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Research article

Stacking wheat (*Triticum aestivum* L.) seeding rate and pre-emergent herbicide choice for annual ryegrass (*Lolium rigidum* Gaud.) control in dry-seeded crops

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ABSTRACT

Dryland crop production is increasingly exposed to drier, more variable climatic conditions. To mitigate these constraints, growers in southern Australia have adopted dry seeding, in which wheat (*Triticum aestivum* L.) is seeded into dry soil before season-breaking rainfall. With widespread resistance in annual ryegrass (*Lolium rigidum* Gaud.) to in-crop selective herbicides, and potential constraints on glyphosate and paraquat, there is increasing reliance on soil-applied pre-emergent herbicides and greater crop competitiveness. Despite this increased reliance, detailed studies characterising the efficacy and persistence of important pre-emergent herbicides remain limited, and their persistence under dry-seeded conditions is poorly quantified.

From 2019 to 2021, nine multi-factor field experiments were conducted in the Western Australian grainbelt, comprising two times of seeding (early May "dry" and early June "delayed"), three target wheat densities (100, 150 and 200 plants m^{-2}), and key pre-emergent herbicides (trifluralin, metolachlor/prosulfocarb, cinmethylin, pyroxasulfone, aclonifen/pyroxasulfone/diflufenican, bixlozone, plus a nil-herbicide control). Herbicide persistence, crop and weed growth parameters, grain yield and annual ryegrass seed production were assessed.

Across sites and seasons, herbicide persistence consistently ranked: trifluralin $\leq$ metolachlor/prosulfocarb $<$ cinmethylin $<$ pyroxasulfone $\approx$ aclonifen/pyroxasulfone/diflufenican $<$ bixlozone. Robust pre-emergent herbicides, particularly pyroxasulfone and aclonifen/pyroxasulfone/diflufenican, and to a lesser extent bixlozone and cinmethylin, provided strong and consistent ryegrass suppression with associated yield benefits. In 2019–2020, pyroxasulfone increased grain yield from 2493 kg ha^{-1} in the nil-herbicide, low-density treatment to 2860–2879 kg ha^{-1} at moderate and high wheat densities. Whilst increasing wheat seeding rate reduced ryegrass biomass and seed production, with the greatest improvement in ryegrass suppression observed at a target wheat density of 150 plants m^{-2}. These findings demonstrate that using effective residual herbicides in combination with higher wheat seeding rates provides a practical strategy to suppress annual ryegrass in dry-seeded wheat.

1. Introduction

Dryland wheat (*Triticum aestivum* L.) production globally is increasingly constrained by more variable and declining in-season rainfall and a higher risk of terminal heat stress around flowering (Harries et al., 2015; Fletcher et al., 2016). In Australia, to effectively seed larger cropping programs within a short optimum time window, growers are adopting dry seeding techniques, where crops are seeded into dry soil ahead of the season-breaking rainfall (Kirkegaard et al., 2010; Hunt et al., 2019; Shayanmehr et al., 2020).

Dryland cropping is a rainfed crop production system where crops are grown without irrigation, commonly under conservation agriculture practices into retained crop (Ashworth et al., 2010). Within dryland crops, annual ryegrass (*Lolium rigidum* Gaud) is one of the most problematic weeds. Its high seed production, flexible phenology and long history of evolving resistance to multiple herbicide modes of action make control difficult (Bajwa et al., 2021). Traditionally, the time of seeding in weedy fields was delayed to allow multiple weed cohorts to

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emerge and be killed using non-selective pre-plant "knockdown" herbicides. This essentially reduced the number of weeds to emerge within the crop. This was further refined as the double-knock technique, where a glyphosate application is followed by paraquat to improve ryegrass control and slow herbicide resistance evolution (Borger and Hashem, 2007; Owen and Powles, 2010; Walsh et al., 2013). However, these tactics require delayed crop seeding, which shortens the growing season, reduces crop yield potential and weakens crop competitiveness against subsequent weed germinations (Gomez-Macpherson and Richards, 1995).

The expansion of dry seeding in Australia has fundamentally changed this weed control paradigm. When crops are seeded into dry soil before the season-breaking rainfall, growers forego the opportunity for effective pre-plant knockdown herbicide applications (Harries et al., 2020). As a result, larger ryegrass cohorts may emerge concurrently with the crop and must be controlled by crop-selective herbicides (Colbach and Cordeau, 2022). This is being further exacerbated by delayed and protracted germination in many annual ryegrass populations (Owen et al., 2011, 2015; Maity et al., 2022). At the same time, resistance to in-crop selective herbicides in wheat is now widespread (Owen et al., 2014; Broster et al., 2022), necessitating an increased reliance on residual, soil-applied pre-emergent herbicides.

However pre-emergent herbicide over-reliance without integrated weed management will rapidly lead to resistance (Busi and Powles, 2013; Busi et al., 2018; Chen et al., 2022; Goggin et al., 2022, 2025a, 2025b). Where there is sufficient stored soil moisture to support crop establishment, very early seeded wheat can emerge and develop a dense weed suppressive canopy (Kirkegaard and Hunt, 2010). Increasing wheat seeding rate is also a well-established cultural tactic to further improve crop competitiveness against weeds (Lemerle et al., 2004). Thus, for effective weed management in dry seeded crops, there is a strong rationale for combining higher crop seeding rates with effective residual herbicides to create a more asymmetric competitive environment against later emerging weed cohorts.

The efficacy of soil-applied pre-emergent herbicides depends on their persistence. Herbicide activity declines over time due to dissipation, sorption–desorption processes and microbial degradation, which are influenced by soil moisture and temperature (Minkey and Ashworth, 2012; Khalil et al., 2020). In early seeded crops, herbicides are often applied to dry soil for extended periods before rainfall, likely resulting in reduced subsequent bioavailability and in crop persistence once crop germinating rains occur (Khalil et al., 2020). Recently released pre-emergent herbicide chemistries in Australia offer improved residual activity; however, their performance is still expected to interact with seasonal conditions and soil properties. Despite the widespread adoption of dry seeding and the increasing dependence on soil-applied residual herbicides, there is limited field evidence examining how pre-emergent herbicide choice interacts with crop competitiveness in dry seeded crops.

This study addresses this gap by evaluating the combined effects of wheat seeding time, wheat density and pre-emergent herbicide choice on the control of annual ryegrass in dry seeded wheat, with particular emphasis on quantifying herbicide persistence. The findings are intended to inform practical seeding and herbicide strategies to ensure sustainable ryegrass management in dry seeded wheat crops.

2. Material and methods

A series of experiments studying the effect of crop seed rate, time of seeding and herbicide on annual ryegrass competition with wheat were conducted, from 2019 to 2021, in the Western Australian grainbelt. The sites were Miling (−30.29 S, 116.22 E), York (−31.87 S, 116.76 E) and Kojonup (−33.84 S, 117.15 E) in 2019, Tammin (−30.29 S, 116.22 E), Pingelly (−32.48 S, 116.96 E) and Dandaragan (−30.87 S, 115.70 E) in 2020 and Esperance (−33.44 S, 121.48 E), Beverley (−31.85 S, 117.22 E) and Dandaragan (−30.87 S, 115.70 E) in 2021. The soil characterization and site rainfall are outlined in the supplementary information.

2.1. Trial design and establishment

A randomized complete block design (RCBD) was used with three factors, including crop seed rate with three levels, pre-emergent herbicide (four levels in 2019-20 and seven in 2021), and time of seeding (TOS) with two levels (Table 1). Due to experimental restrictions, the two times of seeding were established as separate blocks. For each trial, each time of seeding consisted of four replicates, each containing a full set of crop seed rate and herbicide treatment combinations.

At each site, the first time of seeding (TOS1) took place in the first week of May and the second time of seeding (TOS2) in the first week of June in each year (Table 1). Each trial was direct seeded into cereal stubble (3-4.5 t ha^{-1}). The wheat variety used was Scepter (Intergrain, Bibra Lake Western Australia, Australia) which is a high yielding, mid-late maturing variety, seeded at 25 cm row spacing, with the density treatments outlined in Table 1. The site was seeded with no tillage tine openers with press wheels to provide sufficient seed soil packing and promote good weed germination. All plots were planted at one seeding depth (approx. 2 cm) to minimise the confounding effects of emergence rate and seeding depth differences on biomass and grain yield. Immediately prior to seeding, the whole experimental area was treated with 2 L ha^{-1} Roundup Ultramax (Glyphosate 540 g L, Sinochem Australia), 100 ml ha^{-1} Lontrel (Clopyralid 750 g L, DowAgrosciences Australia), to control all germinated weeds; followed by the application of each individual plot's pre-emergent herbicide treatment (Table 2). To control dicotyledonous species, all plots had a post emergent application of 800 ml/ha Velocity (210 g L Bromoxynil + 37.5 g L Pyrasulfotole, Bayer Australia). For the duration of this study, no additional annual ryegrass control was applied. All herbicides were applied using a motorized sprayer calibrated to deliver a carrier volume of 120 L water ha^{-1} at 275 kPa pressure. Each plot size was 2.5 m wide by 10 m long. To ensure

Table 1
Factorial combinations of wheat density, pre-emergent herbicide treatment and TOS of wheat at the Miling, York and Kojonup sites in 2019, Tammin, Pingelly and Dandaragan sites in 2020 and the Esperance, Beverley and Dandaragan sites in 2021.

Treatments	Comments
Factor 1 - Crop density treatment description	
Low	Seed rate: 45 kg/ha (Target density 100 plants/m^2)
Moderate	Seed rate: 68 kg/ha (Target density 150 plants/m^2)
High	Seed rate: 90 kg/ha (Target density 200 plants/m^2)
Factor 2 - Time of seeding treatment description	
Time of seeding 1 (TOS1)	Early seeded (1–4th May) predominantly into dry soil, prior to season opening rains.
Time of seeing 2 (TOS2)	Standard seeding time (1–3rd June), predominantly into moist or drying soils after season opening rains.
Factor 3 - Pre-emergent herbicide treatment description (all treatments receive pre-seeding glyphosate application)	
Nil (knockdown only)	Nil residual herbicide applied (Control)
Trifluralin 480 gai/L	Trifluralin™ (Nufarm Australia Laverton North, Victoria, Australia) 2.0 L/ha
s-metolachlor 120 gai/L + Prosulfocarb 800 gai/L	Boxer Gold™ (Syngenta Australia, Macquarie Park NSW, Australia) 2.5 L/ha
Pyroxasulfone 850 g ai/kg	Sakura™ (Bayer Australasia, Bentley, Western Australia, Australia) 118 g/ha
Cinmethylin 750 g ai/L	Luximax™ (BASF Australia, Southbank, Victoria, Australia) 500 mL/ha
Aclonifen 400 g ai/L, Pyroxasulfone 100 g ai/L, Diflufenican 66 g ai/L	Matino complete™ (Bayer Australasia, Bentley, Western Australia, Australia) 1.0 L/ha
Bixlozone 400 g ai/L	Overwatch™ (FMC Australasia North Ryde, NSW Australia) 1.25 l/ha

Table 2
Mean herbicide persistence in the soil with early and standard seeding (across three sites within each year (2019, 2020 and 2021). Persistence assessed, by bioassay, as weeks after herbicide application until 90%, 70% and 50% of annual ryegrass shoot length obtained compared with ryegrass grown in soil from Nil/control plots. Sites were Miling, York and Kojonup in 2019, Tammin, Pingelly and Dandaragan in 2020 and Esperance, Beverley and Dandaragan in 2021.

Herbicide	2019			2020			2021		
	90%	70%	50%	90%	70%	50%	90%	70%	50%
Early seeding (max sampling weeks)	**12**			**18**			**17**		
Metolachlor/Prosulfocarb	1.8	5.2	8.5	[a]	3.5	10.0	[a]	1.8	7.7
Pyroxasulfone	4.6	10.8	[a]	6.9	16.0	[a]	4.6	10.2	14.8
Trifluralin	[a]	3.6	8.4	1.0	4.1	9.2	[a]	1.8	4.7
Aclonifen/Pyroxsasulfone/Diflufenican	-	-	-	9.9	18.1	[a]	7.0	12.9	16.6
Bixlozone	-	-	-	14.6	[a]	[a]	11.7	16.6	[a]
Cinmethylin	-	-	-	3.8	11.0	15.5	7.4	6.9	9.9
Std seeding (max sampling weeks)	**10**			**14**			**13**		
Metolachlor/Prosulfocarb	0.5	4.2	6.9	[a]	3.7	10.0	[a]	3.8	10.0
Pyroxasulfone	2.9	8.0	[a]	4.6	[a]	[a]	9.8	[a]	[a]
Trifluralin	[a]	3.3	7.1	4.9	7.9	14.8	[a]	1.2	4.5
Aclonifen/Pyroxsasulfone/Diflufenican	-	-	-	7.6	[a]	[a]	8.0	[a]	[a]
Bixlozone	-	-	-	16.9	[a]	[a]	12.1	[a]	[a]
Cinmethylin	-	-	-	10.0	[a]	[a]	1.7	6.3	8.5

[a] The % reduction in growth either not reached (for 90%) or outside bounds of the sampled weeks (for 70% and 50%).

optimal wheat growth, 130 kg ha^{-1} of MacroPro Extra (CSBP Fertilisers, Australia, N – 9.5%, P- 10.7%, K- 12.8%, S- 8.8%, Cu- 0.1% and Zn-0.2%) was drilled 3 cm below the seed to minimise contact with the germinating wheat seed. To optimise crop growth, 100 kg/ha of supplementary nitrogen fertiliser in the form of Urea (CSBP fertilisers Australia; N- 46%) was applied to the soil surface immediately after seeding with an additional 150 kg/ha applied at GS24 (Zadoks et al., 1974). At GS32 (Zadoks et al., 1974), 100 L ha^{-1} of Flexi-N urea ammonium nitrate liquid fertiliser (CSBP fertilisers Australia; N- 34%) was applied to all plots.

Above ground biomass of annual ryegrass was assessed at GS59 of wheat (Zadoks et al., 1974) by sampling three 0.25 m^2 quadrats per plot. Biomass samples were dried at 60°C and weighed. At wheat GS85 (Zadoks et al., 1974), mature annual ryegrass panicles were collected within a 1 m^2 quadrant to be threshed to extract seed. The number of seeds extracted were counted using an S-25 optical seed counter (Data Technologies, Kibbutz Tzora, Israel).

At wheat GS99 (Zadoks et al., 1974), the whole plot (10 m length with 6 by 25-cm rows) was machine harvested to determine grain yield.

2.2. Herbicide bioassay

Soil for the bioassays was collected for each herbicide treatment from the moderate seed rate treatment across the two times of seeding (TOS), starting at the time of pre-emergent herbicide application (week 0). Subsequent soil samples were collected at 14-day intervals (except for Esperance which was sampled at 28-day intervals) by collecting six 30 mm diameter cores from central interrow of the plot, to a depth of 8 cm. Soil samples were immediately transferred into sealed plastic trays with no holes to prevent leaching and stored at <15°C for no more than 24 h before seeding. Upon receipt at the University of Western Australia, all soil samples were moistened using 75 ml de-ionised water containing TWEEN 20 ionic surfactant (Polyethylene glycol sorbitan monolaurate, Sigma Aldrich Australia) to overcome hydrophobicity. Fifty seeds from a known herbicide susceptible annual ryegrass biotype (VLR1) were seeded at 1 cm depth into the premoistened soil, before being placed in a temperature controlled naturally lit glasshouse (15°C night 25°C Day). All trays were watered daily to maintain soil field capacity. At 21 days after seeding, above ground shoot length was measured (n = 20), with the percentage shoot length inhibition calculated as per Khalil et al. (2018) using formula [1].

$$\text{Inhibition (\%)} = 1\text{-}(Lt/Lo) \times 100\% \quad [1]$$

Where: Lt is the shoot or root length measured in the herbicide-treated soil and L0 is the shoot or root length in the untreated soil.

2.3. Statistical analysis

The experiments were conducted over three years (2019-2021) at multiple sites (YearSite). The objective of the experiment was to assess the effects of crop seed rate (Seed Rate), pre-emergent herbicide (Herbicide), time of seeding (TOS) and their interactions on annual ryegrass factors such as density, biomass and seed production, as well as wheat factors such as crop emergence, wheat biomass and yield. The persistence of the herbicides in the field was also assessed by assessing bioassays over time.

Trials established in 2019 had four herbicide treatments, Trifluralin™, Boxer Gold™, Sakura™ and a nil control (Table 1). Due to the limited release of new herbicides in 2020, three additional herbicides were included (Luximax™, Matino complete™ and Overwatch™) at the moderate crop density (Table 1). In 2021, all six herbicide treatments, including a nil control were assessed across all seed rates (Table 1). Hence the three herbicides were excluded from the data in 2020, and a separate analysis was conducted for the weed and crop data sets in 2019-20 and 2021, the latter having the seven herbicide treatments. For the assessment of herbicide persistence, the additional herbicides were included in 2020 and 2021 analysis.

All analyses were performed in R (v 4.5.1, R Core Team, 2025; https://www.R-project.org/) within RStudio (build 513, Posit team, 2025, https://posit.co/). The crop and weed data analyses were conducted with the R-package Asreml-R (Gilmour et al., 1995; Butler et al., 2017).

Linear mixed models were used for the analyses, with year, site and time of seeding combinations treated as twelve different environments for 2019-2020 data sets. As such, a multi-environment trial (MET) analysis was conducted, assuming heterogeneous variance for all year, site and time of seeding combinations. The factorial structure YearSite * TOS * Seed Rate * Herbicide was fitted as a fixed term with removing non-significant terms, and the blocking structure at each environment was fitted as random. For the 2021 data set, the factor Site was fitted as environment, and Site * TOS * Seed Rate * Herbicide was fitted as a fixed term with removing non-significant terms, and the blocking structure at each environment was fitted as random. The spatial variability (local and global trends) was accounted for in the model.

The weed data were zero-inflated and skewed at the 2020 and 2021 sites. A Generalised Linear Mixed Model (GLMM) were used. The best models were obtained using negative binomial with link functions [1];

$$\mu = e\hat{}X\beta = \phi(1\text{-}p)/p. \quad [1]$$

The fits were reasonable and the overdispersion was controlled. The test for the fixed effect was performed using the Wald-test.

The herbicide persistence data were modelled using a Generalised Linear Model (GLM) with a quasibinomial error distribution and logit link function (glm, family = quasibinomial (link = “logit")). The response variable was the ryegrass shoot length as a percentage of the untreated control (nil herbicide), with replicate counts included as weights. The model included the fixed effects of time (weeks after herbicide application), herbicide and their interaction. The time for ryegrass shoots to only reach 10%, 30%, and 50% of the untreated control (T_{10}, T_{30}, T_{50}; corresponding to 90%, 70%, and 50% growth reduction, respectively) was estimated by inverse prediction from the fitted curves, with standard errors determined using the delta method on the link scale and back transformed.

3. Results

3.1. Herbicide persistence

In 2019, trifluralin and metolachlor/prosulfocarb had similar persistence, with 50% loss of activity evident after approximately 6-8 weeks after treatment, compared with the equivalent 50% loss in activity for pyroxasulfone occurring after 11–14 weeks (Table 2). The herbicides were generally most persistent in 2021, which was associated with higher rainfall (mean annual rainfall across the three sites in each year was 297 mm in 2019, 360 mm in 2020 and 517 mm in 2021) (Supplementary information). In 2020, trifluralin and metolachlor/prosulfocarb reached 50% efficacy between 9 and 14 weeks after treatment with cinmethylin, lost a similar 50% efficacy in the top 80 mm of soil after 15 weeks in the early seeded treatment. The treatments pyroxasulfone, aclonifen/pyroxsasulfone/diflufenican, and bixlozone did not reach 50% efficacy after 18 weeks of assessment with early seeding and after 14 weeks with late seeding. A similar pattern of persistence was evident in 2021, although trifluralin was the least persistent, reaching 50% efficacy at approximately 4–5 weeks after treatment, compared with 7–10 weeks for metolachlor/prosulfocarb. Cinmethylin reached 50% efficacy after 8–10 weeks, with the treatments pyroxasulfone and pyroxsasulfone/aclonifen/diflufenican having a similar persistence of 14–17 weeks with early seeding. Bixlozone was the most persistent herbicide in the study and did not reach 50% efficacy following 17 weeks of assessment for early seeding or 13 weeks for late seeding. Although there were some differences in ranking of herbicide persistence between sites, the overall persistence trend was trifluralin ≤ metolachlor/prosulfocarb < cinmethylin < pyroxasulfone = aclonifen/pyroxsasulfone/diflufenican < bixlozone.

3.2. Weed density, biomass and seed production

There was generally lower weed pressure in 2020 and 2021 compared with 2019, especially at Dandaragan in 2021 (Fig. 1). Across the sites, significant site-year × TOS interactions occurred for annual ryegrass density, biomass and weed seed production in 2019–20 and 2021 (Tables 3 and 4). The main effect of herbicide on weed density was significant in 2019–20, and on weed density, biomass and seed production in 2021. There were also significant site-year × herbicide interactions for weed biomass and seed production in 2019–20. Crop seed rate had significant main effects on weed density, biomass and seed production in all site-years (Tables 3 and 4).

The results for annual ryegrass density, biomass and seed production were similar; therefore, only weed biomass and seed production are discussed here, with the annual ryegrass density data presented in the supplementary data. In 2019–20, there was a site-year × TOS interaction with early seeding having higher weed biomass and seed production than late seeding at Kojonup, Dandaragan, Pingelly and Tammin, whilst there was no difference between early and late seeding at Miling and York (Fig. 1a and 2a). In 2021, early seeding had a higher annual ryegrass biomass at Beverley and higher seed production and Beverley and Esperance, while there were no differences between TOS at Dandaragan (Figs. 1 and 2b–b).

Herbicide treatment had no significant effect on annual ryegrass biomass or seed production compared with the nil control at Kojonup in

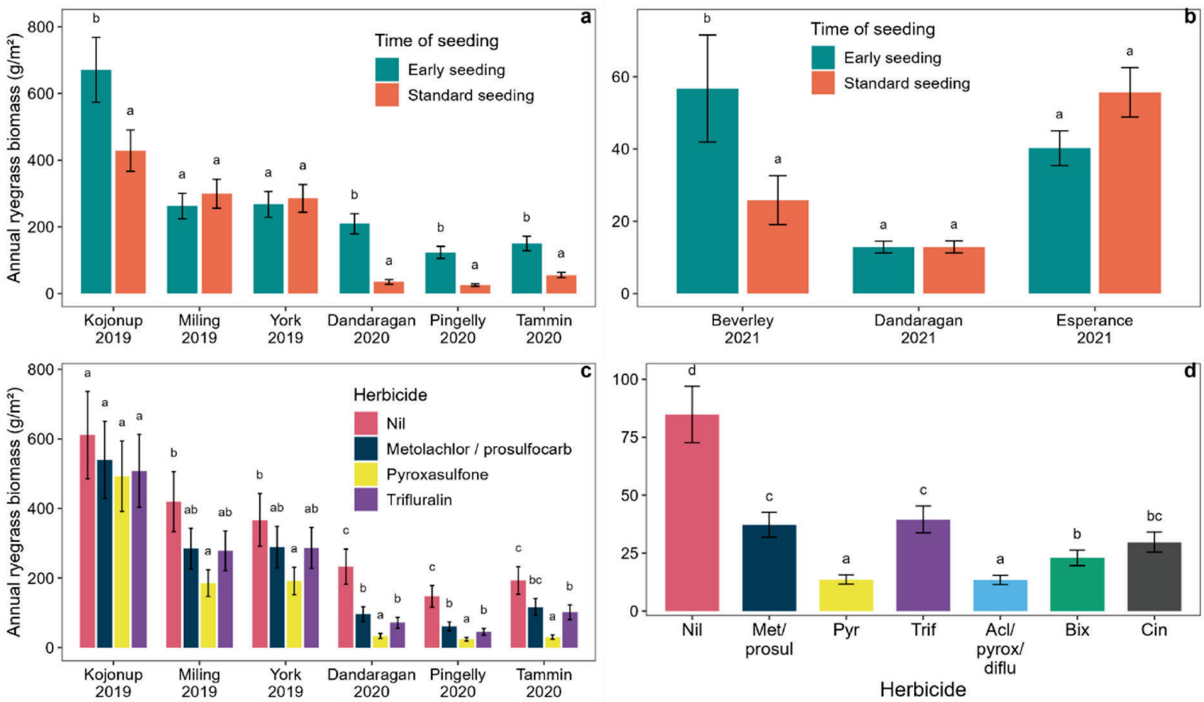

Fig. 1. The effect of site-year and time of seeding (TOS) on annual ryegrass biomass in 2019-20 (a) and 2021 (b). Effect of site-year and herbicide on annual ryegrass biomass in 2019–20 (c) and main effect of herbicide in 2021 (d). Different letters show significance at $P \leq 0.05$, within each site-year (i.e., time of seeding and herbicide comparisons). Bars show ± SE (n = 4). Herbicide list includes nil, metolachlor/prosulfocarb, pyroxasulfone, trifluralin, aclonifen/pyroxasulfone/diflufenican, bixlozone and cinmethylin.

Table 3
Significant P-values (P ≤ 0.05) from linear mixed model for weed number, biomass and seed production and wheat establishment, biomass and yield in 2019-20 (over six site-years). *NS* denotes not significant (P > 0.05).

Effect	Weed			Wheat		
	Number	Biomass	*Seed production*	Establishment	Biomass	Yield
Site-year (S)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
TOS	<0.001	<0.001	<0.001	0.013	<0.001	<0.001
Seed rate (SR)	0.010	<0.001	<0.001	<0.001	NS	<0.001
Herbicide (H)	<0.001	<0.001	<0.001	NS	NS	<0.001
S × TOS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
S × SR	NS	NS	NS	0.001	NS	NS
S × H	NS	0.002	0.004	NS	NS	NS
TOS × SR	NS	NS	NS	0.042	NS	NS
TOS × H	NS	NS	NS	NS	NS	NS
SR × H	NS	NS	NS	NS	NS	<0.001
S × TOS × SR	NS	NS	NS	<0.001	NS	NS
S × TOS × H	NS	NS	NS	NS	NS	NS
S × SR × H	NS	NS	NS	NS	NS	NS
TOS × SR × H	NS	NS	NS	NS	NS	NS
S × TOS × SR × H	NS	NS	NS	NS	NS	NS

Table 4
Significant P-values (P ≤ 0.05) from linear mixed model for weed number, biomass and seed production and wheat establishment, biomass and yield in 2021 (over three sites). *NS* denotes not significant (P > 0.05).

Effect	Weed			Wheat		
	Number	Biomass	*Seed production*	Establishment	Biomass	Yield
Site-year (S)	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
TOS	NS	NS	NS	0.001	<0.001	<0.001
Seed rate (SR)	0.021	<0.001	<0.001	<0.001	0.019	<0.001
Herbicide (H)	<0.001	<0.001	<0.001	NS	<0.001	<0.001
S × TOS	0.005	0.001	0.002	0.006	0.002	<0.001
S × SR	NS	NS	NS	NS	NS	NS
S × H	NS	NS	NS	NS	NS	<0.001
TOS × SR	NS	NS	NS	<0.001	NS	0.002
TOS × H	NS	NS	NS	NS	NS	<0.001
SR × H	NS	NS	NS	NS	<0.001	<0.001
S × TOS × SR	NS	NS	NS	0.004	NS	0.022
S × TOS × H	NS	NS	NS	NS	0.008	<0.001
S × SR × H	NS	NS	NS	NS	NS	NS
TOS × SR × H	NS	NS	NS	NS	0.024	NS
S × TOS × SR × H	NS	NS	NS	NS	NS	NS

2019–20 (Fig. 1c and 2c). However, herbicides did reduce weed biomass at the other sites in 2019-20, having the lowest annual ryegrass biomass and seed production with pyroxasulfone, followed by the metolachlor/prosulfocarb and trifluralin (Fig. 1c and 2c). In 2021, herbicide treatment significantly reduced weeds across all sites, with lowest annual ryegrass biomass and seed production following treatment with pyroxasulfone and aclonifen/pyroxasulfone/diflufenican, followed by bixlozone and cinmethylin, with trifluralin and metolachlor/prosulfocarb having the least control, apart from nil herbicide (Fig. 1d and 2d).

Across all sites in 2019/2020 and in 2021, that annual ryegrass biomass and seed production decreased when the seeding rate was increased, with the greatest change occurring when the seeding rate was increased from the low crop density (Target: 100 plants/m^2) to the moderate establishment density (Target: 150 plants/m^2) (Table 5).

3.3. Wheat emergence, biomass and yield

There were significant site-year × TOS × seed rate interactions for wheat plant count (establishment) in 2019–20 and 2021 (Tables 3 and 4). There was a positive relationship between crop seed rate and crop establishment, except for early seeding at Kojonup and late seeding at Tammin in 2019-20, where the highest seed rate was not different from the lowest (Table 6). The effect of TOS on establishment was variable; with early seeding having higher crop establishment than the late time of seeding at some sites, but the reverse at others. Herbicide treatment had no significant effect on wheat establishment (Tables 3 and 4).

There was a site-year × TOS interaction for wheat biomass in 2019-20, where early seeding produced greater biomass than late seeding at Dandaragan 2020 and Pingelly 2020, with the reverse occurring at Kojonup 2019, with no significant differences at Miling 2019, York 2019 and Tammin 2020 (Table 3, Fig. 3a). Crop seed rate and herbicide treatment had no significant effect on wheat biomass in 2019-20 (Table 3). In 2021, there were site-year × TOS × herbicide and TOS × seed rate × herbicide interactions for wheat biomass (Table 4). Generally, early seeding produced greater wheat biomass than late seeding at Dandaragan 2021; Esperance 2021, whereas at Beverley 2021the differences between TOS were small (Fig. 3b). The effect of herbicide on wheat biomass was variable in 2021, with few effects at Dandaragan and more significant effects at Beverley and Esperance (Fig. 3b). At the two latter sites, wheat biomass was significantly lower in the nil controls, compared with herbicide treatment, except for bixlozone with early seeding and cinmethylin with standard seeding at Beverley 2021. At Esperance 2021 with standard seeding, wheat biomass was greatest with pyroxasulfone and aclonifen/pyroxasulfone/diflufenican and lowest with trifluralin, metolachlor/prosulfocarb and bixlozone. In 2021, the effect of crop seed rate on wheat biomass was inconsistent, although for most herbicides and TOS, a small, positive increase was observed (Supplementary information).

In 2019-20, there were site-year × TOS and seed rate × herbicide interactions for wheat yield (Table 3). Early seeding produced greater yield than late seeding at York and Pingelly with the reverse at Kojonup, Dandaragan and Tammin, with no significant differences at Miling

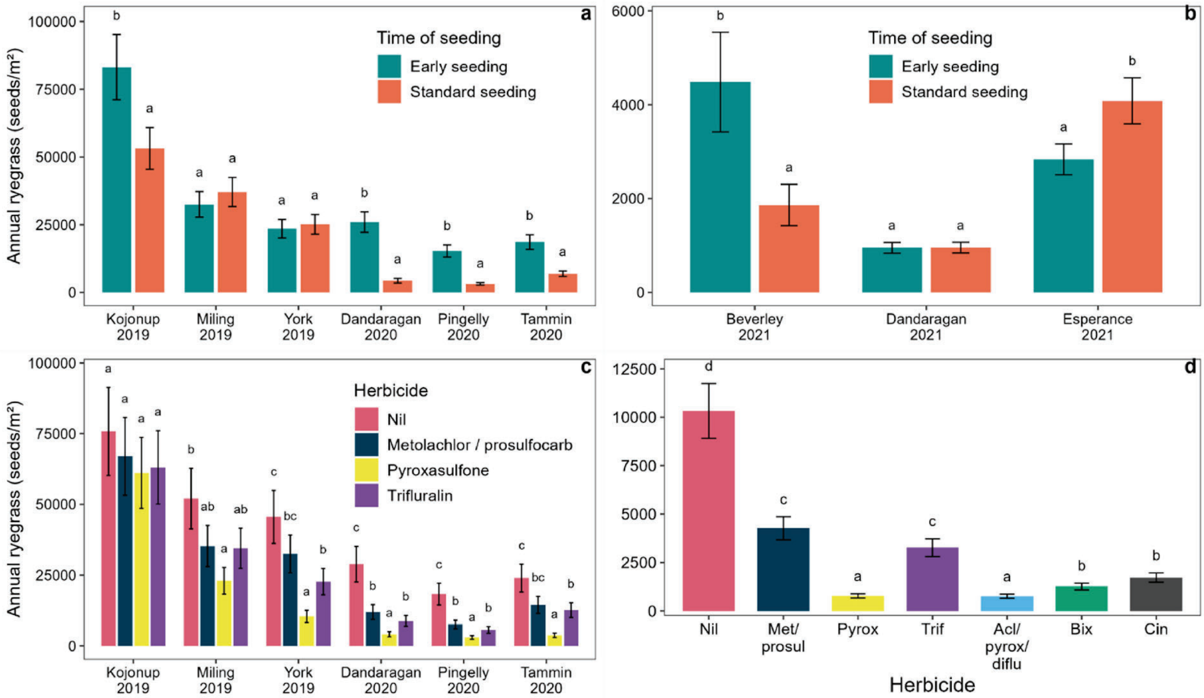

Fig. 2. The effect of site-year and time of seeding (TOS) on annual ryegrass seed production in 2019-20 (a) and 2021 (b). Effect of site-year and herbicide on annual ryegrass seed production in 2019–20 (c) and main effect of herbicide in 2021 (d). Different letters show significance at P ≤ 0.05. TOS and herbicide comparisons are within each site-year. Bars show ± SE (n = 4). Herbicide list includes nil, metolachlor/prosulfocarb, pyroxasulfone, trifluralin, aclonifen/pyroxasulfone/diflufenican, bixlozone and cinmethylin.

Table 5
Main effect of crop seed rate on annual ryegrass biomass and seed production in 2019-20 and 2021. Different letters show significance at P ≤ 0.05 within each analysis year.

Seed rate	2019-20	2021
Biomass (g m^{-2})		
Low	202 b	41 c
Moderate	156 a	27 b
High	135 a	21 a
Seed production (seeds m^{-2})		
Low	23,755 b	3073 c
Moderate	18,207 a	1981 b
High	15,788 a	1537 a

(Fig. 4a). For all herbicide treatments in 2019-20, yield increased with crop seed rate, with the largest change from the low to moderate seed rate, particularly in the nil herbicide treatment, with little further yield increase at the high seed rate (Table 7). Where herbicide was applied, yields were significantly higher than nil herbicide, and differences between herbicides were small, although pyroxasulfone produced the highest yield with the low crop seed rate (Table 7).

In 2021, there were site-year × TOS × herbicide and site-year × TOS × seed rate interactions (Table 4) As in 2019-20, early seeding produced higher yield than standard seeding at some of the sites (Dandaragan and Esperance) but not all (e.g., Beverley) (Fig. 4b). Like wheat biomass, herbicide had little effect on wheat yield at Dandaragan at both TOS and Beverley with standard seeding in 2021. Pyroxasulfone and aclonifen/pyroxasulfone/diflufenican had the highest yield with early seeding at Beverley and both TOS at Esperance (Fig. 4b). In 2021, as seeding rate increased there was a similar increase in wheat yield at three of the six sites, especially from the low to moderate crop seed rate; however, this effect was variable across sites and TOS (Table 8).

4. Discussion

Across diverse environments and seasons, this study demonstrates that integrating herbicide choice and cultural weed management strategies can significantly reduce ryegrass growth and competitiveness at early seeding times in wheat crops.

Table 6
Effect of crop seed rate (Low, Moderate and High) and time of seeding (Early and Standard) on wheat establishment (plants m^{-2}) at the 2019-20 and 2021 sites. Different lower-case letters show significance between times of seeding (within each site and seed rate) and upper-case letters show significance between crop seed rates (within each site and time of seeding) at P ≤ 0.05.

Seed rate	**Kojonup 2019**		**Miling 2019**		**York 2019**		**Dandaragan 2020**		**Pingelly 2020**		**Tammin 2020**	
	Early	*Std*	*Early*	*Std*	*Early*	*Std*	*Early*	*Std*	*Early*	*Std*	*Early*	*Std*
Low	120b A	96a A	113a A	113a A	104b A	82a A	106a A	111a A	123a A	125a A	114a A	137 b A
Moderate	133a B	142a B	131a B	143a B	126b B	109a B	126a B	136a B	122a A	136 b AB	144a B	133a A
High	119a A	177 b C	147a C	160a C	142a C	135a C	130a B	151 b C	155a B	143a A	162 b C	135a A
	Beverley 2021		**Dandaragan 2021**		**Esperance 2021**							
	Early	*Std*	*Early*	*Std*	*Early*	*Std*						
Low	105a A	112b A	84a A	107b A	108b A	90a A						
Moderate	138a B	153b B	108a B	124b B	133b B	108a B						
High	155a C	179b C	123aCA	141b C	141b C	120a C						

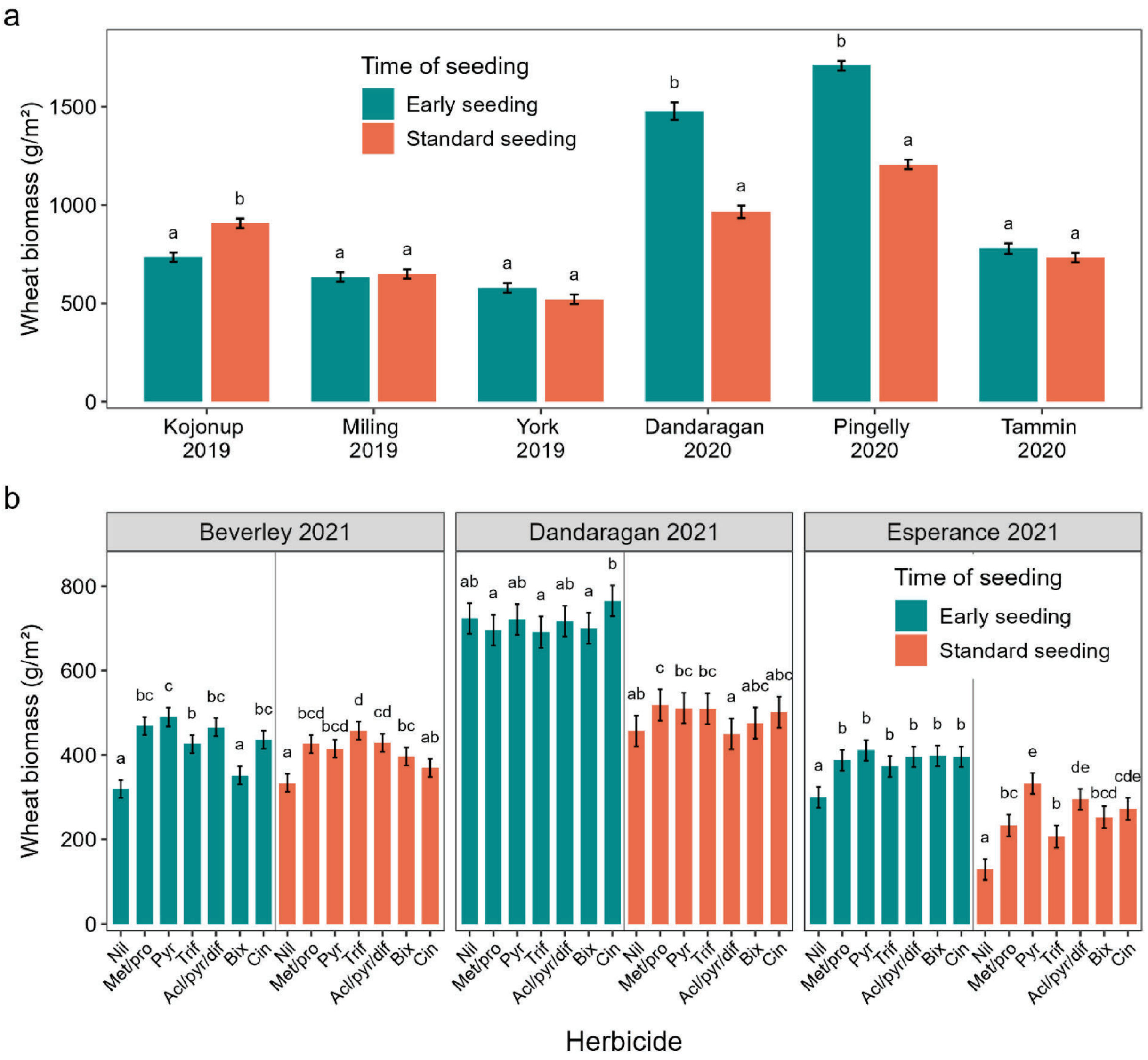

Fig. 3. Effect of site-year and time of seeding (TOS) on wheat biomass in 2019–20 (a) and site-year, TOS and herbicide on wheat biomass in 2021 (b). Different letters show significance at $P \leq 0.05$. TOS comparisons are within each site-year (a) and herbicide comparisons within each site and TOS (b). Bars show ± SE (n = 4). Herbicide list includes nil, metolachlor/prosulfocarb, pyroxasulfone, trifluralin, aclonifen/pyroxasulfone/diflufenican, bixlozone and cinmethylin.

4.1. Herbicide persistence and efficacy

Weed control before seeding of cereal crops has predominantly relied upon nonselective herbicides such as glyphosate to reduce weed densities. Many farmers in Australia are now seeding their crops early, often before season-opening rains (Fletcher et al., 2015, 2016; Harries et al., 2020). In these early/dry-seeding situations, pre-seeding knockdown applications are mostly forgone, increasing reliance on pre-emergent herbicides for weed management. Light showers with early seeding can make soil moisture in the surface layers transient. Therefore, pre-emergent herbicide choice for dry seeding is challenging, particularly when long periods between sowing and rainfall can lead to herbicide degradation and a shorter period of weed control following germination. Knowledge of the persistence of pre-emergent herbicides applied to different soil types and under different seeding conditions (times) is important in choosing an appropriate herbicide for dry seeding systems.

This study demonstrated substantial differences in pre-emergent herbicide persistence under Mediterranean-type conditions, along the lines of Walsh et al. (2005) and Ribeiro et al. (2021). In this study, large year–site and time-of-seeding differences in herbicide persistence in the field were evident, as measured by a ryegrass bioassay. The herbicides were generally more persistent in 2020 than in 2021, the latter being associated with higher rainfall. Trifluralin and metolachlor/prosulfocarb were least persistent, followed by cinmethylin with moderate persistence, while pyroxasulfone, aclonifen/pyroxasulfone/diflufenican and bixlozone were most persistent. The persistence of the herbicides mostly matched the weed control across both early and late seeding times, with trifluralin and metolachlor/prosulfocarb having the poorest control of annual ryegrass, followed by cinmethylin, then bixlozone, with pyroxasulfone and aclonifen/pyroxasulfone/diflufenican having the best weed control, even though bixlozone was slightly more persistent.

Whilst trifluralin mobility in soil is low, it rapidly volatilizes requiring, immediate incorporation into the soil (Horowitz et al., 1974; Kleemann et al., 2014). Confounding this, trifluralin is also tightly bound when it comes into contact with surface retained crop residue, reducing it's effective rate in the soil (Ashworth et al., 2010; Borger et al., 2013). The mixture of metolachlor/prosulfocarb also demonstrated poor persistence achieving a GR70 value of 1.8 weeks after application (i.e., 1.8 weeks to reach 70% growth reduction in annual ryegrass compared with untreated control) with the GR50 value of

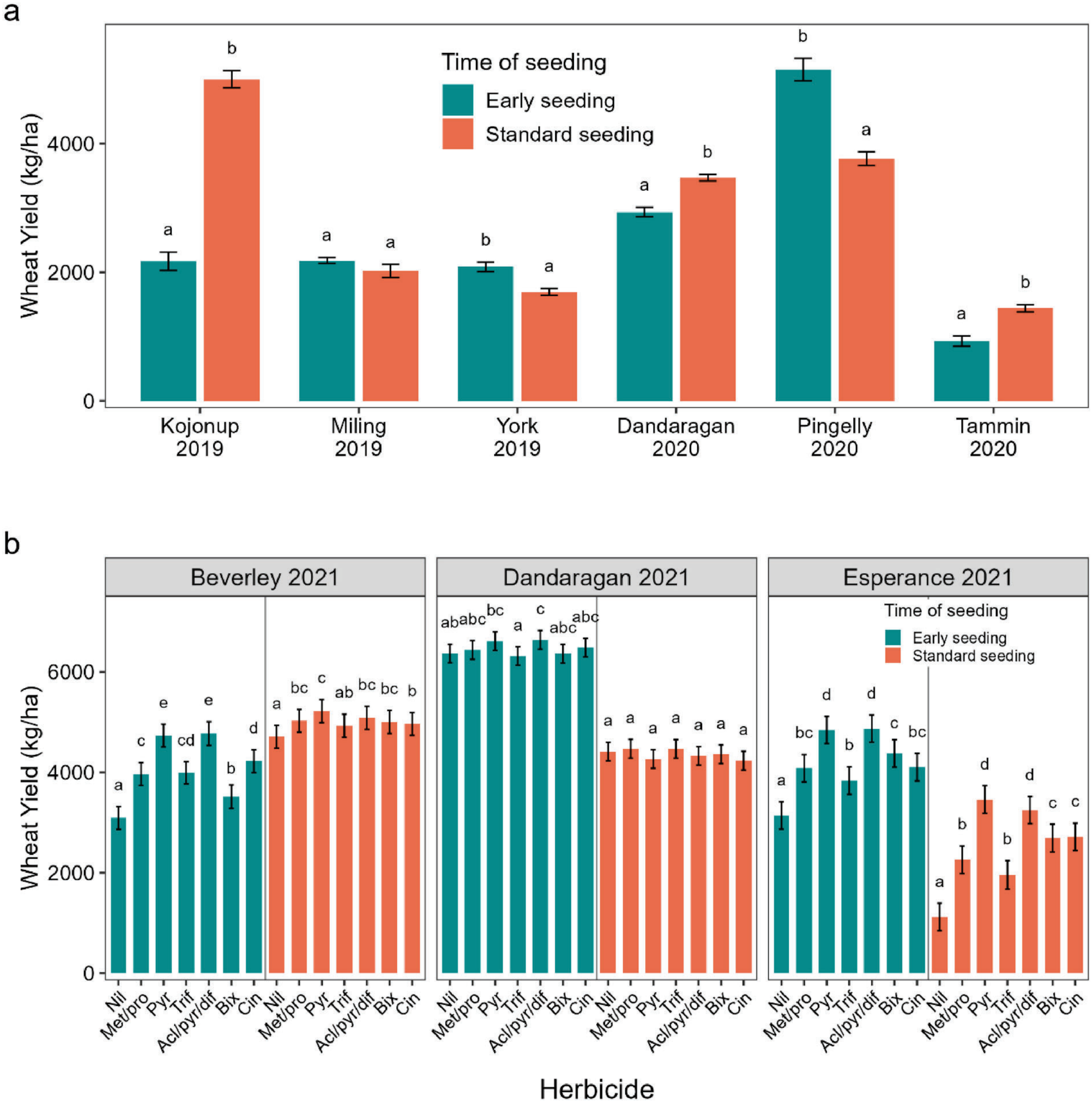

Fig. 4. Effect of site-year and time of seeding (TOS) on wheat yield in 2019–20 (a) and site-year, TOS and herbicide on wheat yield in 2021 (b). Different letters show significance at P ≤ 0.05. TOS comparisons are within each site-year (a) and herbicide comparisons within each site and TOS (b). Bars show ± SE (n = 4). Herbicide list includes nil, metolachlor/prosulfocarb, pyroxasulfone, trifluralin, aclonifen/pyroxasulfone/diflufenican, bixlozone and cinmethylin.

Table 7
Effect of crop seed rate and herbicide on wheat yield (kg ha^{-1}) in 2019-20. Different lower-case letters show significance between herbicides (within crop seed rate) and upper-case letters show significance between crop seed rates (within each herbicide) at P ≤ 0.05.

Seed rate	Nil	Met/prosul	Pyrox	Trif
Low	2476a A	2671b A	2763c A	2692 b A
Moderate	2618a B	2745b A	2847c AB	2872c B
High	2651a B	2812b B	2868 b B	2829 b B

Table 8
Effect of crop seed rate, time of seeding (early, standard) and site on wheat yield (kg ha^{-1}) across 2021 sites. Different lower-case letters show significance between times of seeding (within each site and seed rate) and upper-case letters show significance between crop seed rates (within each site and time of seeding) at P ≤ 0.05.

Seed rate	Beverley 2021		Dandaragan 2021		Esperance 2021	
	Early	Standard	Early	Standard	Early	Standard
Low	3981a A	4710 b A	6363 b A	4270a A	3995 b A	2237a A
Moderate	4113a A	4960 b B	6509 b A	4404a A	4207 b AB	2497a B
High	4038a A	5310 b C	6518 b A	4424a A	4342 b B	2750a C

7.7–10.0 weeks after application, depending on conditions. While metolachlor/prosulfocarb was slightly more persistent than trifluralin, it is highly mobile in soil leading to significant dissipation following rainfall events (Scherner et al., 2018), likely resulting in higher ryegrass seed production (Boutsalis et al., 2010).

Cinmethylin demonstrated intermediate persistence of between 6.3 and >14 weeks for GR70 and 8.5–>14 weeks for GR50. Cinmethylin is mobile in soil with a solubility of 63mg L^{-1} and a binding coefficient (KOC) of 300ml g^{-1}. This relatively high solubility makes cinmethylin effective for controlling annual ryegrass when rainfall is limited after sowing (Figueiredo et al., 2023). However this solubility can also be a disadvantage as significant rainfall after application can lead to unintended movement into the crop row, potentially resulting in herbicide injury or leaching of the herbicide deeper into the profile, likely limiting its persistence, as measured in this study (Russell et al., 1990, 1991). Cinmethylin is also moderately volatile with a vapor pressure of 10.2 mPa, similar to trifluralin at 9.5 mPa. As a result, cinmethylin should be incorporated within 3 days of seeding to maximise persistence (Grayson et al., 1987).

The herbicides pyroxasulfone, the premix aclonifen/pyroxasulfone/diflufenican and bixlozone consistently exhibited prolonged residual activity across sites and seasons, maintaining >50% reduction in ryegrass (bioassay) growth up to 15 weeks after application. Pyroxasulfone achieved a GR70 value of 8.0–16.0 weeks after application, with the GR50 value of 14.8–>18 weeks. Previous studies conclude that pyroxasulfone is persistent in soil (Kaur et al., 2025; Mueller et al., 2025), with resultant weed control and persistence influenced by application rate (Mueller and Steckel, 2011; Contreras et al., 2024). Pyroxasulfone is a highly effective pre-emergent herbicide for dry sowing, with a solubility of 3.5mg L^{-1}, limiting unintended vertical leaching following rainfall, and a low binding coefficient (K_{OC}) of 223 ml g^{-1}, reducing the amount bound to organic matter or stubble, ensuring herbicide effectiveness in moist soil (Khalil et al., 2018). When pyroxasulfone was used in combination with aclonifen and diflufenican, the GR70 value was similar at 12.9–18.1 weeks and the GR50 value increased at 16.6–>18 weeks. The mixing partner, Aclonifen is not mobile in the soil with a solubility of 1.4 mg L^{-1} and a binding coefficient (KOC) of 7126 ml g^{-1}. The lower solubility of aclonifen may contribute to the increased persistence of aclonifen/pyroxasulfone/diflufenican mixture compared to pyroxasulfone alone, making this mixture useful in higher rainfall locations.

Across years and locations in this study, bixlozone was consistently persistent, achieving a GR70 value 16.6–>18 weeks after application with the GR50 not being reached during the sampling duration. Bixlozone has been reported to have long persistence compared to many other herbicides, with a published DT50 (days to 50% dissipation) of 93 days when left on the soil surface. As bixlozone can be degraded by UV light exposure and has a vapor pressure of 2.3 mPa, incorporation into the soil at seeding further increases bixlozone persistence to a DT50 value of 179 days (Lewis et al., 2016; APVMA, 2020).

Across all site-years, pyroxasulfone and aclonifen/pyroxasulfone/diflufenican had the best control of annual ryegrass, followed by bixlozone, cinmethylin with trifluralin and meotolachlor/prosulfocarb having the least control.

Following application, rainfall is a key factor in pre-emergent herbicide performance. However, with lower and more variable rainfall conditions becoming more common during the crop growing season in Australia, the risk of dry soils not mobilising less soluble herbicides should be considered. Mixtures of non-persistent herbicides such as trifluralin or cinmethylin, that activate with less moisture, with highly persistent herbicides such as pyroxasulfone or bixlozone may be an effective strategy for ensuring effective weed control in dry-seeded conditions, requiring further research.

4.2. Shifting the competitive balance through herbicide use

The competitive interaction between crops and weeds is a key determinant of crop yield. This competitive effect is most pronounced when weeds emerge concurrently or soon after the crop. With long residual pre-emergent herbicides, the crop develops a competitive advantage, enabling crops to disproportionately utilise available resources through increased density and earlier establishment (Weiner, 1990). This effect was demonstrated in this study, with ryegrass growth and seed production reduced by using more residual preemergent herbicides such as pyroxasulfone or the premix aclonifen/pyroxasulfone/diflufenican significantly delayed weed establishment, whilst crop competitiveness was increased through early seeding and increased seeding rate.

This study found that increasing crop seed rate significantly reduced weed density, biomass, and weed seed production and increased yield, demonstrating the effectiveness of this non-chemical weed control tactic. The greatest reduction in ryegrass density, biomass and seed production occurred as the target wheat density was increased from the low target density (100 plants m^{-2}) to the moderate target density (150 plants m^{-2}), which was near the optimum wheat density for yield in Western Australia (Anderson et al., 2004). The effect of increasing wheat density in decreasing ryegrass seed production was consistent across all site-year combinations as previously demonstrated by Lemerle et al. (2004). The competitive effects of increased crop density can be further increased by reducing the spacing between crop rows, adopting more vigorous and higher biomass wheat cultivars, improved fertiliser rates at seeding to maximise early vigour, adopt north south row orientation to optimise interplant shading and even soil amelioration to overcome soil constraints such as subsoil acidity or compaction that can retard wheat growth (Shackley and Anderson, 1995; Lemerle et al., 2001; Borger et al., 2010, 2016, 2020; Kolb et al., 2012; Feledyn-Szewczyk and Jonczyk, 2017). Furthermore, in this study, treatments with lower target wheat density (100plants m^2) tended to have the lowest yield, indicating that sub-optimal plant populations constrain yield formation and underscore the need to match crop density to expected water supply (Anderson, 2010; Anderson et al., 2012).

The effect of seeding time varied across sites and seasons. At five of the nine sites tested from 2019 to 2021, early sowing (TOS1) resulted in higher ryegrass density and seed production than later seeding treatments. This was likely due to the lack of seedbank depletion from the lack of pre-seeding non-selective herbicide use, leading to more weeds establishing within the crop. These early emerging weeds disproportionately contribute to yield loss as increasing competition with the crop. The only site that demonstrated that early seeding reduced ryegrass seed production was Esperance in 2021. This result likely occurred due to excessive rainfall and cold conditions which limited wheat growth in the delayed seeding treatments (Supplementary information), thereby affecting crop growth and competitiveness. These results demonstrate the value of delayed seeding and the use of non-selective pre-plant “knockdown” herbicides in achieving excellent weed control on sites with high weed seed banks. In 2021, early seeding yield higher compared with delayed seeding at Dandaragan and Esperance.

This study found that early seeding increased wheat biomass and yield compared to delayed seeding at the Dandaragan, Tammin, York and Pingelly sites in 2019-20, and at the Dandaragan and Esperance sites in 2021. With sufficient stored soil moisture available for growth, early crop establishment allows crops to accumulate greater biomass due to increased soil temperatures and through improved access to light, water, and nutrients, whilst reducing temperature stress at maturity. However, earlier seeding times may increase exposure to frost in some environments, reducing yield (Rebetzke et al., 2005; Kirkegaard and Hunt, 2010; Crimp et al., 2016; Hunt et al., 2019).

5. Conclusion

This study demonstrates that integrating long-residual pre-emergent herbicides with increased wheat crop density provides a robust foundation for annual ryegrass control in early, dry-seeded wheat systems. However, at sites with high ryegrass seedbanks, these strategies may be insufficient, underscoring the importance of selecting fields with low weed seedbanks for dry seeding. Given the short seed dormancy of annual ryegrass, soil weed seedbanks can be effectively reduced through an integrated weed management approach. This includes diversified crop rotations that utilise a broad range of herbicides from different modes of action, coupled with competitive cropping and seed set elimination tactics through herbicidal crop-topping and harvest weed seed control. Successful weed management also requires systematic field scouting and prompt control of survivors to prevent any seedbank replenishment, to reduce subsequent infestations, and limit herbicide resistance selection.

CRediT authorship contribution statement

Mike Ashworth: Conceptualization, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Roberto Lujan Rocha:** Investigation, Visualization. **Shane Baxter:** Investigation. **Yaseen Khalil:** Investigation. **Zhanglong Cao:** Formal analysis. **Ken Flower:** Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr Michael Ashworth reports financial support was provided by Grains Research and Development Corporation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2026.107769.

Data availability

Data will be made available on request.

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