Herbicide mixtures and crop competition provide excellent control of annual ryegrass and mitigate herbicide resistance

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## Key words herbicide mixture, crop competition, annual ryegrass, wheat density, seed production

## Key messages

* A herbicide mixture of pyroxasulfone and prosulfocarb increased the control of resistant annual ryegrass by 11% (vs prosulfocarb alone) and 29% (vs pyroxasulfone alone) and reduced seed production of surviving resistant plants by 88% when compared to an untreated control.
* Crop competition helps to reduce annual ryegrass seed production. The effect of a competitive wheat crop at 150 plants/m2 reduced seed production of resistant plants by 56%. Increasing plant density to 300 plants/m2 did not further reduce seed set or production.

## Aims

## This research aimed to assess the effect of combined pre-emergence herbicide treatments and wheat competition on the survival and seed production of annual ryegrass plants resistant to pre-emergence herbicides, in order to incorporate this tactic into integrated weed management programs and delay the evolution of pre-emergence herbicide resistance.

## Introduction

Herbicide resistance evolution is challenging the sustainability and profitability of most world cropping systems (Walsh & Powles 2004). This problem also affects Australian farmers by increasing their costs and crop revenue losses. By far, annual ryegrass (*Lolium rigidum* Gaud.) is the most prevalent weed in southern Australian cropping areas, with $50.3 million annual revenue losses in Western Australia (WA) alone (Llewellyn & Ronning 2016).

Ryegrass resistant to post-emergence herbicides occurs across the WA wheatbelt, but resistance to pre-emergence herbicides like trifluralin, pyroxasulfone and prosulfocarb remains relatively low (Owen & Powles 2018). In South Australia (SA), a population was confirmed resistant to all of these pre-emergence herbicides (Brunton et al 2018, Brunton et al. 2019).

Resistance to pyroxasulfone in annual ryegrass has been described as metabolism-based (Busi et al 2018). Moreover, a multiple-resistant population obtained after three generations of low-dose recurrent pyroxasulfone selection by Busi et al (2012) was also cross-resistant to prosulfocarb and triallate (Busi & Powles 2013).

In this context of herbicide resistance, research on the most effective use of pre-emergence herbicides in an integrated weed management program is needed. The research in the current study was focused on crop competition as affected by wheat plant density. While other practices, like reducing row spacing, E-W sowing, competitive variety selection and harvest weed seed control are also important, they were not the focus of this research.

The impact on general weed performance (as opposed to specifically herbicide-resistant populations) of increasing the crop seeding rate has been studied by several authors. For example, Lemerle et al (2004) studied increasing wheat seeding rates in nine experiments across the states of New South Wales (NSW), SA, WA, and Victoria (Vic). On average, doubling the crop density from 100 to 200 plants/m2, reduced annual ryegrass biomass from 100 to 50 g/m2. Having evaluated wheat densities from 50 to 400 plants/m2, they concluded that at least 200 plants/m were needed to suppress annual ryegrass.

Similarly, in a multi-site experiment conducted during 2010 and 2011 in three locations across WA, Borger et al (2016) found significant annual ryegrass fecundity reduction when crop densities were increased from 117 to 178 plants/m2 in three of six experiments. The reduction in weed seed production ranged from 34 to 75%.

In the current study, the focus was on the response of annual ryegrass that is resistant to pre-emergence herbicides, to determine if a combination of crop competition and herbicide mixtures could improve the control of resistant populations.

## Method

### The research was conducted in the plant growth facilities at The University of Western Australia. It consisted of a factorial experiment in pots where herbicide-resistant ryegrass biotypes were treated with different herbicide treatments under different wheat densities. The following factors were tested:

### - Two ryegrass populations (“SLR31” and “M3/54”) obtained from SA and WA fields and then selected after four cycles of pyroxasulfone recurrent selection to develop herbicide resistance (Busi & Powles 2013, Busi et al unpublished).

### - Two genotypes per population (parental resistant and an F1 obtained after the crossing with a susceptible population).

### - Four herbicide treatments (pyroxasulfone at two different dosages, prosulfocarb, a two-way mixture of pyroxasulfone + prosulfocarb versus an untreated control) (Table 1).

### - Three wheat densities (0, 150 and 300 plants/m2).

**Table 1. Herbicide treatments**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Treatment | Herbicide | Commercial name | A.I concentration | Formulation | Rate applied g a.i. ha-1 | Figure code |
| 1 | Untreated control |  |  |  |  | Control |
| 2 | Pyroxasulfone | Sakura® | 850 g ai/kg | WG | 102 | Pyr 51 |
| 3 | Pyroxasulfone | Sakura® | 850 g ai/kg | WG | 51 | Pyr 102 |
| 4 | Prosulfocarb | Arcade® | 800 g ai/l | EC | 2500 | Pros 2500 |
| 5 | Pyroxasulfone + Prosulfocarb | Sakura® + Arcade® |  | WG + EC | 102 + 2500 | Mix |

*Wheat competition*

Three wheat densities were sown using a target-neighbourhood model to test the effect of wheat competition on annual ryegrass (Vila‐Aiub et al 2009). The densities were a control with no wheat plants, a commonly used plant density of 150 plants/m2 and double that density (300 plants/m2) (Figure 1).

0 plants/m2

150 plants/m2

300 plants/m2

**Figure 1. Target-neighbourhood model used to test the effect of wheat competition on annual ryegrass. Closed circles () represent ryegrass plants and open circles () represent wheat plants. Wheat density is shown**.

*The experiment*

The wheat (var. Scepter ) seeding date was 06 June 2019. Ryegrass seeds were pre-germinated in a 0.6% (w/v) agar medium. When the primordial roots were starting to be visible, 20 seeds per pot were transplanted on 11 June 2019. Herbicide was sprayed directly onto the weed seeds one day later, to simulate a post-sow, pre-emergence application (PSPE) in the field, using a dual-nozzle (Teejet® XR11001) cabinet sprayer delivering herbicide at 106 L/ha.

To guarantee a uniform wheat plant number, 20 seeds per pot were sown and then one week after their emergence, extra plants were manually eliminated. Ryegrass survival was assessed 28 days after the herbicide application. At that time, every pot that exceeded four surviving plants had the remaining plants removed to ensure a constant ryegrass density of 80 plants/m2. During the experiment, all the pots were irrigated and fertilised as needed.

Harvest was done when the plants reached maturity, on 22 October 2019 (wheat) and on 14 November 2019 (ryegrass). After harvest, heads were threshed and seeds counted. Final plant biomass was oven-dried and then weighed.

*Statistical analysis*

Survival data was analysed with a GLM binomial analysis. Seed production, biomass and wheat yield were analysed using ANOVA. To compare significant treatments, we performed Tukey post-hoc analysis. A linear regression was done to test the effect of surviving annual ryegrass biomass on wheat yield. All the analyses were done using the open-source statistical software *R* (version 3.5.1).

## Results

### Survival

No differences were observed between the two analysed populations. Therefore, all of the analyses were performed on combined population data. On the other hand, there was a significant interaction between the herbicide treatments and the genotypic condition (i.e. parental F0 vs. crossed F1) effect (p<0.0001).

The mixture of prosulfocarb and pyroxasulfone was the most effective treatment, regardless of the genotypic condition of the population (Figure 2). Prosulfocarb also showed high efficacy and resulted in a survival lower than 20% for both the parental and F1 populations. Pyroxasulfone treatments showed an interaction effect, where the F1 crosses had fewer surviving plants when compared with the F0 resistant parents. In the full-dose treatment, the survival of the F0 was 43% on average and 23% for the F1. This observed difference may be explained by the semi-dominant inheritance of pyroxasulfone resistance (Busi et al 2014).

**Figure 2. Annual ryegrass survival (28 days after herbicide application), where white bars represent the parental resistant population (F0) and grey bars the crosses with a susceptible population (F1). Refer to Table 1 for herbicide treatments. Different letters indicate significant differences after a post-hoc Tukey test (p<0.05). Error bars represent standard error.**

### Seed production

Ryegrass seed production was affected by wheat density and by the herbicide treatment. When analysed by the wheat density factor alone, Figure 3 shows that there was a significant reduction in seed production in the presence of wheat, but that doubling its density did not further reduce seed production (p<0.0001). On average, the 150 wheat plants/m2 treatment reduced the ryegrass seed production by 56% when compared to the control with no wheat present.

**Figure 3. Effect of wheat density on ryegrass seed production (all herbicide treatments are pooled). Different letters indicate significant differences after a post-hoc Tukey test (p<0.05). Error bars represent standard error.**

While the average of the prosulfocarb and the full-dose pyroxasulfone treatment reduced seed production by 46%, the mixture of both herbicides reduced it by 88% when compared to the untreated control (Figure 4). Although the interaction is not shown in order to clarify the analysis, the treatment that resulted in the lowest seed production was the herbicide mixture with 300 wheat plants/m2 (4 208 seeds/m2).

**Figure 4. Effect of herbicide treatment on ryegrass seed production (all wheat densities are pooled). Refer to Table 1 for herbicide treatments. Different letters indicate significant differences after a post-hoc Tukey test (p<0.05). Error bars represent standard error.**

### Wheat yield

In the case of wheat yield, there were no significant interactions between sowing density and herbicide treatment. Wheat density was a significant factor (p<0.0001), with the highest yields obtained with the highest density. On average, the 300 plants/m2 treatment yielded the equivalent of 6.2 t/ha, while the density of 150 plants/m2 yielded 5.3 t/ha. However, these yields should not be extrapolated to a farming situation, since water and nutrition were never limiting factors.

Herbicide was the other significant factor that affected wheat yield (p<0.0001). Due to the irrigation effect, the differences between treatments were reduced with only the mixture differing significantly from the untreated control (Figure 5). This can also be seen in the regression analysis (Figure 6), where wheat yield was primarily explained by ryegrass biomass (R2 = 0.6; p<0.0001).

**Figure 5. Wheat yield of the different herbicide treatments. Refer to Table 1 for herbicide treatments. Different letters indicate significant differences after a post-hoc Tukey test (p < 0.05). Error bars represent standard error.**

**Figure 6. Regression of annual ryegrass biomass effect on wheat yield (p<0.0001). The equation relating ryegrass biomass and wheat yield without factoring by herbicide treatment is presented in the graph.**

## Cost analysis

Based on the above research findings, a brief economic analysis is presented where we compared the cost of doubling the wheat density with the cost of using herbicide mixture rather than single products (Table 1). Only direct costs are compared, with associated costs such as impact on machinery efficiency by increasing wheat density (and therefore being able to sow fewer hectares per day) not considered. Since this is just an example based on some cost assumptions a deeper analysis considering each farming procedure is recommended.

From a herbicide resistance perspective, herbicide mixtures are recommended (Beckie & Reboud 2009). If the cost of the mix is a limitation, these research findings can help growers to decide whether to partially compensate for the cost of the mix by saving on the cost of increasing the wheat density. The ideal wheat density for each farm situation should be considered when making these calculations.

Table 1. Cost comparison between the analysed herbicide treatments and the two analysed wheat densities.

|  |  |  |  |
| --- | --- | --- | --- |
|   | **Pyroxasulfone** | **Prosulfocarb** | **Mixture** |
| Cost ($/L or $/kg) | 340 | 9.45 |  |
| Dose (L/ha or kg/ha) | 0.12 | 3.125 | 0.12 + 3.125 |
| Cost per ha ($/ha) | 40.80 | 29.53 | 70.33 |
|  |  |  |  |
|   | **150 pl/m2** | **300 pl/m2** |  |
| Seeding rate (kg/ha) (1) | 76 | 152 |   |
| Cost ($/t) (2) | 278 |   |
| Seed treatment cost ($/kg) (3) | 0.04 | 0.04 |   |
| Cost ($/ha) | 24.14 | 48.29 |   |

## Comments:

## (1): Considering 90% of establishment, 90% of germination, and a 1000-seed weight of 41 g (Wheat variety sowing guide for Western Australia. Trainor et al 2019).

## (2): APW Five-year average price (From Farm gross margin and enterprise planning guide 2019, SA Rural Solutions, 2019).

## (3) Smuts and Bunts treatment (From Farm gross margin and enterprise planning guide 2019, SA Rural Solutions, 2019).

## Conclusion

Crop competition is a crucial tool to compete against weeds. However, in this experiment doubling the wheat plant density did not help significantly and had a differential cost of approximately $24.

Use herbicide mixtures! Besides the fact that they do slow the chance of developing herbicide resistance within a population, this trial also showed that by mixing two herbicides with different sites of action the survival and seed production of pyroxasulfone-resistant populations can be significantly decreased.

## Acknowledgements

This research was part of my Masters thesis carried out at UWA during 2019. I would first like to thank the Argentinian government and the BEC.AR program that are supporting my studies in Australia. The research grant was supported by Royalties for Regions. I also thank the invaluable help provided by my supervisors, Dr Roberto Busi, Prof. Hugh Beckie and Dr Danica Goggin.

It was a big experiment! I could not have done it without the help of Carolina Martino, Martina Badano, Emanuel Gomez, Ci Sun, Justina Serrano, Sergio Banchero, Santiago Bertacca, Shane Baxter, and Paula Reeve. All of them provided some help to conduct the experiment.

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