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*CORRESPONDENCE Bhagirath S. Chauhan S. chauhan@uq.edu.au

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Novel use pattern of pendimethalin for annual ryegrass (*Lolium rigidum* Gaudin) control in wheat and barley

Gulshan Mahajan^{1,2} and Bhagirath S. Chauhan^{3*}

¹Queensland Alliance for Agriculture and Food Innovation (QAAFI), The University of Queensland, Gatton, QLD, Australia, ²Department of Agronomy, Punjab Agricultural University, Ludhiana, India, ³Queensland Alliance for Agriculture and Food Innovation (QAAFI) and School of Agriculture and Food Sustainability (AGFS), The University of Queensland, Gatton, QLD, Australia

Annual ryegrass (Lolium rigidum Gaudin) is a problematic weed in most of the cropping regions in Australia. Evolved herbicide resistance to multiple modes of action groups, the lack of new herbicidal molecules, and the emergence of late cohorts in winter season crops necessitate the use of existing herbicides in an innovative way to control L. rigidum. Pendimethalin as pre-emergence (PRE) is used for L. rigidum control; however, it is unable to control late cohorts of L. rigidum in winter season crops. Pot studies were conducted from 2019 to 2021 to evaluate the effect of a post-emergence (POST) application of pendimethalin with six doses (0, 220, 440, 880, 1,760, and 3,520 g a.i. ha⁻¹) on *L. rigidum* control in addition to crop safety in wheat, barley, and sorghum. POST application of pendimethalin was found to be safe for wheat and barley, and the biomass reduction in both crops at varied rates (220 to 3,520 g ha⁻¹) of pendimethalin ranged from 10% to 20%. A dose-response study revealed that the pendimethalin dose required for 50% mortality and growth reduction of L. rigidum was 179 and 144 g ai ha⁻¹, respectively. Pendimethalin at 880 g ai ha⁻¹ completely killed late cohorts of L. rigidum. These results suggest that the POST application of pendimethalin can be used for the effective control of late cohorts of L. rigidum in wheat and barley crops. POST application of pendimethalin suppressed the biomass of sorghum plants compared with the non-treated control treatment, and biomass reduction in sorghum at different pendimethalin doses ranged from 32% to 40%. Results implied that POST applications of pendimethalin in wheat and barley can diversify herbicide programs in managing L. rigidum and may help in delaying the evolution of resistance in this weed.

KEYWORDS

barley, crop suppression, GR₅₀, LD₅₀, mortality, wheat, sorghum

1 Introduction

Annual ryegrass (*Lolium rigidum* Gaud.) is the most prevalent weed across the southern cropping region of Australia (Gill, 1996; Peterson et al., 2018). This weed infests a large area (~8 m ha) in Australia and is responsible for a revenue loss of approximately AU \$ 93 million each year to Australian grain growers (Llewellyn et al., 2016). *Lolium rigidum* has evolved resistance to herbicides in multiple modes of action groups in Australia, and the additional cost involved in resistance management of *L. rigidum* was estimated to be AU\$ 103 million annually (Llewellyn et al., 2016).

The adoption of no-till production systems has favored the infestation of *L. rigidum* throughout Australia (Goggin et al., 2012). Tillage and residue-burning practices for weed control were mostly eliminated in no-till production systems, and the frequent use of herbicides for weed control in these systems has led to herbicide resistance in this weed (Pearce and Holmes, 1976; Walsh et al., 2019). Currently, this weed has evolved resistance to 11 different herbicide modes of action groups, including acetyl coenzyme-A carboxylase (ACCase) inhibitors, acetolactase synthase (ALS) inhibitors, and the 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase inhibitor glyphosate, and the first unique case of herbicide resistance in *L. rigidum* was reported from Australia (Heap, 2021). This suggests that it is the most predominant herbicide-resistant weed in Australia.

The seed production potential of *L. rigidum* is very high, and it has been reported that in a wheat (*Triticum aestivum* L.) crop, a single plant of this weed can produce 45,000 seeds m⁻² (Rerkasem et al., 1980). Multiple cohorts of *L. rigidum* in paddocks make the control of this weed difficult, resulting in plants not being exposed to either pre-emergence (PRE) or post-emergence (POST) herbicide control in the winter season (Chauhan et al., 2006; Owen et al., 2011). *Lolium rigidum* seeds have innate dormancy, which prevents the complete germination of *L. rigidum* in the summer season (Gramshaw and Stern, 1977; Steadman et al., 2003). A reduction in wheat yield by 50% was reported with a heavy infestation (200 plants m⁻²) of *L. rigidum* compared with weed-free conditions (Wu et al., 1998). Similarly, a 40% reduction in barley (*Hordeum vulgare* L.) yield was reported with *L. rigidum* infestation of 300 plants m⁻² (Lemerle et al., 1995).

With the onset of a favorable environment (autumn and start of winter seasons) for germination, 40%–60% of seeds germinate and residual viable seeds increase their infestation in succeeding winter crops by producing multiple cohorts. Australian farmers, in general, sow winter crops early to utilize the residual soil moisture, and in that scenario, late cohorts of *L. rigidum* after crop emergence make its management difficult as they escape from herbicide application (Gill, 1996; Chauhan et al., 2006).

Recently, it has been observed that this weed is emerging in summer seasons due to changing seasonality (Thompson et al., 2021). The occurrence of this has been noticed in the cotton (*Gossypium hirsutum* L.) crop of eastern Australia (Thompson and Chauhan, 2022). The introduction of glyphosate-tolerant cotton in Australia and the heavy reliance on glyphosate for weed control in fallows have resulted in the occurrence of glyphosate resistance in this weed (Pratley et al., 1996; Powles et al., 1998). In winter crops, POST herbicides (most commonly used groups 1 and 2) are not able to provide effective control of *L. rigidum* (Boutsalis et al., 2012). The literature suggests that PRE herbicides (e.g., dinitroaniline) are relatively more effective for *L. rigidum* control compared with POST herbicides (Brunton et al., 2018). However, PRE herbicides exhibit varying residual activity, consequently offering different durations of control, some of which may encompass the period of *L. rigidum* emergence. It was hypothesized that if PRE herbicides are to be applied as POST to crops (e.g., at the 5–6-leaf stage of crops), they may provide effective control of late emerging *L. rigidum*. However, information is lacking regarding the phytotoxicity in crops with the use of PRE herbicides if applied POST to crops.

Pendimethalin, a dinitroaniline herbicide, is widely used for the control of grass and broadleaf weeds in cereal crops (Vighi et al., 2017). It is mainly applied as a PRE but is sometimes also used as a POST (Vighi et al., 2017). There is no information available on the control of late emerging *L. rigidum* by pendimethalin if used as POST in crops and the application rates that are safe to crops, such as wheat, barley, and sorghum [*Sorghum bicolor* (L.) Moench]. Wheat is the most dominant winter cereal and sorghum is the most dominant summer grain crop grown in Australia. This study investigated the novel use pattern of pendimethalin and its phytotoxicity to wheat, barley, and sorghum. This study also evaluated the effect of the rate of application of pendimethalin on *L. rigidum*.

2 Materials and methods

The studies were conducted at the research facilities of the University of Queensland, Gatton, Australia, from 2018 to 2020. The seeds of *L. rigidum* used in this study were collected from Griffith, New South Wales. Appropriate permissions for the collection of weed seeds were obtained. After collection, seeds were dried in sunlight for 3 days and then stored at room temperature ($25^{\circ}C \pm 2^{\circ}C$) in the Weed Science Laboratory of the Queensland Alliance for Agriculture and Food Innovation (QAAFI), The University of Queensland, Australia, until used for experimental purpose.

The first experiment on wheat (variety: Spitfire), barley (variety: Commander), and L. rigidum was conducted in April 2019, and the second experiment was conducted in April 2021. The first experiment on sorghum (variety: Sentinel IG) was conducted in March 2019, and the second experiment was conducted in August 2019. All pendimethalin treatments in each species (wheat, barley, sorghum, and L. rigidum) were evaluated in a randomized complete block design. In each experiment, there were three replications in the first experiment and four replications in the second experiment. The studies were conducted in pots (20 cm diameter and 20 cm height) filled with potting mix containing biological organic-based products and natural rock substrates (Centenary Landscape, Australia). The potting mix had a pH of 6.3 and an electrical conductivity of 1.42 dS m⁻¹. No fertilizer was added to the plants. The pots were kept on benches and maintained in an open environment and regularly watered with an automatic sprinkler

system of irrigation (two times per day). Wheat and barley were sown with 15 seeds per pot. Sorghum was planted with six seeds per pot. *Lolium rigidum* was sown with 20 seeds per pot, and these pots were sown 3 weeks after wheat and barley planting to simulate the emergence of late cohorts.

The wheat, barley, and sorghum were sprayed with different pendimethalin rates (0, 220, 440, 880, 1,760, and 3,520 g ai ha⁻¹) 3 weeks after planting (5–6-leaf stage). Pots of *L. rigidum* were also sprayed (as PRE) during that time. As there is no post-emergent recommendation for this herbicide, the rates were chosen based on the pre-plant recommended rate (440 g ai ha⁻¹) of pendimethalin for *L. rigidum* in wheat and barley. Herbicide spray was done with a research track sprayer using Teejet XR 110015 flat fan nozzles and a spray volume of 108 L ha⁻¹ was used at 200 kPa. Pots were kept without watering for 24 h after herbicide application and thereafter regularly watered.

The mortality percentage for pendimethalin-treated plants was assessed based on a comparison with non-treated control plants. Plants were considered dead if no new growth or green tissue appeared after herbicide application. For aboveground biomass measurements, plants in each pot were cut close to the base at 28 days after treatment, ovendried at 70°C for 72 h, and weighed by individual pots. The biomass data were converted into percent biomass reduction compared with the non-treated control (see Equation 1).

Percent biomass reduction =
$$[(C - D)/C] * 100$$
 (1)

where C is the mean biomass of the non-treated control pot and D is the mean biomass of the pendimethalin-treated pot.

Data were subjected to analysis of variance (ANOVA) using Genstat (16th Edition; VSN International, Hemel Hempstead, UK). There was no interaction between the two experiments and treatments; therefore, data were pooled for the two experiments. The biomass reduction of wheat, barley, sorghum, and *L. rigidum* was assessed at $P \leq 0.05$ using Fisher's protected least significant difference (LSD) test.

Mortality and biomass reduction of *L. rigidum* (as a percentage compared with the non-treated control) data were regressed over herbicide treatments using a non-linear regression of a four-parameter log-logistic model (see Equation 2) using SigmaPlot 14.0 (Systat Software, San Jose, CA, USA).

$$y = y_0 + \left[\frac{a}{1} + \left(\frac{x}{x_{50}} \right)^b \right]$$
(2)

Where y = mortality (%) or biomass reduction (%), $y_0 =$ bottom of the curve, a = difference between the top and bottom of the curve, $x_{50} =$ dose required to kill 50% plants (LD₅₀) or plant growth (GR₅₀), b = slope of the curve, and x = pendimethalin dose.

3 Results and discussion

Pendimethalin effectively controlled *L. rigidum*. The mortality of *L. rigidum* at pendimethalin 220 g ai ha^{-1} was 62%, which increased to 88% at 440 g ai ha^{-1} and 100% at 880 g ha^{-1} (Figure 1A). A similar response was found for biomass reduction of *L. rigidum* with pendimethalin application. The lethal dose of

pendimethalin for 50% control of *L. rigidum* was 179 g ai ha⁻¹ (Figure 1A). Similarly, the pendimethalin dose required for 50% biomass reduction of *L. rigidum* was 144 g ai ha⁻¹ (Figure 1B). These observations suggest that pendimethalin effectively controlled *L. rigidum*. No mortality in wheat, barley, and sorghum crops was observed when pendimethalin was applied at different rates as POST (21 days after planting).

In wheat and barley crops, the biomass reduction with pendimethalin doses varied from 10% to 20% (Figures 2A, B). Pendimethalin at 880 g ha⁻¹ caused a 16% biomass reduction in both crops; at this dose, 100% control of *L. rigidum* was achieved. In sorghum crops, phytotoxicity of pendimethalin was observed in terms of biomass reduction (Figure 2C). Pendimethalin caused a biomass reduction of 38%–44% in sorghum and was the highest at 3,520 g ha⁻¹

Pendimethalin kills susceptible plants by inhibiting mitotic cell division, and tolerant species grow through by having a root system below the treated zone (Vencill, 2002). A previous study reported that a POST application of pendimethalin 1,000 g ai ha⁻¹ at the 2-leaf stage of barley was safe for the crop and effectively controlled Italian ryegrass (*Lolium multiflorum* Lam.) (Alshallash, 2015).



The dose–response curve of *Lolium rigidum* for **(A)** mortality percentage and **(B)** biomass reduction percentage. The curve is a log-logistic regression model fitted to the data.

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POST application of pendimethalin is not recommended for wheat, barley, and sorghum crops in Australia. As pendimethalin POST caused major reductions in sorghum biomass, it is unlikely to be recommended in this crop. However, these results confirm that the POST application of pendimethalin is safe for wheat and barley and can provide effective control of late cohorts of *L. rigidum*. There was no visual injury at any rate of pendimethalin to wheat and barley. The biomass reduction in both crops was 16% at pendimethalin 880 g ai ha⁻¹. As the crop biomass was recorded 4 weeks after pendimethalin application, little suppression was expected. This minor level of suppression is expected to recover at a late stage of the crop; however, this needs verification under field conditions. The minor suppression also needs to be tested under field conditions for a trade-off relationship between grain yield and *L. rigidum* control.

Glyphosate and paraquat are widely used herbicides for L. rigidum control in fallows or as pre-plant control in crops. Under crop situations, ACCase- and ALS-inhibiting herbicides are mostly used for the control of L. rigidum (Owen et al., 2011; Loureiro et al., 2017). However, widespread resistance of L. rigidum to these herbicides in the no-till system has shifted the herbicides' pattern toward PRE herbicides (Boutsalis et al., 2014). During the last decade, farmers have diverted their attention to sowing winter crops early to avail the opportunity of residual soil moisture (Anderson et al., 2005). In that situation, late germinating L. rigidum plants resistant to ACCase- and ALS-inhibiting herbicides make weed management complicated. PRE herbicides can be used in that situation; however, limited residual activity for a certain period of these herbicides is a crucial factor to provide season-long weed control. It has been reported that the surface-applied half-life of pendimethalin may occur in 4-6 days due to photochemical, volatilization, and other degradation processes (Savage and Jordan, 1980; Vencill, 2002). Under these circumstances, a POST application of pendimethalin could be another strategy for seasonlong L. rigidum control; therefore, registration of pendimethalin as POST in these crops needs to be considered. The risk of resistance increases if pendimethalin is allowed to be used twice in one season or as a POST with no follow-up control of survivors.

Overreliance on ACCase and ALS inhibitor herbicides has led to resistant populations as these herbicides create a very high selection pressure (Powles et al., 1998; Saini et al., 2016). Moreover, the continuous use of a herbicide with a single mode of action could lead to the evolution of resistance to that specific herbicide, necessitating the use of a herbicide with another mode of action in the herbicide rotation program (Jasieniuk et al., 1996). Mixing and rotating herbicides with different modes of action is a useful strategy to slow down the evolution of resistance (Vogwill et al., 2012; Lagator et al., 2013). Hence, diversifying weed control options with the use of pendimethalin as a POST could provide sustainable control of *L. rigidum*.

This research was conducted in pots; therefore, further testing of pendimethalin POST for crop safety is required under field conditions. It is important to assess the trade-off relationship between crop suppression due to the POST application of pendimethalin and grain yield under field situations and various soil and climatic conditions for robust recommendations. There is also a need to assess the yield losses in different crops with late cohorts of *L. rigidum*. We exclusively utilized one variety per crop species. Hence, future studies should encompass multiple varieties to ascertain whether there exists a variety-specific response to the POST application of pendimethalin.

In conclusion, the POST application of pendimethalin (880 g ai ha^{-1}) can be successfully used in wheat and barley and could provide effective control of late cohorts of *L. rigidum*. Further

information is required regarding the environmental impact of POST-application pendimethalin, its potential for soil pollution reduction, the absence of herbicide residues in grains, and its impact on beneficial soil microorganisms. Additionally, investigation is needed to determine the viability of tank mixing pendimethalin with group 1 or 2 herbicides for season-long, broad-spectrum control of *L. rigidum* in wheat and barley. Cost-effectiveness assessments are necessary to evaluate if these combinations can control emerged weeds with POST herbicides while utilizing pendimethalin for residual activity against late-germinating *L. rigidum*. Moreover, testing the effectiveness of POST-application pendimethalin in combination with non-selective herbicides like glufosinate, glyphosate, and paraquat for the season-long control of *L. rigidum* in fallow areas and along fence lines is also imperative.

Data availability statement

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Author contributions

GM: Formal analysis, Investigation, Methodology, Writing - original draft. BC: Conceptualization, Funding acquisition,

Investigation, Methodology, Project administration, Writing – review & editing.

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