163	Wheat(A)	4.1 ± 0.16	663	1185
1.3-Canola(C)	1.6 ± 0.08	348	Wheat(C)	2.5 ± 0.08	274	Wheat(A)	4.0 ± 0.05	605	1227
1.4-Canola(C)	1.6 ± 0.08	348	Wheat(A)	2.7 ± 0.06	23	Wheat(A)	4.4 ± 0.05	681	1052
1.5-Canola(A)	1.9 ± 0.19	171	Wheat(C)	2.5 ± 0.03	309	Wheat(A)	4.5 ± 0.07	566	1046
1.6-Canola(A)	1.9 ± 0.19	171	Wheat(A)	2.6 ± 0.02	36	Wheat(A)	4.1 ± 0.08	609	816
1.7-Lupin	**2.6 ± 0.11**	**724**	**Wheat(C)**	**2.1 ± 0.11**	**222**	**Wheat(A)**	**3.4 ± 0.09**	**696**	**1642**
1.8-Lupin	**2.6 ± 0.11**	**724**	**Wheat(A)**	**2.6 ± 0.05**	**42**	**Wheat(A)**	**4.1 ± 0.05**	**697**	**1463**
1.9-Pea BM	(5.0 ± 0.22)[A]	−204[B]	Wheat(C)	2.9 ± 0.14	421	Wheat(A)	3.9 ± 0.09	695	912
1.10-Pea BM	(5.0 ± 0.22)[A]	−204[B]	Wheat(A)	2.8 ± 0.08	114	Wheat(A)	4.2 ± 0.14	654	564
1.11-Fallow	Nil	−72[B]	Wheat(C)	3.0 ± 0.02	442	Wheat(A)	4.3 ± 0.05	519	889
1.12-Fallow	Nil	−72[B]	Wheat(A)	2.7 ± 0.06	115	Wheat(A)	4.0 ± 0.07	715	758
2.1-Canola(C)	**1.6 ± 0.08**	**348**	**Wheat hay**	**(7.9 ± 0.12)[A]**	**933**	**Wheat(A)**	**3.7 ± 0.02**	**638**	**1919**
2.2-Canola(A)	**1.9 ± 0.19**	**171**	**Wheat hay**	**(7.9 ± 0.06)[A]**	**937**	**Wheat(A)**	**4.3 ± 0.06**	**587**	**1695**
2.3-Lupin	**2.6 ± 0.11**	**724**	**Canola(A)**	**1.7 ± 0.07**	**157**	**Wheat(A)**	**4.6 ± 0.03**	**753**	**1634**
2.4-Pea BM	(5.0 ± 0.22)[A]	−204[B]	Canola(A)	1.9 ± 0.07	242	Wheat(A)	4.7 ± 0.05	634	672
2.5-Fallow	Nil	−72[B]	Canola(A)	1.9 ± 0.09	285	Wheat(A)	4.8 ± 0.06	705	918

Grain yield data represent the mean ± s.e. The bold treatment codes indicate the five most profitable systems.
A treatment code commencing with '0' indicates 3 years of continous wheat grain crops. Codes commencing with '1' or '2' represent sequences with either a single- or double-break from wheat grain production over 3 years respectively. See Table 4 for a full description of treatment strategies and sequences.
[A]Values in parentheses represent measures of above-ground dry matter for field pea BM in Year 1 and wheat cereal hay in Year 2.
[B]Because there was no income, the negative GM represents the full value of the production costs.

Year 3: pre-sowing soil mineral N varied considerably at the start of the final cropping year (Table S7) and the resulting top-dressed rates of urea ranged from 0 to 66 kg N/ha across the various systems (Table S8). The final wheat(A) crops benefited from the above-average rainfall (Table 3) and grain yields ranged from 3.4 to 4.8 t/ha (Table 6). As a result, the Year 3 wheat gross margins (A$519–$753/ha) were much higher than those for the previous 2 years.

The cumulative gross margins calculated over the full 3 years of Experiment 2 (Table 6) had some similarities to those in Experiment 1 (Table 5). The five systems with the highest cumulative profit again included the two canola–cereal hay double-break sequences, but lupin rather than canola featured as a major contributor to the final economic outcome in Experiment 2. The five systems with the lowest cumulative profit included sequences where field pea BM, fallow, or wheat(C) had been grown in Year 1. However, the canola(A)–wheat(A) sequence was also among the least profitable systems because of the low yield and profit in Year 1, and the impact of frost damage in Year 2 (Table 6). This highlighted the risks associated with the investment in high-input systems.

The impact of treatments on in-crop ARG

Experiment 1

Year 1: the highest incidence of in-crop ARG was measured in the wheat(C) (504 spikes/m^2 and 1.60 t DM/ha). Wheat (A), canola(C) and lupin provided partial control (32–78 spikes/m^2 and 0.08–0.34 t DM/ha), but no surviving ARG plants were detected in the winter fallow, canola(A) and field pea BM plots (Table 7).

Year 2: by the spring of Year 2, the continuous wheat system with two consecutive wheat(C) treatments had the highest ARG spike density and biomass (898 spikes/m^2, 4.72 t DM/ha).

Table 7. The impact of weed-control strategies on annual ryegrass (ARG) spike density and shoot dry matter (DM) measured at the end of spring (November) in each cropping year in Experiment 1, and the subsequent size of ARG seedbanks measured in soil in autumn (April) 2, 3 and 4 years after the commencement of experimentation.

Year 1			Year 2				Year 3				Year 4
Treatment code	Spike number (spikes/m^2)	ARG DM (t/ha)	Crop	Seedbank (seeds/m^2)	Spike number (spikes/m^2)	ARG DM (t/ha)	Crop	Seedbank (seeds/m^2)	Spike number (spikes/m^2)	ARG DM (t/ha)	Seedbank (seeds/m^2)
0.1-Wheat(C)	504	1.60	Wheat(C)	5492	898	4.72	Wheat(A)	13 148	943	1.58	3140
0.2-Wheat(C)	504	1.60	Wheat(A)	5492	71	0.33	Wheat(A)	3412	121	0.29	523
0.3-Wheat(A)	78	0.25	Wheat(C)	777	294	2.38	Wheat(A)	5508	147	0.46	2158
0.4-Wheat(A)	78	0.25	Wheat(A)	777	29	0.12	Wheat(A)	1379	60	0.24	366
1.1-Wheat(C)	504	1.60	Canola(A)	5492	0	0	Wheat(A)	797	22	0.09	332
1.2-Wheat(A)	78	0.25	Canola(A)	777	0	0	Wheat(A)	259	20	0.09	267
1.3-Canola(C)	32	0.34	Wheat(C)	505	383	3.25	Wheat(A)	n.d.[A]	229	0.59	2222
1.4-Canola(C)	32	0.34	Wheat(A)	505	14	0.07	Wheat(A)	n.d.[A]	82	0.36	252
1.5-Canola(A)	0	0	Wheat(C)	208	388	2.89	Wheat(A)	7770	200	0.69	2387
1.6-Canola(A)	0	0	Wheat(A)	208	15	0.08	Wheat(A)	381	29	0.13	219
1.7-Lupin[B]	43	0.08	Wheat(C)	748	200	1.44	Wheat(A)	6614	122	0.88	1167
1.8-Lupin[B]	43	0.08	Wheat(A)	748	8	0.04	Wheat(A)	312	19	0.09	148
1.9-Pea BM	n.d.[A]	(0.70)[C]	Wheat(C)	464	237	1.67	Wheat(A)	7413	157	0.36	3118
1.10-Pea BM	n.d.[A]	(0.70)[C]	Wheat(A)	464	2	0.02	Wheat(A)	496	14	0.11	162
1.11-Fallow	0	0	Wheat(C)	290	60	0.61	Wheat(A)	n.d.[A]	277	0.84	970
1.12-Fallow	**0**	**0**	**Wheat(A)**	**290**	**2**	**0.01**	**Wheat(A)**	**n.d.[A]**	**10**	**0.04**	**118**
2.1-Canola(C)	32	0.34	Wheat hay	505	(790)[D]	(5.00)[D]	Wheat(A)	n.d.[A]	23	0.15	300
2.2-Canola(A)	**0**	**0**	**Wheat hay**	**208**	**(537)[D]**	**(3.70)[D]**	**Wheat(A)**	**124**	**23**	**0.13**	**122**
2.3-Lupin[B]	**43**	**0.08**	**Canola(A)**	**748**	**0**	**0**	**Wheat(A)**	**196**	**6**	**0.01**	**63**
2.4-Pea BM	**n.d.[A]**	**(0.70)[C]**	**Canola(A)**	**464**	**0**	**0**	**Wheat(A)**	**249**	**4**	**0.02**	**142**
2.5-Fallow	**0**	**0**	**Canola(A)**	**290**	**0**	**0**	**Wheat(A)**	**n.d.[A]**	**2**	**0.04**	**56**
P-value Year 1	<0.001	<0.001		<0.001	<0.001	<0.001		<0.001	<0.001	0.002	<0.001
P-value Year 2					<0.001	<0.001		<0.001	<0.001	<0.001	<0.001
P interaction					0.004	0.002		0.105	<0.001	0.006	0.699
l.s.d. (at $P = 0.05$)	147	0.16		815							

The initial year 1 ARG seedbank count prior to establishment of treatments was 1815 seeds/m^2. The bold treatment codes indicate the five most effective systems at reducing ARG seedbanks. Additional statistical details are provided in Tables S10 and S11.
[A]No data available.
[B]The lupin crop in Experiment 1 was spray-topped to kill ARG seed prior to maturity.
[C]The values in parentheses represent the amount of ARG DM present within the field pea crop prior to imposing the BM treatment.
[D]The values in parentheses represent the number of ARG spikes present prior to cutting wheat for hay and amount of ARG DM removed in hay.

Apart from where wheat(C) followed fallow, in-crop ARG spike density and biomass were also high whenever wheat(C) was the second crop in the sequence (200–237 spikes/m^2, 1.44–1.67 t DM/ha; Table 7). By comparison, the Year 2 wheat(A) appeared to provide reasonable control of ARG (2–15 spikes/m^2, 0.01–0.08 t DM/ha), except where the preceding crop had also been wheat (29–71 spikes/m^2, 0.12–0.33 t DM/ha; Table 7). No live ARG plants were detected in canola(A) plots, and 537–790 spikes/m^2 and 3.7–5.0 t ARG DM/ha was removed from the site in cereal hay (Table 7).

Year 3: the final wheat crop in Year 3 of experimentation in this system represented the fifth consecutive wheat grain crop at the farm site. The highest ARG infestations (943 spikes/m^2, 1.58 t DM/ha; Table 7) were recorded in the continuous wheat system where wheat(C) had been grown in the first 2 years of the sequence. The double-break sequences that included canola(A) in Year 2, or those where wheat(A) followed the most effective Year 1 break-control measures, had the fewest ARG spikes and lowest late-spring ARG DM (4–29 spikes/m^2, 0.04–0.13 t DM/ha; Table 7).

Table 8. The impact of weed-control strategies on annual ryegrass (ARG) spike density and shoot dry matter (DM) measured at the end of spring (November) in each cropping year in Experiment 2, and the subsequent size of ARG seedbanks measured in soil in autumn (April) 2, 3 and 4 years after the commencement of experimentation.

Year 1			Year 2				Year 3				Year 4
Treatment code	Spike number (spikes/m^2)	ARG DM (t/ha)	Crop	Seedbank (seeds/m^2)	Spike number (spikes/m^2)	ARG DM (t/ha)	Crop	Seedbank (seeds/m^2)	Spike number (spikes/m^2)	ARG DM (t/ha)	Seedbank (seeds/m^2)
0.1-Wheat(C)	534	3.48	Wheat(C)	6748	532	1.94	Wheat(A)	4930	167	0.48	1693abc
0.2-Wheat(C)	534	3.48	Wheat(A)	6748	130	0.38	Wheat(A)	3216	126	0.37	1567abc
0.3-Wheat(A)	30	0.10	Wheat(C)	1337	173	0.95	Wheat(A)	2722	104	0.28	1316abcde
0.4-Wheat(A)	30	0.10	Wheat(A)	1337	6	0.05	Wheat(A)	593	23	0.09	363fgh
1.1-Wheat(C)	534	3.48	Canola(A)	6748	1	0.03	Wheat(A)	1507	133	0.47	1477abcd
1.2-Wheat(A)	**30**	**0.10**	**Canola(A)**	**1337**	**1**	**0.04**	**Wheat(A)**	**212**	**5**	**0.05**	**115jk**
1.3-Canola(C)	193	0.72	Wheat(C)	3358	166	1.19	Wheat(A)	3415	108	0.28	1720ab
1.4-Canola(C)	193	0.72	Wheat(A)	3358	70	0.22	Wheat(A)	1019	51	0.11	826abcdef
1.5-Canola(A)	1	0.01	Wheat(C)	670	20	0.35	Wheat(A)	819	10	0.06	597bcdefgh
1.6-Canola(A)	**1**	**0.01**	**Wheat(A)**	**670**	**2**	**0.01**	**Wheat(A)**	**350**	**3**	**0.01**	**59kl**
1.7-Lupin[A]	462	1.51	Wheat(C)	4505	537	3.14	Wheat(A)	4251	152	0.37	1951a
1.8-Lupin[A]	462	1.51	Wheat(A)	4505	47	0.38	Wheat(A)	1129	61	0.18	711abcdefg
1.9-Pea BM	(108)[B]	(0.74)[B]	Wheat(C)	897	52	0.18	Wheat(A)	729	26	0.11	437fgh
1.10-Pea BM	(108)[B]	(0.74)[B]	Wheat(A)	897	3	0.01	Wheat(A)	309	8	0.03	218hij
1.11-Fallow	0	0	Wheat(C)	649	44	0.34	Wheat(A)	1112	39	0.21	653bcdefg
1.12-Fallow	0	0	Wheat(A)	649	2	0.02	Wheat(A)	226	5	0.02	223hij
2.1-Canola(C)	193	0.72	Wheat hay	3358	(631)[C]	(1.69)[C]	Wheat(A)	1004	47	0.16	347fghi
2.2-Canola(A)	**1**	**0.01**	**Wheat hay**	**670**	**(99)[C]**	**(0.32)[C]**	**Wheat(A)**	**457**	**15**	**0.07**	**132ijk**
2.3-Lupin[A]	462	1.51	Canola(A)	4505	1	0.03	Wheat(A)	892	46	0.13	638bcdefg
2.4-Pea BM	**(108)[B]**	**(0.74)[B]**	**Canola(A)**	**897**	**1**	**0**	**Wheat(A)**	**104**	**10**	**0.04**	**106jkl**
2.5-Fallow	**0**	**0**	**Canola(A)**	**649**	**1**	**0.01**	**Wheat(A)**	**408**	**22**	**0.08**	**37l**
P-value Year 1	<0.001	<0.001		<0.001	<0.001	<0.001		<0.001	<0.001	<0.001	<0.001
P-value Year 2					<0.001	<0.001		<0.001	<0.001	<0.001	<0.001
P interaction					<0.001	<0.001		0.025	0.037	0.246	0.005
l.s.d. (at $P = 0.05$)	70	0.27		1627							

The Year 1 initial ARG seedbank count prior to establishment of treatments was 2775 seeds/m^2. The bold treatment codes indicate the five most effective systems at reducing ARG seedbanks.
Year 4 values followed by the same letter are not significantly different (at $P = 0.05$). Additional statistical details are provided in Table S12.
[A]Difficulties with site access prevented the lupin crop in Experiment 2 being spray-topped to kill ARG seed prior to maturity.
[B]The values in parentheses represent the number of ARG spikes or amount of ARG DM present within the field pea crop prior to imposing the BM treatment.
[C]The values in parentheses represent the number of ARG spikes present prior to cutting wheat for hay and amount of ARG DM removed in hay.

Experiment 2

Year 1: the general ARG control outcomes in Experiment 2 were similar to those in Experiment 1. The highest spike densities and greatest ARG DM were again measured in wheat(C) (535 spikes/m^2, 3.48 t DM/ha). The best control was achieved by fallow, canola(A) and field pea BM (Table 8). However, in contrast to Experiment 1, there was particularly poor ARG control in the lupin grain crop (462 spikes/m^2, 1.51 t DM/ha; Table 8).

Year 2: the in-crop ARG weed burdens were greatest where there had either been two consecutive wheat(C) crops or where wheat(C) followed lupin (532–537 spikes/m^2, 1.94–3.14 t DM/ha). The lowest ARG populations were recorded in canola(A) and where wheat(A) was grown following Year 1 canola(A), wheat(A), fallow or field pea BM treatments (1–6 spikes/m^2, 0.01–0.05 t DM/ha; Table 8). Between 99 and 631 spikes/m^2, representing 0.32–1.69 t ARG DM/ha, were removed from the site in cereal hay (Table 8).

Year 3: the late-spring measures of in-crop ARG within the Year 3 wheat(A) crops indicated that the systems with the highest incidence of ARG were characterised by wheat(C) in Years 1 and/or 2 (100–167 spikes/m^2, 0.28–0.48 t DM/ha), whereas systems with the lowest ARG tended to be sequences with field pea BM, fallow, canola(A) or wheat(A) in Year 1, and canola(A), wheat(A), or cereal hay in Year 2 (3–15 spikes/m^2, 0.01–0.07 t DM/ha; Table 8).

The impact of treatments on soil ARG seedbanks

Experiment 1

The initial ARG seedbank of viable seeds measured prior to the commencement of Experiment 1 was 1815 seeds/m^2.

Autumn Year 2: the ARG seedbank counts undertaken on soil collected immediately before the Year 2 growing season were highest where wheat(C) was the first treatment (5492 seeds/m^2), which represented a 3-fold increase in ARG seedbank size compared with the initial pre-season seedbank count. The lowest ARG seedbank counts followed canola(A), and winter fallow (208–290 seeds/m^2; Table 7).

Autumn Year 3: there was a further 2.5-fold increase in pre-season ARG seedbank density in the last experimental cropping year following two consecutive years of wheat(C) (13 148 seeds/m^2). High ARG seedbank numbers were also observed in any system where wheat(C) had been used as the second crop (5508–7770 seeds/m^2; Table 7). However, the ARG seedbank values ranged from 124 to 496 seeds/m^2 for systems wherever wheat(A), canola(A) or wheat cereal hay treatments had been imposed in Year 2 (Table 7). Some treatments that achieved near-complete control (0–2 panicles/m^2) in both Year 1 and Year 2, such as canola(A)-cereal hay and field pea BM-canola(A), still had seedbanks of 124–249 seeds/m^2, illustrating the highly persistent nature of the ARG seedbank.

Autumn Year 4: the ARG seedbank count measured 5–6 months after the completion of Experiment 1 indicated that the Year 3 wheat(A) crop lowered ARG seedbanks across all systems (Table 7). The highest final ARG seedbank was recorded in the continuous wheat system with two consecutive years of wheat(C) (3140 seeds/m^2). The most effective single-break systems were where fallow, field pea BM, lupin or canola(A) were followed by 2 years of wheat(A) (118–162 seed/m^2), which represented a 95–96% reduction in soil ARG seedbanks compared with wheat(C)–wheat(C). However, the same single-break treatments were either totally ineffective, or provided <70% reduction in ARG seedbank counts if they were followed by a single year of wheat(C) (Table 7). Four of the five systems with the lowest ARG seedbank counts were double-break sequences consisting of canola(A)-cereal hay, or fallow, lupin or field pea BM followed by canola(A) (56–142 seeds/m^2; Table 8), equivalent to a 96–98% reduction in ARG seedbank.

Experiment 2

The ARG seedbank of viable seeds prior to Experiment 2 was 2775 seeds/m^2.

Autumn Year 2: pre-season determinations in Year 2 indicated that poor weed control by the initial wheat(C) and lupin treatments resulted in a major increase in ARG seedbanks (4505–6748 seeds/m^2). There was a net decline in ARG seedbanks following all other Year 1 treatments except canola(C) (3358 seeds/m^2). Fallow and canola(A) provided the most effective ARG control (649–670 seeds/m^2; Table 8).

Autumn Year 3: seedbank counts were highest where either Year 1 lupin or wheat(C) was followed by wheat(C) (4251–4930 seeds/m^2; Table 8). However, unlike the seedbank measurements undertaken the previous autumn, ARG spike densities of 530–540 spikes/m^2 in the preceding wheat(C) crop did not result in an overall increase in ARG seedbank numbers. This may have been due to a higher depletion of ARG seedbanks as a result of predation, a series of post-harvest germinations of ARG seedlings over summer, and/or the deterioration in viability of both the newly produced ARG seeds and seeds already present in the seedbank in response to the wetter-than-average 2014 December–2015 January period (Table 3; Chauhan *et al.* 2006; Spafford Jacob *et al.* 2006; Walsh *et al.* 2019).

As in Experiment 1, there were several treatments (canola(A) followed by wheat(A) or cereal hay, fallow or field pea BM followed by wheat(A)) that achieved very high levels of ARG control in both Year 1 and Year 2 (0–2 panicles/m^2), but still had seedbanks of 104–457 seeds/m^2.

The benefits of Year 1 weed control by fallow, canola(A) and field pea BM were largely lost where ever wheat(C) was grown after these treatments (729–2722 seeds/m^2). Comparisons of Year 2 and Year 3 seedbank data suggested that Year 2 canola(A), cereal hay and wheat(A) reduced ARG seed numbers when grown after the wheat(C), lupin and canola(C) treatments, but the overall size of ARG seedbanks remained high (892–3216 seeds/m^2; Table 8). Soil ARG seed counts were markedly lower when wheat(A) had been grown in association with single-breaks (212–350 seeds/m^2) either in Year 1 or Year 2, and in double-break systems involving fallow or field pea BM with canola(A) and in the canola(A)-cereal hay sequence (104–457 seeds/m^2; Table 8).

Autumn Year 4: although the Year 3 wheat(A) crop again lowered ARG seedbanks across all systems, the final seedbank counts still remained high in the continuous wheat system with two consecutive years of wheat(C) (1693 seeds/m^2) and where Year 1 canola(C) or lupin grain crops had been followed by wheat(C) (1720–1951 seeds/m^2; Table 8). Other single-break systems lowered ARG seedbanks by between 13% and 74% (437–1477 seed/m^2) if wheat(C) had been the first or second crop in the sequence, but depleted seedbanks by 87–97% when grown in combination with 2 years of wheat(A) (59–223 seeds/m^2).

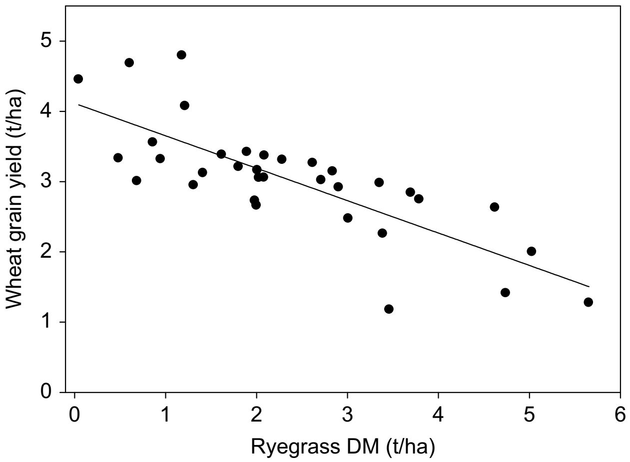

Fig. 1. Relationship between annual ryegrass (ARG) shoot dry matter (DM) and wheat grain yield on the basis of data generated under conservative weed-management treatments derived from the combined data collected from Year 2 and Year 1 wheat grain crops from Experiments 1 and 2 respectively, at on-farm study sites at Eurongilly, NSW in 2013. Regression equation: Grain yield (t/ha) = 4.099 − 0.45 × (ARG DM); $r^2 = 0.61$.

Three of the five systems with the lowest recorded ARG seedbank counts represented double-break sequences consisting of fallow or field pea BM followed by canola(A), or canola(A)-cereal hay (37–132 seeds/m^2; Table 8).

The influence of ARG competition on wheat yield

Analyses comparing the grain yield data generated across all wheat(C) crops from both experiments in 2013, with the amount of ARG DM present within each crop in late spring indicated that ARG competition reduced wheat grain yield by 0.45 t/ha for every additional 1.0 t/ha of in-crop ARG DM measured (Fig. 1).

Discussion

It is well documented that the inclusion of either legume or brassica break crops in cropping sequences can enhance subsequent wheat yields; especially where soil N availability and/or disease are constraints to productivity (Kirkegaard *et al.* 2008; Angus *et al.* 2015; Ladha *et al.* 2022; Reckling *et al.* 2022; Zhao *et al.* 2022). However, the on-farm studies reported here indicated that broadleaf break crops can also contribute to increased grain yields by facilitating the reduction of ARG weed pressure. Although this outcome relies heavily on timely and effective weed control during and following the broadleaf phase.

Despite the high background populations of ARG observed in the current study, sequences that included a break crop were generally more profitable than was continuous wheat in both experiments (Fig. 2). Strategies involving canola contolled ARG effectively (particularly Roundup Ready hybrids), and were frequently the most profitable (Fig. 2), but sequences that incuded lupin grain crops were also profitable. This was due in part to the subsequent improvements in soil mineral N, lowering fertiliser-N input costs for crops following lupin (e.g. by A$74–$118/ha in Experiment 1; Evans *et al.* 2003; Peoples *et al.* 2017; Ladha *et al.* 2022). Unfortunately, lupin's impact on ARG and final seedbank size differed greatly between the two experiments (Fig. 2). The poor control of ARG observed in the lupins in Experiment 2 may have resulted from the failure of the pre-emergent herbicide because of insufficient rainfall at the time sowing in 2013 (only 7 mm monthly total rainfall in April), or resistance to the in-crop herbicide butroxydim, which was present in the population at low levels in Experiment 2, but not in Experiment 1 (Table 1). However, the Experiment 2 lupin crop also failed to receive a late-spring herbicide spray-topping application (which had been applied in Experiment 1) because of site-access problems. This undoubtedly contributed to the poor ARG control because lupin is a weak competitor against ARG (Arnold *et al.* 1985; Lemerle *et al.* 1995; French *et al.* 2015; Bajwa *et al.* 2021). In many respects, the specific difficulties observed in lupin during Experiment 2 were indicative of the inconsistency in weed control often experienced by farmers within any grain crop if the growing-season becomes too wet and timely in-crop weed-management strategies cannot be implemented.

In both experiments, the higher inputs of N and P fertilisers and increased crop density (from ~75 to 150 plants/m^2) used to enhance wheat's competitive ability combined with the alternative pre- and post-emergent herbicide treatments in the aggressive wheat-management strategy greatly improved the efficacy of ARG control compared with that achieved in the conservatively managed wheat grain crops (Lemerle *et al.* 2004; Walsh *et al.* 2019). Indeed, comparisons of the densities of ARG spikes measured in late spring in the Year 1 wheat(C) treatment in Experiment 1 (504 spikes/m^2) with those in the untreated buffer areas surrounding the experimental plots (1042 spikes/m^2; data not shown) suggested that the standard practices used by local farmers were controlling only 50% of the ARG population. Given that each additional ARG spike might contribute 20–30 seeds to the seedbank (Chauhan *et al.* 2007), it was not surprising to find that the higher ARG burdens observed in wheat(C) subplots resulted in substantially higher final soil ARG seedbank size in any system that included wheat(C), regardless of whether a break crop was included in the sequence (Fig. 2). The greater efficacy of pyroxasulfone tank-mixed with triallate used in wheat(A), relative to trifluralin used in wheat(C), was likely to be a major factor contributing to the different levels of ARG control observed in the two different wheat treatments. It is possible that the

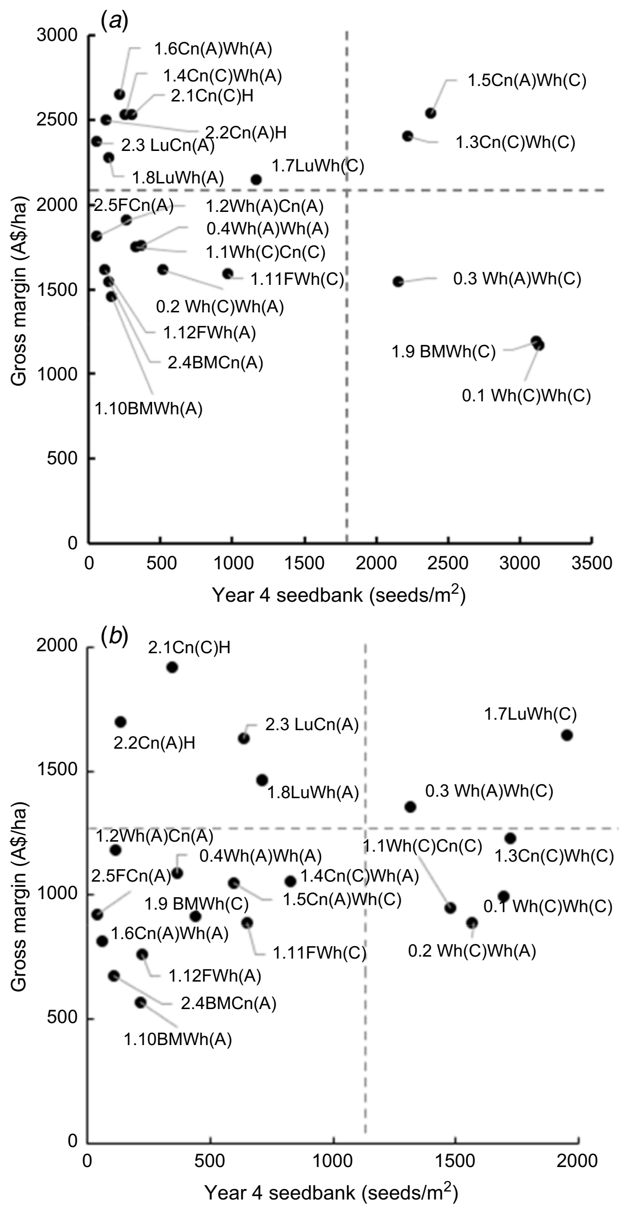

Fig. 2. The effect of weed-control strategies on final annual ryegrass (ARG) seedbank populations and 3-year cumulative gross margins (A$/ha) in (*a*) Experiment 1, and (*b*) Experiment 2. The treatment identity number is given alongside the rotation descriptions next to each data point (see Table 4 for more detail). Acronyms used to describe the first two years of the various crop sequences are as follows: Wh, wheat; Cn, canola; Lu, lupin; BM, brown-manured pea; F, bare-soil fallow; H, wheat hay; (C), conservative weed control; (A), aggressive weed control. Aggressive weed-control measures were used on wheat grown in the third year of all systems.

on-farm ARG populations in this study were resistant to trifluralin because this could not be included in the live plant assay used to test resistances; however, this is unlikely given the low levels of resistance to trifluralin observed in the district at the time of the experiment (Broster *et al.* 2022). Even in the absence of resistance, efficacy of trifluralin is frequently inferior to pyroxasulfone tank-mixed with triallate (e.g. 58% cf. 75% control respectively observed at a susceptible site by Boutsalis *et al.* 2014).

Two consecutive years of near complete ARG control followed by the high levels of control achieved in wheat(A) were necessary to reduce the in-crop density of ARG plants and soil ARG seedbanks to low levels (Fig. 2; Flower *et al.* 2012; Kleemann *et al.* 2016). The most profitable double-break systems also provided some of the most effective ARG management (Fig. 2), with either canola(A)–cereal hay (Experiments 1 and 2), or lupin–canola(A) (Experiment 1) sequences reducing final ARG seedbank numbers by 92–98% over 3 years compared with the conservatively managed continuous wheat system. It is worth noting that in both experiments after 2 years of near complete control (0–2 panicles/m^2) in some treatments, seedbanks still numbered in the hundreds of seeds/m^2 and would have been more than sufficient to re-infest the paddock if high levels of control had not been achieved by wheat(A) in Year 3. This illustrates the importance of a persistent seedbank in maintaining populations of ARG, and highlights the need for very high levels of control over at least 3 years, to manage infestations.

Strategies involving field pea BM cover cropping or chemical winter fallow greatly reduced the Year 2 soil ARG seedbank numbers by 87–95%. They also provided an additional 60–95 kg soil mineral N/ha, lowered subsequent fertiliser-N input costs, and improved soil water reserves at the start of the following growing season, compared with wheat or canola grain crops (data not shown) for the potential benefit of the next crop. This was consistent with the findings of many other studies (e.g. Evans *et al.* 2003; Angus *et al.* 2015; French *et al.* 2015; Peoples *et al.* 2017; Collins and Norton 2019; Cann *et al.* 2020). However, despite both field pea BM-canola(A) and fallow-canola(A) providing some of the lowest ARG seedbank counts in both experiments, these practices incurred income losses and opportunity costs in the year they were used. Even though both BM and fallow treatments enhanced the yield of following wheat and canola crops, the increased grain production was insufficient to fully compensate for the loss of income in the previous year. The net result was that the 3-year cumulative gross margin of sequences that included a fallow or BM were some of the least profitable of all the systems tested (Fig. 2). It is worth noting that the high concentrations of available soil N after both of these treatments also increased the potential risk of 'haying-off' a following wheat crop in a dry growing season (van Herwaarden *et al.* 1998; Kirkegaard and Ryan 2014). Nonetheless, a legume BM crop followed by canola(A) is likely to be particularly beneficial where both high ARG populations and low soil N fertility are problematic, given the high efficacy in weed control, the large inputs of labile legume N, and its subsequent effects on soil N dynamics (Evans *et al.* 2003; Peoples *et al.* 2017; Ladha *et al.* 2022). Evidence from regional NSW growers and advisers has also indicated that the incorporation of a BM legume cover crop followed by canola and then two cereal crops can be a profitable strategy at the whole-farm level (Minehan 2020; Patterson 2022). In addition to reductions in fertiliser N rates, growers have found that less herbicides tend to be required in a second cereal crop grown following a legume BM and increased efficiencies in labour and machinery because the BM legume does not have a critical time of sowing so it can be established when sowing equipment is not required to plant grain cash crops. Despite the impact of bare-soil fallow on cumulative gross margin reported here, replacing a crop with a period of fallow can also play a valuable role in the rainfed cropping systems of south-eastern Australia, especially in semi-arid regions, through its impact on whole-farm economics, owing to the improved timeliness of operations associated with lower areas of the farm dedicated to cropping, reduced input costs and lowered subsequent production risk owing to the accumulation of soil water reserves during the fallow phase (Cann *et al.* 2020).

Collectively, the findings presented here for the on-farm experiments confirmed the results of work undertaken elsewhere in Australia, which have demonstrated the individual merits of either break crops (Moodie 2012; Seymour *et al.* 2012; French *et al.* 2015), or cultural practices such as spray-topping legumes, BM, hay-cutting or chemical fallow in managing weeds (Gill and Holmes 1997; French *et al.* 2015; Kleemann *et al.* 2016; Llewellyn *et al.* 2016; Walsh *et al.* 2019; Bajwa *et al.* 2021). However, the results reported here differ from other studies in that they target specific gaps in local farmer knowledge by linking the efficacy of ARG control with the relative systems profitability of different combinations of crop sequences and intensities of weed management. The main insight provided by the current study was that no single management tool can be expected to consistently or fully control ARG. Rather, it was necessary to combine multiple control methods involving diversified rotations and practices that integrated herbicides with different modes of action and non-chemical control to substantially reduce and maintain low ARG density and seedbanks in no-till wheat-based cropping systems. Further gains could be achieved on farm if the practices evaluated in this study were also combined with harvest weed-seed control techniques (Walsh and Powles 2022).

The detailed data sets generated from the current on-farm studies represent a valuable resource for the future development, and/or validation, of simulation models used for systems analysis of weed dynamics and their impact on dryland crop production, and in the generation of weed-management decision-support tools. The cost–benefit analyses of the different weed-control strategies could also be particularly informative for farmers and advisors considering the adoption of new approaches to manage herbicide-resistant weeds in other grain-growing areas of Australia and elsewhere in the world (Schroeder *et al.* 2018). Similarly, the experimental results indicating that competition from in-crop ARG can reduce wheat grain yield by ~0.5 t grain/ha for every additional tonne of ARG DM present during late spring provides a useful benchmark for educational purposes to alert farmers of the scale of potential yield losses they could be experiencing and to encourage the implementation of alternative management practices to address their weed problems.

Conclusions

Using the registered pre- and post-emergent herbicides available at the time of experimentation, aggressive weed management in wheat successfully lowered ARG weed burden in individual years but was more expensive than strategies involving break crops. Break crops, field pea brown manuring, cutting wheat for hay, and weed-free winter fallow also reduced in-crop ARG infestations and soil ARG seedbanks. Seedbank reductions could be lost by a reinfestation of ARG resulting from a single year of ineffective weed control or a missed application of in-crop herbicide. Compared with the final soil ARG seedbank counts measured following five consecutive wheat crops in the continuous wheat system with 2 years of conservative weed management, the most successful single-break systems achieved >86% reductions in ARG seedbanks over the 3-year sequence when accompanied by two aggressively managed wheat crops. Double-breaks consisting of either two broadleaf crops, or canola(A)–cereal hay depleted ARG seedbanks by >91% and represented some of the most profitable systems. It was concluded that multiple strategies involving intensely managed diverse cropping sequences to disrupt the life cycle of the ARG and deplete ARG seedbanks were required to cost-effectively manage severe ARG weed infestations within a 3-year timeframe.

Supplementary material

Supplementary material is available online.

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Data availability. The data that support this study will be shared upon reasonable request to the corresponding author.

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